

What is the death ?

- An ambiguous term referring to the cessation of life .
- Where life ends and death begins isn't always clear .
- Considering that pronouncing death is usually a physician's responsibility.
- It is important to know the signs of death and the differences between reversible and irreversible clinical death .
- When pronouncing death, it is important to understand the events leading up to death, if it was expected or not, and to conduct a careful examination before declaring the death and its time.

How can we diagnose death ?

• Apparent death

Reduction of vital function to a minimum, creating the appearance of death without signs of certain death, Misdiagnosing apparent death as clinical death can have grave consequences such as postponing vital care, false alarms for organ donation, and unnecessary emotional stress for family members .

- Clinical death : a period during which vital signs are absent but resuscitation is still possible.
- CP death : irreversible cessation of circulatory and respiratory functions
- Brain death : complete loss of function of the entire brain (including the brainstem), even if cardiopulmonary functions can be upheld by artificial life support.
- Intermediary life : the period of time between irreversible cardiopulmonary death and biological death
- **Biological death** : Permanent and irreversible cellular damage with complete cessation of metabolic cell function

Somatic Death vs Molecular Death			
More Information Online WWW.DIFFERENCEBETWEEN.COM			
NA AMONINA IN	Somatic Death	Molecular Death	
DEFINITION	The phenomenon when the brain stops functioning followed by cessation of functional properties of the heart and the lungs.	The phenomenon that takes place after the somatic death where the cells and organs undergo cessation.	
TIME	Immediately followed by brain dead and the cessation of lungs and heart.	Takes place approximately after about a few minutes from somatic death.	
CESSATION TYPES	Organs such as the brain. lungs and heart	Cells, tissues and biomolecules	
SYNONYMS	Clinical death	Cell death	
CONDITIONS FOR CESSATION	Clinical conditions	Unavailability of oxygen followed by clinical death	

Somatic death

- Diagnosis of somatic death :
- 1) Cessation of heart beating (by absent of heart sounds for a continuous period 5-10 and flat ECG
- 2) Cessation of breathing
- 3) Cns(dilated fixed pupil , absent of papillary and corneal reflex , flat EEG)
- ** some condtions mimic somatic death
- 1) Apperant death
- 2) 2) after excess dose of sedatives or hypnotics coma
- 3) Hypothermia in old age

Molecular death

- After 2-4 min after somatic death
- Last tissue that will die is muscle after 3-4 hrs
- Muscles don't respond to strong stimuli
- Pupil doesn't respond to stimuli and drugs

Cadriopulmonary death

- Monitoring of the patient for a specific period of time to confirm continuous apnea, unconsciousness, and lack of circulation
- • Exclusion of factors that may be the cause of the cardiorespiratory arrest, such as:
- • Hypothermia
- • Endocrine dysfunction
- • Metabolic causes
- • Biochemical imbalances (e.g., hyperkalemia)
- No intention of beginning or continuing cardiopulmonary resuscitation (CPR); prohibition of any intervention that might restore cerebral blood flow

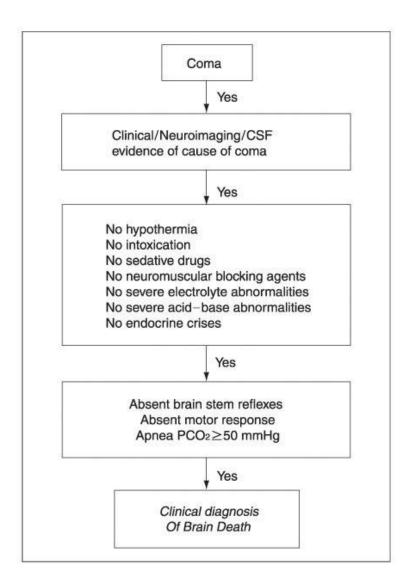
Brain death

- Cortex vs brain stem death
- Cortex : is responsible for voluntary and senseibility , so in cottex death there will be general muscle flaccidity
- ** vegetative state : severe cortex damage without brain stem involvement .

Brain stem death involve (coma , apnea , loss of brain stem reflex like papillary reflex .

Signs of death

(Semulic) Death Changes (Signs of Death)			
Immediate changes	Early changes	Late changes	
Insensibility (Crta Cat)	Postmortem cooling	Putrefaction	
Respiratory arrest	Eye changes	Adipocere formation	
Circulatory arrest	Skin changes	Mummification	
Brain/brainstem death	Postmortem lividity	Skeletonisation	
	Muscle changes		



Cause, mechanism, manner

POST-MORTEM CHANGES

Nour Maher Rand Farhat Bayan Al-Oran

Post-mortem changes

- With the onset of death, all organisms undergo changes, mainly as a result of cell death (decomposition)
- External factors such as climate and location can also affect the state of a cadaver.

Postmortem changes

- Postmortem changes are signs of certain death that can provide information regarding
- 1) the time, cause, mode
- 2) mechanism, and manner of death
- 3)whether the location of the body corresponds to the place of death.
- These changes may be of **medical and/or legal interest**.

Some definitions before we start

- **Decomposition :** breakdown of bone and tissue through aerobic and anaerobic processes.
- Putrefaction : Anaerobic decomposition from colonization of tissue by endogenous and exogenous bacteria and fungi.
- Autolysis: aerobic decomposition through endogenous acids and enzymes in the stomach, pancreas, etc.

Post Mortem Changes

- Immediate:
 - Loss of voluntary power
 - Cessation of respiration and circulation
- Early:
 - Pallor and loss of skin elasticity
 - Eye changes (tache noire)
 - Primary flaccidity
 - Rigor mortis
 - Hypostasis
 - Cooling of the body
- Late (decomposition):
 - Putrefaction
 - Adipocere
 - Mummification

Early Changes (Molecular Death)

Changes in the skin and facial pallor

- Skin becomes pale -white due to stoppage of circulation and drainage of blood from the capillaries and the small vessels.
- The skin loses its elasticity, and the face looks younger due to loss of creases. The lips appear brownish, dry and hard due to drying

Early Changes (Molecular Death)

Primary relaxation or flaccidity of the muscles

 Muscles lose their tonicity and become flaccid, but the muscular tissues are still alive, their chemical reaction is alkaline and <u>responds to electrical stimuli.</u>

Contact flattening and pallor

 The areas which remain in contact with the ground, become flat and the blood from vessels of these areas is pressed out, this continues even after the formation of postmortem staining over the surrounding areas

Early Changes (Molecular Death)

Changes in the eye

1. Loss of corneal and pupillary reflexes

2. Pupils: The **pupils are dilated after death**, because of the relaxation of muscles of the iris. Later, they are constricted

3. pupil can be changed in shape, and the change may persist during the stage of rigor mortis of the muscles.

Changes in the eye

4. Tache noire : If the eyelids remain open for 3–4 h after death, there is formation of **two yellow triangles on the sclera** at each side of the iris, which **become brown and then black**.

 Cause: Drying/desiccation, and deposition of cellular debris, mucus and dust on the exposed conjunctiva and the sclera underneath.

5.Loss of intraocular tension: Intraocular tension falls rapidly after death. It becomes zero in **4–8 h** from 10–22 mmHg during life. The eyeballs look sunken in the orbit.

6.Changes in the retina: The blood in retinal vessels appears fragmented or segmented (cattle trucking or shunting) within seconds to minutes after death and persists for about an hour (Kevorkian sign).

Tache Noire



Kevorkian sign



Early post-mortem changes.

> Algor Mortis (Cooling of the Dead Body)

- The fall of temperature of the cadaver occurs due to cessation of energy production and inactivity of the heat regulating center after somatic death.
- The average rate of fall of the body temperature is 1°C/h, and the body attains environmental temperature in 16–20 h after death
- For the purpose of estimation of time passed after death, the measurement of the inner core temperature is important and is more reliable than the outer surface temperature

Medico-legal Importance of Algor Mortis

1. It is a sign of death.

- 2. It helps in the estimation of the time of death.
- 3. Rapid cooling of a dead body delays the processes of rigor mortis and decomposition. If the heat is preserved for a longer period, then both the processes start early

Early post-mortem changes.

Livor mortis (Postmortem staining or PM staining)

 <u>Definition</u>: purple-red discoloration of dependent areas of skin not exposed to pressure that begins 20–30 minutes after circulation stops due to **blood settling under the force of** gravity (hypostasis)



Livor mortis

✤ Where ?

It is present in the superficial layers of the dermis of areas of dependency under the force of gravity.

- Person died lying face-up: back of the corpse
- Hanging death: feet, fingertips and ear lobes
- Drowning: face, upper chest, hands, lower arms, feet, and calves
- Lividity (hypostasis) is evident on the ear lobes ,the nail beds and visceral organs (e.g., lungs)

Livor mortis

✤ Where ?

- However , there is an exception : the areas directly pressed on the ground or the bed , which do not show any staining and appear rather pale .
- This phenomenon is known as contact pallor



Livor mortis

- ✤ When ?
 - At least 30 minutes to 2 hours after onset of death .
 - In early stages (30 min to 1 h), it consists of discolored patches of 1–
 2 cm in diameter on the dependant parts of the body, having the same color as blood which can be mistaken for bruises.
 - Gradually, in 3–4 h, the small patches increase in size and coalesce with each other to form uniformly stained large areas. It is usually well-developed within 4 h, complete in 5–6 h
 - After complete formation of the postmortem staining, if the body is undisturbed, the staining gets 'fixed' in 8–12 h and persists until putrefaction

Medico-legal Importance of PM Staining

- 1. It is a sign of death.
- 2. The time since death can be roughly estimated from the formation, extension and fixation of the PM staining.
- 3. It indicates the posture of the body at the time of death.
- 4. It may indicate the moving of the body to another position sometime after death.
- 5. Cause of death may be judged from the distribution and color of PM staining.

Medico-legal Importance of PM Staining

6. In the early phase of its formation, it may be confused with bruise when patchy and small

7. It may be confused with congestion of the organs, particularly of the internal organs

8. Hemorrhagic spots on skin due to blood dyscrasias may be mistaken for PM staining.

9. Some extraneous color or stain may be mistaken for PM staining; however, these can be easily wiped or rubbed off or washed out.

Hypostasis vs. bruises

Hypostasis	Bruises (Ecchymosis)
Dependant areas	Any where
Well defined edges	III defined edges
Blood is retained in intact capillaries	Blood escapes through ruptured capillaries
Same level on surface	Raised
Pale over pressure areas	Red
Incision: blood flows from the cut vessel (washable)	Incision: blood coagulates in tissue



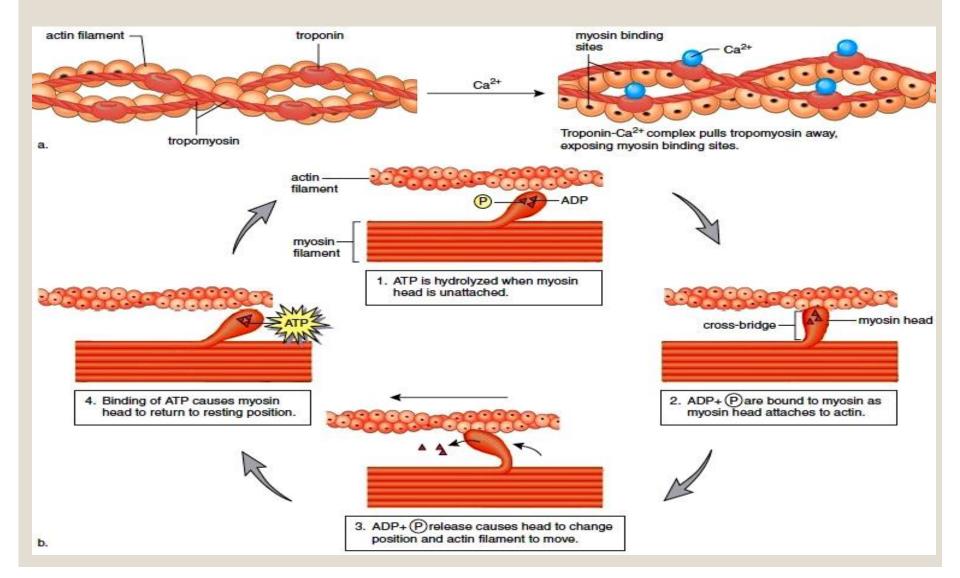
With a bruise, blood will not flow from the cut

Early post-mortem changes.

Rigor mortis

- The stiffening of the muscles after death, potentially with muscle shortening
 - The phase of primary relaxation of the muscles continues for about an hour which is followed by stiffening or rigidity. It indicates molecular death of the concerned muscles
- Usually occurs within 1–2 hours of death
- Starts to reside after about 24 hours
- Caused by persistent attachment of actin to myosin due to lack of ATP

Rigor Mortis



Rigor Mortis Mechanism

- ✓ Muscle fibers contain bundles of myofibrils which consist of two types of protein filaments—actin and myosin.
- ✓ At rest, actin filaments interdigitate myosin filaments only to a small extent and the muscle fibers also appear soft and supple. Maintenance of this condition of muscles is due to the presence of ATP above a certain level.
- ✓On nervous stimulation, hydrolysis of ATP occurs to ADP and phosphate with the liberation of energy which causes contraction of the muscle fibers and extension of the actin filaments more inside the myosin filaments.
 - ✓ <u>After death</u>, there is continuous hydrolysis of the ATP, and as long as glycogen is available in the muscle, there is resynthesis of ATP.
 - ✓ In this process, once the muscle glycogen is exhausted, no further resynthesis of ATP is possible and the muscle loses softness, elasticity and extensibility due to formation of viscid acto-myosin complex giving rise to rigor mortis in the muscle

Rigor Mortis

Muscles Involved ?

- Rigor mortis occurs both in the voluntary and involuntary muscles.
- Occurs earlier in the involuntary or smooth muscles than in the voluntary or striated muscles.

Onset and Duration ?

 in tropical countries like India, roughly, it commences in 1–2 h after death, takes about 9–12 h to develop from head to foot, persists for another 12 h and takes 12 h to pass off.

Rigor Mortis

Order of Appearance Rigor mortis ?

- first appears in the heart muscle within an hour after death
- Among the voluntary muscles, rigor mortis usually develops sequentially and follows a descending pattern
- The rigidity disappears in the same order in which it has appeared. In the whole body, it stays for maximum duration in the muscles of the lower limbs.

Effects of rigor mortis

- There is goose skin appearance of the body due to rigor mortis of the **erector pilae muscles**.
- Rigor in the muscles of the seminal vesicles may cause postmortem ejaculation of seminal fluid.
- Contraction of the heart muscle due to rigor mortis should not be mistaken for myocardial hypertrophy
- Rigor mortis in the uterine muscle cannot expel the fetus from the womb.
- The iris is also affected so that ante-mortem constriction or dilatation is modified.
 - Hence, the postmortem position of pupil is an unreliable indicator of toxic or neurological conditions during life

Effects of rigor mortis



Medico-legal Importance of Rigor Mortis

- 1. Its is a sign of death and indicates molecular death of the muscle involved.
- 2. During the early phase after death, it helps in estimating the time since death.
 - During summer, if rigor mortis has not set in, death might have occurred within 2 h.
- 3. If rigor mortis has involved the whole body, then death might have occurred between 12–24 h back
- 4. It indicates the position of the body at the time of death.
 - For example, if the body is lying on its back with its lower limbs raised in air, it indicates that the body reached full rigidity elsewhere while lying in a position where the legs were flexed.

5. Some conditions occur in dead bodies which may imitate/simulate rigor mortis:

 25 z Cadaveric spasm or instantaneous rigor , Heat stiffening, Cold stiffening, Gas stiffening or putrefaction

Cadaveric Spasm

 It is a condition in which the muscles of the body which were in a state of contraction immediately before death, continue to be so after death <u>without passing through the stage of primary relaxation.</u>

- It is a rare phenomenon of instantaneous rigor, which develops at the time of death with no period of postmortem flaccidity
- Predisposing conditions:
 - sudden death, excitement, fear, severe pain, exhaustion, cerebral hemorrhage, electrocution, injury to the nervous system, firearm wound of the head, or convulsant poisons.

Cadaveric Spasm

✤ Muscles involved ?

- The spasm is primarily a vital phenomenon; it originates by normal nervous stimulation of the muscles.
- It is usually limited to a single group of voluntary muscles, and frequently involve the hands.



Cadaveric spasm in a drowning victim: had grass from the river bank firmly clutched in the hand

Medico-legal Importance Cadaveric spasm

- 1. Being an antemortem phenomenon, reflects the last act of the subject performed before and at the time of his death.
- 2. In case of drowning, the hand may firmly grip sand, mud, gravel or weed which are present in the pond or lake from where the body was recovered "
- 3. In case of firearm/stab injury over an approachable vital part of the body, the pistol/knife may be firmly grasped in the victim's hand which is a strong presumptive evidence of suicide.
- 4. Although, Attempts may be made to simulate this condition in order to conceal murder, but rigor does not produce the same firm grip of a weapon.
- 5. In homicidal cases, the deceased may grasp some part of clothing, button or foreign hair of the assailant(s) with whom he had a struggle prior to his death

Rigor Mortis	Cadaveric Spasm
Onset delayed after death	Onset is instantaneous.
(2-3 hrs.).	Duration is a few hours, until it is
Duration up to 36 hrs.	replaced by rigor mortis.
Intensity comparatively	Intensity comparatively very
moderate.	strong.
Mechanism of formation:	Mechanism of formation
Breakdown of ATP below critical level.	unknown, but predisposing factors: Excitement, fear, fatigue, exhaustion, nervous tension, contraction of M's at time of death.
All muscles of the body are	Selected muscles, which were
affected gradually.	in a state of contraction at the time of death, are affected.

Other simulators of Rigor Mortis

A) Heat Stiffness

Seen in individuals exposed to high temperature, high voltage electrocution, or scalding due to hot liquids and is **characterized by rigidity of the body due to coagulation and denaturation of the tissue proteins**

- If the body is subjected to heat exposure at <u>> 65°C</u>, <u>rigidity</u> is produced which is much <u>more marked than that found in rigor</u> <u>mortis</u>.
- There will be coagulation of the muscle protein in which the <u>flexors are affected more</u>
- A zone of brownish-pink 'cooked meat' is seen under this, overlying normal red muscle.

B) Cold Stiffening

- in individuals found in sub-zero temperatures and results from freezing of the biological fluids and subcutaneous fat, which leads to stiffening of the body
- The body is exposed to freezing temperatures for a reasonable period, the tissues becoming frozen and stiff, simulating rigor.
- It occurs due to:
 - 1. Freezing of body fluids, particularly at the tissue level and in the synovial sacs of the joints

2. Hardening of the subcutaneous fatty tissue

C) Gas stiffening

characterized by rigidity due to the accumulation of putrefactive gases all over the body.

LATE POST-MORTEM CHANGES

Late post-mortem changes

Secondary Relaxation of Muscles

- After some hours of stay, rigor mortis passes away and the <u>body becomes relaxed or flaccid for the second</u> <u>time.</u> This is secondary relaxation or secondary flaccidity of the muscles.
- It occurs with the onset of decomposition or putrefaction of the dead body

Late post-mortem changes

Post-Mortem Decomposition

- In the cycle of life, dead bodies are usually returned, through reduction into their various components, to the chemical pool that is the earth.
- Some components will do this by entering the food chain at almost any level – from ant to tiger – whereas others will be reduced to simple chemicals by the autolytic enzymic processes built into the lysosomes of each cell.

O Putrefaction.

O Mummification.

O Adipocere.

O Skeletonization

Decomposition

 It is a process by which complex organic body tissue breaks down into simpler inorganic compounds or elements due to the action of saprophytic microorganisms or due to autolysis.

Putrefaction

- Anaerobic decomposition from colonization of tissue by endogenous and exogenous bacteria and fungi
- Putrefaction usually follows the designs of putrefaction: <u>marbling</u> <u>outlining vasculature</u>, <u>green discoloration</u>, <u>increase in temperature due</u> <u>to bacterial activity</u>
- appearance of rigor mortis

Putrefaction



Putrefaction

Etiology ?

- After death, the body's protective functions are absent and its defense barrier is lost. Saprophytic microorganisms, which cannot invade the body during life, physical and chemical agents which are present in the environment, all act on the dead body. Further, some body chemicals and enzymes which are helpful in different metabolic processes, in the absence of physiological control after death, start acting adversely
- Clostridium welchii, main organism in putrefaction
- In dead born, maceration—an aseptic autolysis of dead fetus in utero is seen

External Signs of Decomposition

Decomposition changes ('4 Ds')

- Discoloration: Greenish discoloration in the lower abdominal quadrants. h2s
- Distension: Various gases produced during decomposition permeate into skin, soft tissue and organs which manifests as crepitus and distension
- Degradation: Decomposition causes a loss of anatomic integrity of skin and other tissues such as localized peeling of skin ('skin slippage'), loosening of skin of hands and feet ('degloving') and loosening of hair and nails.
- **Dissolution:** Progressive decomposition leads to liquefaction and disappearance of tissues and organs, and eventual skeletonization

Discoloration

- The first external sign of decomposition is usually a greenish discoloration over the right iliac fossa over the region of the cecum which lies superficially, and the contents of the bowel are more fluid and fuller of bacteria.
 - C. welchii are most abundant at the iliocecal zone of the intestinal tract.
- ✓ Internally, this is seen on the undersurface of the liver, where it is in contact with the transverse colon

Marbling' of skin

• The blood vessels provide an important route through which the bacteria can spread with ease throughout the body.

•Their passage is marked by the decomposition of hemoglobin to sulphmethemoglobin in the blood vessels, which causes a greenish or reddish- brown staining of the inner walls of the superficial vessels. rn

- This is seen as linear branching patterns, which gives a 'marbled' ('road map')
- appearance of the skin
- Areas where visible: It appears first in the shoulder, ro thighs,
- $\circ\,$ sides of abdomen, chest and neck



Internal Changes due to Putrefaction

- The organs composed of muscular tissue and those containing large amount of fibrous tissue resist putrefaction longer than the parenchymatous organs, with the exception of the stomach and intestine, which decompose rapidly because of their contents at the time of death.
 - Prostate and uterus being the last organs to decompose, they help to identify the sex of the dead bodies in advanced state of decomposition

Adipocere (Saponification)

- formation of an offensive, sweet rancid smelling, soft, whitish or grayish white, crumbly, waxy and greasy material (similar to soap) occurring in fatty tissues of a dead body. It is a modification of decomposition
- In hot and moist environment, it may occur by the end of 1 week In temperate countries, it starts in 3 weeks and completes in about 3 months.



Mechanism of formation:

- Adipocere consists mainly of fatty acids formed due to postmortem hydrolysis and hydrogenation of body fats. The process needs water which is provided by the body fluid of soft tissues. The chemical reaction essentially involves conversion of unsaturated liquid fats (oleic acid) to saturated solid higher fatty acids, like palmitic
- A warm, moist and anaerobic environment favors adipocere formation.

Medico-legal Importance of adipocere

- 1. Sign of death: It is the surest sign of death.
- 2. Time since death: It gives a rough estimate about the time since death.
- 3. Personal identification: When the process involves the face, the features are well-preserved, which helps in identification.
- 4. Recognition of injuries: The cause of death may be determined, since injuries can be recognized.
- 5. Place of disposable of body: Some idea about the place of disposal of the body can be made, since its formation requires a warm place with high humidity or presence of moisture or water.

Mummification

- It is the rapid dehydration/desiccation and shriveling of the dead body from evaporation of water, with preservation of natural appearances and features of the body.
- It is a modification of putrefaction (dry decomposition)
- The entire body loses weight, becomes thin, stiff, brittle and odorless
- Time required for mummification: It varies between 3-12 months or longer



Factors Favoring Mummification

□Hot environment: As in the deserts.

Dry atmosphere: Mummification cannot occur in humid conditions.

□Free air movement: It helps in rapid evaporation of body fluids.

Contact of the body with absorbing media: A dead body lying in shallow grave, in dry sandy soils mummifies early due to absorption of body fluid rapidly.

Poisoning: Chronic arsenic or antimony poisoning favors the process of mummification

Adipocere

- Also known as "grave wax," adipocere (from the Latin, adipo for fat and cera for wax)
- a grayish-white postmortem matter caused by fat decomposition, which results from hydrolysis and hydrogenation of the lipids (fatty cells) that compose subcutaneous fat tissues.
- Moisture is necessary.
- The optimum conditions for the formation of adipocere:
 - Wet, warm environment (Sometimes original body water being sufficient for adipocere).
 - ✓ Bacterial activity (C. perfiringes).
- It occurs in ?
 - > Subcutaneous fat of the cheeks ,breast, buttocks.
 - \succ May occur in internal organs such as liver, kidney & heart.
- It needs months to occur and occurs partially.

3 stages



> In early stages: Adipocere is a pale, rancid, greasy semi-fluid material with a most unpleasant smell.



> Later: Becomes more brittle and whiter.



> When fully formed, adipocere is a grey, firm, waxy compound which maintains the shape of the body.





Medico-legal Importance of Adipocere

- 1. <u>Preserve the body</u> which can permit identification after death.
- 2. It may give conclusions about the <u>cause</u> of death.
- 3. It indicates that the <u>time</u> interval since death was <u>at least weeks to several</u> <u>months</u>.

Immersion and burial

 Immersion in water or burial will slow the process of decomposition.

 Body in air will decompose twice as fast as a body in water and four times as fast as a body under the ground.

 The first change that affects the body in water is the loss of epidermis. Gaseous decomposition progresses and the bloated body is often lifted to the surface by these gases, most commonly at about 1 week but this time is extremely variable.

Skeletonization

- The **environment** is **more important** than the **time** in this process.
- 12-18 months: Soft tissues will be absent.
 - Tendons, ligaments, hair and nails will be identifiable for some time after that.
- After 3 yrs: the bones will be bare and disarticulated.
- In temperate zones the bones will remain solid & heavy with the preservation of **bone marrow** in long bones for a number of years, that can sometimes be suitable for specialist DNA analysis.
- After 40-50 years:
 - Bone surface becomes dry & brittle.
 - Marrow cavity will be empty.



Estimating the Time of Death

 Unfortunately, all methods now in use to determine the time of death are to a degree unreliable and inaccurate. They usually give vague or answers.

• The longer the postmortem interval, the less precise the estimate of the interval.

Estimating the Time of Death

- 1. Core body temperature:
 - The best and the most commonly used.
- 2. Rigor mortis.

3. Hypostasis:

• Complete after 6 hrs.

4. Chemical changes in vitreous.

- all electrolytes in the body change after death except K.
- There is linear relation b/w K level and time passed after death up to 120 hrs.
- Measured from vitrous humor
- When there is high urea concentration, there is an electrolyte imbalance and K can no longer be used as an indicator
- Measurement of potassium levels in the eyes can vary greatly from left to right eyes in the same corpse in ideal conditions.
- From a medico-legal standpoint, this technique is frowned upon, and so is the most infrequently used method to determine post mortem interval.
- 0

5. Eye pressure:

• Eye balls become softer, and less fluid pressure in the first 3 hrs.

Estimating the Time of Death

6. Gastric emptying:

• Depend on type of meal and emotional status.

7. The entomology of dead:

- <u>Studying insects</u> & their maggots which infest the dead body for estimating the probable time of death.
- <u>Different types of insects infest the dead body at</u> <u>different stages</u> after death occurs.

8. Scene markers

• Though unscientific, is often more accurate than determinations made by scientific means.

https://www.ncbi.nlm.nih.gov/books/NBK539741/

