PG. DIP. IN CRIMINOLOGY & POLICE ADMINISTRATION **PAPER-5**

CRIMINALISTICS AND PHYSICAL EVIDENCES

Department of Distance Education
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Lesson No. :

UNIT-III

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Note : Students can download the syllabus from department's website www.dccpbi.com.

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ARSON

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Why Investigate Fires?
The Problem of Fire Investigations
Then Who Investigates Fires?
Preserve the Fire Scene
Documenting the Scene
Photograph/Videotape the Scene
Identify, Collect, and Preserve Evidence
Prevent Contamination
Establish and Maintain the Chain of Custody

INTRODUCTION

It is a capital mistake to theorize before one has data. Insensibly, one begins to twist facts to suit theories instead of theories to suit facts.

—Sherlock Holmes, *A Study in Scarlet,* by Sir Arthur Conan Doyle

As Sherlock Holmes pointed out, many types of investigations are susceptible to prejudgment, but few as often as fire scene investigations. Fires, by their destructive nature, consume the evidence of their initiation and progress as they grow. Investigations are compromised, and often scenes are further destroyed by the activities of the fire service, whose primary responsibilities are to save lives and protect property against further damage. Fire scenes often involve all manner of public entities: emergency medical, law enforcement, and fire services. Public utilities such as gas and electric companies may be involved. Passers-by, owners, tenants, customers, delivery agents all may have relevant information. The press and curious individuals attracted to large fire scenes can complicate investigations, as they make security a necessity. As has frequently been said, “A fire investigation is like a picture puzzle. Everyone involved with it has some of the pieces, but no one has the whole picture. It is up to the investigator to gather enough of these pieces together to solve the puzzle.”

WHY INVESTIGATE FIRES?

Since Roman times, civil authorities have recognized the threat that fire represents, not only to the well-being of individuals, but also, and perhaps more importantly, to the welfare and security of the community as a whole. In the days of wooden walls and roofs and straw-covered floors, any fire could ravage an entire city. So, it was in the interest of all concerned to investigate fires and establish how they began. Civil

authorities attempted to control the fire risk by assessing penalties if an accidental fire was allowed to get out of control. Dangerous practices, such as leaving cooking fires unguarded, were identified and controlled. William the Conqueror issued an edict that cooking fires be damped or covered after a particular time of evening so that unattended fires could not flare up. This policy of couvre feu (cover the fire) gave rise to the "curfew" of today. If authorities could determine the fire was deliberately set, the perpetrator could be identified and punished. Some of the oldest English common laws regarded arson to be the crime of burning the house or dwelling of another. The crime of arson was considered to be such a danger that it was punishable by death. The same rationale applies today. Fires of accidental cause need to be identified, so that dangerous practices, such as filling kerosene room heaters with gasoline, can be eliminated by public education, or so that defective or dangerous products, such as instant-on televisions or room heaters with no overheating or tip-over protection, can be taken off the market or modified so they no longer pose a significant fire risk. Fires of incendiary (i.e., deliberate) cause must be detected, so that the fire setter can be intercepted before doing more harm and punished as necessary.

THE PROBLEM OF FIRE INVESTIGATIONS

The advantages of accurate and thorough fire investigations are obvious. The United States is one of the few countries where public authorities have statutory responsibility to investigate all fires and determine their origins and causes. While this may appear to be a solution to the problem of fires and arsons, a number of major complications in fire investigations exist in the United States:

- A fire can be a complex event whose origin and cause are not obvious. Investigators may have to expend considerable time and effort before the cause can be identified. This is the area where Holmes' dictum is especially applicable. Without gathering data, the investigator can only guess at what might have caused the fire, based on circumstances alone. The training and preparation of qualified investigators are often costly and time-consuming, requiring dedication to the profession over many years.
- The destructive power of the fire itself compromises evidence from the outset. The larger a fire becomes and the longer it burns, the less evidence of causation will remain. In some fires, sufficient data to establish the origin and cause (i.e., evidence) do not survive, no matter how diligent the search or well prepared the searcher. This destruction may be exacerbated by the normal and necessary duties of fire personnel carrying out rescue, suppression, overhaul, and salvage tasks.
- The complexity of the threat a major fire presents to the health and welfare of the community means that representatives from law enforcement, fire, rescue, and emergency medical services; hazardous materials teams; utility company personnel; health and safety officers; and other public agency personnel may be on hand and may conduct some obligatory official duties. The presence of so

many people, in addition to members of the press and the public who were attracted by the sights and sounds of a major fire, offers yet more chances for scene security to be compromised and critical evidence to be contaminated, moved, or destroyed.

- Responsibility for the investigation of fires is split. While the fire service has the primary civil responsibility to establish a fire's cause, if the cause is determined to be accidental, the scene is released to the owner or the owner's insurance company for further examination. If the conclusion is that the fire was purposely set, a crime has been committed and law enforcement authority is needed to investigate the crime. This often means releasing the scene and evidence to a local law enforcement agency. In a few cases, individuals have both law enforcement and fire authority, thanks to extensive cross-training, so cases are handled from start to finish by a minimal number of trained, motivated investigators.
- A lack of commitment to conduct fire investigations exists on the part of some law enforcement and fire agencies. Because of the demand for rescue, hazardous materials, and emergency medical assistance, in addition to their traditional duties of fire suppression, fire departments often find themselves with fewer resources to stretch to cover all obligations. As a result, the less visible responsibilities of fire investigation and fire prevention are often scaled back. These cutbacks occur despite the advantages that aggressive programs in both areas could provide to the individual department and to the community it serves: Preventing a fire means there is no loss of life or property, no risk to personnel, and no equipment costs; investigating a fire means that potential accidental or criminal threats to the community may be averted in the future. Law enforcement agencies, facing similar overwhelming demands for their time, might prefer not to become involved in cases where the scene is destroyed or at the very least compromised, time-consuming scene examination and interviews are required, and the resulting evidence is often complex and circumstantial (meaning prosecutors may not want to use it even if it is properly and completely collected).

THEN WHO INVESTIGATES FIRES?

As might be gathered from the preceding points, who actually will investigate a fire is not an easy question to answer. In addition to law enforcement and fire authorities, there may be local law enforcement agency, forensic laboratory experts, engineering specialists (fire, chemical, mechanical, or electrical), and private investigators representing insurance companies, owners, tenants, and manufacturers of the myriad ignition sources found in a modern home or business.

PRESERVE THE FIRE SCENE

Principle

Evidence at a fire scene takes many different forms, some of which are transient (i.e., they are not permanent and may disappear quickly, such as impressions in snow or evaporating liquids). First responders must understand how rescue, medical, fire suppression, overhaul, and salvage efforts can adversely affect different forms of evidence and take steps to preserve evidence accordingly. First responders should assess the fire scene to identify potential evidence, take preliminary steps to preserve it, and notify appropriate authorities about its existence.

Procedure

To preserve evidence, first responders should: A. Observe and mentally note evidence that may be present at the scene, such as:

- Fire patterns (including multiple fire locations).
- Burn injuries to victims and fire patterns on clothing.
- Trailers, ignitable liquids, or other unusual fuel distribution (e.g., piles of newspapers, furniture pushed together).
- Incendiary/ignition/explosive devices (e.g., lighters, matches, timing devices). Shoe prints and tire impressions.
- Broken windows and doors.
- Distribution of broken glass and debris.
- Indications of forced entry (tools and tool marks).
- Containers.
- Discarded clothing.
- Trace evidence (e.g., hairs, fibers, fingerprints, blood, other body fluids).
- Evidence of crimes in addition to the possible arson (e.g., weapons, bodies, drugs, clandestine drug laboratory equipment).
- Witnesses, bystanders, and victims.
- Any other unusual items or the absence of normal contents or structural components.

B. Recognize threats to evidence (i.e., its movement, removal, contamination, or destruction) from any of the following sources:

- Fire suppression activities, such as a straight stream applied at the point of origin or deluge applications that may wash away or dilute potential evidence.
- Overhaul activities that destroy fire patterns.
- Salvage activities that involve moving or removing potential physical evidence.
- Use of a tool in any manner that causes destruction of evidence.
- Movement of knobs, switches, and controls on appliances and utilities.
- Weather conditions that affect transient evidence (i.e., wind precipitation, or temperature changes).
- Personnel walking through the scene.
- Witnesses and victims leaving the scene.

- Medical intervention and treatment of victims (e.g., by damaging evidence at the scene or destroying victims' clothing).
- Premature removal or movement of bodies.
- Vehicles at the scene (e.g., that introduce fluid to the scene through vehicle leaks or destroy other evidence, including shoe prints and tire impressions).
- Contamination from external sources, such as fuel-powered tools or equipment.

C. Protect evidence by:

- Limiting excessive fire suppression, overhaul, and salvage.
- Avoiding needless destruction of property.
- Leaving bodies undisturbed.
- Flagging items of evidence with cones or markers.
- Recording observations through written notes or voice recordings.
- Covering items or areas containing evidence with objects that will not contaminate the evidence (e.g., clean boxes or tarpaulins).
- Isolating items or areas containing evidence with rope, barrier tape, barricades, or sentries.
- Retaining and securing clothing items removed from victims and suspects.
- Obtaining information about victims and witnesses (i.e., their names, addresses, and telephone numbers).
- Preserving transient evidence (e.g., trace evidence, shoe prints, tire impressions).
- Removing evidence at risk of imminent destruction by the fire or the structural collapse of the damaged building.
- Ensuring that later arriving investigators are fully apprised of the evidence discovered.

Summary

First responders should recognize items that may have evidentiary value in a subsequent investigation and take steps to protect them from damage that could result from the fire, fire suppression, or rescue efforts.

DOCUMENTING THE SCENE

PHOTOGRAPH/VIDEOTAPE THE SCENE

Principle: Photographic documentation creates a permanent record of the scene and supplements the written incident report(s), witness statements, or reports on the position of evidence. The investigator should create and preserve an accurate visual record of the scene and the evidence prior to disturbing the scene. Additional photography or videography should be done as the investigation progresses.

Procedure: The scene should be photographed prior to the disturbance or removal of any evidence and throughout the scene investigation. The investigator (or other individual responsible for evidence) should:

- Photograph and/or videotape the assembled crowd and the fire in progress.
- Remove all nonessential personnel from the background when photographing the scene and evidence.
- Photograph the exterior and interior of the fire scene (consider walls, doors, windows, ceilings, floors) in a systematic and consistent manner. (Videotaping may serve as an additional record but not as a replacement for still photography.)
- Photograph any points or areas of origin, ignition sources, and first material ignited.
- Photograph any physical reconstruction of the scene.
- Maintain photo and video logs. Record the date, the name of the photographer, and the subject.
- Determine whether additional photographic resources are necessary(e.g., aerial photography, infrared photography, stereo photography, photogrammetry).

Summary: Photographic documentation provides a permanent record of the scene.

IDENTIFY, COLLECT, AND PRESERVE EVIDENCE

Principle: Collecting evidence at a fire scene requires attention to documenting and maintaining the integrity of the evidence. The investigator should ensure that evidence collectors identify and properly document, collect, and preserve evidence for laboratory analyses, further investigations, and court proceedings, in accordance with *NFPA 921* and other recognized national guidelines, including American Society for Testing and Materials standards E860, E1188, and E1459. This will ensure that critical evidence is not contaminated or lost prior to analysis and that the chain of custody is maintained.

Procedure: To optimize the recovery and evaluation of physical evidence, evidence collectors should:

- Take precautions to prevent contamination. (See “Prevent Contamination.”)
- Document the location of evidence using written notes, sketches, photographs, photo and video logs, the evidence recovery log, evidence tags, and container labels. (See appendix A.) When evidence is excavated, additional photographs may be of value.
- Take special care to collect evidence in any areas of origin (such as the first fuel ignited and ignition source) in cases where the fire is not accidental.
- Pack evidence in labeled containers for transportation and preservation. Evidence collected for laboratory identification of ignitable liquids must be immediately placed in clean, unused, Vapor tight containers (e.g., clean,

unused paint cans; glass jars; laboratory-approved nylon or polyester bags) and then sealed.

- Label each container so that it is uniquely identified. Labeling may include the name of the investigator, date and time of collection, case number, sample number, description, and location of recovery.
- Collect and preserve suitable comparison samples but recognize that such samples may be unavailable.
- Package evidence in accordance with their laboratories' policies and procedures.
- Recognize the presence of other physical evidence, such as blood stains, shoe prints, latent prints, and trace evidence, and use proper preservation and collection methods or seek qualified assistance.

Summary: Proper collection and packaging preserve the value of physical evidence.

PREVENT CONTAMINATION

Principle: Preventing contamination during evidence collection protects the integrity of the fire scene and evidence. The investigator should ensure that access to the fire scene after fire suppression is controlled and that evidence is collected, stored, and transported in such a manner that it will not be contaminated.

Procedure: To prevent contamination, personnel (e.g., evidence collectors) should:

Note: *In cases where the fire appears to be accidental, evidence should not be needlessly disturbed, but the property owner or insurer should be notified to avoid issues of spoilation.*

- Establish and maintain strict control of access to the scene.
- Recognize that fuel-powered tools and equipment present potential contamination sources and should be avoided. When it is necessary to use these tools and equipment, the investigator should document their use.
- Wear clean, protective outer garments, including footwear.
- Use clean disposable gloves for collecting items of evidence. (To avoid cross-contamination, gloves should be changed between collection of unrelated items of evidence or when visibly soiled.)
- Use clean tools for collecting items of evidence from different locations within a scene. (Disposable tools also can be used.)
- Place evidence in clean, unused containers and seal immediately.
- Store and ship fire debris evidence containers of evidence collected from different scenes in separate packages.
- Package liquid samples to prevent leakage and ship them separately from other evidence.
- Store and ship fire debris evidence separately from other evidence.
- Follow any specific laboratory requests, such as submitting an unused sample container or absorbent medium for detection of any contaminants.

Summary: Attention to scene control and evidence collection and packaging helps to prevent contamination and ensures the integrity of the evidence.

PACKAGE AND TRANSPORT EVIDENCE

Principle: Preventing changes in the condition of a sample after it has been collected ensures the integrity of the evidence and requires controlled packaging and transportation. The investigator should ensure that packaging, transportation, and storage procedures are followed to prevent any destructive changes in the condition of samples.

Procedure: To minimize changes in the condition of samples, the personnel responsible for packaging and transport should:

- Take precautions to prevent contamination. (See "Prevent Contamination.")
- Package fragile items carefully.
- Freeze or immediately transport items containing soil to the laboratory.
- Transport all volatile samples to the laboratory in a timely manner.
- Comply with shipping regulations.

Summary: Adherence to approved packaging and transportation procedures safeguards the condition of the evidence and ensures its continued integrity.

ESTABLISH AND MAINTAIN THE CHAIN OF CUSTODY

Principle: Establishing and maintaining a chain of custody verifies the integrity of the evidence. The investigator should ensure that the chain of custody is maintained.

Procedure: Personnel responsible for the chain of custody should:

- Maintain written records documenting the sample number, description of the evidence, date and location where it was found, collector's name, and miscellaneous comments.
- Document all transfers of custody, including the name of the recipient and the date and manner of transfer.
- Document the final disposition of the evidence.

Summary: Maintaining the chain of custody for evidence, from collection through final disposition, ensures its integrity.

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DRUGS OF ABUSE AND DRUG ADDICTION

Introduction
Drug addiction and its problems
Classification of Abused Substances
Introduction to field tests and laboratory tests

INTRODUCTION

Humans have used drugs of one sort or another for thousands of years. Wine was used at least from the time of the early Egyptians; narcotics from 4000 BC; and medicinal use of marijuana has been dated to 2737 BC in China. But not until the 19th century AD was the active substances in drugs extracted. There followed a time when some of these newly discovered substances—morphine, laudanum, cocaine—were completely unregulated and prescribed freely by physicians for a wide variety of ailments. They were available in patent medicines and sold by traveling tinkers, in drugstores, or through the mail. During the American Civil War, morphine was used freely, and wounded veterans returned home with their kits of morphine and hypodermic needles. Opium dens flourished. By the early 1900s there were an estimated 250,000 addicts in the United States.

Drug addiction and drug abuse chronic or habitual use of any chemical substance to alter states of body or mind for other than medically warranted purposes. Traditional definitions of addiction, with their criteria of physical dependence and withdrawal (and often an underlying tenor of depravity and sin) have been modified with increased understanding; with the introduction of new drugs, such as cocaine, that are psychologically or neuropsychologically addicting; and with the realization that its stereotypical application to opiate-drug users was invalid because many of them remain occasional users with no physical dependence. Addiction is more often now defined by the continuing, compulsive nature of the drug use despite physical and/or psychological harm to the user and society and includes both licit and illicit drugs, and the term "substance abuse" is now frequently used because of the broad range of substances (including alcohol and inhalants) that can fit the addictive profile. Psychological dependence is the subjective feeling that the user needs the drug to maintain a feeling of well-being; physical dependence is characterized by tolerance (the need for increasingly larger doses in order to achieve the initial effect) and withdrawal symptoms when the user is abstinent.

Definitions of drug abuse and addiction are subjective and infused with the political and moral values of the society or culture. For example, the stimulant caffeine in

coffee and tea is a drug used by millions of people, but because of its relatively mild stimulatory effects and because caffeine does not generally trigger antisocial behavior in users, the drinking of coffee and tea, despite the fact that caffeine is physically addictive, is not generally considered drug abuse. Even narcotics addiction is seen only as drug abuse in certain social contexts. In India opium has been used for centuries without becoming unduly corrosive to the social fabric.

The United States has the highest substance abuse rate of any industrialized nation. Government statistics (1997) showed that 36% of the United States population has tried marijuana, cocaine, or other illicit drugs. By comparison, 71% of the population has smoked cigarettes and 82% has tried alcoholic beverages. Marijuana is the most commonly used illicit drug.

DRUG ADDICTION AND ITS PROBLEMS

Motivations for Drug Use

People take drugs for many reasons: peer pressure, relief of stress, increased energy, to relax, to relieve pain, to escape reality, to feel more self-esteem, and for recreation. They may take stimulants to keep alert, or cocaine for the feeling of excitement it produces. Athletes and bodybuilders may take anabolic steroids to increase muscle mass.

Effects of Substance Abuse

The effects of substance abuse can be felt on many levels: on the individual, on friends and family, and on society.

Effects on the Individual

People who use drugs experience a wide array of physical effects other than those expected. The excitement of a cocaine high, for instance, is followed by a "crash" : a period of anxiety, fatigue, depression, and an acute desire for more cocaine to alleviate the feelings of the crash. Marijuana and alcohol interfere with motor control and are factors in many automobile accidents. Users of marijuana and hallucinogenic drugs may experience flashbacks, unwanted recurrences of the drug's effects weeks or months after use. Sudden abstinence from certain drugs results in withdrawal symptoms. For example, heroin withdrawal can cause vomiting, muscle cramps, convulsions, and delirium. With the continued use of a physically addictive drug, tolerance develops; i.e., constantly increasing amounts of the drug are needed to duplicate the initial effect. Sharing hypodermic needles used to inject some drugs dramatically increases the risk of contracting AIDS and some types of hepatitis . In addition, increased sexual activity among drug users, both in prostitution and from the disinhibiting effect of some drugs, also puts them at a higher risk of AIDS and other sexually transmitted diseases . Because the purity and dosage of illegal drugs are uncontrolled, drug overdose is a constant risk. There are over 10,000 deaths directly attributable to drug use in the United States every year; the substances most frequently involved are cocaine, heroin, and morphine, often combined with alcohol or other drugs. Many drug users engage in criminal activity, such as burglary and

prostitution, to raise the money to buy drugs, and some drugs, especially alcohol, are associated with violent behavior.

Effects on the Family

The user's preoccupation with the substance, plus its effects on mood and performance, can lead to marital problems and poor work performance or dismissal. Drug use can disrupt family life and create destructive patterns of codependency, that is, the spouse or whole family, out of love or fear of consequences, inadvertently enables the user to continue using drugs by covering up, supplying money, or denying there is a problem. Pregnant drug users, because of the drugs themselves or poor self-care in general, bear a much higher rate of low birth-weight babies than the average. Many drugs (e.g., crack and heroin) cross the placental barrier, resulting in addicted babies who go through withdrawal soon after birth, and fetal alcohol syndrome can affect children of mothers who consume alcohol during pregnancy. Pregnant women who acquire the AIDS virus through intravenous drug use pass the virus to their infant.

Effects on Society

Drug abuse affects society in many ways. In the workplace it is costly in terms of lost work time and inefficiency. Drug users are more likely than nonusers to have occupational accidents, endangering themselves and those around them. Over half of the highway deaths in the United States involve alcohol. Drug-related crime can disrupt neighborhoods due to violence among drug dealers, threats to residents, and the crimes of the addicts themselves. In some neighborhoods, younger children are recruited as lookouts and helpers because of the lighter sentences given to juvenile offenders, and guns have become commonplace among children and adolescents. The great majority of homeless people have either a drug or alcohol problem or a mental illness—many have all three.

CLASSIFICATION OF ABUSED SUBSTANCES

There are many levels of substance abuse and many kinds of drugs, some of them readily accepted by society are

Legal Substances

Legal substances, approved by law for sale over the counter or by doctor's prescription, include caffeine, alcoholic beverages (see alcoholism), nicotine (see smoking) and inhalants (nail polish, glue, inhalers, gasoline). Prescription drugs such as tranquilizers, amphetamines, benzodiazepines, barbiturates, steroids, and analgesics can be knowingly or unknowingly overprescribed or otherwise used improperly. In many cases, new drugs prescribed in good conscience by physicians turn out to be a problem later. For example, diazepam (Valium) was widely prescribed in the 1960s and 70s before its potential for serious addiction was realized. In the 1990s, sales of fluoxetine (Prozac) helped create a $3 billion antidepressant market in the United States, leading many people to criticize what they saw as the creation of a legal drug culture that discouraged people from learning other ways to deal with their

problems. At the same time, readily available but largely unregulated herbal medicines have grown in popularity; many of these are psychoactive to some degree, raising questions of quality and safety. Prescription drugs are regulated by the Food and Drug Administration and the Drug Enforcement Administration.

Illegal Substances

Prescription drugs are considered illegal when diverted from proper use. Supplies are sometimes stolen from laboratories, clinics, or hospitals. Morphine, a strictly controlled opiate , and synthetic opiates, such as fentanyl, are most often abused by people in the medical professions, who have easier access to these drugs. Other illegal substances include cocaine and crack, marijuana and hashish, heroin, hallucinogenic drugs such as LSD, PCP (phencycline or "angel dust"), "designer drugs" such as MDMA (Ecstasy), and "party drugs" such as GHB (gamma hydroxybutyrate).

CLASSIFICATION

The following drug classification system divides frequently abused drugs into categories according to how they imitate or interfere with messages within your brain and moving between your brain and body.

1.Depressants	*Depressants slow things down.*  ***Effects*** • Slows down the central nervous system. • One desired effect is a feeling of relaxation and feeling more at ease in social situations. • Another desired effect is a release from inhibitions, enabling us to “let loose” and enjoy ourselves. • Slowed down messages from the brain to muscle impair our reflexes, reduce reaction time and impair our coordination, and our ability to drive is impaired. • You would experience this as a slurring of speech, stumbling when you walk, or weaving and a loss of balance. • Hand-eye coordination is reduced. • Thought and judgment are impaired because messages between the neurons in the brain are slowed down. • Reduced inhibitions and impaired judgment can lead to increased risk for violent behavior. ***Examples*** **Alcohol, Seconal, Nembutal, Amytal, Tuinal, Mandrax, Dalmane, Halcion, Valium, Librium, Serax, Ativan, Xanax, Inhalants**

2. Narcotics	*Narcotics slow things down and block pain.*
	 
	Effects
	• Have the same effects as depressants in that they slow down the central nervous system.
	• They have other effects that depressants do not have, which would include:
	  ○ Pain relief
	  ○ Suppress cough reaction
	Examples
	Opium, Codeine, Morphine, Heroin, Methadone, Demerol, Dilaudid, Novahistex-DH, Novahistine-DH, Novahistine, Expectorant, Percodan, Talwin, Lomotil

3. Stimulants	*Stimulants speed things up.*  ***Effects*** • Speeds up the central nervous system. • Desired effects would include a sense of well being or euphoria, or an enhanced ability to think and function. • Other effects include anxiety, paranoia, increased heart rate, increased blood pressure, reduced appetite, restlessness, insomnia, and a feeling of being "shaky." ***Examples*** **Cocaine** **Dexedrine** **Methedrine** **Tenuate** **Ionamin** **Ritalin** **Fastin** **Tobacco** **Caffeine**

4. Hallucinogens	*Hallucinogens mix things up, speeding up and slowing down.*  ***Effects*** • "Mixes up" the central nervous system, speeds things up and then slows things down randomly. • Distorts messages within the brain, and this can be felt as a distortion in perception. • Can cause hallucinations. • Milder hallucinogens are experienced as an enhancement of the senses: more sensitive to touch, pain can be magnified, music sounds better, hearing is altered, vision can be enhanced or blurred. • Perception of time can be affected. • Thought processes are affected: poor short term memory, alternating inability to focus and enhanced ability to focus, reduced ability to learn, and giddiness (everything is funny). • Other effects would include increased blood pressure, increased heart rate, and increased appetite. ***Examples*** **LSD** **PCP** **MDA** **Mescaline or Peyote** **DMT** **Psilocybin** **STP or DOM** **PMA** **Cannabis**
5. Designer Drugs	These are the drugs which are prepared by the underground chemists. • These are more toxic than their precursors. • Produce hellucinogenic and stimulant effect. • User may experience panic, anxiety, tremors, rapid heart rate, loss of coordination and psychotic behaviour. • **Examples : MPPP, MDA, Ecstasy**
6. OTC (over the	Drugs which can be purchased from local chemist shops without

counter drugs)	the prescription of doctor. • OTC must be safe and effective when the product information is followed. • Weight loss aid have potential harmful effects. • Ephidrine can produce high bloodpressure, sleepiness, irregular and rapid heartbeat. • Numerous herbs and plant extracts contain substances that can produce harmful side effects when ingested.

INTRODUCTION TO FIELD TESTS AND LABORATORY TESTS

With the increase in rapid diversification of drugs of abuse it involves an increase both in frequency and volume of seizures of different forms of abused drugs. Owing to ingenuity of producers and promoters combination of drugs appearing in latest kinds, an additional challenge has been founded with forensic analyst. Thousands of drugs are currently available licitly, either over-the-counter or upon presentation of a prescription form from medical store. Considerable number of drugs with potential for abuse is further controlled by the law. New products appear continually often in a variety of presentations. The forensic analyst examiner maybe asked to examine many products ranging from tablets and capsule bearing easily identifiable markings to illicit products of poor quality and unknown composition. The amount of drug varies from traces in clothing, motor vehicles, syringes or smuggling concealments to seizures of many tones. Samples may consist of synthetic drugs or vegetable matter or a mixture of two. Purity may vary from pharmaceutical grade substances to fractions of samples found at street level, which maybe adulterated (cut) with physiologically inactive materials or mixed with other drugs.

A number of methods are available for the detection and identification of drug of abuse, from sample field testing procedures to the use of most sophisticated instruments in analytical chemistry. These methods are discussed as follows:-

1. CHEMICAL SPOT TESTS:

Before the advent of more sophisticated and readily available equipment, colour tests were often employed for identification, Hider in 1971 described a system for rapid identification of unknown drugs using colour and microcrystal tests. Gupta et al considered that identification using only colour test was likely to lead to misidentification in many instances. Velapoldi and Wicks and John et al have published valuable reports or the use of colour test for analyzing drug of abuse. Both these groups considered that the primary purpose of colour test was to narrow down the list of substances possibly present in unknown sample. Both groups discussed multiple reagents testing schemes using up to reagents and numerical codes for possible identification. Masuad and Stevens has discussed many colour tests and Clarke has presented a comprehensive list of more important results.

2. FIELD TESTS:

Police and custom investigators frequently need a rapid sorting test for identification and detection of controlled drugs at the time and place of seizure. Such a test should, where negative minimize the risk of unnecessary detention of a person or goods and where positive justify professional analysis. An account has been given for the development of a composite field test kit, difficulties encountered with packaging and storage under various climatic conditions and philosophy of testing. The tests and others requiring greater discrimation or involving simple chromatographic producer, can assist greater scientists attending the scene of crime working group collected information on field test for drug of abuse commonly found in illicit traffic, there selection was based on the relative merits of stability , specificity , simplicity and safety. With the increase in rapid diversification of drugs of abuse it involves an increase both in frequency and volume of seizures of different forms of abused drugs. Owing to ingenuity of producers and promoters combination of drugs appearing in latest kinds, an additional challenge has been founded with forensic analyst. Thousands of drugs are currently available licitly, either over-the-counter or upon presentation of a prescription form from medical store. Considerable number of drugs with potential for abuse is further controlled b the law. New products appear continually often in a variety of presentations. The forensic analyst examiner maybe asked to examine many products ranging from tablets and capsule bearing easily identifiable manufacturers and markings to illicit products of poor quality and unknown composition. The amount of drug varies from traces in clothing, motor vehicles, syringes or smuggling concealments to seizures of many tones. Samples may consist of synthetic drugs or vegetable matter or a mixture of two. Purity may vary form pharmaceutical grade substances to fractions of a percent in samples found at street level, which maybe adulterated (cut) with physiologically inactive materials or mixed with other drugs.

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3. MICROCRYSTAL TEST:

Crystal or microcrystal test finds little current application in forensic analysis of drugs of abuse. Clarke considered that although useful for supporting a provisional diagnosis, being simple, rapid and specific such tests were unsuitable as a primary means of identification as they did not lend themselves to forming the basis of scheme of identification. Der Marderosion and Chao recognized that although some tests were very specific, others were more general in application, and that there was a tendency for related compounds to give similar crystals with the same reagent Clarke has discussed the topic in detail and described tests on many individual drugs.

4. UV SPECTROSCOPY :

The ultraviolet absorption spectrum information about the chemical functional groups involved in electronic transition. More data may sometimes be obtained by using different polarity differences in solute-solvent interactions may give recognizable shift in the UV absorption spectra and help to distinguish between compounds. Clarke has listed UV absorption data for many drugs of abuse in different pH. The drug identification Atlas contains UV absorption spectra recorded at pH 2.0 and pH 12.0 for many drugs of abuse.

5. IR SPECTROSCOPY

Organic molecules absorb IR radiation under transitions involved in rotational vibration energy levels in electronic ground state of the molecule. The fundamental overtone and combination IR absorption bands produce a very characteristic spectra, ensuring good selectivity and capable of identifying a pure compound in almost all cases. For screening purposes IR spectrophotometery may yield good results provided that not too many interfering compounds are present. There are collections of infrared spectra of many drugs of abuse in Clarke and the identification Atlas.Computer-aided chemistry with digital storage of the spectra and rational programmes for search, identification and quantification maybe of great help to forensic analyst handing and documenting large amounts of spectrophotometric data.

6. CHROMATOGRAPHIC TECHNIQUES:

Among various chromatographic methods, thin-layer chromatography (TLC) is comparatively simple, rapid and convenient for identifying many chemicals. It has been applied to the identification of drugs which induce poisoning or abuse. TLC may be run qualitatively with many samples in parallel, two dimensionally, quantitatively or as a preparative technique. Many types of TLC systems have been used for analysis of drugs of abuse; comprehensive TLC data has been collected by Clarke and Sunshine giving details of adsorbents, solvent systems, spray reagents and Rf values.

Benzodiazepines and zopiclone often induce coma at large doses, and may mask the actions of other drugs taken concomitantly. In emergencies, it is most important for clinicians to identify specific drugs rapidly, to make decisions as to treatment. The conventional method for identification in emergencies has been the ToxiLab system, including TLC. However, the detection limits in serum are low enough to identify several benzodiazepines . A useful method for identifying benzodiazepines in human serum in emergencies using rapid and sensitive high-performance thin layer chromatography (HPTLC) was developed. The thin-layer chromatography (TLC) method was proposed for separation and identification of drugs in mixture aminazine: nitrazepam : barbamylum. However TLC has shown little interest in the more recent publications because of lack of sensitivity and specificity. This technique may be used to predict the HPLC retention behavior and, to isolate metabolites from the biological samples. Flinn et al. described a simple, inexpensive TLC method in which rigid and fragile developing chambers were replaced by flexible

polyethylene bags for developing the chromatogram. They tested the analytical capability with theophylline in the experimental system.

Gas chromatography is one of the most widely used methods of analysing drugs. The technique is reliable, highly selective and very sensitive.

Capillary gas chromatography/mass spectrometry (GC/MS) is the most reliable technique for drug identification due to its excellent chromatographic resolution and the availability of library-searchable spectral information using electron ionization. As a result, a number of screening methods for sedative-hypnotics that involve the use of GC/MS have been reported. However, some of the sedative-hypnotics have low volatility and require derivatization prior to analysis.

HPLC, high performance liquid chromatography is a technique of major importance of the analysis of drugs of forensic interest. It is particularly suitable for drugs that can be troublesome to analysis with GC because they are thermally degradable, non volatile or polar. The technique is non destructive and compounds can be isolated for identification by other methods. Several detectors are available such as UV detector, the fluorimetric detector and electrochemical detector. The forensic application of HPLC to the analysis of wide range of drugs of abuse has been reviewed by Jane. HPLC techniques were capable of detecting most of the reported compounds using UV absorbance detection.

Liquid chromatography/mass spectrometry (LC/MS) is an alternative technique for drug identification. The high sensitivity and wide applicability of LC/MS has led to dramatic changes in drug analysis and a number of drug screening methods using LC/MS (/MS) have been reported

To establish the window of detection of drugs in urine, oral fluid and hair using LC-MS/MS, appears today as the most suitable tool to test for the low concentrations of benzodiazepines.

A simple and sensitive method for the determination of benzodiazepines in whole blood is solid-phase extraction and gas chromatography/mass spectrometry (GC/MS).

GC methods using fused-silica capillary columns were most commonly used with nitrogen-phosphorous detector, although some papers found electron-capture detector as a more useful alternative. GC–MS methods were also reviewed allowing, as expected, interesting results in metabolism study. Derivatization improved spectral definition and reduced thermal degradation on-column. Fast atom bombardment measurement was used to confirm the structures of 2, 3-BZ metabolites.

7. MASS SPECTROMETRY

Lawson considered that the high cost and complexity of mass spectrometry (MS) equipment was a considerable drawback to its routine use. Very few laboratories have the resource to use MS as a screening tool and the principal use of technique, at least in the analysis of drugs above track levels , is in the confirmation of the identity of a substance tentatively by some another technique.

8. NUCLEAR MAGNETIC RESONANCE SPECTROMETRY:

Until recently, NMR spectrometry has found only limited application on forensic analysis of drugs. However, the increasing popularity of ^{13}C NMR spectrometry, particularly in pharmaceutical analysis suggests that this technique may play significant role in the future.

9. IMMUNOCHEMICAL METHOD

Frequent procedures employed for the screening of benzodiazepines are the immunochemical methods, whose main advantage lies in a direct urine analysis without any sample preparation. However, in the case of flunitrazepam or its metabolite, the applied systems demonstrate false negativity, thus being unsuitable for the screening of this metabolite in urine.

REVIEW QUESTIONS :

1. Define Forensic Chemistry?
2. Explain Types of cases which require Chemical Analysis?
3. Write an essay on the importance of various methods of chemical analysis?
4. What do you mean by contact traces?
5. Mention the Importance of contact traces in crime Investigation?
6. What do you mean by detective dyes?
7. Define arson?
8. Mention various problems encountered during arson investigation?
9. Discuss importance of proper collection of clue materials from a scene of arson?
10. Define drug of abuse mention examples?
11. Discuss drug addiction and its problems with special reference to society?
12. Write an essay on the importance of field tests, and laboratory tests for drugs of abuse examination?

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COLLECTION, PRESERVATION AND SUBMISSION OF VISCERA SAMPLES FOR FORENSIC TOXICOLOGICAL ANALYSIS

- **Overview :**
  - I) General Introduction
  - II) Collection of various types of the viscera samples
  - III) Methods and Techniques for the collection of viscera samples
  - IV) Preservation, packing and forwarding of viscera samples
  - V) Submission of Samples to the Forensic Laboratory
  - VI) Post- mortem examination report and opinion
- **A list of important questions**
- **REFERENCES**

I) General Introduction:

It should always be remembered that except in corrosive poisons, the pathological findings are rarely conclusive; hence an appropriate portion of viscera will have to be submitted for chemical analysis. It is therefore vital to collect and preserve the relevant material in the right way and should preferably be kept in cold storage. The selection of visceral tissues to be preserved for chemical analysis is based on the history of poisoning or its distribution in various organs and tissues. The detection and distribution of a poison or drug in different body fluids and tissues is of great importance because,

a) There are various routes of entry of poisons into the body viz., by internal route e.g. by mouth or rectum, peritoneal e.g. by injection either subcutaneous, intramuscular, intravenous, inhalation, by external application on wounds or skin, introducing into natural orifices such as rectum, vagina, urethra, nose, eye etc. and by sublingual route.

b) The poison might have passed from one organ to another.

c) The poison might have been introduced into one organ like stomach after death to mark a homicidal act.

Secondly, after death, the supply of energy from metabolic processes is dramatically reduced, the integrity of the different compartments within the body breaks down at different rates, complex molecules tend to breakdown into their simpler subunits and move down the concentration gradient that were maintained in life by the expenditure of metabolic energy. Obviously, these processes do not occur at once. Thus, for a

variable length of time after death, the analysis of appropriate samples may yield useful information about the metabolic state of the poison in the period just before death. Once death takes place, many drugs are released from their binding sites in tissues as pH decreases on death and as the processes of autolysis proceed. These phenomena can make the interpretation of drug concentration after death less than straight forward.

Ideally, the decision to take viscera samples for toxicological or biochemical analysis should be made before conducting the postmortem examination by studying the police report, death report and the treatment report. In the cases, where cause of death is not apparent at PM, it becomes necessary to take tissue samples for toxicological analysis. Ideally, all the stomach contents should be submitted. In some circumstances samples of small bowel content should also be submitted for toxicological analysis. Normally, about 100gms of individual tissue samples are recommended to be preserved viz. brain, adipose tissue, liver and kidney. The amount of urine obtained in bladder should also be preserved as a routine practice. The doctor should have suitable containers and materials in hand for preservation of viscera. He should ensure that the chain of evidence is maintained properly labeled and submitted to laboratory without delay. In difficult cases, whenever necessary, the expert should be consulted.

II. Collection of various types of viscera samples

Since, the poisons or drugs after absorption into the system pass through the liver and spleen and are excreted in urine through kidney; hence, the following materials should be routinely preserved in all cases irrespective of the nature of poison.

1) Stomach and whole of its contents or stomach wash whichever is available and one feet of proximal part of small intestine along with its contents. It is preferable to send these two organs separately.
2) 100 gms of liver in pieces, preferably the portion containing gall bladder and its contents, half of each kidney.
3) Blood about 50 ml obtained from the femoral artery or vein by percutaneous puncture with a wide bore needle. It is never advisable to collect spilled blood or blood from body cavities.
4) Spleen - half in adult and whole in children.
5) 100 ml of urine or the amount available in bladder.

Additional materials:

1. In case of injected poison, injection site skin, subcutaneous tissues along with needle tract weighing about 100 gms should be collected. Similar material from opposite area is also taken as a control in separate container.
2. In case of inhaled poison like carbon monoxide, coal gas, hydrocyanic acid, chloroform or other anesthetic drugs etc; lung tissues, brain and blood from the cavity of the heart should be preserved.

3. Bile should be taken in the case of narcotic drugs, cocaine and paracetamol poisoning etc.
4. Shaft of long bones (8-10 cms of femur), a tuft of head hair, finger and toe nails and some muscles should be taken in suspected cases of chronic poisoning by heavy metals like arsenic, lead, antimony etc. In cases of prolonged use of drugs e.g. barbiturates; hair & finger nails are useful.
5. In fatal cases of suspected criminal abortion, the genital organs together with the bladder, rectum and foreign bodies should be preserved.
6. Blood from peripheral vein, lung tissue and a cerebrospinal fluid should be preserved in a suspected case of poisoning by alcohol. In alcohol poisoning, blood should never be collected from heart, pleural or abdominal cavities as it always gives higher results due to proximity of stomach and seepage. The blood from heart or body cavities may be taken for grouping.
7. The heart, portion of brain and spinal cord should be preserved if poisoning by nux vomica or strychnine is suspected. Brain and urine should be preserved in suspected cases of poisoning by barbiturates, opium or anesthetics.
8. Feaces may sometimes be useful especially if porphyria is suspected.
9. Urine should be collected for catecholamines estimation in a suspected case of hypothermia.
10. In highly putrified bodies, larvae, maggots, pupa and other entomological samples should be preserved.
11. When the body is partially skeletonised and it is not possible to have soft tissues from any part of body, it may be possible to have soft tissues from the foot because, to some extent foot wear protects the advancement of the putrification. Bone marrow from long bones in skeletonised bodies may also serve the excellent purpose of toxicological analysis.
12. In embalmed bodies, the vitreous humour usually remains uncontaminated by the process and may serve the purpose of analysing urea, creatinine (biochemical) and ethyl alcohol. For toxicological analysis, skeletal muscles and bone marrow are the only materials available in such cases.
13. In the cases of deaths by drowning where the study of diatoms is required, then the spleen, rather than bone marrow may be the most useful material to preserve. Control sample of the water in which the body was recovered should also be taken in separate container. Care should be taken to ensure that the control sample do not contaminate the spleen or bone marrow during the collection or transportation. Spleen tissue may be useful for DNA analysis.
14. Cerebro-spinal fluid may be taken in suspected case of alcohol poisoning.
15. If the death is suspected due to inborn error of metabolism, the advice of local reference laboratory should be sought as to what samples are required.
16. Fatty tissues from abdominal wall or perinephric region in the cases of pesticides.

17. About 2.5 cm square from the affected skin area and similar portion from opposite area as control in cases of corrosive poisons (acids and alkalies).
18. Soil samples from above, beneath and sides of the dead body and control soil samples from some distance away should be taken in cases of exhumed skeletalised dead bodies.

III. Methods and techniques for the collection of viscera samples

1) Blood:

1.1) When biochemical analysis is required to be carried out on serum, the blood sample taken at postmortem is centrifuged as soon as possible. If red cells are required in the investigations like haemoglobinopathies or glycated haemoglobin, then red cells should be taken separately; and if not required, then dispose off. The samples should be sent in frozen condition.

1.2) Blood samples are best obtained from the femoral artery or vein by percutaneous puncture using 50 ml syringe with a wide bore needle. Blood should not be collected by being milked from a limb. This process can endanger changes in drug concentration. Cardiac blood and blood from paracolic gutter is not suitable for quantitative toxicological analysis. The paracolic gutter blood may be contaminated with gut contents, urine or other body fluids. Try to collect about 50 ml of blood.

1.3) Blood for alcohol estimation in living should be taken from peripheral vein.

2) Urine:

Urine in dead may be collected by direct puncture with needle and syringe of the exposed bladder after the abdomen has been opened. It may also be obtained by insertion of urethral catheter before starting the PM.

3) Cerebro-Spinal fluid:

It should be obtained by cisternal puncture or by aspirating with pasteur pipette from the base after reflecting the frontal lobes. The maximum possible amount should be withdrawn,

4) Bone and Bone marrow:

8 to 10 cms. portions of the shaft of femur should be taken. The required amount of bone marrow should be withdrawn from the sternum or femur.

5) Hair:

About 10 gm or less, if available. The head and pubic hair should be plucked out along with the roots, and not by shaving.

6) Muscles:

About 10 gms wedge of thigh or chest muscles are collected before the abdomen is opened. 100 to 200 mg. of muscles can be the ideal tissue for DNA extraction.

7) Nails:

All the nail should be removed entirely and collected in separate clean envelops.

8) Skin:

A piece of at least 2.5 cms square from the affected area in the case of corrosives or skin applications and from thigh or back in suspected metal poisoning. If there is a puncture like injection or animal bite, the whole needle track or bite mark along with the surrounding tissues with 5 cm. square skin should be excised. Control specimen should be taken from similar of the opposite side of the body and preserved separately as control. In firearm injury cases, the affected portion of skin around entrance and exit should be excised and sent.

9) In case of poisoning in living patients, vomit, stomach wash, blood and urine are collected.

10) The articles collected from scenes of crime in poisoning cases are vomits, purged materials, urine or fecal stains, cloths, bed sheets and covers, medicines and their empty containers or poisons used by the deceased. Remains of food and drinks, containers of food or drinks, cooking utensils etc and the solids and liquids contained in the traps of wash basin. While sending stained clothes, surrounding unstained portion should be sent as control.

IV) Preservation, packing and forwarding of viscera samples

In order to avoid putrefaction of viscera samples, they are taken in clean wide mouthed containers properly. They should be preserved and packed in a suitable container to avoid any breakage during transportation. The samples should be sent at the earliest possible to the forensic laboratory.

The stomach with its contents should be preserved in one wide mouth glass bottle, small intestine with its contents in another and liver, spleen and kidney should be preserved in the third. When additional material is required, it should be preserved separately.

1) In all cases of poisoning including carbolic acid but excluding other acids, saturated solution of common salt should be used. The sufficient quantity of the preservative should be added so as to cover the visceral material.

2) In cases where poisoning by acids is suspected, except carbolic acid, rectified spirit should be used. Denatured alcohol or formalin should never be used while preserving the samples for toxicological analysis.

2.1 BLOOD:

2.1.1 When blood is taken for the estimation of volatile poisons viz. various alcohols, ethers, chloroform, hydrocyanic acid etc. and if the analysis is to be taken up immediately, then it should be sent as such in air tight container under frozen condition without adding any preservative.

2.1.2 Post mortem blood taken in suspected cases of poisoning including alcohols but excluding flouride, carbon monoxide and oxalic acid, if immediate analysis is not possible, it should be preserved with fluoride preservative to reach concentration of 1.5% by weight.

2.1.3 Blood for alcohol estimation in living should be preserved with 10 mg sodium fluoride (enzyme inhibitor) and 30 mg of potassium oxalate (anticoagulant) for 10 ml of blood.

2.1.4 The liquid blood when taken for blood grouping should be preserved with an equal quantity of 5% (w/v) solution of sodium citrate in water containing 0.25% v/v formalin or the PM blood for grouping may be soaked in a starch free clean cotton bandage cloth piece folded several times from heart chambers or body cavity. The piece should be air dried under shade and then packed in a paper packet. This can be an ideal method for the preservation of blood stain from crime scene, body on cloths for DNA test. To avoid bacterial decomposition, airtight containers or polythene bags should never be used for packing such exhibits.

2.1.5 In the suspected case of poisoning by carbon monoxide, a layer of 1-2 cms of liquid paraffin should be added immediately over the blood sample to avoid exposure to atmospheric oxygen.

2.1.6 In case of oxalic acid and ethylene glycol poisoning 30mg of sodium citrate should be used for 10 ml of blood and in the case of fluoride poisoning; 10 mg of sodium nitrite should be used. 2-3 ml of intravenous blood is thoroughly mixed with anticoagulant by shaking in a sterile glass container, sealed and forwarded to laboratory in ice-box without any delay.

Heparin and EDTA should never be used as anticoagulants because they interfere with the methanol detection.

2.1.7 The blood serum or the vitreous humour, when biochemical analysis is required, should be sent in frozen conditions.

2.1.8 0.5% w/v of EDTA or heparin (anticoagulant) preservation of liquid blood is preferred when taken for DNA test.

2.2 URINE: If the urine is taken for alcohol analysis, then it should be preserved with 30 mg. of phenylmercuric nitrate per 10ml of urine or by adding a few grains of thymol. For toxicological analysis, the urine may be preserved by adding 2.5 ml conc. HCl drop wise and shaking for 100 ml of urine or it should be acidified to attain a pH of less than 2.0 by adding conc. HCl dropwise. This preservation is also applicable, if the urine sample is taken for biochemical analysis i.e. the determination of the concentration of catecholamines in deaths due to hypothermia. For routine toxicological analysis, urine may be preserved by making it saturated by adding sufficient amount of sodium chloride crystals or it may be preserved by adding equal amount of rectified spirit.

2.3 TISSUES: The tissues taken for histopathological examination may be preserved in 10% formalin. 100 to 200 mg. of tissue taken for DNA extraction may be preserved in 20% solution of DMSO (Dimethyl sulphoxide) saturated with sodium chloride.

2.4 STOMACH CONTENT AND VOMIT: The stomach contents, vomit samples or the food materials taken for bacteriological examination (food poisoning), should never be preserved. The samples are to be sent in frozen condition.

2.5 FEACAL MATTER: The feacal matter or any other body fluid like saliva or seminal stains collected for toxicological analysis or biological examination from crime scene, body or clothes should be air dried under shade and sent to the laboratory. The saliva or seminal stains for DNA tests may be preserved by drying as described earlier in case blood stains. Such samples should never be packed in airtight containers or dried under direct sunlight. After packing, the samples should be kept at room temperature. The samples of medicines or poisons recovered from scene of crime or from the possession of the deceased or from any other place should be sent as such in the same original form without adding any preservative. They are required to be sent in airtight and leak proof containers.

2.6 Highly purified bodies, larvae, maggots and other entomological samples may be preserved by refrigeration for toxicological analysis. They should not be preserved in any fixative. They serve as useful substitute for viscera and blood group identification. For entomological examination larvae is put alive to boiling absolute alcohol and then preserved in 50% ethyl alcohol. This technique keeps the larvae in extended state.

2.7 In case of the suspected death due to rabies, 1-2 cm cube of hippocampus, cerebral cortex, cerebellum and medulla should be preserved in 50% glycerol saline for negri bodies and virus isolation.

The stomach and intestine are opened before they are preserved. The liver and kidney are cut into small pieces to ensure penetration of the preservative. The bottle should not be filled up with the preservative, but it is necessary that the viscera should completely immerse under preservative. As an additional precaution, some preservative (25-50 gm) of sodium chloride or a layer of about 2 cm of rectified spirit (as the case may be) should be added.

V) Submission of Samples to the Forensic Laboratory

The samples taken in sterile and suitable containers should be well stoppered to avoid any leakage and then tied with a string or adhesive tape to resist pressure if developed by gases in the container. Each container should be sealed with sealing wax with a personal authenticated seal. The seal should remain intact and its impression should be legible. A facsimile seal impression should be sent along with the PM or in the cases of stomach wash, blood or samples taken from living persons, it should be sent along with the forwarding letter. If the articles are sealed by the IO, the same procedure should be adopted and the legible facsimile seal impression should be sent along with the forwarding letter. Each bottle should be properly labelled and the label should bear the name of the deceased, PM No. or the case registration number, the name, age, sex of the deceased, approximate quantity of the organ, the date, time and place of autopsy, followed by the signature, rubber stamp indicating place and

designation of the doctor. About 25ml of preservative should also be taken, sealed and sent separately for analysis to rule out any poison being present as a contaminant. The sealed bottles are then put in viscera box with padded compartments into which bottles snugly fit. The box is then locked, the key of the box and the specimen of the seal used are put in a separate sealed envelope. The sealed box and the envelope are then handed over to police constable after taking a receipt to deliver those to the forensic laboratory.

The Important Documents which should be submitted along with the Viscera box are as follows.

1) A copy of the panchnama and FIR if available, brief facts of the case submitted by police and case sheet viz., name, sex and age of the deceased and time at which PM examination was carried out, an account of final illness and a list of drugs to which the deceased had access.
2) A copy of the autopsy report with detailed observations and findings. It should also clearly mention the specific name of the poison or drug for the detection, estimation for which the viscera is sent and also mention if any additional analysis is required. If there is any previous analysis done, reference should also be mentioned.
3) A forwarding letter by the IO requesting to examine the submitted sample and informing the findings to the medical officer and indicating the viscera box is transported by the constable authorized to do so.
4) An authority letter from a magistrate or relevant officer for chemical examiner to examine the viscera.
5) If possible the statement of the witness relating the symptoms developed and the behaviour of the deceased before death. This is not mandatory but can save the time and material of the expert.

   Once, the viscera samples are preserved for chemical analysis but not sent for analysis, they should be destroyed with the assent of the Magistrate or if the investigating officer informs that the case has been closed or if further analysis is not required.

VI) Post- mortem examination report and opinion

It is a signed document containing a written record of post mortem findings and can be used as proof for the cause of death, manner of death and time since death. It help the reader visualize what has been seen at autopsy and how the conclusions have been drawn. Accordingly, it must contain all the positive and not all but important negative findings. It should be an accurate, complete and objective record of all autopsy finding from which legally valid conclusions can be drawn. It should be written in a simple language without using technical terms whenever possible.

The report should state the authority ordering the post mortem, name of the deceased, the date, place and the time the body was received, the date, place and time of commencing and completion of postmortem. The name of the person or the means

by which the body was identified. An overall account of external examination, a list of clothings and their conditions, detailed description of injuries including their age. The injuries should be numbered and summarized to provide an overview of the entire injury pattern. This is followed by the internal examination. It contains a detailed description of stomach and its contents and general condition of other organs. If special studies like microscopy and radiography are performed, the findings are recorded. Any disease, if present, should be described along with its note in the cause of death. Details of samples, tissues and organs removed and preserved are also recorded along with the name of the preservative used.

This is followed by conclusion as to the cause of death, manner of death and time since death based on autopsy findings. There should be no confusion between the cause and manner of death. The cause of death, means the condition or disease that brought the end of life. The manner of death must fall within any one of the categories viz., [1] natural [2] suicide [3] homicide [4] accident [5] undetermined. The opinion regarding the cause and manner of death should be concise and clear, without which the report has no legal value, i.e., [1] cause of death; meningitis; manner of death: natural [2] cause of death: gun shot injury in the forehead, self inflicted; manner of death: suicide [3] cause of death: asphyxia by throttling; manner of death: homicide [4] cause of death: shock and hemorrhage due to multiple fractures, run over by a truck; manner of death: accident [5] cause of death: indeterminate – no disease, no injury, no poisoning; manner of death: undetermined. The time since death can be determined from body temperature, rigor mortis, PM lividity, progress of putrefaction, state of digestion of food etc. Opinion regarding the cause of death should not include any non-medical facts or discussions. If the opinion is based on the statement of the police, history of the case or due to natural causes, the fact should be mentioned in the report. Other conditions contributing the death should also be mentioned. The opinion is given in the form of a certificate by filling in all its columns. This should be followed by the signature, qualifications and designation of the medical officer.

The postmortem certificate is issued within 24 hours after conducting the postmortem. In cases of poisoning, decomposed bodies or where the cause of death requires further examination such as chemical, histopathological or microbiological investigations, the opinion as to the cause of death is reserved pending such examination or the probable cause of death can be given and final cause of death may be given after receiving the CA or HP reports. In some cases, in spite of a thorough post mortem examination, chemical analysis, histopathologic or microbiological examination, the cause of death cannot be arrived at. Under such circumstances, the medical officer is justified in mentioning the cause and manner of death as undetermined. In such type of cases, the investigating officer may still proceed depending on the circumstantial or physical evidences. In poisoning cases with a positive history, some times, negative analytical findings may be obtained from chemical analyser because of many reasons like [1] the poison may have been vomited out, excreted, neutralized, metabolized, detoxified or the poison is in such small

amount which may not be detected by the available methods of chemical analysis, [2] the absorbed poison is not evenly distributed in various organs which varies with different poisons, [3] the distribution of poison may also vary with the mode of administration, [4] if the victim has been treated, the medication may alter the poisonous substance and makes its detection difficult, [5] for the reasons not known, in certain deaths due to intravenous narcotism, sometimes, the victims is found with the needle in the vein, no narcotic is found, [6] delayed deaths, [7] adding irrelevant preservatives in the tissues, or preservation of irrelevant tissues etc; [8] decomposed tissues and [9] if the analysis fails to test the suspected poison. Beside considering such limiting factors, non detection of poisons in PM tissues need not necessarily be interpreted as no poisoning has been occurred. Besides, there are some standard opinions which can be given in various types of cases.

In the cases of snake bites, the doctor should see the fang marks, rule out other causes of death, study the case history where treatment is given and study the circumstantial evidence or information given by the police. In the cases of snake bites, the 'no poison' given by the toxicologist rules out the presence of other poisons in the tissues than snake venom. Because, it is not possible to detect the venom by chemical analysis as the venom is destroyed very fast. In the cases of snake bite, the organs or the tissues should not be sent for histopathology as there will be no pathogenic findings.

The doctor must submit the PM report to the investigating officer and a copy on demand to the judiciary department. If the lawyer or relative of deceased demands PM report, as in practice, the doctor can direct them to police. The police can issue a copy of the report after filing the case in court. In some states, the relatives of victims can have a copy of the PM reports from medical officer after depositing the prescribed fee in his office.

Viscera samples & Preservatives

Sr. No.	Sample	Type of analysis	Preservative
1.	BLOOD	For estimation of volatile poisons	Rectified Spirit
		Postmortem blood including alcohol estimation	Solution of sodium fluoride to reach concentration of 1.5 by weight.
		Blood for alcohol estimation	10mg of Sodium fluoride+ 30mg of Potassium Oxalate for 10 ml of blood
		Liquid Blood for grouping	5% solution of Sodium Citrate + 0.25% formalin
		Poisoning by Carbon monoxide	1-2Cms of liquid paraffin wax.
		Oxalic acid and ethyl glycol poisoning	30mg of Sodium Citrate

		For Fluoride poisoning	10ml of sodium nitrate Heparine and EDTA are not used as these interfere with methanol detection.
		For biochemical analysis (blood serum and vitrous humor)	Frozen sample
		For DNA analysis	0.5% EDTA or Heparin
2.	Urine	For alcohol analysis	30mg of phenyl mercuric nitrate for 10ml of urine OR reduce ph less than 2 with HCl.
		For routine toxicological analysis	by making it saturated by adding sufficient amount of sodium chloride crystals or it may be preserved by adding equal amount of rectified spirit.
3.	Tissue	For histopathological examination	10% formalin
		For DNA extraction	20% DMSO (Dimethyl Sulphoxide)
4.	Stomach Contents	For food poisoning	Frozen state.
5.	Entomological samples		Preserved by refrigeration. **OR** by boiling larva in absolute alcohol to keep them in extended form.
6.	Cerebral Cortex, cerebellum & medulla	In case of death by rabies virus	Preserve in 50% gycerol
		For virus isolation	Preserve in saline

- **A list of important questions.**
  1. Describe in detail the medico legal procedures in India.
  2. Write a note collection of viscera and its importance in determining the cause of death in poisoning cases.
  3. What are the precautions to be taken during the collection and packing of blood samples.

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LESSON NO. 2.4 **AUTHOR : JASWINDER SINGH**

METHOD FOR COLLECTION OF VARIOUS PHYSICAL EVIDENCES

SPECIMEN	TECHNIQUE FOR HANDLING	METHOD OF MARKING	SAMPLE	PACKING & FORWARD-ING	EVIDENTIARY VALUE
ARSON & EXPLOSIVE					
I .Liquid	Keep the liq-uid in the original cont-ainer and check for finger prints	Label on the outside of the containe r indicating place, description of specimen, date,time, FIR	upto 4 Oz In case only little amount or traces available,t he same may also be sent to the Lab for analysis.	May be packed in a wooden box taking care to see that the bottle does not move during transit and the sides of the bottle do not touch the box	To determine whether the liquid is inflammable/expl o sive material.
2.Non Liquid i.e. solids, pastes, ashes, debris etc. from the spot including electric wires	Material may be collected by means of forceps If ash is present,the same may be collected by sliding paper	-do-	All specimen including traces.	Place the specimen in a dry vial and handle with care to keep burnt material intact.	To fmd out the cause of fire/explosion thereby modus operandi.

BLOOD					
1.liquid	Known and unknown blood samples may be collected on a clean dry cloth. Later the cloth may be dried and kept in a paper envelope. If liquid blood	-do-	5 ml in sterile vial.	The vial may be placed in a cloth bag & sealed properly. Label on the cloth bag.	Blood grouping, blood alcohol/ poison detection/
2. Blood stained clothing	Care must be taken not to loose trace materials from the garments.	Tags must be used. Indicating relevant data.	Entire clothing.	The clothes should be packed after drying. Each garment should be separated by means of paper sheets, & may be kept- in a cloth bag, indicating the contents on the bag.	To determine presence, species of origin & group of body fluids trace evidence etc. from the clothings.

DOCUMENTS					
Letters,notes, cheques etc.	May be collected by means of forceps, if needed care should be taken to preserve latent finger prints.	No marking should be made on the document. The documents may be kept in an envelope and the envelope may be labelled outside.	All documents in cases of forgery standard and admitted writings may be supplied if there are any suspects	As for as possible documents should not be folded or rolled. If it cannot be avoided may be done along the old folding lines. The envelope containing the documents may be sealed by cello tape. Relevant details of case may	To determine authorship identity, to check finger prints, alterations, erasures or any other type of forgery.

FIBRES.					
	May be collected through vacuum sweeping with filter attachment or by means of cello tape.	May be placed in a small container with a label indicating place/article from where the fibres have been	All fibres alongwith original garments, if available.	Packed in folded papers and place = in an envelope or . pill box. Seal tightly to prevent loss.	'To link the suspect with the scene of occurrence or a vehicle :i

FINGER PRINTS.					
1. On polished surfaces.	Articles like tumblers, glass panes, polished surfaces etc. may be developed at the scene of crime, photographed & lifted by means of special finger print lifters. In case experts are not available at the scene of crime, the articles may be packed in such a way that the packing doesn't wipe out the latent prints.	The tag on the exhibit should contain date, case No., place of occurrence, name of the expert etc.	All latent prints.Names of all the persons associated with the scenes of crime visit and their elimination prints.	The prints may be transferred from the object in a non porous card. The card is then sealed in an envelope.	To determine positively who was at the scene of crime.
2. On paper surface	The paper should be handled as little as possible. No	-d-	-do-	-do-	To determine the presence of latent finger
FIRE ARMS					
1. Hand gun	Should be handled by the Knurled portion of the hand grips until processed for finger prints.	Put a signature or an identification mark in inconspicuous place	All	Attach a string tag and place the weapon in a heavy envelope.	To determine if the weapon was fired or for fire arms identtion.

2. Rifle/shot gun	Handle by the trigger guard or the serrated parts.	-do-	-do-	-do-	
AMMUNITION.					
1. Fired Bullet	Use forceps with taped ends. Avoid damage to rifling marks on the circumference	Mark with initials either on base or nose of bullet. Do not mark onsides	-do-	.	
2. Bullets and cartridges(unfired)	Use forceps with taped ends, Avoid damage to the bullet and	Initial or put signature date and no. of rounds recovered from the weapon should	-do-	-do-	
3.Fired cartridge case.	Pick up at the open end by means of forceps. Avoid Scratching	-do-	-do-	-do-	To determine make, calibre, type of fire arm. Future comparison if weapon not received immedia
4.Shot Shell (fired)	-do-	Mark/signature on the side of brass cap. Do not scrap,nick or disturb the base of the shell.	-do-	Roll individually in paper and place in paper envelopes	To determine gauge of gun, and comparison of weapon marks.

5. Shot Pellets	Use forceps with taped ends. Avoid damage to the pellets.	Pack in a small pill box, seal and mark properly	-do-	-do-	The size of the shot may be consistent with other ammunition found with
6. Wads	Use forceps, avoid damage to the wads.	Mark with signature of I.O. or initial for identification.	-do-	Place in paper envelope	To determine size of shot, gauge or fire arm and manufacture of the ammunition
GLASS					
1. Fragments	Use fingers on the edges of the longer pieces, do not	Mark the container with signature of I.O. Separate	-do-	Wrap each piece in cotton or soft paper, pack to	To identify fragments collected from the
2.Small Particles	Use forceps or paper sheet for collection.	-do-	-do-	Place in pill box seal and protect from further damage.	-do-
HAIR					
	Carefully remove hair from the scene by means of forceps.	-do-	All portions of hair found at the scene. Twenty or more full hair from head, body possible.	Fold in clean paper and place in an envelope.	For identification and comparison purposes.

IMPRESSIONS					
1. Foot Prints 2.Tyre impressions. 3. Tool Marks.	First photograph the impressions including a scale in the picture. The impression should be carefully preserved till they are lifted by means of plaster of paris/plastacin/m-seal.	Label on the back of the impression with the signature of the I.O.	Sufficient photographs to show the impressions from various angles.	Wooden Box.	Comparison with suspects/comparison in criminal cases.

LIQUID					
Poison, acid etc.	Leave in original container. Examine the container for latent prints.	Outside the container label with requisite information	All liquid available	Wooden Box taking care to see that finger prints are not disturbed.	To detect the poison qualitatively and quantitatively.
Liquor	-do-	Label outside	-do-	As far as possible in original container.	To identify the drug.
SUSPECTED NARCOTICS AND DANGEROUS DRUGS.					
1. Liquids	do	do	do	do	do

2.Tablets,powder and solids	Use forceps or small brush to transfer	-do-	-do-	Pack in a cotton wool/thermocol packing, labels FRAGILE'	To identify the drug and to classify it as narcotic/dangerous /Psychotropic drug.
PAINT FLAKES					
	Handle with forceps of scoop with a piece	Mark sealed container with data	-do-	Place in pill box or rigid container	Chips found at the scene may be traced to suspect and used for comparison
FIBRES, STRINGS, ROPES ETC					
	Handle small pieces with forceps and transfer carefully to paper container	-do-	-do-	Place the envelopes or secure firmly by wrapping in paper sheet	For comparison in various types of criminal cases.

TOOLS				
Handle by side of tool after the same has been examined for latent prints.	Mark on side of tool. Do not mark on the face of tool. Use string tag.	-do-	Place the evidence bag. Prevent damage to the tools.	To compare tool marks

TOOL MARKS				
Cover tool marks with soft paper to avoid damage to the marks	Inscribe name/initial on the article containing tool marks. Do not disturb the tool mark.	Complete tool marks	Keep from contaminating the mark.	Comparison purposes.
SOIL SAMPLES AND OTHER TRACE MATERIAL				
The soil samples from the garments, floors etc. may be collected on a clean sheet of paper without further contaminating the material.	May be kept in an envelope labelled outside.	Complete Material	Keep from contaminating the material	To link a suspect to a place or to find out the profession

LESSON NO. 2.5 AUTHOR : DR. MUKESH KUMAR THAKAR

HAIR

INTRODUCTION

The biological material/ stains that are encountered in forensic investigation may be derived from human, animal or plant sources, The most common stain of human or animal origin is blood. Other stains may be due to secretions and excretions or derived from them e.g. as such or materials from Pollens , Fiber wood semen, saliva, sweat, urine, faces, etc.,. The stains of plant origin may be derived from fruits, flowers leaves etc. Hair, Fiber, Pollens and wood also constitute an important biological evidence.

The examination of biological materials constitutes one of the most important evidence of a forensic science laboratory not merely because of the number of such exhibits it has to handle but of the great significance associated with the results which reflects some very stable character of the source that is involved. The character of the biological stains like blood, semen , saliva etc. generally encountered in forensic practice, are intimately associated with the genetic make up of the individual concerned and if their examinations are performed meticulously and with precision these are likely to offer unmistakable evidence to associate a criminal with the crime or vise-versa. The identification and characterization of all the different types of biological stains likely to be encountered in connection with the scientific investigation of crimes are highly significant Through their examination (biological material), nature source and individual involved can be established, if not, in certain cases , they can provide a very important corroborative evidence in forensic investigations.

Mutual transfer of hairs, due to contact, is classical example of Locard's principle of mutual exchange of traces. Hair, if properly searched, are found in a large number of criminal cases. It is one of the earliest types of physical evidence, which was considered, collected, evaluated and utilized in forensic work.

The identification of hair is not conclusive at the present stage of development except through DNA profiling, if hair roots are available or through mitochondrial protein analysis. Without these it cannot be said that the hairs in question belong to a particular individual and could not come from any other person. In extremely rare cases, the presence of some individual dye, contamination, some extraordinary defect or disease in the hair, may permit individualization of the source.

On the other hand, it is possible to exclude some persons as the possible sources of questioned hairs. This exclusion, the possible corroboration and its identification,

now, with the new techniques, make the hair evidence one of the important pieces of physical evidence.

The evidence establishes the possible presence of a culprit at the scene of occurrence. It indicates the contact if transfer of hairs has taken place. It indicates the route of ingress or egress if some hairs are retained at the points of entrance or exit. It helps to identify the weapon of offence if some hairs get attached to it. It also indicates the vehicle used in the commission of crime. It may also establish the ownership of the articles left at the scene.

The hair evidence is often not easily seen. Hence it goes unnoticed and it does not bother the culprit. Yet if they carry blood or body cells, through DNA profiling they permit definite identification of the culprit. Hair evaluations have also been utilized.

- To determine the nature, quantity and the period over which the poison had been administered in slow poisoning cases. In case of Napoleon, the nature and quantity was determined after about 140 years.
- The hair evidence as corroboratory evidence is available in most of the serious cases, especially in offences against person.

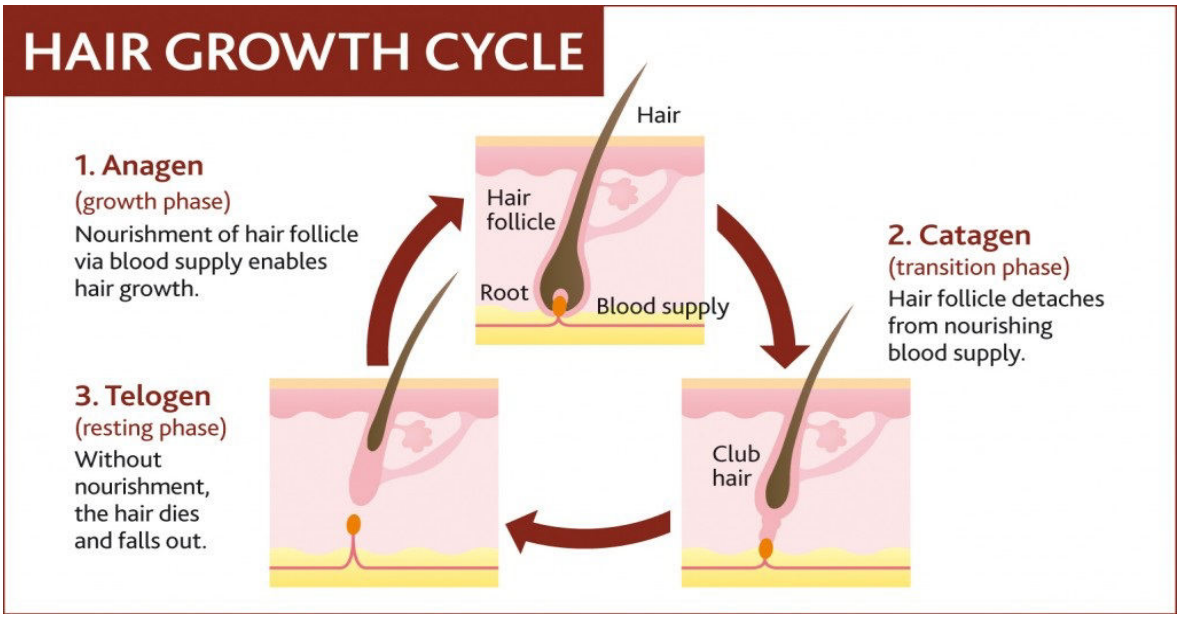
The falling rate of hair increases if the person is under emotional stress. The hairs are, therefore, likely to fall at the scene in rape or murder cases. They can form important corroboratory link if collected correctly. The hair are ordinarily available in murders, assaults, road accidents, rapes, abortions, bestiality, house breaking, maiming, poaching, worrying and thefts of furs and animals. Identification of hairs is not an easy task. It requires extensive experience in the field, because there are variation in the hairs of the same person at same as well as at different times. Further, there are similarities in the hairs of the some persons. However, recent advances, especially DNA profiling of hair root cells and mitochondrial proteins hold promise for definite acceptable identifications, whenever body cells are available.

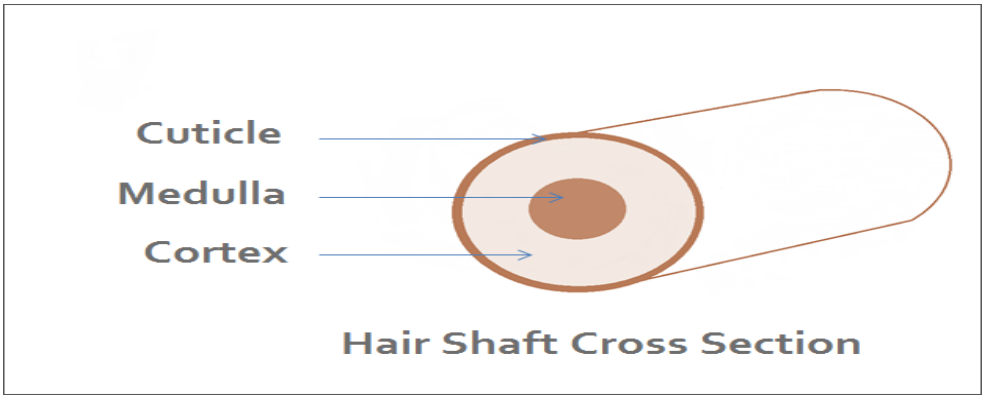
NATURE

Hair grows on most of the limbs of human beings and animals. Mammalian species (those who suckle their young one) have two types of hairs: long and stiff hairs and fine fuzzy or wooly hairs.

Hair grows from the hair centers called follicles in the body. They are in the shapes of fine rods. They may or may not have fine tips. Some hairs, which have fine tips to start with, acquire almost uniform diameter later on, others do not. Hair are multicellular structures produced by specialized organs in the skin known as hair follicles During its active growth phase ,hair is produced at a constant rate by the follicle. Deep within an active follicle, the cells making up the hair shaft in its formative stages are alive and active. Once the hair reaches the skin surface, these cells are no longer living but become cornified or keratinized at this stage, hair is composed mainly of a particularly tough protein, called keratin.

Human hair follicle go through three distinct growth phases i.e. anagen phase, telogen phase, catagen phase .In the anagen phase, the follicle is actively producing hair. In the dormant or resting period, the follicle is said to be in telogen phase. The transition period from anagen to telogen is short in human beings and is called the Catagen phase. After a period of inactivity, the follicle enters the anagen phase again and resumes activity. A telogen hair remaining in the follicle is shed at this point, if it has not been lost already. At the onset of catagen (inactivity), the pigment-producing cells in the follicle contract and cease functioning. The last segment of hair produced before telogen is, therefore, white. Most of hairs coming to the laboratories are fallen (telogen) hairs. Human hair follicles are independent of one another ;each determines its own growth and resting period cycles.



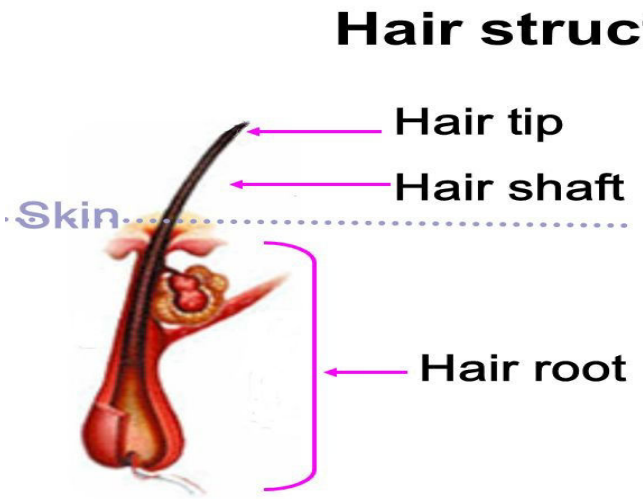


Hair grow to certain lengths and then they fall of if they are not cut. New hairs may come up in their place. The growth rate of hair from other parts of the body is less.

Chemically hairs are made of proteins, mostly keratin. Hair pigments usually consist of melanin proteins, uric acid, cholestrol, vitamins and other organic materials are also constituents of hairs. The elements like arsenic, lead, iron and silicon are also found in the hairs. Slow lead or arsenic poisoning is detected through the excess of the poisonous metal in hairs.

Due to the external contamination, the hair may be found covered with oily substances, dust and dirt.

Morphologically, hair consist of root, shaft and tip, which have characteristic shapes and structures. Pigment distribution and the presence of air bubbles (Vacuoles) in the hair give additional points for characterization.



The structure of hair is characteristic in another way. It has three parts: the central, called medulla, the middle part, surrounding medulla is the cortex and the outer part is the cuticle. The nature of medulla and its diameter with reference to total diameter of the hair are highly characteristic and often indicate the species of the source of hairs. Further, the structure of cuticles, which is like the scales of a fish or shingles of a roof, are also characteristic of the origin. Physical properties of hair like refractive index, birefringence and the density also differ not only from one species to another but also from individual to individual.

The hair undergoes changes. These changes in the same individual depend upon his food, habits, climate conditions and age. Besides, the external treatment, bleaching, dyeing, cutting and combing have definite effects on the hair.

In the evaluation of the hair evidence, answers to the following questions are needed:

1. Is the given fibre a hair?
2. Is the hair of human or of animal origin?
3. Does the hair come from a male or a female?

4. From which part of the body the hair in question has come?
5. What is the age of the individual to whom the hairs belong?
6. Do the given two sets of hair come from the suspected common source?
7. What is the species of animals to which the hairs in questions belong?
8. Was the hairs cut, fallen or pulled out?
9. Has the hair been dyed or bleached? if so, can the dye be Identified?
10. Are the hair cut, if so, when were cut last?
11. Do the hair have evidence of chronic poisoning?
12. Are the hair contaminated if so, what is the nature of the contamination?

LOCATION

The Location of hair evidence is a difficult task because of their dimensions, quantity and colour. The search is tedious and time consuming. It is facilitated by a strong torchlight and magnifying glass.

The following are the sources of the evidence, which should be checked for hair evidence.

Culprit and Victim

The victim might have grappled with the culprit or come in contact with him in some other way. Thus exchange of hair might have taken place. The victim may carry hair in his hand, in his or her sexual parts (in sex offences) or on his clothes. If the victim has hit the accused with shoes or something else the same is likely to carry hair.

COLLECTION

Collection of hairs involves collection of incriminating and sample hair.

Incriminating Hair

Incriminating hairs are found in a variety of situations. They are minute and are not easily visible. They are collected by the following techniques :-

1. Hair are located under strong light. They should be picked up with hands by wearing gloves or with a tweezer, placed in a paper fold, on which identification marks and place of collection is mentioned. They are then placed in paper or plastic envelops.
2. The hair found attached to immovable articles are picked up with tweezers and handled as in first.
3. Hairs from different places are put in separate envelops. The place of collection and identification data should be put thereon. These envelops are enclosed in bigger paper envelops and sealed.
4. If an article like clothing or weapon of offence is suspected to bear hair, the article itself is placed in clean envelope and sent to the laboratory.
5. The use of adhesive tape to lift hair from suspected articles is often convenient. A convenient length of tape is unfolded and pressed against the suspected surface.

The tape picks up the loose fibers, hair and dust. Covering the lifted material with fresh tape projects the lifted hair. Stereomicroscopic examination of the tape indicates the location of the hairs. If the hair is picked up from the tape, a drop of xylene is put on the place. It dissolves the adhesive and the hair are free. It can be picked up with a tweezer. The technique is useful both for visible and invisible hair and other fibres. There is very little chance of losing the evidence by this technique.

6. The lifting with the tape fails only in those cases where the tape does not come in contact with the evidence, for example in crevices or depressions; it is useful to collect the hair from these places where they are retained for longer periods and are available in large numbers.
7. When the large areas like the inside of a car, furniture or floor is to be searched for hairs, vacuum cleaning apparatus, especially adapted for the collection of hair, dust and dirt is found particularly suitable. Dust containing hairs from different items and places is collected separately by using different filter discs for different areas. They should be kept in the folds of a paper and properly labeled. They are kept in transparent petri dishes in laboratories. Plastic boxes can be used for preservation of the evidence.

   The technique is useful in sexual offences committed in cars, on carpeted floors, on sofas or in beds or, in those cases where a large area is involved in the struggle.
8. If the hair are fixed in mud, blood, paint or semen stains, they are pulled or scratched out.

Sample Hairs

Sample hairs are collected from both the victim and the accused in the following ways:

1. Combing or brushing

Combing or brushing (with a stiff brush) is the best way to collect the sample hair of a person. Representative samples from various parts of the body (crown, temple, nape, eyebrows, eyelashes, moustaches, beard, sexual and other parts) are separately collected. Combing gives hair of all types complete with roots, broken hair, hair ready to fall, etc. They are also of all shades and have varying characteristics. They are usually the best representative hair. About 100- specimen hair are adequate for comparison.

2. Pulling Out

Pulled out hairs are the best sample hair. Samples from animals and dead persons should invariably be pulled out. Some living persons may not allow pulling out of the hairs.

Pulled out hairs sample are necessary where the incriminating hair have been allegedly pulled out. When hair are pulled out in ones or two's it does not pain much.

3. Clipping

Collection of hairs sample by clipping is the least satisfactory method and should be adopted only when other methods cannot be applied. The hair should be clipped as close to the skin as possible.

Precautions

The following points in addition to usual ones, should be kept in mind while collecting hair.

1. The investigating officer should not clean the collected hairs. They are forwarded in the original condition. If they are wet, smeared with blood, semen or other fluids, they should be dried before packing. The investigating officer should send all the collected incriminating hairs :-

(1) Sample hairs of the victim should invariably be collected in fatal, or even in non-fatal important cases.

(2) Samples of hairs from the suspect and the victim should invariably be collected in rape cases.

(3) The site of origin of the hair samples collected from various parts should be specified on the paper folds of the package.

EVALUATION

The hairs are examined visually and microscopically. The cross-sections are studied after microtomy using appropriate instruments, studying physical density, refractive index and bi-refringence. Neutron activation analysis is helpful in the detection of traces of metals. The evaluation methods briefly are:

1. Visual Examination

Visual examination is useful to determine the colour, length, character and contamination of the hair. It may give an idea whether the hair has been dyed or not. The presence or absence of contaminants and their nature is also indicated.

Visual examination is supplemented by a hand lens and stereomicroscope. The latter examination gives better appreciation of the colour and contaminants of the hair.

2. Microscopy Preliminary microscopic examination is carried out without cleaning or mounting the hairs. It reveals their colour, contamination and character, whether they are curly, wavy, soft or coarse, whether the ends are frayed or have tips or whether roots are present or not. it is possible to say whether the hair have been pulled out, cut or fallen. Pulled out hairs have live 'bulbs' or are found with shrunken. The detailed microscopic examination of hairs is done after cleaning them in alcohol-either mixture (or acetone). They are then mounted in Canada Balsam on a microscopic slide and examined with a magnification of about 400x. The examination reveals;

1. Actual colour of the hairs. The contamination, which may be masking the real colour of the hair, is removed in the cleaning process.
2. Whether the hairs have been dyed or bleached. Natural colour of hairs is found near the root end. Content of dye in hair depends upon the period elapsed between the dyeing or bleaching and collection (from the head or the fallen hair) of the hairs for examination.
3. The part of the body from which the hairs have come. The head hairs are like rods, whereas the hairs from eyebrows or eyelashes have pointed tips. The beard hair may be somewhat flattened.
4. The morphology of the hair. It indicates the presence or absence and nature of the medulla and the pigmentation pattern.
5. The Medullary index- The ratio of diameters of the medulla and the hair measured at the thickest point is known as medullary index. Its determination establishes the species of origin. The medullary index of the human hair is commonly less than 0.3 and in animals generally it is more than 0.5.
   Medullary index varies somewhat in male and female hairs and in the hairs from different parts of the body. Ordinarily, the medullary index is greater in woman when hair from the corresponding parts of the body are compared. The male beard hairs have greater medullary index than hairs form other parts.

   Medulla may be continous, fragmentary or it may be absent in human beings it may be masked sometimes by the hair pigment. It is made visible by bleaching the hair.
6. Pigment distribution. It is helpful in the study of species of origin and in the identification of hairs of an individual. It is often an important characteristic and permits elimination of certain suspected sources of hairs.
7. The roots and the ends- They reveal whether the hair have been pulled out or not. Whether they have been cut, and if so, when they were last cut. When tips are freshly cut, with the passage of time, the sharp ends gradually become round. The possible nature of weapon with which the hair has been severed is also revealed. With blunt weapons the cut end is found frayed and irregular. With sharp edged instrument the cut ends have sharp edges.
8. Any deformity or disease in the hair can be found.
9. Vacuoles- They give characteristic formations in certain species, which are useful in their identification. They are also known as air bubbles.
10. The special treatment (for example. burning) hairs may have undergone.

3. Microtomy

The cross-sections of hairs are obtained with the help of an instrument called microtome, clean hair is embedded in hard wax, plastics or flesh (hardened by special treatment) and sliced. The cross sections of hairs, obtained, are placed on a microscope slide treated with albumen. The embedding material is removed with a

suitable solvent and the section is fixed in Canada Balsam. Microscopy reveals the cross- sectional structure of the hair.

Microtomy is helpful to determine pigment distribution, medulary shape medullary index of the hair. In human hair the pigment is found concentrated near the periphery of the cortex close to cuticles while in animals, pigment concentration are near the medulla and in the cortex. It also permits proper study of the shape of the cross-sectional area.

4. Scale Count

The cuticle scales of hairs are difficult to study directly under a microscope. The casts of the hair are, therefore, prepared on plastic sheets or in clear finger nail polish. The hair being cast is placed on a plastic sheet slightly stretched, covered with a glass slide and evently pressed with a weight. It is then warmed with a table (or an infrared) lamp for suitable length of time. it is allowed to cool. The pattern of the scales is imprinted on the plastic sheet. If the nail polish is used, it is spread over a slide and the hair is fixed over it when it is still wet. When the polish has hardened, the hair is removed. The scale pattern is printed in the nail polish. The number of scales per unit length and their shapes and pattern vary with different species and individuals. They, therefore, help to identify the source of hair.

5. Density

Density is one of the most important physical properties of hairs. It is best studied and compared by density gradient tube method. The question and sample hair are placed in the columns of two similarly prepared gradient tubes and allowed to settle. They settle at the levels where the densities of the hairs correspond to the liquid mixtures. If the hairs settle at the same height in the two columns, a common source is indicated.

6. Refractive Index

Refractive index of hair is determined by Beckline method. There are minor variations in the refractive indices of hairs of the same individual; the variations are statistically ignored.

Refractive index of hair also gives rough idea about the age and sex of the source of origin.

The phenomenon of deviation of light passing through some transparent materials, differently at different angles is called **birefringence.** The deviation is characteristic of the materials. The hairs also show the phenomenon but due to inconsistency in the deviations of different hairs of the same person, it has not been used to any appreciable extent to identify or individualize hairs. Other properties such as elasticity, tensile strength and elongation under stress have been studied in textile

industry. They can also be adopted in the evaluation of hair evidence in criminal investigation.

7. Neutron Activation Analysis

Many substances when bombarded with neutron in an atomic reactor become radioactive. The induced radioactively is highly specific of the elements present and can be used to identify them quantitatively and qualitatively. The technique can determine these elements in quantities, which defy analysis with the conventional chemical and physical methods.

Carbon, hydrogen oxygen and nitrogen elements form over 95% of the bulk of the biological samples. They do not interfere in the determination of other trace elements in biological materials in the technique. In initial stages of hair analysis by this technique it was hoped that individualization of hair is within reach. But extended work in the field showed variations in the hair composition of the same individual at the same time and at different times due to diet, climate, contamination and diseases.

Quantitative and qualitative examination of the element such as arsenic, iron, lead and silicon, which are present in hair, permit identification of the possible common source of hair, because the composition varies from one individual to another, It does not lead to individualization of the person.

The technique is simple in principle. The hairs under examination are exposed to neutron bombardment. The trace elements in the hair become radioactive. They are studied by Gamma- rays-spectrometry to give qualitative and quantitative analyses. Comparison of the analyses of the questioned and the standard samples indicates the possible common source.

Scanning electron microscopy is playing an increasingly important part in the evaluation of hair structures. The instrument has been particularly utilized for the study of cuticles, especially the scale structures. They are studied with the instrument in both ways, direct or after preparing the casts of the hairs.

PROBLEMS

(a) Species

The species of the source of hairs can be identified from morphology, nature of medulla, cortex, cuticle and pigment distribution, It is desirable to have extensive experience and compiled data before such identification are made.

(b) Racial Identification

With the intermarriages among the persons of different races, the problem of identification of the racial origin of the person from the hair is becoming increasingly difficult, However, hair studies do provide some guidance.

There are some features, which help in distinguishing the hairs of the different races. Blood grouping and isozyme tying of the hair roots is also proving useful for individualization, in recent times.

(c) DNA Profiling

If the skin, flesh, mitochondria or blood is sticking to the hair root, definite identification of the person can be made through DNA profiling.

(d) Age

A rough estimate of the age of person from the hair can be made. In growing children the hair are soft and fine. They become coarser with age. The pigment distribution, refractive index and density also undergo changes but no definite co-relation between the age and these properties has been established so far.

Male or Female : It was difficult to say whether the given sample of hair comes from a male or female only a few years ago. However, research in the field has proved fruitful. Now identification of the sex from two sources on the pulled out hair can be made. They may carry:

1. Body Cells
2. Mitochondria. These body materials carry DNA, which permit sex determination.

(e) Cut, Pulled or Fallen

Microscopic examination of the root ends indicates whether the hair has been pulled out, fallen or cut. Healthy roots indicate pulling out the healthy hair, dried root bulbs indicate fallen hair and absence of roots indicates cut or broken hair. The shapes of the edges indicate whether the hair has been cut. The rough idea of the duration of the cutting can also be given from the extent of rounding of the cut edges.

Bleached or Dyed

Microscopic examination of hairs reveals whether they were dyed or bleached. Chemical examination of the dye or of the bleach reveals the identity of the dye or bleach. TLC helps in the identification of the dye or bleach

(f) Part of the Body

Hair from some parts of the body have characteristic morphological structures and contamination and can indicate the part of the body from which they have originated. For example, nasal secretion may indicate that the hair have originated from the nose. Semen and similar secretions, may show pubic origin of the hair and waxy contamination may show ear as the source of hair The various varying characteristics, given for the hair from different parts, are indicative only. The identity of the parts is not absolute. Therefore, unless there are some highly characteristic features in the hair, the part of the body should not be given in positive terms.

(g) Slow poisoning

The concentration of metals, in slow poisoning cases, increases in the hair with the passage of time. It is detected and estimated in the roots and the shafts near the root.

(h) Contamination

The dust and other contamination in the hair are identified by micro chemical and instrumental methods.

(i) Individualization

It is possible to individualize hair and say that they belong to a particular person in case where external contamination, defects or diseases may so permit. In addition, blood groups, isozyme typing and DNA analysis can individualize the hair, if there is some blood, skin or flesh sticking to the hairs, i.e. if roots are present with the hairs. The discovery of mitochondrial DNA, however, has further facilitated the individualization of the hair as well as its source.

Suggested Questions:

1. Define hair?
2. How will you differentiate hair and a fibre?
3. Discuss methods to determine the whether hair belong to human or animal?
4. What type of information can be sought from hair?
5. Discuss about the collection, packaging and transportation of hair samples recovered from scene of crime?
6. How the microscopic examination hair is done?
7. Is individualization from hair possible?

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LESSON NO. 2.6 AUTHOR : DR. MUKESH KUMAR THAKAR

FIBRES

The forensic investigation of fibre has traditionally been carried out by biologist since the classical fibre materials all had biological origin.

Transfer and cross transfer of fibres frequently take place among all the persons, places and paraphernalia which are involved in the criminal cases through wearing apparel, carpet, upholstery, bedding, textile mills, draperies, furriers, etc. Fibres surround us all around. So much so that it is difficult to imagine a person who would not transfer and or pick up fibres even in his day-to-day functioning. This transference and cross transference has also to take place in criminal situations. The fibrous evidence, therefore, should be available, most of the time, in most of the crime situations, on most of the persons and materials involved. Mutual exchange once established clinches the case against the accused, especially in cases of offences against person, to the main evidence of the victim and the eyewitnesses.

Fibres are valuable evidence because of large variety and frequent exchange. It has been calculated that if six different common fibres (different in colour, sizes, material, treatment or extent of use), have been exchanged, they provide the linkage. The probability of reoccurrence of such exchange, on a conservative estimate is one in $64X\ 10^6$. A Similar exchange, of eight fibres reduces the probability of reoccurrence to one in $256x10^8$. If the fibres are of rare types, they provide a still higher linking probability.

Fibrous evidence is often inconspicuous. It remains unnoticed. The culprit, therefore, does not destroy it. For the same reason the evidence also remains unhampered. But all the fibrous evidence does not remain attached to the substrata. In fact most of the fibres are shed off soon. Some, however, resist shedding and even withstand dry cleaning or washing. They provide excellent evidence even after long periods:

Unfortunately the fibre evidence has been considered to be weak evidence as it was supposed to offer no individualizing features earlier. It is no longer true. The fibres can be highly individualistic due to the following characteristic:

1. Chemical composition
2. Manufacturing Process.
3. Dimensions.
4. Dyes (TLC, FTIR analysis can identify the dyes.)
5. Optical properties

6. Impurities from the manufacturer, wearer, partial melting, paint smear or other stain.
7. Changes in the material due to ageing.

The fibre evidence can help innocent suspects:

The advanced technologies, new and ever increasing number of materials, more and newer modes of manufacture and ever- increasing sophistication in examination techniques can give near individualistic identity. Besides, cross transfer and variations in transfer fibres can fix the culprit- victim contact. We have no quantitative probability values for all types of fibres. However, it is believed that cross-transfer of two or more fibres of uncommon nature should clinch the contact issue.

Nature

Fibre is defined as a material that has much greater length than width or diameter. Fibres are varied in nature. There are **: Plant fibres, animal fibre, mineral fibres. metal fibres, and modified fibres.**

1. Plant fibres

The commonest and important plant fibres, in India, are cotton, flax, hemp, jute and coir. But there are other fibres obtained from other plants though they have no commercial status. The latter can also occur sometimes in crimes. Formerly plant fibres dominated the clothes upholstery industry. Synthetic fibre are taking over the trade increasingly now.

2. Animal fibres

The frequently utilized animal fibres, in India, are wool, mohair cashmere, angora, featheres (down feathers for pillows, quills, etc.). Camel, goat and other animal hairs have also been utilized.

Natural silk is another variety. The three common varieties are:

- Cultivated silk,
- Tassar (Tussore, tussah)
- Cultivated and analyse silk.

Animal furs are also used as dress materials. Their hair are animal fibres.

3. Mineral fibres

Asbestos and glass fibres are the most important mineral fibres. They are extensively utilized.

4. Metal fibres

Metal fibres are extensively used in textiles and for some other purposes, In India. Gold, Silver, Brass, Aluminium fibres are common. Increasing use is being made of metal-coated plastic fibres, and plastic-coated metallic fibres. They and their fragments will be encountered more frequently in the coming times.

5. Modified fibres

Rayons are modified cellulose fibres. Important varieties of the rayon are Acetates, Viscose, Cuprammunium. They are extensively utilized in textile industry. They are also man-made fibres but their base is natural fibre, mostly cotton and wood. Plastic coated natural fibres, like plastic coated coir is also used in mattresses and sofas.

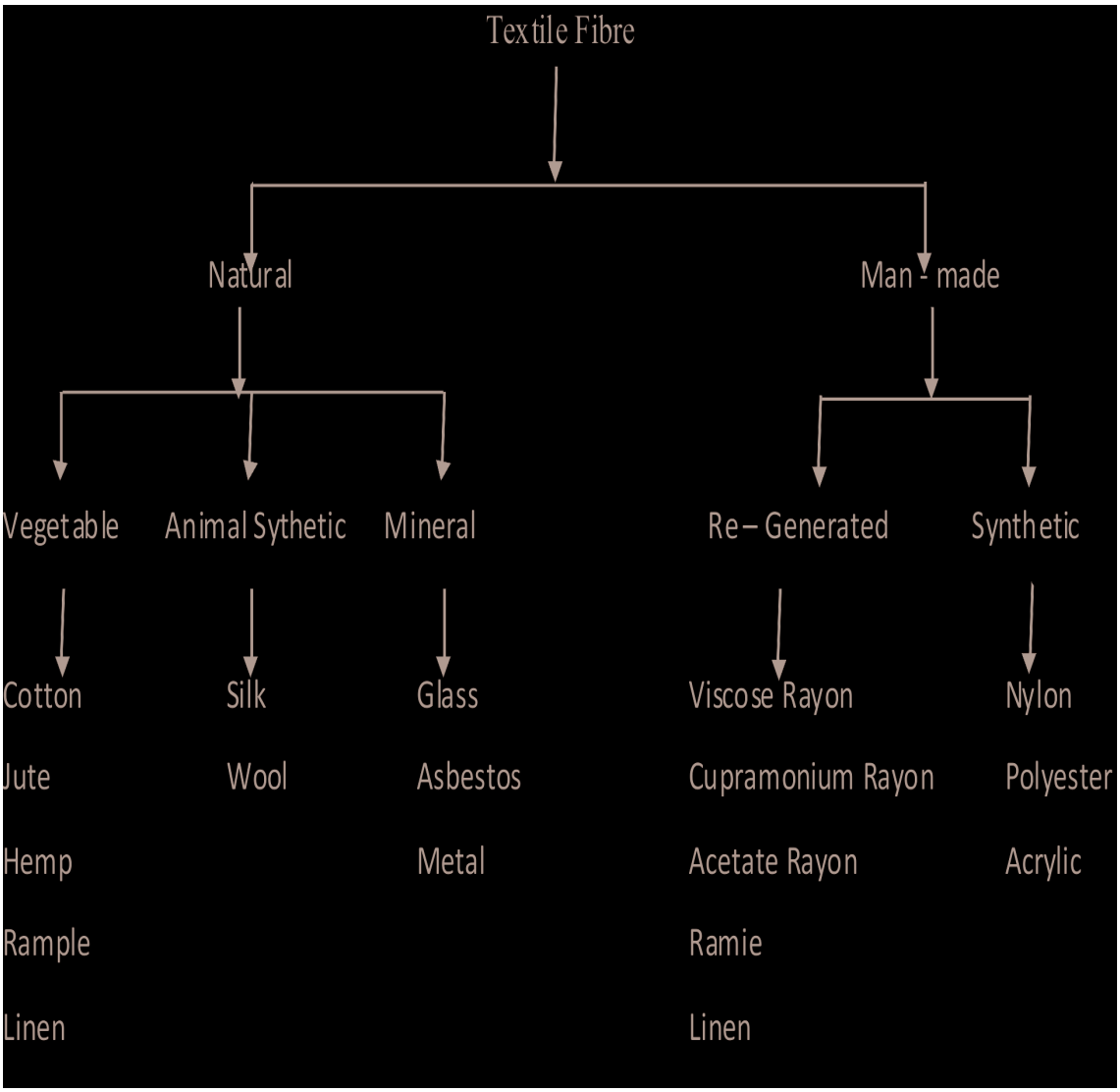
The paper is manufactured from wood, rags and or grasses. Disintegrated paper provides fibre evidence. The paper fibres are sometimes strengthened with plants, plastic, or metal fibres. They are used in some industries.

Naturally occurring proteins have also been used to produce modified fibres. For example casein fibre is obtained when alkaline solution of certain protein is extruded through acidic solution.

Synthetic fibres dominate the scene and it is doing so increasingly. The number of different synthetic fibres is already a legion and the number is increasing with vehemence with the passage of time. There are about 25 varieties or more. The main types (and there are many sub-types to most of them) are:

1. Acrylics - a big family.
2. Polyamides- numerous nylons
3. Polyesters - varieties are increasing in number.
4. Polyvinyl
5. Fluorocarbons- Teflon
6. Olefins

Other synthetic fibres, which are likely to invade the industry increasingly, are chlorofibres, flurocarbon and polyurethane elastomers etc.



Evidentiary Clues

Fibres provide the following variety of clue materials.

1. Individual fibres.
2. Threads.
3. A piece of cloth.
4. A clothing.
5. A cord, string, rope or their piece(s).
6. Hairs from different sources, woolens.
7. Furs.

Location

The main sources of fibrous evidence are the clothing, the bedding, vehicular upholstery, carpets, household furnishing, human and animal bodies, skins, furs, fur

garments, etc. Fibrous evidence may be sticking to the weapon of offence (sticks, knives, hammers, axes, firearms, and the like). The evidence may be found at the scene, with the culprits, with the victims, on the floors and on the objects at the scene. The search, as in other types of microtraces, should be carried out systematically and thoroughly. The search should be rewarding most of the times as transfers and cross transfers of fibres take place almost everytime.

Location and collection of the fibrous evidence is rather troublesome, being almost 'invisible' against the background substrata. It is, therefore, suggestive to send the article suspected to bear the fibrous evidence to the laboratory whenever feasible. Alternatively, the laboratory specialist should be called to help in the collection of the evidence. If the investigator has to collect the fibres, he should:

1. Fix the location of the evidence - Photography! Sketch! written record.
2. Ensure Contamination free collection- use transparent plastic small envelopes. Small fibres are kept in paper folds.
3. Utilise simple gadgets-hand magnifier, bright light, UV light, fine-tipped foreceps. Hand collection is best mode, use it whenever possible
4. Collect a small piece sticking to the substrata by scarping with spatula or as the situation requires.
5. Use separate packing for each garment. Fibres get easily exchanged and thus get contaminated easily.
6. Ensure extreme cleanliness for containers, storing place, examination table, etc.

Questions in relation to Fibre :

Study and evaluation of the fibrous evidence can answer a variety of questions:

1. Has the fibre come from a particular garment, Carpet, or any other given source?
2. What is the nature of the fibres? Are they foreign to the scene or the person where they are found? Or, are they part of the back ground where they have been found?
3. What is the evidentiary value of the given fibrous evidence? Definite linkage or de-linkage? High probability? A weak Corroborative piece?
4. Do the location, nature and pattern of the evidence indicate the sequence of events that may help in the verification of versions or identification of the real or fake nature of the scene?
5. Does the fibre, piece of cloth, string, and rope recovered from the scene match the corresponding material recovered from the suspect or the victim?

Evaluations:

The methods employed were accordingly morphological in character. The physical appearance of the material was noted and compared with the standards whenever

possible both by macroscopic and microscopic means**.** The appearance of synthetic fibre did not, to begin with, change this mode of investigation. However, the introduction of chemical and physical micromethods of this century soon had a pronounced influence also on the examination of fibres in the forensic laboratory. At present, a large number of morphological,physical and especially chemical methods are available-and are used- for fibre identification and comparison.

Preliminary examination

The main evaluation techniques for the fibrous evidence follow the general pattern of evaluating the microtraces. However, there are specific determinations to be made. The following techniques, processes and determinations are made:

The fibres are examined visually, with a hand magnifier and under stereomicroscope. The study indicates:

1. The twist of the thread, string, rope, or cord.
2. The number of strands.
3. The number of thread in the string.
4. The number of fibres in each thread.
5. The defect in the thread or weave pattern
6. The thread counts of cloth, both in the weft and wrap.
7. If recovered torn piece of cloth originally formed part of the standard provided, a mechanical fit may indicate the common source.

Microscopic examination

Microscopic examination is useful to understand the following characteristics of the fibres:

1. The structure of the fibre
2. The nature of the fibre materials
3. The diameter of the fibre.
4. The presence or absence of contamination
5. Colour of the fibre
6. Cross sectional structure
7. Inclusions, including de-lustring agents
8. Adhering materials
9. Process failure defects
10. Damages, if any
11. Abrasion due to wear and tear

Using ultraviolet rays (for fluorescence) and polarized light (for phase contrast) extends the microscopic examination and provides additional discriminating data.

Physical properties

Physical properties like density, refractive index, melting or softening point and tensile strength give important information about the fibres.

Cross sectional study of fibres through microtomy reveals structural features that are otherwise difficult to comprehend. The technique is similar to one employed in the study of hair.

Chemical test

Burning, solvent action and staining techniques are employed for the evaluation of the evidence. .

The methods are roughly grouped into two types- **non destructive** and destructive procedures. This enables the forensic scientist to choose a correct sequence when progressing from one method to another. Among the Non destructive micro methods of analysis-visual comparison, microscopic examination, density determinations, micro spectrophotometry, x-ray differaction and electron spectroscopy chemical analysis. The **destructive** micro methods of anaylsis of fibres are burning test, solubility test, hot stage microscopy, staining techniques, microscopy of cross sections, extraction of dyes, pyrolysis gas chromatography, differential thermal analysis, mass spectrophotometry etc. The selection of the appropriate tests in the evaluation of the fibers depends upon the choice and expertise of the scientist working in the field.

Fibers depend upon the choice and expertise of the scientist working in the field.

Suggested Questions:

1. Define fiber?
2. How will you differentiate hair and a fibre?
3. Elaborate the classification of fibres.
4. Discuss methods to determine the whether fibre is synthetic or animal origin?
5. What type of information can be sought from fibre?
6. Discuss about the collection, packaging and transportation of fibre samples recovered from scene of crime?
7. How the microscopic examination fibre is done?
8. Is individualization from fibre possible?
9. Discuss about the forensic significance of fibre.

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LESSON NO. 2.7 AUTHOR : DR. MUKESH KUMAR THAKAR

POLLENS

Pollens are more significant in criminal investigations than any other form in which plant remains occur in evidence. This assumption follows from the fact that pollen grains are so tiny that they adhere to almost anything and will invariably be found in clothing, sweeping, earwax and other locations. Because their presence is not suspected by the person carrying them. No special effort is made to eliminate them, and if it were, it is more than likely to be unsuccessful.

Pollens like other plant parts are highly individual as to species, although the distinction between some species is difficult. The identification of the species of a plant which contribute pollens to a suspect is of value in direct relation to the uncommonness of the species, and its proximity to the environment in question. Evidence based on pollen identification is more likely to aid in defining the individual's normal habitat than his brief presence at the scene of crime. This would not be the case if he brushed against pollinating flowers in entering or leaving a particular questioned location. If this happened, he might carry away so much pollen of a certain type as virtually to prove direct contact with the exact type of flower. The ordinary distribution of pollens to be located on an individual's clothing or in his earwax is the distribution to which he is ordinarily exposed Viz. that of his normal environment.

The chief source of pollen in physical evidence is sweeping of clothing. Pollens will be recognized microscopically in the fine dust that remains after removal of hairs, fibres and the more conspicuous forms of debris.

Pollens generally contributes information that is supplemental to that available from other evidence, but it is valuable in confirming such indications and in narrowing the possibilities, even when the pollen alone is of only limited value. The value of pollen as evidence is not limited to rural areas, because urban environments, having fewer sources of pollen, give to any pollens that are present in evidence a greater value than they would have in a locale where pollen is plentiful and diverse.

Palynology, the study of pollens is now gaining importance in forensic casework analysis. They not only help to link the particular type of flowers found at a crime scene but also to the time (season) when they are in bloom. Forensic palynology refers to the use of pollens and spores evidence in legal cases. It also includes legal information derived from the analysis of a broad range of microscopic organisms that can be found in both fresh and marine environments. Because of their microspic nature, pollens are different type of trace evidence found at the scene of crime.

One of the earliest reported cases using palynology occurred in Sweden 1959. A women was killed in May during a trip in Central Sweden. Palynological examination was done to determine whether or not the women was killed where she was found or the murder took place elsewhere and the body dumped at the site of the alleged crime scene. The examination suggested that the murder took place elsewhere because the dirt lacked pollen from plants common in the area where the body was found (i.e Plantago, Rumex and grasses).However the later interpretation was that murder took place in May because the pollination during that time was over.

Location : Pollens may be present in the dirt, collected from the clothing's, skin, hair, shoes or car of a victim and might prove links between the victim and the scene of crime.

Collection: Collection of pollens should be made with clean implements. All samples should be well labeled and sealed in sterile, zip lock plastic bags. Collect the relevant samples as soon as the crime is discovered .These spores can be collected by sweeping or using cellophane tape.

Importance : A stolen vehicle can be linked with crime after comparing the mud found on that vehicle. The pollen evidence may be useful in linking with specific crime or a specific geographical area. The pollen grains from bodies of ancient people were examined which provided clues to their diet and domicile. Pollen and spores are abundant in the soil, dirt and dust samples.

1. Soil samples for palynological analysis are very useful in forensics.
2. Pollen sample within a localised area are similar and show the difference from distant localized area.
3. Pollen fingerprints can be very useful in determining the source of origin of the cocaine samples.
4. Pollen samples from the stomach contents or excrements have provided a clue to death as due to the use of, poisonous honey.
5. Pollens as evidence to link suspects to crime scene.
6. The forensic pollen fingerprints have been highlighted very prominently in a murder mystery called the probable cause (Pearson,1992)

   More recently, forensic palynology has been used regularly as evidence in criminal trials in Australia, New Zealand and the U.K.

Application : Forensic palynology can be used in cases as broad as rape, homicide, genocide, terrorism, drug dealing, assault, robbery, hit and run, counterfeiting, and illegal importation as well as civil cases involving geopreservation, illegal fishing, and pollution.

In general palynology can be usd to :

1. Relate a suspect to the scene of a crime or discovery scene.
2. Relate an item left at the crime scene or discovery scene to a suspect.
3. Relate an item at the discovery scene to the crime
4. Prove or disprove alibis.
5. Narrow down a list of suspects.
6. Determine the travel history of items, including drugs.
7. Provide information as to the environment that an item has come from.
8. Provide information as to the geographic source of items.
9. Aid police in their lines of inquiry.
10. Help locate clandestine graves and human remains.
11. Help to determine the perimortem fate of a victim and
12. Help to determine the deposition period of human remains.

Therefore, pollens and spores can be obtained from an extremely wide range of items, including bodies. Their usefulness lies in a combination of their abundance, dispersal mechanism, resistance to mechanical and chemical destruction, microscopic size and morphology. Their often complex morphology allows identification to an individual parent plant taxon that can be related to a specific ecological habitat or a specific scene. Pollen and spores assemblages characterize different environments and scenes and can easily be picked up and transported away from scene of interest without providing any visual clue to a suspect as to what has occurred. Pollen evidence can remain at a scene for many years after the event under investigation occurred.

Evaluation : Morphology features that can be used for the identification are ;

1. Size
2. Shape(spherical,ovoid ,triangular)
3. Sculpture patterns on the surface of the grains (granules, spines, papillae, reticulation etc.)
4. Aperture type and number (pores colpi or both; inaperture,1,2,3,4 apertured or multiapertured)
5. Wall structure (columellae, tectate, perforate, etc.)
6. Wall thickness
7. Variations in thickness around the pollen grains.

Most pollen examinations and identification are done using the transmitting light microscope but additional details of the surface sculpture can be obtained, if required by examination of specific pollen grains using scanning electron microscope (SEM). The outer layer (exine) of the pollen is resistant to acetolysis, physical and biological degradation because of this, pollen can be found well preserved at a crime scene for a long period. Forensic palynologists need access to good pollen reference collection, not only of their native taxa but also of other species from all around the world. If due

attention is given to this important aspect of biological material many cases of adulteration of food and other eatables such as honey, milk etc. and cases of rape, murder, kidnapping can be solved easily. Pollen evidence has also become significant in determining the manner and time of death, source of origin of illegal drugs and their route through which it has probably been transported. Forensic palynology can provide scientific comparisons that may be used as associative or exclusionary evidence in court.

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LESSON NO. 2.8 AUTHOR : PROF. R.K.GARG

BLOOD

One of the important types of physical evidence that can be associated with serious crimes is serological evidences. Serological evidence consists of blood and body fluids found at the scene of crime. Bloodstains are commonly encountered in crimes like murders, physical assaults, rape abortions, etc. If they are properly analysed, they can help in determining the individual involved in the commission of crime.

Blood is one of the most important frequently encountered types of evidence in criminal investigation. Blood can be found at the crime scene or on the victim, the weapon and possibly the suspect. Other types of serological evidence that can be recovered from the scene include semen, saliva, urine etc. These types of evidence are most often found in association with violent crimes against persons, but their presence should not be overlooked at other types of crimes.

Blood is a fluid (normally red in colour that circulates throughout the body, transporting oxygen, nutrients, and waste materials. The volume of an average human being is six to eight quarts and accounts for 6 percent to 8 percent of the total body weight. If a tube of whole blood is allowed to stand with the addition of a chemical to prevent clotting, it will separate into two distinct parts. The upper yellow liquid portion is called the plasma, and the bottom solid red portion contains the blood cells.

The plasma, or liquid portion of the blood, makes up 55 percent of the total blood volume. Plasma is composed of approximately 90 percent water, the source of which is food and the water produced by metabolism. This supply of water is adjusted by elimination through breathing and through the excretory system. By these processes the total water content of the body, and the volume of the blood, is kept under control. The materials carried by the plasma include glucose (blood sugar), hormones, vitamins, proteins, minerals, the chemicals necessary for blood clotting, and the blood cells. Some of the proteins contained in the plasma are called antibodies. These antibodies are useful in forensic identifications. Serum is a term used to describe blood plasma that has had all of the chemicals used in blood clotting removed.

There are three types of cells contained in blood: the red cells or erythrocytes, the white blood cells, and the platelets. Red blood cells are produced in the bone marrow and are disc shaped with indentations on each side. A cubic millimeter of blood contains approximately 5 million red blood cells. The primary function of the red blood cells is to transport oxygen from the lungs to the body tissues and then transport carbon dioxide produced as a waste product back to the lungs, where it is

exhaled. This function is accomplished by a chemical, called hemoglobin, which is contained in the red blood cell. This chemical is also responsible for the red colour of the blood. Hemoglobin is composed of two parts, a pigment containing iron, heme and a protein component, globulin.

The major function of white blood cells is to protect the body against infection. Leukocytes, one type of white blood cell, are capable of moving through the walls of the blood vessels and traveling to the site of an infection. Once they have reached the site of an infection, they will engulf and destroy any bacteria present. The accumulation of white cells at the site of an infection is called pus.

Platelets are cell fragments formed in the bone marrow. These plateles will rupture on the edges of a torn blood vessel and initiate the blood clotting mechanism. Upon rupture, the platelets release a chemical which in combination with other chemicals found in the blood, will form a blood clot. The actual blood clot is formed from a protein in the plasma called fibrin. Fibrin forms tangled network of long strands, which traps blood cells. This fibrin network covers the wound opening and prevents excessive bleeding. A pure blood clot is yellowish–white in colour, although the fibrin network traps red blood cells giving the clot a red colour.

Prior to 1985 blood did not provide a definite type of evidence like fingerprints, firearms or tool marks. Up to that stage of development, it was not possible to say positively that a particular blood sample belongs to a particular person only and could not belong to another. Tremendous progress, however, have since been made in this progress direction and the individuality of blood is a reality in the field of forensic investigation.

Formerly, it was possible only to say whether a given stain is of blood or not. Later, it became possible to identity whether the given stain is of human or animal-origin. Further progress made it possible to classify blood into four main groups, O, A, B, and AB. Later, the division was extended further. Determination of M, N and MN types, the study of P factor and eight 'Rh' types, Duffy, Lutheran, Lewis Gm, Gc serum group and enzymatic groups like PGM,AK, etc, divide the blood group into hundreds of groups. Discovery of additional antigens, antibodies and behaviour of blood in electric field (electrophoresis) has increased the division and the probability figures relating to identification of blood. However, it was the discovery of DNA profiling which has increased the importance of the blood evidence tremendously. The evidence today is on the same footing as the fingerprint. It is now possible to individualize a given sample of blood and to say that it belongs to the given individual and to nobody else, except with one exception, that of an identical twin if one exists. In spite of the fact that DNA profiling has made the blood evidence definite, the classical system still continues in almost all the laboratories in India. However, the technology is so important that no laboratory worth the name can afford its non-induction. In fact it is

the most important discovery of the recent times, say of all times in criminal justice system.

The proper study of the evidence helps in the investigation of a number of different types of offences against persons in the following ways:

1. It establishes the occurrence of crime
2. It permits reconstruction of the occurrence of crime from the distribution of blood.
3. It is possible to guess the approximate time when the crime was committed from the study of the age of stains.
4. It helps the investigation of poaching, maiming and worrying.
5. It helps to establish the paternity of a child in disputed cases.
6. It establishes an inter se link between the criminal, the crime, the victim and the scene.

In view of the tremendous importance of blood evidence it is necessary that the investigating officers, lawyers and the judges who have to deal with the evidence should understand its nature and possibilities, so that its value is properly utilized in the dissemination of justice and it is not over or under estimated.

The detection and evaluation of blood evidence is a delicate process. The same is interfered by external factors: contamination, putrefaction, over-heating, bacterial growth and action of chemicals. The surface on which the blood evidence is found also affects it. Like wise, the age deteriorates the bloodstains. Thus the results are often not what they could be.

The blood evidence in criminal investigation is almost always in the form of dried stains. It is, therefore, necessary to understand what happens on drying.

The first change that takes place in blood on exposure, is clotting of the blood. Further, complex changes take place on drying. The cell structure is often destroyed. The proteins lose some of their activities. Bacteria, fungi, heat and light may bring other changes. The process of change continues and with the passage of time the blood may lose all its identifying characteristics. It is, therefore, always suggested that blood should be examined at the earliest.

The colour of the stain also undergoes successive changes with passage of time and the factors affecting drying of the stains are temperature, humidity, quantity, surface bearing the stain and atmospheric conditions including rain and wind

The following are some of the major questions about blood/bloodstain which a scientist can be asked to answer in the court of law:

1. Does the given article bear bloodstain?
2. Is it human or animal blood?
3. What is the blood group of the human bloodstain or the given body fluid?.
4. How much blood has been shed?
5. What is the age of the stain?
6. Is the Child an issue of the alleged parent?
7. Does the blood contain alcohol? if so, how much?

8. Does the blood contain carbon monoxide, hydrogen cyanide, etc?
9. Does the blood contain poisons?
10. Does the blood contain narcotic drug?
12. Did the person suffer from any disease like syphilis or leukaemia ?
13. From which part of the body has the blood come?
14. What is the occupation of the person?
15. Can you individualise the stain?

LOCATION

The location of blood evidence is ordinary not problematic. In searching for bloodstain evidence at the crime scene, the investigator should rely on the circumstances and any information available concerning the crime. As a general rule, any blood that has been shed will be visible to the investigator. This may be in the form of large pools of liquid blood or smaller stains. Whenever large liquid pools of blood are observed, they should be protected until they can be properly photographed and collected. This can be accomplished by covering them with a glass, pan or some other suitable object. This protects against an officer inadvertently stepping in the blood and tracking it to other areas of the scene. Most often blood will be in the form of dried stains located on the victim, on the suspect, if present, and possibly on any object located at the scene. Special attention should be given to deposits that are separated from the general deposits, as they may be from a different source, such as the suspect. However, in some cases where the search is started after a lapse of time it becomes a difficult task because the stain may change colour so as to be in distinguishable with the substrata or the wind, rain, heat and light may wholly or partially cover or wipe it off. Human beings, animals or traffic may also wholly or partially destroy the stains. The search for the location of stains is carried out in the following ways:

Techniques applied

1. The area, articles and clothing suspected to bear bloodstains are examined with strong oblique light. Most of the stains become visible. The light is used even during daytime. If the article is a cloth it should be spread on a table and examined under a shadow-less lamp.
2. Use of coloured lights, red, green and yellow, sometimes proves useful.
3. Ultraviolet rays are very useful to locate bloodstains. The article suspected to carry bloodstains should be examined preferably in the dark under the rays. Even washed stains become visible.
4. Infrared rays are useful in locating the bloodstains on coloured objects, where the bloodstains become invisible, because of the colour of the background. A photograph with infrared rays reveals the blood spots.
5. Luminol, a chemical has been used to locate the bloodstains. The articles suspected to bear stains is sprayed with luminol reagent. It reacts with blood to give luminescence. The bloodstains are, thus made visible. Even decomposed

blood reacts with the reagent. The reagent does not interfere with subsequent blood tests. Blood gives strong luminescence.

The reaction is interfered by copper salts, which also give luminescence with the reagent. If the result is negative, no blood will ordinarly be found on the articles tested.

Sites for the location of Blood

The important places for the location of blood are the scene of occurrence, the culprit, the victim, the weapon of offence, the vehicle and the route the culprit takes after the commission of crime.

1. THE SCENE

The blood evidence may be found at the scene of crime in the form of stains, smears, streaks, splashes, spray, prints, clots, pools or liquid blood, on various articles. It may be even clots trapped in wash basin trap or it may be blood dissolved in the water of the trap.

In indoor scenes, the floor, walls, doors, windows, furniture (Chairs, tables, beds) and fans are the likely places. The position, size and the pattern of the stain, pools etc. are carefully noted, sketched and photographed. They help in the reconstruction of the scene afterwards.

2. BEDDING

Clothing at the scene : Bedding, curtains, upholstey, carpets, table covers and the like, carry stains either due to spattering or due to wiping of the blood by the culprit.

The culprit may have used sundry items like paper, pieces of cloth, rags, articles of clothing or other tissue material to wipe off the blood. He is likely to discard these items at or around the scene. They may be lying in dustbins, debris heaps, kitchen refuse or in water closets and needs to be searched.

The blood may have been removed from the scene. The culprit may have washed the floor or other stained area, or he may have washed his hands, face, or clothes or the weapon of offence. The culprit thus succeeds in obscuring the blood evidence but it can be located. If floors, walls, doors, windows or furniture have been washed the evidence is available in cracks, crevices and joints. If clothes have been washed, blood may still be found under the seams or underneath the sleeves. If blood has been washed in a wash basin or in a closet, some clots are present in the traps attached to them.

The drawers and under surfaces of the furniture should also be checked for wiped blood. if the culprit gets his hand and feet smeared with blood, he is likely to leave finger, palm, foot or footwear prints at the scene.

In outdoor scenes, the ground, the stones, the grass, the plant leaves, twigs and branches may carry blood. A rich growth of grass or dry or wet leaves may have been used to wipe off the blood or, earth, sand or dust may have been used for the purpose.

The victim, ordinarily, carries his own blood but in some cases. he may carry the blood of the culprit on his person and clothing. If the occurrence is properly

reconstructed it is possible to establish the position and location of the culprit's blood on the person of the victim. If the blood is found to be of the same group as that of the suspect but different from the blood group of the victim, the finding carry a strong presumption of the suspect's guilt.

3. THE WEAPON OF OFFENCE

If the victim has been hit when he was bleeding or if the weapon being a sharp edged weapon, the weapon is likely to carry blood evidence. In certain cases (e.g., Where the weapon has been withdrawn from a deep cut) most of the blood is wiped off. The weapon, however, carries sufficient blood to permit identification. Even if the weapon has been cleaned after the offence, some blood is still retained in the joints, crevices and cracks of the weapon. The weapon should invariably be sent to the laboratory for blood tests.

If a firearm is discharged while it is in contact or from a near contact range, it may get splattered with blood, flesh and skin pieces of the victim. They permit identification of the blood group.

4. VEHICLE

If the culprit has used vehicle in the commission of crime or for the disposal of the body, bloodstains are likely to be retained at the spot where the body was placed. Even if the spot is cleaned some traces of blood are likely to be left at the site. If an injured or blood soaked culprit has escaped from the scene in a vehicle, he is likely to deposit blood at the site where he sat. The amount of blood transferred is likely to be small. It is likely to escape notice on visual examination.

If the culprit has placed some blood smeared objects like weapon of offence, blood covered booty or clothes in the vehicle, some traces of blood are likely to be transferred to the vehicle.

If an occurrence has taken place in the vehicle itself, sufficient evidence is found in the vehicle even when the culprit has tried to remove it.

5. THE ROUTE

The route followed by the culprit should be checked for blood:

- He may discard blood-smeared objects on the way.
- He may be bleeding to leave a blood trail.
- He may carry an injured person who gives a blood trail.
- He may carry blood- smeared objects, which may transfer some blood to walls, stones and trees, etc. which come in their contact.

6. DISPOSAL SITE

The culprit may have thrown, hidden or buried the booty, the body or the in criminating evidence at a particular place. The place is likely to carry blood, fragments of skin, flesh or bones at the site.

Collection

When collecting blood evidence the investigating officer should:

1. Establish the location of the evidence and the position of the article with respect to other stationery objects at the scene through copious notes, sketches and photographs.
2. Note the direction, size and the number of stains.
3. Note the condition of the stains, whether they are dry, sticky, wet, crumbling, puckering, contracting or brittle.
4. Note whether the stains are contaminated or exposed to natural elements or heat. The facts should be mentioned in the forwarding letter of the expert.
5. Write the method of collection: whether the stain was scraped, cut, lifted, dislodged or collected in the form in which it is being sent.
6. Pack the bloodstains from various sources separately in suitable bottles or cellophane envelopes of appropriate sizes.
7. Preserve the purity of the stains. This is achieved by preventing mixing of stains with one another or rubbing of the stain with the container or with other parts or the article.
8. Preserve the continuity of the chain of possession and distinct identity of the evidence through proper packing, labeling, sealing and observing other legal formalities. The possession of the evidence should be accounted for from the time of recovery to the time of its production in the court.

Methods of collection of blood evidence vary with the nature of evidence and the place or article on which it is found.

The following techniques cover most of the situations.

LIQUID BLOOD

1. Liquid blood is found at the scene in the form of blood pools. Two samples, each about five milliliters, are collected in clean vials. In one, sodium fluoride, oxalate or citrate is added and the other is kept without any preservative.
2. It is preferable to collect and send the liquid blood in sealed tubes without preservatives. The tubes are cooled in crushed ice contained in a themos bottle, and sent as such to the expert. A third sample is collected on a piece of clean rag. A stain on a piece of clean white cotton cloth, about three centimeter square, with some unstained part of cloth for control test is sufficient. The stain is dried and packed in a clean envelope or bottle. Needless to mention that materials used must be uncontaminated and dry.
3. If the blood in the pool has dried, about three grams of dried blood is collected.
4. If the blood in the pool is sticky mass, a piece of cloth is stained in the centre of the pool, dried and packed as usual along with three grams of dried blood.

WET STAINS

Some times wet stains are found at the scene. The exact technique for the collection will depend upon the nature of the surface and quantity of the blood.

The following are the general guidelines:

1. A clean piece of rag or filter paper is taken and pressed against the stain. The stain is transferred to the rag. If the stain is partially dry, the piece is moistened preferably with saline water. It is pressed against the stain. The same is transferred on to the piece. A cloth piece is always preferable to filter paper.
2. If the stain is on non-absorbent surface, it is allowed to dry and the article is collected. If the stain contains sufficient liquid blood. It is collected in the same way as liquid blood.
3. If the stain is on an absorbent surface and has penetrated the surface, it is allowed to dry and the article is collected. A control sample of unstained material from the place near to the stained area is also collected.

DRY STAINS

In collection of dried stains from the scene of the crime the following situations are common:

On clothes - Take possession of the clothes bearing dry stains. The number position and size of the stains are properly recorded. The stains on different clothes or on the same clothe are not allowed to contaminate one another through dislodgment during packing or transit.

On absorbent surface - Absorbent surfaces absorb the serum. Therefore, for correct sampling stained and unstained materials are separately collected.

On non-absorbent surfaces- To collect blood from non-absorbent surfaces one should:

1. Collect the article whenever possible.
2. Moisten a white clean piece of cotton rag or filter paper with saline water and press it against the stain. The stain is transferred to the piece.
3. Moisten a piece of rag with inert gum and stick it to the stain. The stain adheres strongly to the surface. Peel off the piece when dry. The stain is transferred to the piece. Dry it and pack it as usual.
4. If there is no alternative possible, scrape off as much blood as possible with a clean razor blade on a paper from the stain and pack it in a clean envelope or vial. Ordinarily scarping of blood stain is not recommended, as it is likely to result in the loss of some blood.

On grass - When the blood is found on grass, one should tie the grass with a thread. Cut the tuft. Dry it and pack it in an envelope or glass jar. Take care that the blood does not get dislodged in the process.

On body - Bloodstains are sometimes found on the body of the culprit or the victim. They should be carefully handled to avoid contamination from the body secretions:

1. If blood is found in the hair, it is collected by combing the hair. If sufficient quantities are not obtained the hair are cut and collected.
2. If blood is found in the fingernails, it is scraped out with a clean tooth pick. Collect control sample from unstained fingers also. The paring of nails is not recommended.
3. If blood is found on other parts of the body, a wet piece of clean rag or filter paper is pressed against the stain. The stain is transferred to the piece. If the stain is adhering strongly the piece of cloth may be moistened with inert gum solution.

In all cases there is danger of contamination from the body secretion or blood or the person from whom the bloodstain is recovered. Control sample of his blood should, therefore, always be sent.

If the blood at the scene or on the clothes has been washed it is still possible for the biologist to detect traces of blood from the washed materials.

Evaluations of the blood evidence requires blood samples from the victim and the suspect. A medical officer collects them. However, investigating officer should ensure that the samples collected and sent to the expert be properly preserved, identified and dispatched.

Evaluation :

1. 1. Presumptive tests : Benzidine test, Phenophthalein test leucomalachite green test, O-tolidine test and Luminol reagent test.
2. Confirmatory tests : Teichmann test and Takayama's test

There are chemical tests that the investigator can use for the presumptive identification of blood at the scene. These tests are called presumptive tests because none of them are specific for blood and therefore cannot be used as a positive identification. A positive result indicates that blood may be present, and the stain should be sent to the forensic science laboratory or further analysis be carried out. A negative result, on the other hand, indicates that blood is not present, and no further testing is necessary.

The benzidine, phenolphthalein, Leuco-malachite green, and Luminol can all be employed at the crime scene for the presumptive identification of blood. The benzidine, phenolphthalein, leuco-malchite are based on the activity of the heme group. Heme is component of hemoglobin, which is a chemical present in human blood cells. This heme group exhibits what is called peroxidase like activity. Peroxidase is an enzyme that can speed up a chemical reaction in the body. Benzidine, phenolphthalein, and leuco-malachite green in the presence of peroxidase will turn a charareristic colour indicating the possible presence of blood. Bezidine is prepared by mixing 0.25 grams of benzidine, 175 ml.of ethanol, and 5 to 10 drops of glacial acetic acid together. Once mixed, this reagent should be stored in a dropper

bottle in the refrigerator until used. For conducting the test another dropper bottle containing hydrogen peroxide is necessary. The procedure for conducting the benzidine Test, Phenolphthlein Test in the field is as fillows :

1. Lighly moisten a cotton swab with water.
2. Rub the swab over a small portion of the suspected stain.
3. Add two drops of the reagent i.e benzidine or phenolphthalein solution (blue or pink colour forming at this stage indicates a negative test.)
4. Add two drops of hydrogen peroxide to the swab.
5. A positive result is the rapid appearance of a blue in case of benzidine or pink colour in case of pheolphthlein.

The investigator should bear gloves and take necessary precautions when using benzidine, as it has been determined to be a cancer causing agent.

Leuco-malachite green is prepared in two parts: a dry mixture and a wet mixture. The dry mixture contains 0.32 grams of sodium perborate and 010 grams of leuco-malchite green. These two ingredients can be thoroughly mixed and stored at room temperature for upto a year. The wet mixture consists of 6.6 ml. of glacial acetic acid diluted with 3.3 ml. of water. Just prior to use, the dry chemicals are dissolved in the wet mixture. The application of the Leuco-malachite green test in the field should consist of the following procedure:

1. Lightly moisten a cotton swab with water.
2. Lightly rub the swab on a small portion of the suspected stain.
3. A few drops of the Leucomalachite green reagent is then added to the swab.
4. A positive result is the appearance of a green colour.

An easier method by far for conducting a presumptive blood identification test in the field is through the use of Hemastix. Hemastix is the trade name for a product manufactured by Miles Laborratories. This product is a small plastic strip with a chemically treated piece of dry absorbent material at one end.This test is also based on the peroxidaselike activity of the heme group. The test is conducted by lightly moistening the absorbent material with water and then gently rubbing it on small area of the questioned stain. A blue colour is a positive result indicating the possible presence of blood. Hemastixs are sold in bottles containing fifty strips and represent an easy method for conducting presumptive blood identification tests in the field.

The forensic analysis of serological evidence is to make a positive identification of the stain. Several techniques have been developed that can be used to positively identify blood stains.. The positive identification of blood in questioned stains are based on the detection of the presence of heme. Confirmatory blood identification tests are specific for the heme component of hemoglobin. A positive confirmatory test result is taken as positive proof of the presence of blood in a questioned stain. There are number of confirmatory blood identification tests, but the microcrystal tests are the most commonly used. There are two microcrystal tests, the Teichmann and the Takayama. Neither of these two crystal tests is as sensitive as the presumptive tests and can generally only detect the presence of 0.001 ml.of blood. Confirmatory tests are also

subject to interference from the surface material on which the stain is deposited. Bloodstains on wood and leather give the most interference and often result in a negative result. For this reason a negative confirmatory test is not absolute proof that blood is not present in a stain. Both the Teichmann and Takayama confirmatory tests are based on the observation that heme,in the presence of certain chemicals, will form characteristic crystals that can be seen microscopically. Both these tests are conducted using this procedure :

1. A few stained threads or a small portion of the dried stain are placed on a microscopic slide.
2. A few drops of either the Teichmann or the Takayam reagent are added to the slide.
3. The slide is heated, then observed microscopically for the presence of characteristics crystals. (Needle shape crystals in Teichmann and pink feathery in Takayama test are observed)

Individualization :

1. Enzyme types ;PGM,AK,GLO-1,LDH.etc
2. Blood groups e.g ABO system, MN system, Rh, system, Kell, Duffy, Luthern etc
3. Serum Proteins: Haptoglobin, Globin types, GC etc.
4. Haemoglobin
5. DNA Profiling

Evaluation of blood evidence is a difficult task. An experienced and trained expert should handle the work.

Basis

The identification and evaluation of blood is based on its composition and behaviour under various conditions.

Blood is composed of red and white cells, platelets and plasma. The cells have definite shapes and sizes, which vary in different species. For example, human blood cells have characteristic non nucleated discs with a diameter of about 0.08mm. Camel family blood has oval blood cells. The birds, fish and reptiles have oval, nucleated and large corpuscles in their blood.

The identification of cell structure is possible only when the blood is fresh and moist, usually less than one day old. When the blood has dried, it is not always possible to completely regenerate the blood cells. The study of cell structure is made through microscopy.

Enzyme activity: Other enzymes have been utilized in electrophoretic techniques. When they are subjected to an electric field due to some polarity, they move towards opposite polarity. The rate of movement varies with their structure (stererochemistry) weight and electric charge. The pattern of separation is characteristic of blood.

Spectrophotometry : Haemoglobin on treatment with acids, alkalis, reducing agents or oxidizing agents gives a variety of products which have characteristic

absorption spectra. They help to identify the blood. The absorption bands are seen in length which are characteristic of blood.

Antigens : The blood corpuscles have certain characteristic proteins called antigens. It is believed that the number of the antigens is very large, over 160. They have complex stererochemical structure. If they are completely identified it may be possible to individualize the blood of a human being or of an animal. In addition there are other proteins and cellular enzymes in blood. Above 150 of these proteins and 250 enzymes have been identified.

The knowledge about these antigens, etc. is increasing with the passage of time as more research is being carried out in the field.

Corresponding to antigens, there are antibodies in the plasma (or serum). They are equally complex and large in number. They have a sort of negative relation with the antigens. For example, if a particular antigen is present in blood corpuscles a particular type of antibody will be absent in the serum. If the antibody is introduced in the blood, it coagulates (agglutinates) the blood cells.

Antigens and antibodies are also known as agglutinogens and agglutinins, respectively.

The study and identification of antigens and antibodies form the basis of classification of blood into various groups and their identification by serological methods.

The most important antigens and antibodies discovered so far are 'A' and 'B' major groups which are now internationally known as O,A,B, and AB.

Further studies revealed that antigen A actually exists in a number of forms. Two of them; A1and A2 can be used for further sub-classification.

M and N antigens : In the late twenties it was found that there are at least two more antigens in the blood corpuscles which can be used independently of the previously known antigens. Thus, a person can have antigen M, N or both, M and N. The six groups can thus, be further divided to make eighteen groups.

It was found that there is no corresponding antibody to antigen N. Antibody M is also rare. Therefore, these antigens do not figure prominently in blood transfusions, but they are important in classification of blood.

P factor : Almost simultaneously another antigen named 'P' was discovered. It was found that blood of certain persons have it, while that of others did not contain it. Antibody relating to antigen P is sometimes found in normal human sera. Antigen P offers further sub-division of the blood groups, each of the eighteen groups is divisible into two groups, P- positive and P-negative.

Rhesus groups

In late thirties the immune sera was obtained from rabbits after injecting red blood cells from rhesus monkey. The anti-sera agglutinated about eighty five percent of blood corpuscles of the white population irrespective of the fact whether or not they contain O, A, B or AB, M, N MN or p antigens. The antigen responsible for this reaction was designated as Rh. It doubled the number of the existing groups. Natural

antibody to antigen Rh was not discovered in human blood. But antibodies were developed by Rh negative persons who were given Rh positive blood transfusions.
All of these blood groups are useful in solving the cases of disputed paternity.
The examination of blood involves a number of techniques, involving physical, chemical and biological operations. All the operations are required in all the cases. The choice of techniques depends upon the nature of the evidence, i.e, condition and size of the blood stains, nature of the surface on which blood is found and the expert who may have a liking for certain techniques. The following operations are common: - Identification, Species origin and Blood group determination.

Visual Examination

Visual Examination is useful to determine whether the given stain is of blood or not. Ordinarily, there is no difficulty in fresh stains but old stains may not be identifiable. The examination permits:

1. Determination of the number and size of stains.

2. Rough estimate of the amount of blood shed.

3. The direction from which the blood has fallen is found from the tip of the elongated stain. The tip of the elongation points out the direction.

4. The shape of the stain indicates the height from which the blood has fallen.
- Bloodstains from a height of up to 50 centimeters are round sharp edges.
- Blood stains from a height of 50 to 150 centimeters have small spike like projections along the edges.

5. From the positions of the stains, the movement of the victim and the culprit can be determined. if the culprit ran away while bleeding, he can be tracked through bloodstains.

6. The degree of fluidity, dryness and changes in colour permit rough estimation of the age of the stains.

7. Gross foreign matter like hair, flesh, bones in the blood may identify the site of injury.

UV and IR examination

Ultraviolet or infrared rays reveal washed or 'invisible' bloodstains from clothes, furniture, earth, floors, walls doors or windows. They also reveal stains on coloured garments or on painted surfaces. They help to reveal minute blood traces.

Microscopy

Microscopic examination of bloodstains is important in many situations:

1. Species of origin of fresh bloodstains can be established through microscopic studies and micro-measurements sometimes.
2. The part of the body from which the blood has come can be found out from the nature of extraneous matter. Thus, blood from nose, mouth, vagina or anus can be identified.
3. Diseases like leukemia or syphilis can be detected microscopically.
4. Menstrual blood can be identified.

5. Blood from an infected site can be identified through the presence of pus.

Staining techniques for blood evidence are also utilized in microscopic examinations.

Spectroscopy

Spectroscopic examination of blood is very useful as well as convenient. The test is usually carried out microscopically.

The blood haemoglobin is changed in two or three forms on the slide itself and characteristic absorption are observed. Usually alkali hematin (obtained by treating the stains with ammonium sulphide) and cyanhaemochromogen (obtained by treating the above hematin with potassium cyanide) are studied for their characteristic spectra.

Spectrophotometry

Like some other methods, spectrophotometry can be used to identify blood. Absorption at certain wavelengths indicates the origin of stain. Spectrophotometry is especially useful for disintegrated blood and when only small quantities of blood are available. Absorption in the lower range. (UV range) is more useful. Spectrophotometry has also been used to estimate the quantities of blood.

Chromatography

Ascending paper chromatography, using acetic acid, methanol and water solvent system has been employed to study the Rf values.

Electrophoresis

The electrophoresis technique being used for the separation of various enzyme systems. It is being adopted to study the body proteins. The technique is becoming important to distinguish between blood samples.

Colour tests

Colour tests are the first series of tests employed after the location and visual study of the stain. If a given stain gives positive colour reactions in any of the colour tests, the stain is possibly a bloodstain. If it fails to give colour reactions in all probability it is not bloodstain or the same can not be established. All these tests are highly sensitive and can be performed at site even by investigating officer himself.

All colour reactions excluding luminol are likely to spoil the blood stains for the other confirmatory tests. It is, therefore, advisable to pick up small amout of blood on a filter paper by pressing wet filter paper to the stained area. The filter paper is tested for blood. Alternatively a small amount of stain is scrapped on to a watch glass and tested.

Control tests should be carried out simultaneously under identical conditions. One control test should relate to the surface on which the stain is found and in the second control test, a known bloodstain is used. All these tests are highly sensitive and require only small amounts of blood.

ORIGIN DETERMINATION

Species origin: Precipitin test, gel diffusion, cross-over electrophoresis and antiglobulin consumtion test.

The bloodstains that are positively identified as blood must be tested further to determine the species of blood that is present.

All of the origin determination tests are based on the immunological principle that all animals have an immune system that among other things, is capable of making a protein called an antibody. Antibodies are specific proteins made to counteract foreign proteins that enter the body and will react only with the foreign protein that caused their production. The foreign protein is called an antigen. Most animals will produce antibodies against the proteins, or antigens, of other animals species. If human blood is injected into an animal of a different species, the animal will produce antibodies specifically for the proteins in human blood.

These principles are used to produce antihuman serum in rabbits. First, rabbits are injected with human blood .This causes the immune system of the rabbit to produce antibodies that are specific for the human blood. Antibodies are formed in the white blood cells of the rabbit and are then released into their serum. The rabbits serum, or liquid portion of the rabbits blood, is then collected. This rabbit serum now contains antibodies that are specific for human blood proteins. When this rabbit serum, now called antihuman serum, comes in contact with human blood, the antibodies will react with the proteins of the human blood. This is called a precipitation reaction and results in the formations of large antigen-antibody complexes that will precipitate, or fall out of solution and be visible.

The three common methods for using this precipitin reaction to identify the species origin of stains are the ring precipitation test, the gel diffusion method and electrophoresis. The ring precipitation test involves layering a dilute saline extract of the bloodstain on top of the antihuman serum in a small test tube. Because of the density of the antihuman serum, the blood extract will layer on top, and the two solutions will not mix, thus forming an interface between the two solutions. The test tube is then incubated or heatd, at body temperature. After heating, the tube is examined. If the stain is human blood, a white line, the precipitate, will be formed between the two layers.

The gel diffusion method is based on the fact antigens and antibodies will diffuse, or move towards each other, on an agar gel coated plate. The stain extract and the antihuman serum are placed in separate holes in the gel, where a white precipite line will be formed between the two materials if the stain is human blood. Another technique, used because it is more sensitive than the others, is cross-over electroporesis. Electrophoresis is a technique involving the use of an electric current. This electric current is passed through a gel plate, causing the movement of materials in the gel. In this method the antihuman antibody and an extract of the stain are placed in separate holes in the gel. An electric current is applied that causes the materials to move toward each other .If the stain is human blood, a white line will be formed between the two materials. In order to conduct the origin determination properly, controls are necessary. One such necessary control is an unstained portion of the material the blood stain is on, to verify that the blood is causing the positive

reaction, and not the surface material on which it is deposited. For this reason, the investigator should submit unstained samples of all the materials on which bloodstains are found. Human blood that has been heated will often result in a negative test result. Therefore, the forensic scientist should never dry liquid bloodstains by heating them.

BLOOD GROUPING TECHNIQUES:

Blood grouping techniques depend upon the existence of antigens and antibodies in human blood. The reactions of the antigen and antibodies permit identification of the blood groups.

The antigen- antibody reactions are carried out by a number of techniques. In the past few decades very sensitive methods have been developed by which blood attached to even one millimeters long fiber of ordinary cotton cloth can be successfully grouped.

The commonly employed techniques for blood groups determination from bloodstains are ;

1. **Absorption inhibition**
2. **Absorption elution**
3. **Mixed agglutination**

Direct methods involving mixed agglutination, or absorption-elution techniques are more popular. They are Simple in principle and participate, but need experience. They may give unsatisfactory results with inexperienced person.

MIXED AGGLUTINATION

The antigen of the questioned sample is flooded with antisera. Excess is removed after some time by washing. Known cells are then introduced. The indicator cells corresponding to the original antigen get attached.

Absorption-Inhibition method : Antisera vary in their strength. Some antisera have more antibodies in them than others do. By doing a procedure called titration of the antiserum, an estimate of the amount of antibody present in the aniserum can be obtained. The absorption-inhibition method depends on being able to estimate the amount of antibody present in an antiserum before and after exposure to a stain extract containing a possible antigen. The test is called absorption-inhibition because it is designed to find out whether the dried blood will absorb some of the specific antibody in the antiserum, thereby inhibiting its strength.

Absorption- elution method

Absorption elution methods involves the following steps:

1. Prepare Anti- A, Anti-B and Anti-H sera of 1:256 to 1:512 titre value.
2. Prepare 0.5% cell Suspension of A, B and O cells.
3. Fix stain bearing thread (2.3 mm.) On a cellulose acetate sheet along with known
   blood stains and control cloth fibre, with an adhensive.
4. Add one drop of appropriate anti-serum on each thread.

5. Incubate in a moist chamber at 4^0C for 12hours.
6. Wash off the excess anti sera with cold saline 6 times.
7. Blot dry the sheet with filter paper.
8. Add one drop of 0.5% indicator cells to each thread.
9. Incubate the sheet at 56^0C for 15minutes in moist chamber.
10. Rotate the sheet for 30 minutes and read the agglutination under a microscope.

If cells of group 'A' are agglutinated,the original blood belongs to group 'A'if cell of 'B' group are agglutinated, the orignal sample is of group 'B' and so on.

Kind (1961) has suggested a slightly modified method for the same.

Absorption- elution technique has been employed successfully for the detection of A, B, H, MN, Rh and for other antigens.

The following points should be kept in view.

1. The original sample should be properly fixed.
2. Absorption should take place at optimum conditions. High temperature may denature the antigen and prevent absorption.
3. The antisera should be of correct strength (avidity).
4. Proper washing is needed. Excessive washing causes elution while in complete washing does not remove the antisera completely.
5. Indicator cells should have low concentration.

Inhibition procedure being less sensitiver require more bloodstain material than elution tests. Certain inhibition procedures are also more involved than the usual elution procedure, but both techniques have their place in bloodstain grouping. Selection of a technique must be based upon the size and nature of the sample and the circumstances of the case. Most of the laboratories in the world use elution technique for most bloodstain samples.

Blood groping from antibodies

Blood groping is usually carried out with the antigens present in the bloodstain. But antibodies can also be used for the purpose.

Antibodies are labile and are destroyed comparatively more easily. When fresh blood is available in sufficient quantity, antibodies in the serum can be used similarly in antibody-antigen reactions.

Pitfalls

Blood grouping is interfered by many factors.

1. Imperfect antisera
2. Auto- agglutination - Auto agglutination is the formation of clumps in the blood extract without adding the antiserum. This can be due to a number of reasons.
3. Pseudo-agglutination - Pseudo-agglutination is the apparent clumping of cells when higher than necessary concentrations of cells of sera are used in the experiments. On dilution the clumps break up. The blood behaves normally.
4. Bacteriogenic agglutination - Bacteriogenic agglutination takes place through bacteria. if blood samples of their solutions are kept in humid climate and at

appreciable temprature the bacterial growth takes place which gives non specific reactions.

5. Atypical antibodies - Atypical antibodies are found only in a few blood sera. They interfere in the antigen-antibody reactions.

In the view of the above, it is essential that blood grouping is carried out under optimum conditions and in addition to using duplicate samples, control and blank samples must also be used . The high sensitivity of the methods requires a high degree of clealiness and strict control of the reaction conditions.

SPECIAL PROBLEMS

Blood in debris:

Dust and debris may contaminate blood. It can be tested only after purification. The techniques evolved for the purpose is:

The debris is placed in a container and covered with water. A filter paper is placed vertically with the flower end dipping in the container. By capillary action blood solution is sucked up. The blood gets concentrated near the upper end of the paper strip.

Age of blood stains

In addition to the colour and appearance, which change with time, blood also diffuses, if the surface on which the blood is found is permeable. The extent of diffusion indicates the time elapsed.

The stained surface is treated with silver nitrate solution. The extent of diffusion may be indicated by the size of the black ring formed.

DISPUTED PATERNITY

Cases relating to disputed paternity are not frequent in India as compared to Western countries due to certain social and cultural patterns. But such cases do come up. In western countries determination of disputed paternity is an important and sizable problem. For example, in the Denmark over 10,000 blood samples are examined annually for disputed paternity alone.

Blood groups in children are inherited from their parents according to Mendelian hereditary law. Consequently if blood groups of father and mother are known it is possible to ascertain what could be the possible groups of the child and vice versa. For example, if the father and mother belong to group 'O'. The Child can belong to group 'O' only. He could not have A,B or AB blood group.

Quantity of blood shed is estimated from the solid residue found at the spot. The solid residue is about twenty percent of the total blood.

1. The distribution of blood stains is useful in the reconstruction of the sequence of the occurrence. The blood drops can lead to the place or places visited by the injured victim or the culprit.
2. The determination of the presence of disease in blood may authenticate the claim for diminished responsibility for the death of the victim

3. The disease organisms are examined microscopically, by staining techniques and by biological methods.
4. The presence of alcohol, carbon monoxide and other poisons in blood are important clues in criminal investigations.

Suggested Questions :

1. Discuss various tests to identify Blood/Blood Stains.
2. Discuss different techniques to identify the species from blood stains.

LESSON NO. 2.9 **AUTHOR : MR. JASWINDER SINGH**

SEMEN

IMPORTANCE AND NATURE

Semen is the most important body fluid in the investigation of crime relating to sex offences, indecency, rape, sodomy, bestiality and the like.

Semen is a complex fluid produced by the male reproductive system. Semen is usually white to yellowish in colour and is ejaculated or expelled from the penis. The average human ejaculate is approximately 3.5 ml. or about one teaspoonsful and contains about 350 million spermatozoa. Semen is composed of two fractions : the liquid portion called the seminal plasma ,and the cellular portion, the spermatozoa. The liquid portion, seminal plasma, is thick complex mixture of secretions from the male reproductive organs and serves as the transport medium for the spermatozoa cells. One of the chemical components of semen is flavin. This chemical is responsible for the somewhat yellowish colour of semen, which causes the semen to fluoresce, or glow, in the presence of ultraviolet light. This characteristics can serve as an important means of locating semen at crime scenes.

The cellular portion of the semen is the spermatozoa. Spermatozoa are the male reproductive cells and contain one half of the genetic information necessary for the formation of fetus. Spermatozoa are approx.1/500 of an inch in length and are structurally composed of four parts. The head of the cell contains the genetic information and is usually teardrop shaped. The shape of the head is the most striking difference among the sperm cells of various animal species. The neck joins the head and the midpiece of the cell. The midpiece produces the energy necessary for the movement of the cell, and the tail is necessary for the mobility. The tail of the spermatozoa accounts for nine-tenths of the cell's overall length. The identification of one intact sperm cell, by the biologist on a submitted stain, is positive proof of the presence of semen.

Semen may be found in liquid form, smears or stains or, it may be found in vagina, anus or rectum. Fresh semen is a gel like fluid, which liquefies on exposure to atmosphere. The dry weight is about seven percent of the liquid weight.

The sperm has a definite morphological structure. Its identification in a stain establishes the presence of semen.

The shape and size of the human spermatozoon is characteristic. But the morphology alone does not permit individualization.

Semen of Some persons does not contain any spermatozoon. It is called aspermic semen. This may be due to some disease or it may be due to vasectomy operation. In such cases this criterion for the identification of semen is lost. However

immunological test using anti-semen sera against seminal plasma are increasingly being accepted as reliable test for aspermic semen. Electrophoresis is becoming popular for the identification semen.

Chemically semen is a complex mixture of organic and inorganic compounds. Important Constituents of semen, from identification point of view are proteins including enzymes, blood group factors, choline, fructose, cirtic acid, uric acid and Zinc. The composition varies from individual to individual.

Enzyme, acid phosphatase, found in semen, is in concentrations which are significantly higher than those found in any other body fluid. Acid phosphatase therefore, offers a very delicate test for the identification and location of semen stains, though positive identification of the semen are not based upon the acid phosphatase test alone. Choline, another substance, in semen is used to get crystal tests. Fructose, citric acid and Zinc are more less absent in other body fluids and hence their detection in semen should permit its identification but these substances have not been utilized to any appreciable extent so far.

The blood group factors and other proteinous substances have been used in carrying out blood grouping test of semen. They are also used to carry out precipitin reactions with semen stains using anti-semen serum prepared in the way anti- human blood serum is prepared.

Semen stains are located by the following techniques:

Visual examination: The suspected place or article is examined visually. A semen stain, if fresh, is colourless, or pale yellow. It gives characteristic smell. A freshly dried stain on wetting also gives the characteristic odour. The stain becomes stiff and rough to feel on drying. The stiffness does not wear off unless it is rubbed.

Ultraviolet rays : The surface or the articles suspected to bear the seminal stains when examined under ultraviolet rays in darkness usually gives fluorescence. Some other substances also give fluorescence, while some others mask it. Therefore, the technique, though useful in most of the cases, is not infallible.

The fluorescence of semen depends upon the quantity and freshness of the semen. Therefore, even when a stain shows weak fluroscence, it could be further explored.

Stereomicroscopy : Stereomicroscopic examination of the suspected stain may indicate the characteristic contours and layers like formations in a thick crust of a semen stain.

Phosphatase method : The presence of acid phosphatase in semen helps to search large area and garments for semen stains. In this method, large filter paper sheets are dipped in saline solution. Excess of the solution is removed and the garment to be examined is pressed against the paper or vice versa. The outline of the piece of cloth is marked on the paper to facilitate location of the semen. Filter paper sheet is then sprayed with acid phosphatase reagent. Colour spots indicate the location and size of the seminal stains.

QUESTIONS

The following questions are raised about the evidence:

1. Does the given article bear any seminal stains?
2. Are the seminal stains from human source. Can the human source of the semen be individualized?
3. What is the age of the stains?
4. How long can the spermatozoon survive?

EVALUATION

Systematic Study of seminal stains involves the following steps:

1. Smell. The smell of fresh or wet seminal stains is characteristic.

2. Feel. Dry stains have a rough feels, like dried starch solution and have uneven surface. The contours of the stains are characteristic.

3. Flourescene. Semen stains give strong characteristic fluroscence. Handling, substrata, the quantity and the age of the stain affect it.

It is difficult to determine the age of the stain as the changes with age are largely determined by the environmental conditions.

The semen is a gel like mass after ejaculation. It liquefies on standing. On drying it slowly changes to an opaque white mass with rough feel. After two or three weeks the colour changes to pale yellow and then to brown.

Spermatozoon in fresh semen is complete with head and tail. On drying and with passage of time it begins to disintegrate, the tail separates out from the head. The head is also affected. The sharp division in differential staining slowly disappears.

Survival of spermatozoon - The Spermatozoon remains motile only for a few hours under normal conditions. The motility seldom continues after twenty- four hours. But if the semen is frozen, the spermatozoon may remain alive for long periods. It shows motility, when the sample is brought to normal temperature.

In humid and warm climate, the spermatozoon gets destroyed in a short while. In dry and cool climate the dead spermatozoon may be identified even after months.

In vaginal swabs of a living person, spermatozoon may be detected up to about five to ten days, though the number of spermatozoa detected goes on decreasing with passage of time.

THE COLLECTION OF SEMINAL STAINS

All sexual assault scenes should be searched for the presence of seminal stains. Once possible stains are located, care is necessary in their collection. Seminal stains are very fragile when dry and can easily be destroyed.

When a sexual assault occurs indoors, the exact location of the assault should be examined. Bed sheets should be marked indicating the head and foot. The reason for these markings is that sexual assaults are often unnatural acts and the location of seminal fluid may provide valuable information about the nature of the assault.

Due to the fragile nature of seminal stains, sheets should never be folded or rolled up. Friction from careless folding may destroy the stain. This can be prevented by attaching a piece of clean carboard to the stain before folding the sheet around the carboard and placing it in an evidence bag. Each item should be packaged in a separate paper bag to prevent transfer of the stain to an unstained portion.

Seminal stains located on wood floors should be carefully dislodged with a clean razor blade. This should be done very carefully so as not to destroy any spermatozoa cells present.

The victim of a sexual assault should immediately be transported to a hospital. A doctor will use a rape kit at the hospital.

THE IDENTIFICATION OF SEMEN

When the evidence of a sexual assault is submitted to a crime laboratory, the first step in the analysis is to identify the presence of semen. The positive identification of seminal fluid and spermatozoa cells from a stain can provide the investigator with valuable information. These identifications can provide the investigator with information concerning the events of the crime, and if the crime is rape, the identification of spermatozoa may tend to corroborate the victim's story. For this reason all items that may possess seminal fluid should be carefully collected and submitted to the science laboratory for analysis.

Considering the large amount of spermatozoa cells obtained in the ejaculated seminal fluid, that in seminal fluid, one would think that the chance of locating a single spermatozoa cell in a seminal stain is very good. However, spermatozoa cells are very brittle when dry and are easily destroyed. For this reason extreme care is necessary when collecting and packaging suspected seminal stains.

Both the seminal plasma and the spermatozoa cells can be identified from dried stains. Dried seminal stains are stiff and crusty to the touch and depending on this method for their identification is often unreliable, as other stains can also have this texture. The best method for locating the presence of seminal stains is the Acid. Phosphatase color Test. Acid phosphatase is an enzyme secreted by the prostate gland of the male reproductive system. The concentration of acid phosphatase in seminal fluid is approximately 20 to 400 times greater than that found in other body fluids. For this reason the detection of high levels of acid phosphatase is the method most commonly used to screen possible seminal stains. This test is relatively simple and consists of dropping a solution of sodium alpha-naphthylphosphate on a small portion of the dried stain. This solution is allowed to soak into the stain for approximately one minute. Then a drop of fast blue B dye is added. A blue-violet color appearing in thirty seconds is not considered a positive proof for the presence of semen, as other substances may produce positive results, but it is highly indicative of the presence of semen.

A stain that gives a positive acid phosphatase reaction is then examined for the presence of spermatozoa. The stain is usually soaked in a small amount of saline to

dissolve it. A few drops of this saline solution is then placed on a slide and observed microscopically for the presence of spermatozoa cells. Human spermatozoa cells are characteristic in appearance and differ greatly from spermatozoa cells of other species of animals.

The presence of semen in a questioned stain does not always mean that sperm cells will be present in the sample. There are many reasons why semen can lack sperm cells. The individual may have an abnormally low sperm count (ogligospermia) or the complete absence of sperm in the seminal fluid (aspermia). The absence of sperm may also be due to the techniques used for the collection of the stain. Finally, a doctor in the rape treatment center of the hospital may have missed any spermatozoa cells present when the vaginal swabs were taken. In cases such these the forensic serologist must rely on the results of the acid phosphatase test in reporting the results of the analysis, Forensic serologists differ in their interpretations of the acid phosphatase test. Some believe that the presence of high levels of acid phosphatase is positive proof of the presence of seminal fluid, while others do not. Opinion differences result from the fact that vaginal secretions also contain acid phosphatase. These vaginal secretitions are normally lower in concentrations than seminal fluid. This level varies from female to female, though no standard level can be determined.

SPECIES ORIGIN AND BLOOD GROUPS

Precipitin reaction with anti- human semen serum (or anti- Human blood serum in the case of secretors only) and specific blood group anti- sera are employed. The former determines whether it is human semen and the latter determines the blood group of the secretor.

The techniques employed are similar to those employed in blood grouping. The recent discovery of a protein called p30 in seminal fluid may provide a positive means of identifying seminal fluid. Current research indicates that this protein is formed in the prostate gland and is present only in seminal fluid. This protein can be identified by an immunological technique known as electrophoresis. Some of the forensic laboratories in the country are using the electrophoretic technique to identify the presence of p 30, thereby identifying the presence of seminal fluid.

With classical methods it was not possible to establish the individuality but DNA profiling has made it possible to pin point the source of origin even from the tiniest speck or even when it is contaminated with vaginal fluid or mixed with semen from other sources.

ORIGIN DETERMINATION

Species origin: Precipitin test, gel diffusion, cross-over electrophoresis and antiglobulin consumtion test.

The bloodstains that are positively identified as blood must be tested further to determine the species of blood that is present.

All of the origin determination tests are based on the immunological principle that all animals have an immune system that among other things, is capable of making a

protein called an antibody. Antibodies are specific proteins made to counteract foreign proteins that enter the body and will react only with the foreign protein that caused their production. The foreign protein is called an antigen. Most animals will produce antibodies against the proteins, or antigens, of other animals species. If human blood is injected into an animal of a different species, the animal will produce antibodies specifically for the proteins in human blood.

These principles are used to produce antihuman serum in rabbits. First, rabbits are injected with human blood .This causes the immune system of the rabbit to produce antibodies that are specific for the human blood. Antibodies are formed in the white blood cells of the rabbit and are then released into their serum. The rabbits serum, or liquid portion of the rabbits blood, is then collected. This rabbit serum now contains antibodies that are specific for human blood proteins. When this rabbit serum, now called antihuman serum, comes in contact with human blood, the antibodies will react with the proteins of the human blood. This is called a precipitation reaction and results in the formations of large antigen-antibody complexes that will precipitate, or fall out of solution and be visible.

The three common methods for using this precipitin reaction to identify the species origin of stains are the ring precipitation test, the gel diffusion method and electrophoresis. The ring precipitation test involves layering a dilute saline extract of the bloodstain on top of the antihuman serum in a small test tube. Because of the density of the antihuman serum, the blood extract will layer on top, and the two solutions will not mix, thus forming an interface between the two solutions. The test tube is then incubated or heatd, at body temperature. After heating, the tube is examined. If the stain is human blood, a white line, the precipitate, will be formed between the two layers.

The gel diffusion method is based on the fact antigens and antibodies will diffuse, or move towards each other, on an agar gel coated plate. The stain extract and the antihuman serum are placed in separate holes in the gel, where a white precipite line will be formed between the two materials if the stain is human blood. Another technique, used because it is more sensitive than the others, is cross-over electroporesis. Electrophoresis is a technique involving the use of an electric current. This electric current is passed through a gel plate, causing the movement of materials in the gel. In this method the antihuman antibody and an extract of the stain are placed in separate holes in the gel. An electric current is applied that causes the materials to move toward each other .If the stain is human blood, a white line will be formed between the two materials. In order to conduct the origin determination properly, controls are necessary. One such necessary control is an unstained portion of the material the blood stain is on, to verify that the blood is causing the positive reaction, and not the surface material on which it is deposited. For this reason, the investigator should submit unstained samples of all the materials on which bloodstains are found. Human blood that has been heated will often result in a

negative test result. Therefore, the forensic scientist should never dry liquid bloodstains by heating them.

BLOOD GROUPING TECHNIQUES:

Blood grouping techniques depend upon the existence of antigens and antibodies in human blood. The reactions of the antigen and antibodies permit identification of the blood groups.

The antigen- antibody reactions are carried out by a number of techniques. In the past few decades very sensitive methods have been developed by which blood attached to even one millimeters long fiber of ordinary cotton cloth can be successfully grouped.

The commonly employed techniques for blood groups determination from bloodstains are ;

1. **Absorption inhibition**
2. **Absorption elution**
3. **Mixed agglutination**

Direct methods involving mixed agglutination, or absorption-elution techniques are more popular. They are Simple in principle and participate, but need experience. They may give unsatisfactory results with inexperienced person.

MIXED AGGLUTINATION

The antigen of the questioned sample is flooded with antisera. Excess is removed after some time by washing. Known cells are then introduced. The indicator cells corresponding to the original antigen get attached.

Absorption-Inhibition method : Antisera vary in their strength. Some antisera have more antibodies in them than others do. By doing a procedure called titration of the antiserum, an estimate of the amount of antibody present in the aniserum can be obtained. The absorption-inhibition method depends on being able to estimate the amount of antibody present in an antiserum before and after exposure to a stain extract containing a possible antigen. The test is called absorption-inhibition because it is designed to find out whether the dried blood will absorb some of the specific antibody in the antiserum, thereby inhibiting its strength.

Absorption- elution method

Absorption elution methods involves the following steps:

1. Prepare Anti- A, Anti-B and Anti-H sera of 1:256 to 1:512 titre value.
2. Prepare 0.5% cell Suspension of A, B and O cells.
3. Fix stain bearing thread (2.3 mm.) On a cellulose acetate sheet along with known blood stains and control cloth fibre, with an adhensive.
4. Add one drop of appropriate anti-serum on each thread.
5. Incubate in a moist chamber at 4^0C for 12hours.
6. Wash off the excess anti sera with cold saline 6 times.
7. Blot dry the sheet with filter paper.
8. Add one drop of 0.5% indicator cells to each thread.

9. Incubate the sheet at 56^0C for 15minutes in moist chamber.
10. Rotate the sheet for 30 minutes and read the agglutination under a microscope. If cells of group 'A' are agglutinated,the original blood belongs to group 'A'if cell of 'B' group are agglutinated, the orignal sample is of group 'B' and so on.

Kind (1961) has suggested a slightly modified method for the same.

Absorption- elution technique has been employed successfully for the detection of A, B, H, MN, Rh and for other antigens.

The following points should be kept in view.

6. The original sample should be properly fixed.
7. Absorption should take place at optimum conditions. High temperature may denature the antigen and prevent absorption.
8. The antisera should be of correct strength (avidity).
9. Proper washing is needed. Excessive washing causes elution while in complete washing does not remove the antisera completely.
10. Indicator cells should have low concentration.

Inhibition procedure being less sensitiver require more bloodstain material than elution tests. Certain inhibition procedures are also more involved than the usual elution procedure, but both techniques have their place in bloodstain grouping. Selection of a technique must be based upon the size and nature of the sample and the circumstances of the case. Most of the laboratories in the world use elution technique for most bloodstain samples.

Blood groping from antibodies

Blood groping is usually carried out with the antigens present in the bloodstain. But antibodies can also be used for the purpose.

Antibodies are labile and are destroyed comparatively more easily. When fresh blood is available in sufficient quantity, antibodies in the serum can be used similarly in antibody-antigen reactions.

Suggested Questions :

1. Discuss various technique to locate the semen stains?
2. Discuss in detail various tests used to identify the semen stains.

LESSON NO. 2.10 AUTHOR : DR. MUKESH KUMAR THAKAR

SALIVA /SALIVA STAINS

Saliva stains may be found in murders, sexual assault cases along with the other items left at the crime scenes like cigarette but ends, music instruments, handkerchieves, vessels used for drinking, glasses etc. In forensic cases if they are examined thoroughly can provide highly significant evidence to established the presence of the individual in the crime. Unlike the other stains investigated saliva stains can rarely be seen, and their detection relies on chemical tests.

Saliva stains are very difficult to identify positively due to the lack of sufficient amounts of detectable substances present. Saliva stains may be distinguished from other stains by the high concentration of Alpha-amylase, the enzyme concerned with hydolysis of starch. Some individuals produce saliva with little or no amylase activity. Therefore, the presence of amylase alone is not a conclusive test for saliva, and may need to be coupled to other tests e.g. the presence of buccal epithelial cells. Two methods for detecting the enzyme are available-starch iodide test and the Phadebas test. The former is less specific and may possibly give false positive results if the stain is contaminated with proteinaceceous material. If this is the case the sensitive Phadebas test is recommended. These tests are based on the ability of amylase to break down starch in foods. The tests are conducted by cutting a small portion of the suspected stain and placing it in a test tube. A starch solution is added to the test tube and is incubated, or heated. if amylase is present in the stain, the starch will be broken down. This is tested by the addition of iodine to the solution. No change in color is considered a positive result. The examination for buccal epithelial cells should be carried out in conjunction with the amylase test.

Saliva can be valuable evidence, because it is sometimes possible to determine an individual's ABO blood group from an analysis of his saliva. These determinations are due to the fact that approximately 80 percent of the human population are secretors.

Secretors are individuals who secrete their ABO blood group substances in their saliva. Male secretors also secrete their ABO blood group substances in their seminal fluid. A forensic analysis of semen and saliva stain can, therefore, indicate the individual's ABO blood group. This information may be valuable in identifying or eliminating suspects.

Examination:

Identification : Physical and chemical tests –starch iodine test, phadebas and epithelial cells

Species origin : Precipitin, Gel diffusion and Cross over elctrophoresis.

Individualization : Blood group determination, enzyme typing, and DNA typing.

For ABO grouping absorption-inhibition and absorption elution are used in parallel. Dilution experiments are undertaken for absorption-inhibition and absorption elution to eliminate non specific reactions.

ORIGIN DETERMINATION

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The bloodstains that are positively identified as blood must be tested further to determine the species of blood that is present.

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These principles are used to produce antihuman serum in rabbits. First, rabbits are injected with human blood .This causes the immune system of the rabbit to produce antibodies that are specific for the human blood. Antibodies are formed in the white blood cells of the rabbit and are then released into their serum. The rabbits serum, or liquid portion of the rabbits blood, is then collected. This rabbit serum now contains antibodies that are specific for human blood proteins. When this rabbit serum, now called antihuman serum, comes in contact with human blood, the antibodies will react with the proteins of the human blood. This is called a precipitation reaction and results in the formations of large antigen-antibody complexes that will precipitate, or fall out of solution and be visible.

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Suggested Questions :

1. Discuss importance of saliva stains and how do you evaluate them.

LESSON NO. 2.11 AUTHOR : DR. MUKESH KUMAR THAKAR

URINE STAINS

The examination of urine stains may occasionally be necessary in cases of murder, sexual assaults and in decency cases.

The stains may be present on the undergarment or on the body of the victim. If these are searched and analysed properly can help in linking the criminal with the scene of crime.

Identification of urine

Identification of urine and urine stains is based on physical characteristics and the chemical constituents present.

Physical Characteristics

1. Fluorescence

Urine stains on fabric are usually colourless or very pale yellow in visible light owing to the presence of urinary pigments, particularly urochrome (a compound of unrobilin or urobilinogen and a peptide).When viewed by U.V. light they may fluoresce.

a) Smell

The characteristic smell of urine,due partly to the presence of ammonia formed from the bacterial breakdown of urea,can be more easily discerned if the stains extract is concentrated .

Chemical tests:

Urea is the chief product of protein metabolism and is found in high concentrations in urine. Detection of urea is effected by means of the enzyme urease. There are a number of colorimetric methods for demonstrating this reaction.

a) Urastrat method-Blue colour in the upper band indicate a positive result.
b) DMAC method (.p-dimethyaminocinnamaldehyde) –magneta colour produce.
c) Creatinine – deep orange colour.
d) Acid phosphatases

Urine from females exhibits little or no phosphatases activity while that from males may show weak activity with same elecrtophoretic mobility as seminal acid phosphatase. Therefore it is thought that urinary AP is due to the presence of prostatic secrections.

Species origin - Species origin can be determined by :

1. Precipitin Tube Technique
2. Gel Diffusion

3. Cross over electrophoresis.

Blood groups :

ABO blood group substances can be detected in urine, but they are present in very small quantity.

DNA Typing:

DNA typing can performed on the urine or the stains samples.

Fortunately for forensic scientists, with a little help, DNA molecules are able to make copies of themselves. By adding an enzyme called polymerase to the DNA sample, then placing it in a device called a thermocycler, scientists create a chain reaction in which the DNA copies - or amplifies - itself. Basically a very precise heater, the thermocycler repeatedly raises and lowers the temperature of the DNA and, after several cycles, there's enough of it to test

Suggested Questions :

1. Discuss Forensic Significance of urine stains and how these can be evaluated in the laboratory.
2. Discuss usefulness of DNA Test in the evaluation of urine/urine stains.

LESSON NO. 2.12

DIATOMS AS PHYSICAL EVIDENCES

Content

- **INTRODUCTION TO DIATOMS**
- **CLASSIFICATION OF DIATOM**
- **STRUCTURE OF DIATOM**
- **FORENSIC DIATOMOLOGY**
- **FORENSIC SIGNIFICANCE OF DIATOM TEST**
- **ANTE-MORTEM AND POST-MORTEM DROWNING**
- **FATE OF DIATOMS INSIDE THE BODY**
- **DROWNING ASSOCIATED DIATOMS**
- **WHY ONLY DIATOMS**
- **SAMPLES REQUIRED**
- **VALIDITY OF DIATOM TEST**
- **VARIOUS METHODS USED FOR THE EXTRACTION OF DIATOMS**
- **PREPARATION OF SLIDES & IDENTIFICATION OF DIATOMS**
- **DIATOM IDENTIFICATION**
- **SUGGESTED QUESTIONS**
- **REFERENCES**

INTRODUCTION TO DIATOMS

Before discussing details of the diatom and its test, it is important to have a brief introduction about types and structure of diatom. Diatoms are unicellular microscopic algae, which belong to Kingdom *Protista* and class *Bacillariophyceae*. More than 200 genera and approximately 100,000 species of diatoms have been reported in the literature so for.

Diatoms usually have yellowish or brownish tint, which is due to the presence of photosynthetic plastids. Diatoms are found in fresh water, salt water and in moist soil. There is large variation not only in the size (which varies from few micrometers to more than hundred micrometers) but also in their shape, which varies greatly from box-shaped to cylindrical, and symmetrical to asymmetrical.

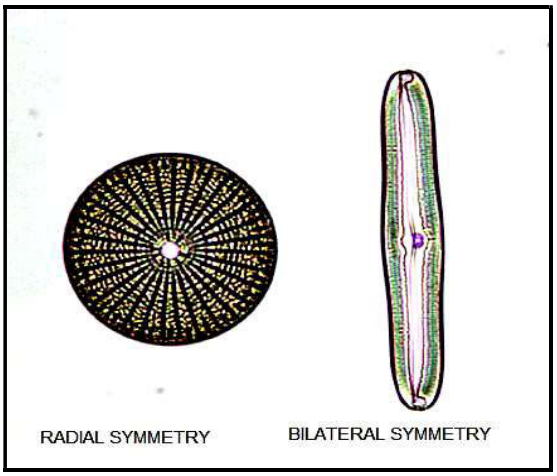
Diatom growth takes place ordinarily by mitotic cell division as well as through the formation of an auxospore by sexual reproduction. Living diatoms often have specific salinity, temperature and other environmental tolerances; therefore growth of diatoms

is directly related to the temperature, light intensity and physiochemical characteristics of the water i.e. pH, salinity, concentration of silicon and the presence of organic matter etc. Their population increases during spring and autumn seasons (because of the favourable climatic conditions) while during summer and winter seasons it reduces**.**

CLASSIFICATION OF DIATOM

On the basis of their symmetry, diatoms can be divided into two orders:

1. **Centrales (Biddulphiales) -** which have valve striae arranged basically in relation to a point, and appear radially symmetrical.
2. **Pennales (Bacillariales) -** which have valve striae arranged in relation to a line and appear bilaterally symmetrical.



STRUCTURE OF DIATOM

All the diatoms are covered by a *frustule*, which is made up of two valves, which are of different size. Larger valve is called *epitheca*, which connects smaller valve or *Hypotheca* with the help of a *cingulum* (also called set of girdle bands (as shown in girdle view-Diagram-1).

- **Cell wall:** the cell wall is composed, for the most part, of silica (sio2).

- **Frustule:** the frustule consists of two valves that fit within each other (one valve is slightly smaller than the other. The frustule can vary greatly in shape, ranging from box-shaped to cylindrical, symmetrical as well as asymmetrical.

- **Epitheca:** this term is used to refer to both the larger, older valve (epitheca) of the frustule as well as the girdle elements (epicingulum) that are connected with it.

- **Hypotheca:** this term is used to refer to the smaller, younger valve (hypotheca) of the frustule as well as the girdle elements (hypocingulum) that are connected with it.

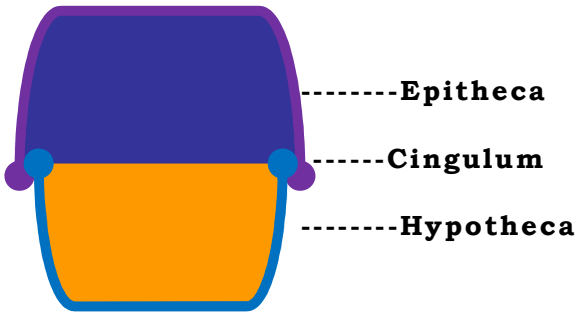
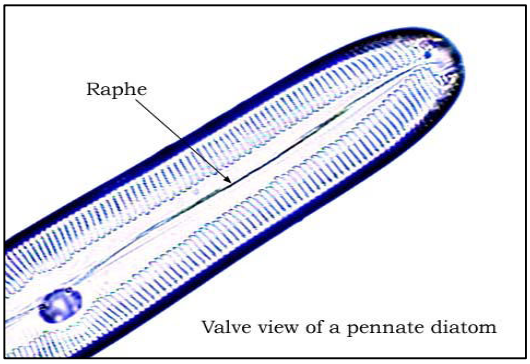


Diagram-1; Diagrammatic representation Girdle view of diatom



The valves are composed of silica (SiO_2), which is a characteristic feature of their silica shells and can be used for their identification. The face side of valve is decorated with various identifying features like pores (areolae), processes, spines, hyaline areas etc. Another important characteristic feature of diatoms is *raphe*, which is found mostly in pennate diatoms, and it divides the valve into two (as shown *in* ***Picture-1)***

FORENSIC DIATOMOLOGY

It is defined as the study of diatoms for solving drowning cases pertaining to medicolegal purposes.

Pollanen (**1996, 1997 and 1998**) and his colleagues contributed significantly by conducting various experiments for utility and validity of diatom test and proposed various factors that could interfere in the proper application of this test.

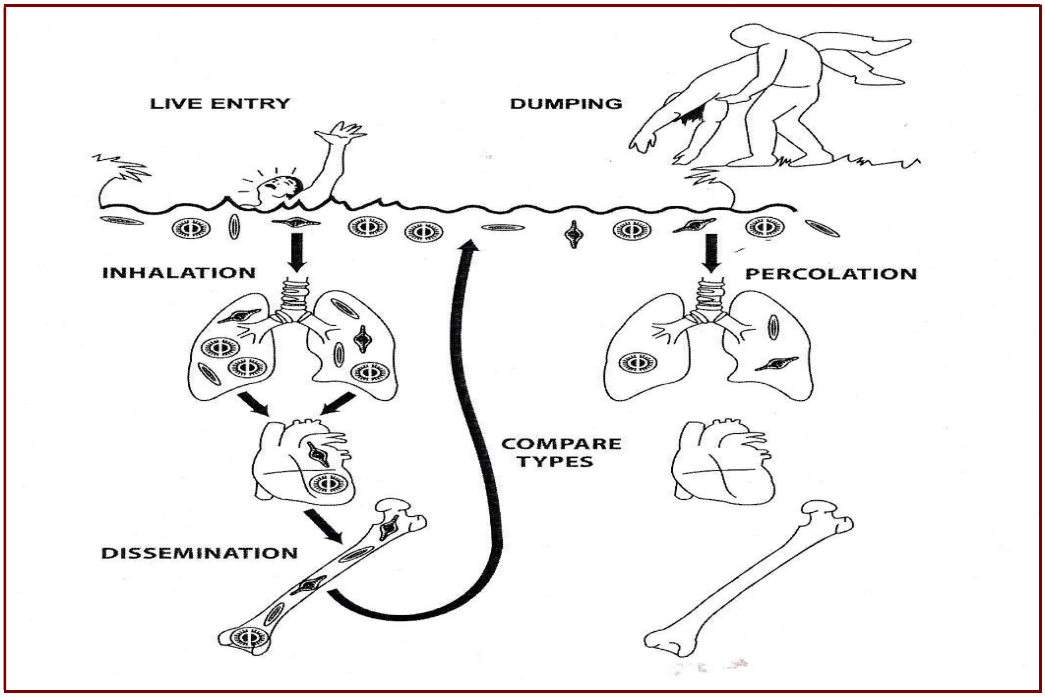
FORENSIC SIGNIFICANCE OF DIATOM TEST

Diatoms can act as very sensitive and significant forensic evidence in drowning cases. The diatom test helps in differentiating between ante-mortem and post-mortem drowning or simply to establish:

1. *Whether the cause of death is drowning or not*
2. *Whether person was living at the time of drowning or not*

ANTE-MORTEM AND POST-MORTEM DROWNING

Basically ante-mortem drowning means that the person drowned when he was still alive. Post-mortem drowning on the other hand means that the person was first killed by someone and then thrown in water to conceal crime. While post-mortem drowning almost certainly points to homicide, ante-mortem drowning could be anything; accidental, suicidal or homicidal, roughly in that order.



- First discovery of diatoms in lungs was made by Hofmann (1878)
- A successful attempt was made by Revenstorf (1904) who correlated this presence of diatoms in lung in solving the drowning mystery.

FATE OF DIATOMS INSIDE THE BODY

When a person breathes inside the water (only in anti-mortem drowning), during this process some water enters the lung cavity with force and exerts pressure on the lung walls. Due to this pressure, lung alveoli get ruptured and water reaches into the blood circulation. Till the last beat of heart, water keeps on mixing with the blood and during this course some diatoms (if at all present in the water) particularly the smaller one are also pushed into various vital organs like liver, spleen, kidneys, brain and even bone marrow. So it is only the respiratory pressure, which helps diatoms to reach these body organs. On the other hand, chance of entering diatoms in distant vital body organs remains negligible, particularly when a dead person (post-mortem drowning) is thrown into the water body. In certain instances, the Diatoms can be detected in the lungs even in case of post-mortem drowning i.e. due to the passive absorption of water, diatoms can reach inside the lung cavity, and their journey ends here only.

DROWNING ASSOCIATED DIATOMS:

Since the diameter and thickness of lung alveoli remains very small therefore it is not impossible for all the diatoms to penetrate into the body organs through the lung cavity but those diatoms which can penetrate through this capillary network are called "*Drowning Associated Diatoms*" (DAD).

WHY ONLY DIATOMS

- Because they are numerous
- Microorganisms with acid resistant frustules
- To are too small to penetrate various distant organs of the body.
- They can be easily classified and identified.
- Found even in case of decomposed/skeletonized bodies.

SAMPLES REQUIRED

Post-mortem samples-

Usually Post mortem samples sent to Forensic Science Laboratories for diatom examinations are of two types

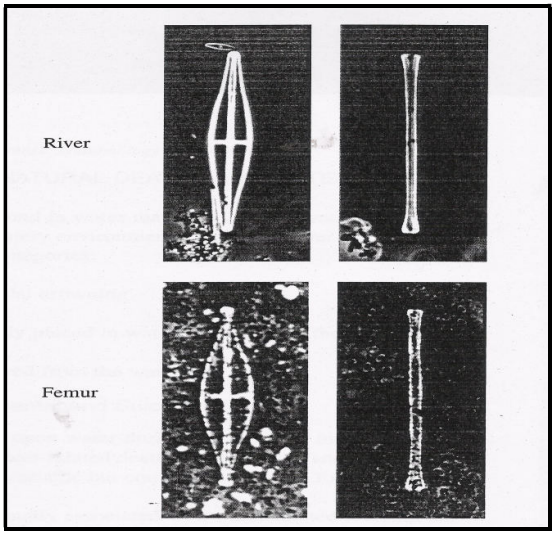
- Hard bones (sternum and femur)
- Soft tissues like lungs, Brain and liver.

Water sample-

- 1 litre of water sample from the putative site of drowning

VALIDITY OF DIATOM TEST

To make a positive diagnosis of drowning, the number and species diversity of diatoms sampled from the lungs and other circulatory organs should match that of the aquatic flora where the body was recovered. **Ludes et al.** (1994) mentioned about the required number of diatoms in different vital body organs of a drowned deceased. They concluded that diatom analysis should be considered positive only when minimal **established limit number of diatoms is present in the different vital body organs i.e. 20 diatoms/ 100 µl of pellet (obtained from 10 gm of lung samples) and 5 complete diatoms from other body organs.** According to **Pollanen (1998**), the validity of diatom test can be checked by applying the guidelines of *"criterion of concordance"* given by Pollanen in 1998. According to this criterion, the significant number of diatoms should be present inside the distant vital body organs and they should match with the species of diatom found in the water body from where body was recovered. This refutes the doubt of many pathologists, who claim that diatoms are always present in human tissues. Therefore, application of the "*Criterion of Concordance"* is thought to be satisfactory enough to convince the most critics in a court of law.



Picture showing matching of diatoms recovered from water body drowned body and drowned body

Various methods used for the extraction of diatoms are as follows: -

- Acid digestion method (Nitric acid)
- Ultrasonic radiation to solubilize tissue
- Enzymatic digestion with proteases -such as Proteinase K
- Strong anionic detergents such as sodium dodecyl sulphate and Hydrogen peroxide
- Membrane filtering method (blood samples)

PREPARATION OF SLIDES & IDENTIFICATION OF DIATOMS

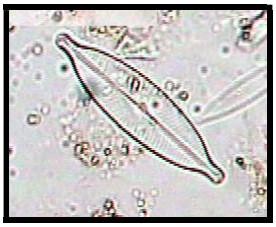
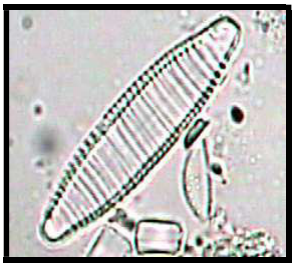
Diatoms once extracted needs to be properly preserved for their noticeable visibility through microscope. For preservation diatoms are permanently mounted on microscopic slide.

Scheme- Residual material obtained after centrifugation is put on a microscopic slide and allowed to fix. Then a drop of DPX mountant is poured on the center of a clean pre-labeled slide and covers it with a cover slip and then examined under the microscope for possible diatoms.

DIATOM IDENTIFICATION

Slides are examined using a Binocular Compound Light Microscope on 1000X (oil immersion) to maximize resolution.

Table-2 Showing Photomicrographs of Some Commonly Occurring Diatoms

SOME DIATOM GENERA		
Navicula 	***Nitzschia*** 	***Diatoma*** 
Cymbella 	***Cocconeis*** 	***Pinnularia*** 

SUGGESTED QUESTIONS:

1. What are diatoms? Give structure of diatoms.
2. What is the forensic significance of diatoms?
3. What tests can be employed for diatom identification?
4. What is diatomology?

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