Papanicolaou staining:

- Standard stain for vaginal wet mount

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Papanicolaou staining reagent:

1. Descending and ascending grade alcohol
2. Hematoxylin stain
3. 0.5% acid alcohol
4. Sodium hydrogen carbonate
5. OG-6
6. EA-36 (eosin azure)
7. Mounting media

Pap stain:

Fix in absolute alcohol and put in hematoxylin for 12-15 mts

Wash and differentiate in 0.5% acid alcohol

Wash and bluing in $NaHCo_3$ for 2 minutes

Dehydrate and put in OG-6 for 5 mts

Dehydrate and put in EA 36 for 7 mts

Dehydrate dry and mount

Results:

- Cells blue to black,
- nucleoli black to red,
- Cytoplasm pink-red,
- erythrocytes bright red

- **REAGENT:**
- Harris Alum Hematoxylin.
- 50%, 60%, 70%, 80%, 95%, alcohol.
- 0.5% OG (Orange-Green)-36.
- EA (Eosin Azur)-6.
- DPX.

PROCEDURE:

- Fix the smear in 95% ethanol either.
- Hydrate the smear in running water.
- Dip the slide in hematoxylin for 2-5 minute.
- Wash in running tap water.
- Give 3-5 quick dip in 0.5% acid alcohol for decolorizes.
- Slide truff put in running tap water until it takes blue color.

- Dip slides in 70%, 90% and absolute alcohol every step for one minute.
- Flood the slide with OG-6 for 3-5 minute.
- Wash in absolute alcohol for 2-3 minute.
- Flood the slide by EA (Eosin Azur)-36 for 3 minute.
- Wash in absolute alcohol.
- Blot, dehydrate, clear and mount with DPX.

Result:

- Nucleus blue
- Cytoplasm various color according to material.

MGG

- 5% MGG IN absolute alcohol

1% giemsa solution

- Fixative alcohol
- Procedure
- 2 mts giemsa in 1:1 for 2 minutes
- 2 minute MGG 2 minutes
- Dilute with d/w and leave for 8-10 minutes
- Dry and examine

Normal cell structure, function, cytological criteria of malignancy

Types of specimen, methods of collection and prepertion of cell block

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History:

- Cell research began in the 17th century
- 3 researchers are credited with developing the cell theory:
  - **Schleiden & Schwann:** all living things are composed of cells
  - **Virchow:** all cells come from pre-existing cells

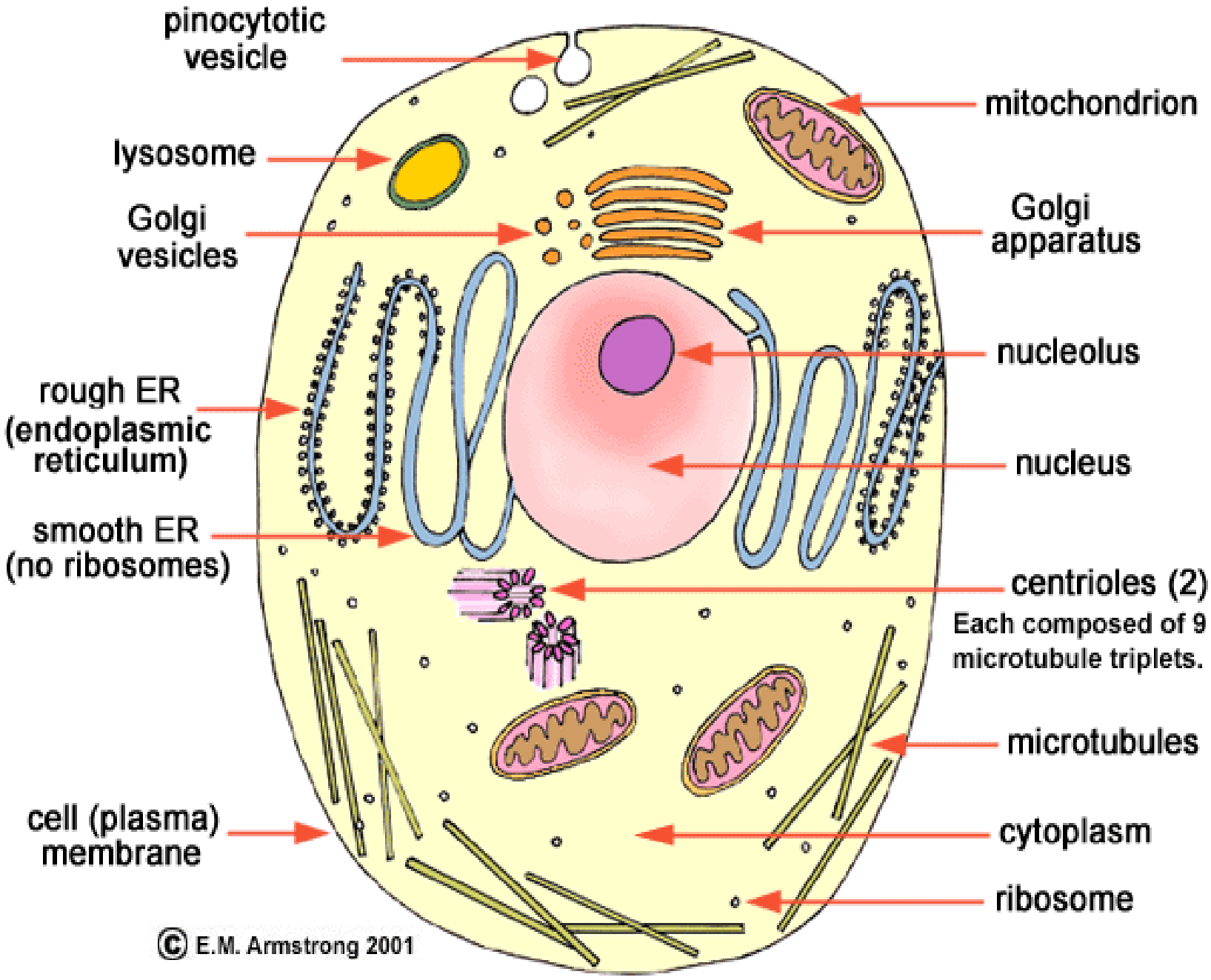
Some Cell Terms & Basic Info:

- **There are 2 basic types of cells:**
  - Prokaryotic: (no internal membranes → no nucleus or membrane-bound organelles)
  - Eukaryotic: (nucleus & membrane-bound organelles)
- **Organelle:** a distinct sub-cellular structure which carries out a specific function
- Ultrastructure: the inner makeup of a cell

Meet the Organelles:

4 groups:

1. cell membrane
2. nucleus-related structures
3. endomembrane system organelles
4. energy-producing organelles


pinocytotic vesicle
lysosome
Golgi vesicles
rough ER (endoplasmic reticulum)
smooth ER (no ribosomes)
cell (plasma) membrane
mitochondrion
Golgi apparatus
nucleolus
nucleus
centrioles (2)
Each composed of 9 microtubule triplets.
microtubules
cytoplasm
ribosome
© E.M. Armstrong 2001

The Cell Membrane:

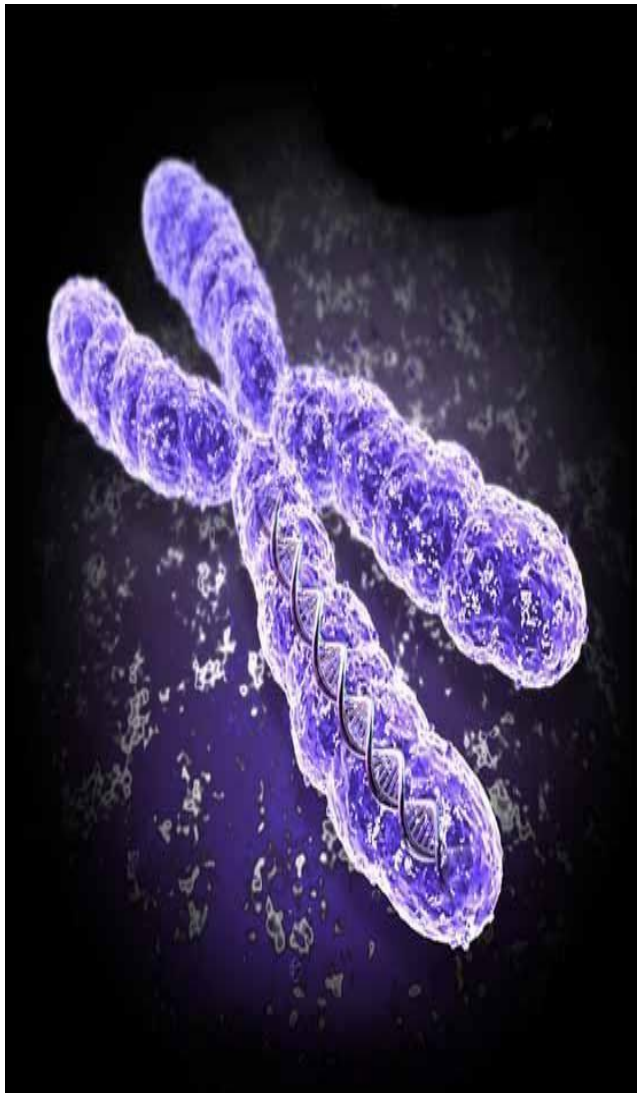
Cell membranes have 4 functions:

1. Act as a **barrier** to pathogens, toxins, etc.
2. To **mark** the cell as self
3. To act as a **receptor** for messages from other cells in the body
4. To allow things to be **transported** in and out of cell

The Nucleus-related Organelles& Structures

- The Nucleus contains the cell's chromosomes and therefore controls the cell's ability to:
  - make proteins
  - undergo cell division
- Related structures: nuclear envelope, nucleolus, nucleoplasm, chromosomes, chromatin, and ribosomes.

Chromosomes:



- When the cell is in its normal state (not dividing) the cell's genetic information (encoded in DNA) is dispersed within the nucleus → chromatin
- When a cell is replicating (mitosis or meiosis) the chromatin condenses to form chromosomes
- Each chromosome consists of 2 sister chromatids

There Are Two Types of Ribosomes:

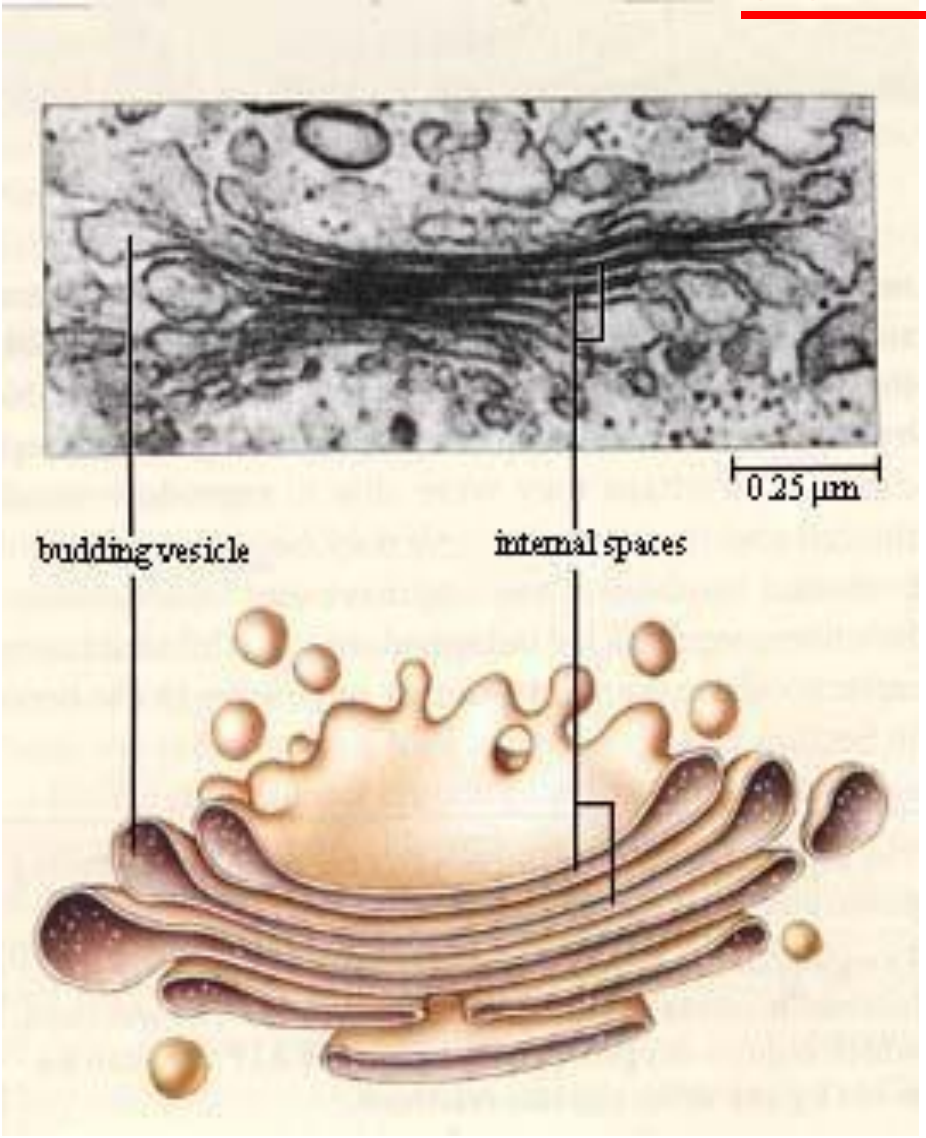
Cytoplasmic

- Free-floating in the cytoplasm, make proteins (such as enzymes) needed within the cell

Endoplasmic

- Attached to endoplasmic reticulum
- When attached to ER the ER is called Rough ER (RER)
- Proteins made here are often secreted (e.g. a peptide hormone)

Golgi Apparatus: processing, packaging, secretion:



- A stack of curved vacuoles
- Receives protein or lipid-filled vesicles from ER
- GA modifies the contents of the vesicles
- Vesicles leave GA for plasma membrane (secretion) others become lysosomes

The Energy-related Organelles:

- Mitochondria and chloroplasts are both related to obtaining energy
- Chloroplasts are only found in plant cells. They are the site of photosynthesis
- Mitochondria are found in both plant and animal cells and make ATP—the cell's main energy currency (how energy requiring processes are "paid" for by the cell)

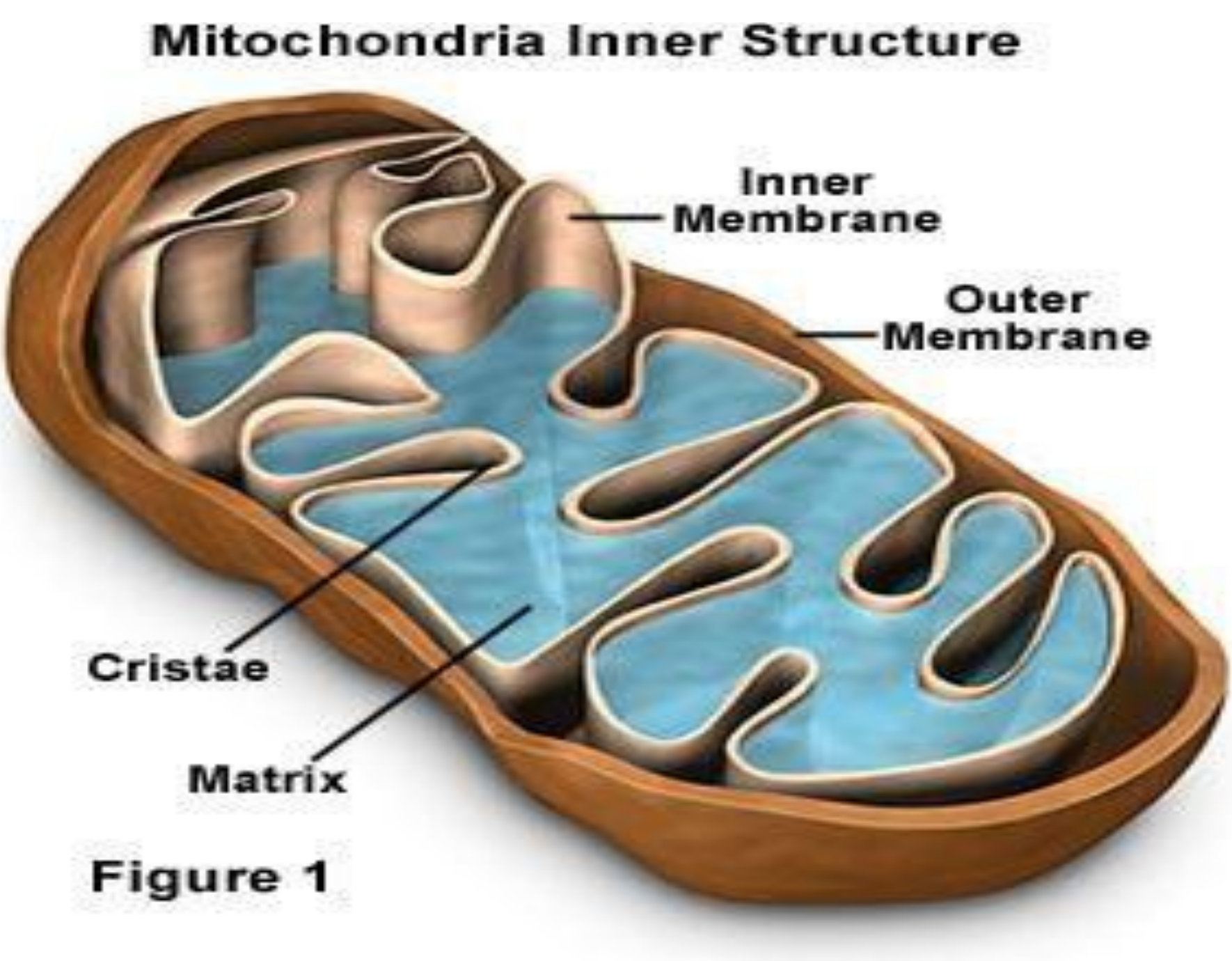

Mitochondria Inner Structure
Inner Membrane
Outer Membrane
Cristae
Matrix

Figure 1

Malignancy:

Malignancy is the most familiar characterization of cancer. A malignancy is not self-limited in its growth, and may be capable of spreading to distant tissues.

Malignant cell:

A cell that has undergone malignant change in which state of proliferation and capable of metastasis.

Cytological Criteria for Malignant Cells:

1. Enlarged nuclei
2. Variation in nuclear size and shape
3. Irregular nuclear border
4. Increased nuclear- cytoplasmic ratio (decreased cytoplasm)
5. Decreased intracellular potassium and calcium.

Cytological Criteria for Malignant Cells:

5. Multiple prominent and irregular nucleoli
6. Hyperchromatism (increased nucleoproteins)
7. Abnormal chromatin pattern and distribution
8. Discrepancy in maturation (extreme variations)
9. Ectopic hormone production
10. Swelling of mitochondria and flooding of matrix.

Defference between normal cell and malignant cell:

Normal cell

- Normal cell have uniform shape
- Cells stop dividing when population are too much
- These cells grow in a controlled manner.

Malignant cell:

- Malignant cell have large variety of size and shape
- Nucleus have irregular structure and small cytoplasm
- The cells growth is uncontrolled manner

Defference between normal cell and malignant cell:

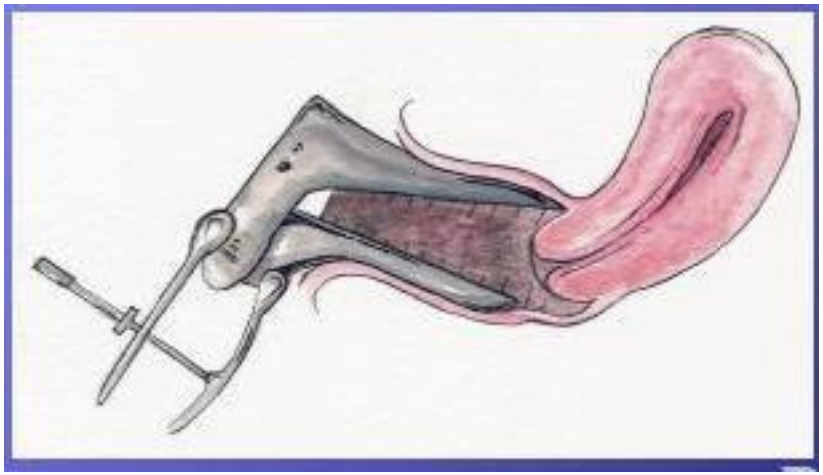
- Normal cells communicate with each other for proper functioning
- These cells have external membrane that allow them to bond with other cells
- After a life, these cells go through apoptosis

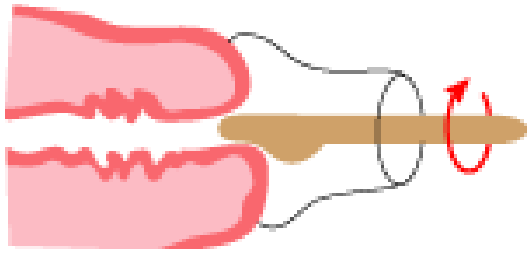
- Malignant cell do not communicate with other cells
- These cells have ability to invade or spread to other parts of body by travelling through blood
- Malignant cells do not mature and dead through apoptosis

Characteristic and features of cancer:

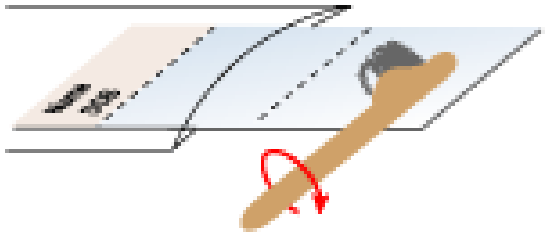
Gynecological Specimen Collection:

Pap Smears

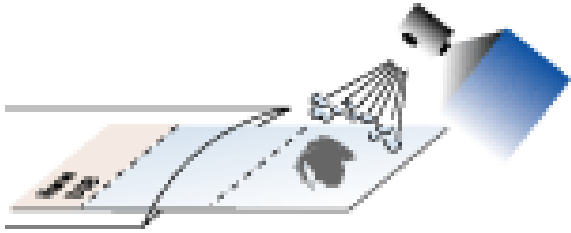




Collection



Smearing



Fixation

Non-Gynecological Specimen Collection

- Respiratory Tract
- Urinary Tract
- Oral Cavity / Gastrointestinal Tract
- Effusions
- Cerebral Spinal Fluid
- Many other body sites

Prepertion of cell block:

PAS Stain for Carbohydrate

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PAS reactive cells and tissue components:

1. Glycogen
2. Starch
3. Mucin (neutral mucin)
4. Basement membrane
5. Fungi
6. Thyroid colloid

Requirements:

Reagents and stain:

1. Periodic acid solution

- Periodic acid 1g
- Distilled water 100 ml

2. Counter stain:

- Harris hematoxylin

2. Schiff reagent preparation:

- Dissolve 2 gm of basic fuchsin in boiled distilled water in which and cool at 50^{o} then add 2 gm of sodium metabisulfite and cool at room temperature then add 2.0 ml of hydrochloric acid (HCL). Shake well the solutions at intervals 10 times and add 2 gm of activated charcoal. Filter before use and store in dark.

Specimen:

- Cut 3-5 um thin section of the tissue that have fixed in **Neutral Buffered Formalin** because glycogen is soluble in aqueous based fixative. **Rossman's fluid** has also been recommended for glycogen fixation because it containing alcoholic formalin with picric acid.

Principle:

- Periodic acid oxidize the carbohydrate substance to form reactive aldehydes. These aldehydes have the reactivity with schiff reagent to form a bright red or magenta color where as the nuclei of the cells are stained as blue.

Procedure:

1. Dewax in xylene and hydrate through descending grade of alcohol.
2. Oxidize with periodic acid for 5 minutes.
3. Rinse in several changes of distilled water.
4. Cover the section with Schiff reagent for 15 minutes.
5. Rinse in running tap water for 5-10 minutes.
6. Stain the nuclei with counter stain for 1 minute.
7. Dehydrate with ascending grade of alcohol and clear with xylene.
8. Mount in DPX.

Result:

- Glycogen and glycoproteins — Magenta
- Nuclei — Blue

Staining for Reticular fibers

Methods:

1. Gordon and Sweets' method

2. Gomori's method

Gordon and Sweets' method for reticular fiber:

- Tech for demonstration of reticular fiber may be divided into those using dyes and metal impregnation methods. There are discuss metal impregnation tech that provide better contrast.

REAGENT:

- 1% potassium permagnate.
- 1% oxalic acid.
- ammonia silver nitrate solution.
- 10% formalin.
- 2.5% iron alum.
- 5% sodium thiosulphate.
- 1% Eosin stain

Ammonium silver solution:

- 10% aquous silver nitrate sol (5ml) in which add concentrated ammonia drop by drop until precipitation.
- Add 5ml 3% sodium hydroxide sol.
- Re-dissolved with concentrated ammonia.
- Add 1 ml 10% silver nitrate sol.
- Filter before use. Store in dark bottle.

Section:

In routine histopathological work paraffin section cut at 3-5 um in thickness are used. Frozen, cryostat and celloidin embedded section are also recommended.

PROCEDURE:

1. Deparafinize the section and hydrate.
2. Cover the slide by 1% potassium permanganate for 5 minute.
3. Rinse in tap water.
4. Cover slide by 1% oxalic acid for one minute.
5. Rinse in tap water.
6. Fill the slide by 2.5% iron alum for 15 minutes.
7. Rinse in several changes of D/W.

8. Place the slide in coplin jar containing silver solution for 2 mts.
9. Rinse in several changes of D/W.
10. Flood the slide in 10% aqueous formalin for 2 mts.
11. rinse in tap water.
12. Dip the slide in 0.2% gold chloride for 3 mts.
13. rinse in tap water.
14. Treat with 5% sodium thiosulphate for 2 mts.
15. rinse in tap water.

16. Dip the slide in eosin or in nuclear fast red for 30 seconds (as counterstain).
17. Dehydrate through ascending grade of alcohol.
18. Clear in xylene and mount.

Results:

- Reticular fiber — black
- Nuclei — black or unstained
- Other components — pink

Staining for Microorganisms (bacteria, fungi)

ZN STAIN: (for Mycobacterium sp.)

Corbel fuchsin:

Basic fuchsin	0.5 gm
Absolute alcohol	5 ml
5% aquous phenol	100 ml

Mix well and filter before use.

0.3% methyline blue:

- Methylene blue 1.4 gm in 100 ml 95% alcohol (stock sol)
- Methylene blue (stock sol) 10 ml in 90 ml tap water.

Decolorizing agent:

- **5% sulfuric acid** used for the **Mycobacterium leprosy**.
- And **20% sulfuric acid** used for **Mycobacterium tuberculosis**.

Specimens:

- Cut section 3-5 um in thickness of the specimen that received in 10% formalin, and be embedded in paraffin wax.
- Also take positive, negative and test sections slide.

Procedure:

1. Dewax and hydrate through ascending grade of alcohol to water with control section.
2. Treat section with hot corbel fuchsin for 3-4 minutes.
3. Wash in running tap water, till the excessive stain remove from slide.
4. Decolorize by 20% and 5% sulfuric acid for Mycobacterium tuberculosis and lepropsy respectively.
5. Rinse in water.
6. Counterstain by methylene blue for 1 minute.
7. Dehydrate, clear and mount.

Results:

Positive Control	shows pink bacilli
Negative control	no bacteria on blue background
Test slide	may or may not

Gram stain:

Crystal violet:

- Crystal violet 10% alcoholic 2 ml
- Distilled water 18 ml
- 1% Ammonium oxalate 80 ml

Mix and store always filter before use.

Gram's iodine:

- Iodine 2 gm
- Potassium iodide 4 gm
- Distilled water 400 ml

Acetone

Carbol fuchsin (as counter stain)

Specimen:

- Cut section 3-5 um in thickness of the specimen that received in 10% formalin, and be embedded in paraffin wax.
- Also take positive control section, with the test slide.

Procedure:

1. Dewax and hydrate through descending grade of alcohol to water.
2. Stain with filtred crystal violet solution for 1 minute
3. Rinse in distilled water
4. Cover slide with iodine solution for 1 minute.
5. Rinse in distilled water and blot slide but not the tissue section.
6. Decolorize with apply acetone untill the blue color drops running.
7. Cover the slide with counter stain as carbol fuchsin for 1 minute.
8. Rinse in water, dehydrate, clear and mount.

RESULT:

Gram positive organism	blue
Gram negative organisms	red
Cells nuclei	red
Other tissue	yellow

Staining for Lipids

Oil Red 'O':

- For demonstration of fat globules

0.5% Sudan IV stain:

Oil-red 'O'	0.5 gm
absolute isopropyl alcohol	100 ml

Allow to stand overnight.

Dextrin solution:

Dextrin	1 gm
Distilled water	100 ml

Working solution:

Stock Oil red O	60 ml
dextrin solution	40 ml

Specimen:

- Cut 5 um section, mount on slide and air dry.
- Also take positive, negative and test fatty tissue on the slide by frozen tech.

Fixation:

- Fresh frozen or Neutral buffered formalin fixed tissue used.

Procedure:

1. Place slide directly into filtered 0.5% Oil red O solution for 20 minutes.
2. Rinse in running water briefly.
3. Counter stain with Gill's hematoxylin for 20-30 second.
4. Rinse in water. Mount in aqueous mounting media.

Result:

- Fat brilliant red
- Nuclei blue

SPECIAL STAINING

- Special stains are used to identify certain normal and abnormal substance present in the cells an tissue, which cannot be differentiated by routine haemotoxylin and eosin staining.

PRINCIPLE

- Trichrome procedure are three dyes
- Sections are first stained with an acid dye such as Biebrich scarlet.
- All acidophilic tissue elements such as cytoplasm. muscle and collagen will bind with the acid dyes.
- The section are then treated with phosphotungstic and phosphomolydic acid.

Contd.....

- Because cytoplasm is much less permeable than collagen the phosphotigstic and phophomolydic acids cause the Biebrich scarlet to diffuse out of the collagen but not out of the cytoplasm.
- Phosphotigstic and phosphomolydic have numerous acidic groups.
- Finally a dye of large particle size such as light green is used that is able to enter collagen.

REAGENT REQUIRMENT

- Weigerts iron haematoxylin
- 5% phosphomolydic acid
- 5% phosphotungstic acid
- 2% light green
- Biebrich scarlet acid fushin solution

PROCEDURE

Depparaffinize section in xylene

↓

Clean xylene by alcohol

↓

Rinse with distilled water

↓

weigerts iron hemotoxylin
5-10 mint;

↓

Contd.....

Rinse well in d/w

↓

Biebrich scarlet acid fuchin
5-10 mint;

↓

Rinse in d/w

↓

Phosphotungstic – phosphomolydic acid solution
10-15 mint;

↓

Light green solution
2-5mint;

↓

Dehydrate in absolute alcohol

↓

Clear in xylene

↓

Mount in synthetic resin

↓

Microscopy

Results

- Nuclei.....black, blue
- Muscle.cytoplasm,fibrin.....red
- Collagen.....green


ThankYou

DECALCIFICATION

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DECALCIFICATION:

Decalcification is a process of complete removal of calcium salt from the tissues like bone and teeth and other calcified tissues following fixation.

Decalcification is done to promise that the specimen is soft enough to allow cutting with the microtome knife. Unless the tissues in completely decalcified the sections will be torn and ragged and may damage the cutting edge of microtome knife.

Decalcification step:

1. To ensure adequate fixation and complete removal of the calcium it is important that the slices are 4-5 mm thick. Calcified tissue needs 2-3 weeks only, for complete decalcification to be achieved so it in necessary to check the decalcification after 2-3 days.
2. Fixative of choice for bone or bone marrow is Zenker formal or Bouin's fluid. Unfixed tissue tends be damaged 4 times greater during decalcification than a properly fixed tissue.

Decalcification is effected by one of the following methods:

1. Disbanding of calcium by a dilute mineral acid.
2. Removal of calcium by used of dilute mineral and along with ion exchange resin to keep the decalcifying fluid free of calcium.
3. Using Chelating agents EDTA.
4. Electrolytic removal of calcium ions from tissue by use of electric current.

The aim of a good decalcifying agents are:

1. Complete removal of calcium.
2. Absence of damage to tissue cells or fibres.
3. Later staining not altered.
4. Short time required for decalcification.

Removal of calcium by mineral acids – Acid decalcifies subdivided into- Strong acid, weak acid. Strong acid - eg. Nitric and hydrochloric acid.

- **Nitric acid- 5-10% aqueous solution used.**

  They decalcify vary rapidly but if used for longer than 24-48 hrs. cause weakening of stainability specially of the nucleus.

- **Hydrochloric acid - 5-10% aqueous solution decalcification slower** than nitric acid but still rapid. Fairly good nuclear staining.

Formalin Nitric acid:

Formalin	10 ml
Distilled water	80 ml
Nitric acid	10ml

Nitric acid causes serious deterioration of nuclear stainability which partially inhibited by formaldehyde. Old nitric acid also tends to develop yellow discoloration which may be prevented by stabilization with 1% urea.

Aqueous nitric acid

Nitric acid	5-10 ml
Distilled water to	100 ml.

Procedure:

1. Place calcified specimen in large quantities of nitric acid solution until decalcification is complete (change solution daily for best results).
2. Washing running water for 30 minutes
3. Neutralize for a period of at least 5 hours in 10% formalin to which excess of calcium or magnesium carbonate has been added.
4. Wash in running water over night
5. Dehydrate, clear and impregnate in paraffin or process as desired.

Note: Over contact to nitric acid impairs nuclear staining. Nitric acid is the solution of choice for decalcifying temporal bones.

Decalcification of Bone marrow biopsy.

Tissue after fixation in Bouin's or Zenker's fixative is decalcified for 2½ hours followed by an hour of washing. The tissue in then dehydrated beginning with alcohol.

Chelating agents:

Chelating agents are organic compounds which have the power of binding certain metals. Ethylene-diamen-tetra-acetic acid, disodium salt has the power of capturing metallic ions. This is a slow process but has little or no effect on other tissue elements. Some enzymes are still active after EDTA decalcification.

EDTA	10 gm
10% formalin	100 ml
	(pH 5.5 to 6.5)
	Time 7-21 days

Electrolytic method:

This is based on the principle of attracting calcium ions to a negative electrode in to addition to the solution.

Decalcifying solution

HCL (Conc.)	80ml
Formic acid 90%	100 ml
Distilled water	1000 ml

Ultrasonic decalcification:

Ultrasonic waves are produced from an ultrasonic generator situated in a metal jaket. This is known a ultra sonic bath. In which fluid consist 7.5% acetic acid. In which temperature accelerate the reaction and vibration produced decalcification.

Determination of end point of decalcification

1. Flexibility method:

Bending, needling or by use of scalpel if it bends easily that means decalcification is complete.

Unreliable, causes damage and distortion of tissue.

2. X-ray method:

Best method for determining complete decalcification but very costly. Tissue fixed in mercuric chloride containing fixatives cannot be tested as they will be radio opaque.

3. Chemical Method:

It is done to detect calcium in the decalcifying fluid when no further calcium is detected, decalcification in considered complete.

Procedure:

1. Take 5 ml of decalcifying fluid from the bottom of container which has been in contact with the tissue for 6-12 hrs.
2. Add 5 ml each of 5% ammonium oxalate and 5% ammonium hydroxide.
3. Mix and let it stand for 15-30 min.
4. A cloudy solution caused by calcium oxalate indicates that specimen is not thoroughly decalcified. Absence of turbidity indicates completeness of decalcification.

Tissue is ready for blocking

Museum techniques

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History of Museum:

- **Rudolf Virchow**, opened museum in 1899 as "The Pathological Museum", that was filling with 23,066 histopathological specimens by the end of 1901.
- **Rudolf Virchow** is also known as the father of pathology.

Definition of Museum:

A museum is a non-profit, permanent institution in the service of society and its development, open to the public, which acquires, conserves, researches, communicates and exhibits the tangible and intangible heritage of humanity and its environment for the purposes of education, study and enjoyment.

Introduction:

- The preserving of pathological specimens, in addition to their teaching value, play a role in recording the history of medicine. For this is essential the original shape & color of such specimens is retained along with accurate record of patient's medical history.
- All teaching hospitals and colleges of Pathology have Museums which serve many functions: permanent exhibition of common specimen for undergraduate and postgraduate teaching purposes, permanent source of histologic material and for gross and microscopic photography

Objective of Histopathological Museum:

- To explain the methods used in handling museum specimens.
- To describe the techniques of specimen preservation.

Terms in Histopathological Museum

1. Casting
2. Tissue
3. Organ
4. Dissection
5. Grossing
6. Injection
7. Viscera
8. Embedding

Casting:

- It is the manufacturing process by which a liquid material is usually pour into a mould, which contains a hollow cavity of the desire shape, and then allow to solidify. The solidified part is also knows as casting, which is ejected or broken out of the mould to complete the process.

Tissue (museum):

- A specified group of cells are known as tissue that tissues are preserved for the coming era (future) to become a history of present histological and histopathological changes.

Grossing for museum:

- It includes all aspects of gross pathology, beginning with the collection of the specimen in the laboratory, progressing through fixation, gross examination, grossing techniques, differential diagnosis and finally mounting of the specimen in the museum.

Injection technique (Museum):

- Injection techniques are the fixation methods for injecting the fluid material (fixative) into the viscera, organ or whole body. These are two types:

1. For microscopic examination
2. For macroscopic examination

Viscera:

- The internal organs of the body, specifically those with in the chest (as the heart or lungs) or abdomen (as the liver, pancreas or intestine or spleen).

Basic museum techniques:

Any specimens for museum are handled by following steps:

1. Reception
2. Preparation
3. Fixation
4. Restoration
5. Preservation
6. Presentation

Reception:

- Any specimen received in the museum should be recorded in a reception book and given a no. followed by year (for instance 32/2018) as histopathology registration number.
- Number will stay with specimen even after it is catalogued in its respective place.
- Reception book should contain all necessary information, About the specimen (clinical gross and microscopic findings).

Preparation:

- An ideal specimen is received fresh in unfixed state. However, it is mostly obtained from pathology lab. After being examined, thus will already be formalin fixed.
- If planning to use a specimen for museum, part of it can be kept without disturbing for museum, e.g. in kidney it can be dissected and one half kept aside for museum.

Fixation:

- Inject with fixative where ever possible. In fixation never wash specimen containing much blood before or after fixing.

Kaiserling's Technique:

- The specimen needs to be kept in a large enough container which can accommodate specimen along with 3-4 times volume of fixative. Specimen is stored in the Kaiserling-I Solution for 1 month depending on the size of the specimen. The specimen should not rest on bottom or an artificial flat surface will be produced on hardening due to fixation.

Kaiserling's fluid – I (fixing solution):

- Formalin 1L
- Potassium acetate 45 g.
- Potassium nitrate 25 g.
- Distilled wate Make up to 10 litres

Specimen is stored in the solution for 1 month depending on the size of the specimen.

Restoration of specimen:

- It is required to restore the specimens, as they lose their natural color on fixation. The recommended method is the Kaiserling's-II method. It involves removing the specimen, washing it in running water and transferring to 95% alcohol for 10 minutes to 1 hour depending on the size of specimen. The specimen is then kept and observed for color change for around 1-1.5 hrs. After this step, specimen is ready for preservation.

Kaiserling II Solution:

- Alcohol 80%
- Store specimen in this solution for 10 minutes to 1 hour depending on size of specimen.

Rejuvenator Solution:

Pyridine	100 ml
Sodium hydrosulphite	100 gm
Distilled water	4 litres

Formalin decreases the natural colour of the specimen. However, rejuvenator solution restores the colour.

Preservation of specimen:

- The recommended solution for this step is Kaiserling III. This is the final solution in which the specimen will remain for display. It is based on glycerine solution.

Kaiserling's-III Solution:

Potassium acetate	14.16 g.
Glycerine	4 litres
Distilled water	Make up to 10 litres

Thymol crystals added to prevent moulds.

- Leave solution to stand for 2 – 3 days before using to ensure proper mixing of chemicals.
- Add 1% pyridine as **stabilizer. This solution acts as permanent fixative. This** solution easily turns yellowish and needs to be replaced to restore colour of the specimen. The specimen will initially float to surface but later sink to bottom.

Fixation of specimens the precaution:

1. The specimen should be injected with fixative to ensure adequate fixation.
2. The specimens containing much blood should be washed in saline or formal saline (not by water) before or after fixation.
3. Fresh soft specimen should be fixed individually.
4. The specimen, together attached structures should be fixed & pinned to cork boards by using rustles pins.
5. To maintain the natural shapes, the cystic cavities if open, should be packed with cotton wool soaked in fixative.
6. Bile stained or bile contaminated specimen must be fixed & stored separately.

Preservation:

- Specimen together with the duplicate label is wrap in a gauge and the label attached with the piece of linen thread.
- Specimen are preserved in large, rectangular glass jar.

Mounting:

- Specimens are trimmed to the desired size and shape, so that it fits into the jar.
- All unwanted and non-representative tissue removed by careful dissection this process is known as mounting.

Presentation:

- Museum specimen should be clearly labeled and system of cataloging should be employed which allowed easy and rapid access.

Museum Material:

- Those histopathological specimens received from **biopsy** section or from **operation theater** for the diagnostic purpose (gall bladder) and some of these received for museum, these specimens require special fixation and caring.
- Some of the histopathological museum specimen received from **autopsy** section after post-mortem findings (heart).
- From cadaver (whole body fixation)

Mounting of museum specimen:

- Slight irregularities may have developed on the surface of the specimen during fixation. Before mounting it is necessary to take following precautions are.

1. Removal of irregular surface of the specimen.
2. Trimming of outer edges of the specimen such as skin & intestine.
3. Filling the cavities by arsenious acid – gelatin after removing cotton wool.
4. Friable specimens should be covered with a thin layer of arsenious gelatin.
5. Bile stained specimen should be soaked in a saturated solution of calcium chloride for 24 hours.

Arsenious acid – gelatin prepared:

- Boil 20 g arsenious acid in 1 liter of D.W .
- Cool & add 120 g gelatin & dissolve by steaming.
- Filter through filter paper.
- Add 100 ml of glycerin.

Routine mounting specimen:

- Perspex boxes are used for mounting the specimen .
- The specimen is placed by allowing ½ inch clearance at the top & 1 inch at the bottom.
- The center plate is thoroughly washed in a detergent & dried on a cloth.
- The specimen is arranged in the preferred position on the center plate by linen or nylon thread at the appropriate cites to hold it properly.
- The center plate, with specimen attached is placed firmly in the proper position.

- To support the specimen within its jar, it is attached to the specimen plate or
- rectangular bent glass rods. It can be done by tying the specimen with nylon
- threads. Double knots should be made by threads, on the specimen surface

Making of Perspex jars:

- Perspex or methacrylate is a high quality transparent plastic sheet imported from the united kingdom. High quality of Perspex is used because.
- It is easily band & can withstand heat up to 110 – 150 ˚C.
- Its surface is smooth & easily bounded using chloroform or ethylene dichloride.
- It is easily cut & polish.
- The thickness varies from 1.5 mm, 3 mm, 6 mm, to 12 mm.

Thank you

Autopsy Technique

Mr. Ashish Dhuan

Lecturer in DMLT

Autopsy:

- An autopsy is a post mortem examination preformed on a cadaver to determine the cause, manner and time of death. It is carried out by trained pathologist or doctor of forensic science deptt.

Purpose:

- Confirming the cause of death
- Establishing the final diagnosis
- Study the therapeutic response of the subject to the treatment
- For determine the missed clinical diagnosis like carcinoma, acute pancreatitis
- Study of demographic and epidemiology of disease
- For educational purpose

Forensic Autopsy:

- Forensics autopsies are preformed when the cause of death of a victim may be a criminal case, often involving foul play.
- A forensic autopsy applies science to legal law.

Classification:

- In a forensic autopsy, death is placed into five different categories.
  - Natural
  - Accident
  - Homicide
  - Suicide
  - Unknown

Equipments for autopsy:

- Autopsy house with built in autopsy table
- Autopsy permission
- Death certificate
- Blank paper for noting rough description
- Lead pencils
- Paper pins
- Autopsy requisition form
- Autopsy report form
- Autopsy report register
- Protective cloths
- Gloves, apron, mask and caps

Equipments for autopsy:

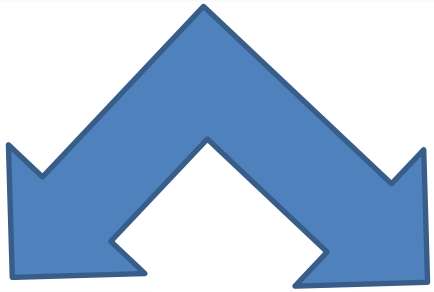
- Forceps
- Surgical blades
- Needle
- Scissors both sharp and blunt
- Straight probe
- Linen thread
- Scale
- Weighting machine
- Labels
- Sealing wax
- Empty container (plastic)
- Fixative (formal saline or 10% formalin)

Care and maintance of autopsy items:

- The sharpness of all the sharp items is to be maintained
- All the cloth items are to be kept neat and clean
- All items are to be washed thoroughly after performing autopsy
- Items are to be kept packed when not in use
- Rubber items are to be kept in dusting powder
- Consumable items are to be replaced
- Proper counting and maintance of adequate stoke to be under taken
- As for as possible, all the items to be sterilized


VIEWIMAGES™

Autopsy protocol



Block extraction of abdominal and thoracic organs

In situ organ-by-organ dissection

For pathologic autopsy:

- To obtain permission for conducting autopsy including restrictions.
- To obtain much history and clinical data about the body.

above 2 requirements can be incorporated in a Autopsy Requisition Form so that entire information has to be compulsorily filled.

Autopsy Requisition Form:

GOVERNMENT MEDICAL COLLEGE HOSPITAL
SECTOR-32, CHANDIGARH

DEPARTMENT OF PATHOLOGY

Referred by

Date of admission

Date of death

Name
Age/Sex
CR No.
Ward/OPD
Date Income

HIV status Autopsy No.....

Permission for autopsy: Yes/No

Restrictions on permission:
(tick mark √)

Abdominal with brain or without brain
Thoracoabdominal with brain or without brain
Brain only
Spinal Cord/eye/ear/others

Laboratory data and radiological findings:

Operative procedures:

Provisional diagnosis:

Previous cytology/histopathology number, if any:

Date and time

Requested by
Doctor's name & signature

Prosection:

- Prosection means carefully programmed dissection for the demonstration of anatomic structures.

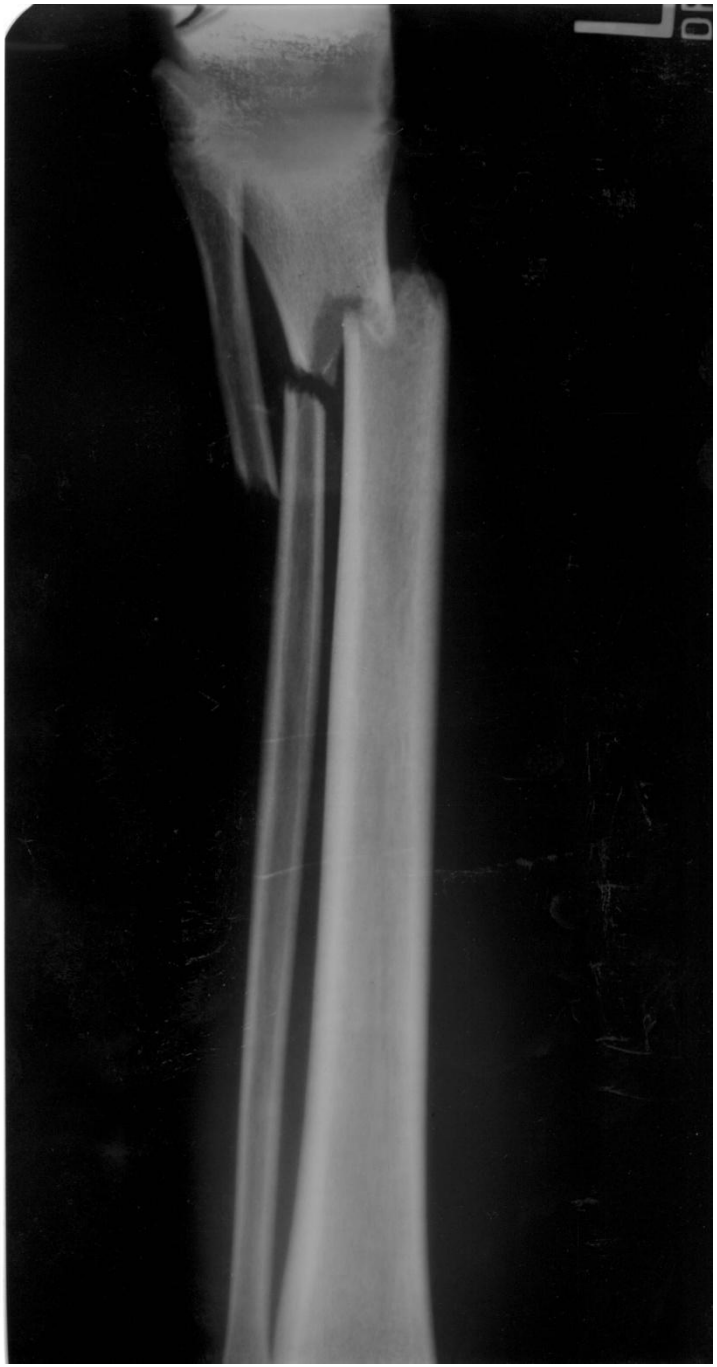
1. Collect the data about age, sex, race and general figure.
2. External examination including injuries (trauma, surgery and burn).
3. Internal examination by incision from neck to pubis avoiding the umbilicus, because this has fibrous tissue that create difficulty in sawing.
4. Examine peritoneal cavity like blood, fluid and pus and bowel organs.
5. Incision in neck up to floor of mouth and base of tongue.

6. Cut sterno-clavicular joints and lift the sternum.
7. Examine lungs and pleural cavity (for blood, pus and fluid).
8. Cut the kidney tissue as specimen from back.
9. All the thoraco-abdominal organs are removed like liver piece, pancreas, spleen, intestine.
10. Make the bitemporal incision, then using electric saw or hand saw cut the skull and remove brain (whole brain is removed in case of meningitis).

Used to Collect Vitreous

Gross examination of organs:

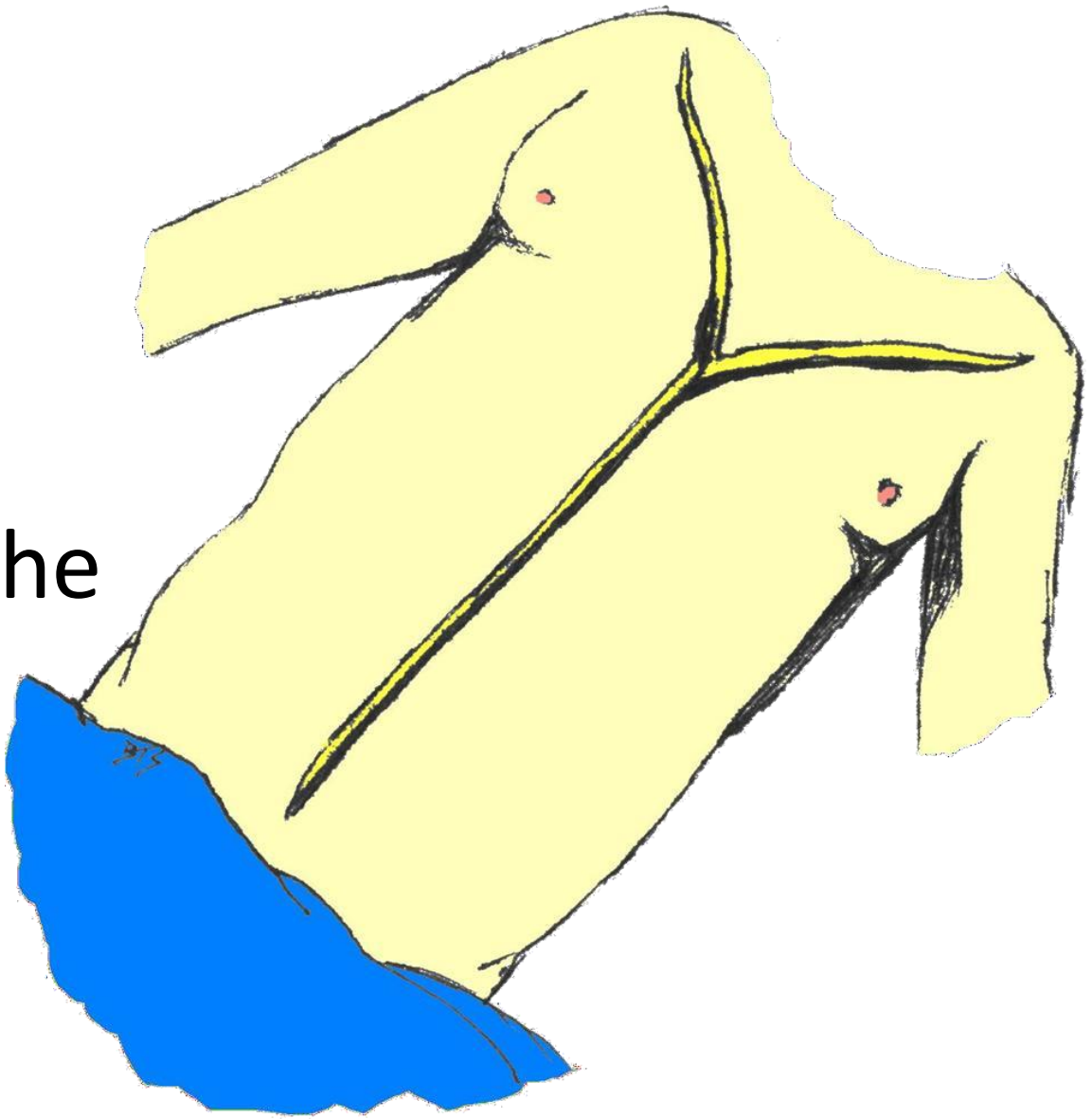
1. Examine neck structure with tongue to larynx.
2. Examine lungs by cutting horizontally section from apex to base.
3. Examine heart, chambers and weighted after removal.
4. Stomach is examine.
5. Examine adrenals and spleen are removed.
6. Examine kidneys by cutting from hilum.
7. Liver is examine after weighting with parallel incision.
8. Examine of brain is after fixation



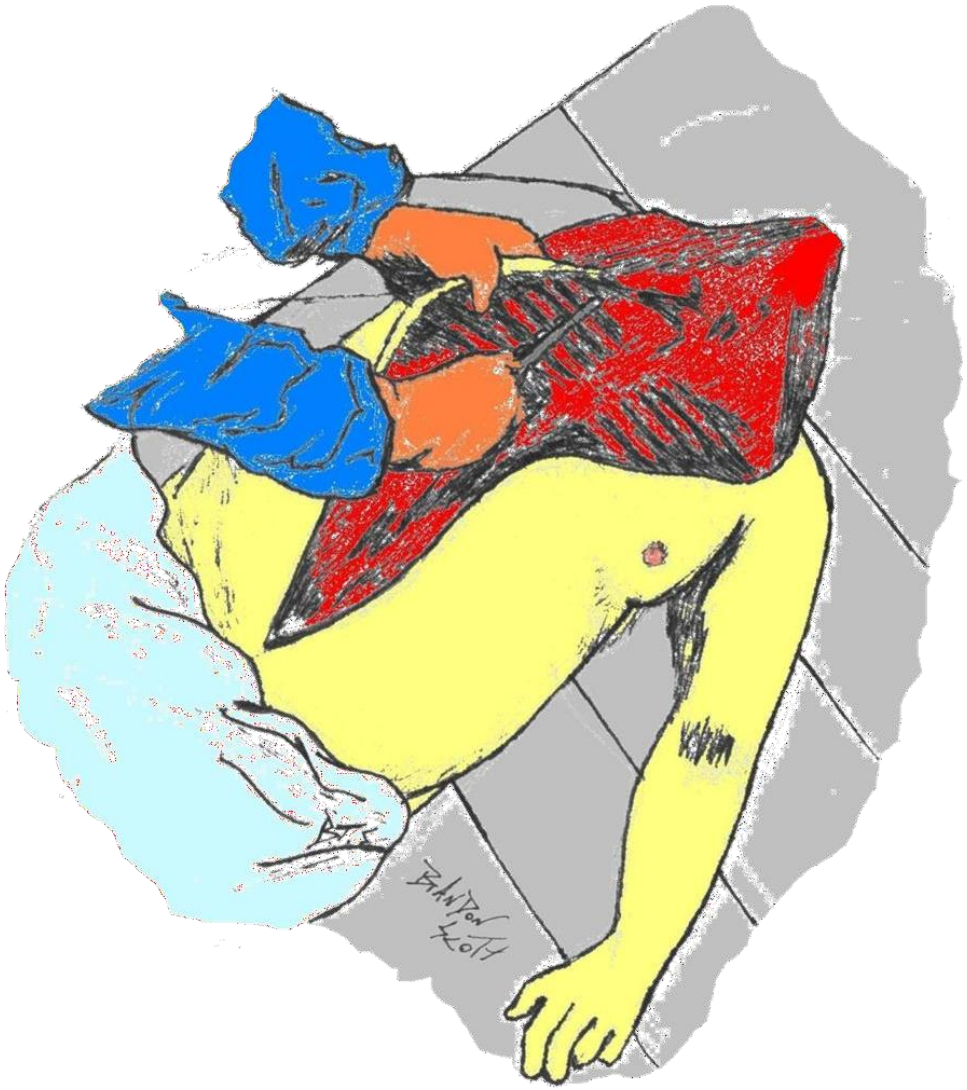
X-Ray

Trunk Dissection

- Y-shaped incision
- From the shoulders to the pelvic bone



Opening the Chest



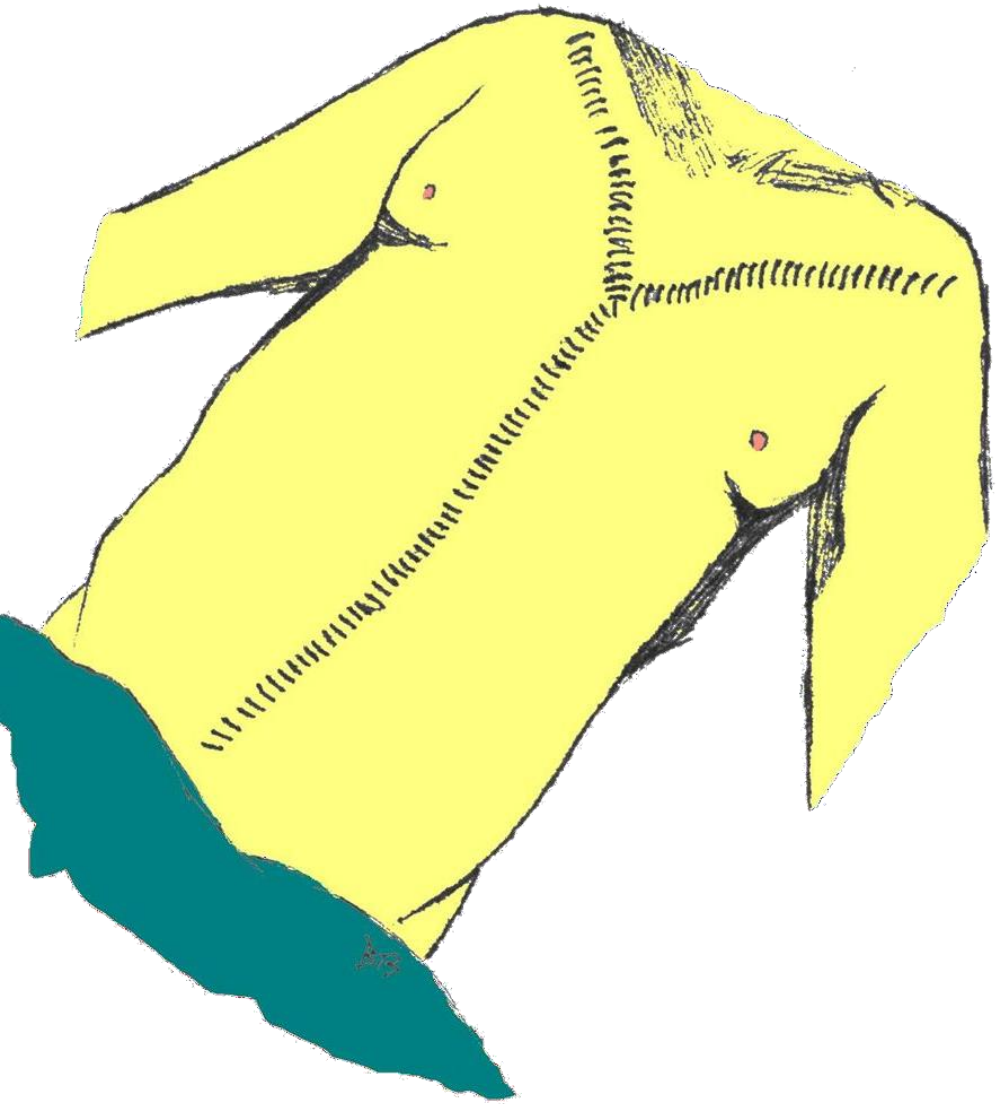
- Skin & muscle, are pulled from the chest wall
- Chest Plate is extracted
- Heart is extracted


URINE
BRAIN
KIDNEY
LIVER
STOMACH CONTENTS
BILE
CONTAMINATED

Samples

Organ Containers

Close Up



- Skull cap is replace
- Skin pulled back in place
- Body Organs may or may not be replaced
- Incisions are sown up with the use of a baseball stitch

Autopsy report:

- Based on external, internal, gross and histopathologic examination. In which following points are consists:

1. **Permission** for autopsy
2. **Complete data as** Name, age, sex, address, date and time of death, autopsy no, clinical history, radiological finding and operative procedure.
3. **Data pertaining to the pathologist** as prosector name, date and time of autopsy, organs morphology, and gross finding.

Autopsy report:

4. **Post mortem findings** as external, internal, microscopic.
5. **Final diagnosis** is based on pathological finding.
6. **cause of death** pathologist make summary and interpretation of causative sequence of various lesions.

Report Form: (microscopic description)

AUTOPSY PATHOLOGY

GOVERNMENT MEDICAL COLLEGE HOSPITAL
SECTOR-32, CHANDIGARH

DEPARTMENT OF PATHOLOGY

Referred by
Clinical diagnosis

HIV status

Name
Age/Sex
CR No.
Ward/OPD
Date Income

Autopsy No.....

MICROSCOPIC DESCRIPTION (ORGAN-WISE):

FINAL AUTOPSY DIAGNOSIS:

CAUSE OF DEATH:

Date of receiving.....
Date of reporting.....

PATHOLOGIST

Thank you

PAS Stain

Mr. Ashish Duhan
Lecturer in DMLT

PAS reactive cells and tissue components:

1. Glycogen
2. Starch
3. Mucin (neutral mucin)
4. Basement membrane
5. Fungi
6. Thyroid colloid

Requirements:

Reagents and stain:

1. Periodic acid solution

- Periodic acid 1g
- Distilled water 100 ml

2. Counter stain:

- Harris hematoxylin

2. Schiff reagent preparation:

- Dissolve 2 gm of basic fuchsin in boiled distilled water in which and cool at 50^{o} then add 2 gm of sodium metabisulfite and cool at room temperature then add 2.0 ml of hydrochloric acid (HCL). Shake well the solutions at intervals 10 times and add 2 gm of activated charcoal. Filter before use and store in dark.

Specimen:

- Cytological smear prepared from FN Aspirated material or body fluid for cytology

Principle:

- Periodic acid oxidize the carbohydrate substance to form reactive aldehydes. These aldehydes have the reactivity with schiff reagent to form a bright red or magenta color where as the nuclei of the cells are stained as blue.

Procedure:

1. Hydrate the smear through descending grade of alcohol.
2. Oxidize with periodic acid for 5 minutes.
3. Rinse in several changes of distilled water.
4. Cover the semear with Schiff reagent for 15 minutes.
5. Rinse in running tap water for 5-10 minutes.
6. Stain the nuclei with counter stain for 1 minute.
7. Dehydrate with ascending grade of alcohol and clear with xylene.
8. Mount in DPX.

Result:

- Glycogen and glycoproteins — Magenta
- Nuclei — Blue

ZN Stain

ZN STAIN: (for Mycobacterium sp.)

Corbel fuchsin:

Basic fuchsin	0.5 gm
Absolute alcohol	5 ml
5% aquous phenol	100 ml

Mix well and filter before use.

0.3% methyline blue:

- Methylene blue 1.4 gm in 100 ml 95% alcohol (stock sol)
- Methylene blue (stock sol) 10 ml in 90 ml tap water.

Decolorizing agent:

- **5% sulfuric acid** used for the **Mycobacterium leprosy**.
- And **20% sulfuric acid** used for **Mycobacterium tuberculosis**.

Specimen:

- Cytological smear prepared from FN Aspirated material or body fluid for cytology

Procedure:

1. Hydrate through ascending grade of alcohol to water with control section.
2. Treat section with hot corbel fuchsin for 3-4 minutes.
3. Wash in running tap water, till the excessive stain remove from slide.
4. Decolorize by 20% and 5% sulfuric acid for Mycobacterium tuberculosis and lepropsy respectively.
5. Rinse in water.
6. Counterstain by methylene blue for 1 minute.
7. Dehydrate, clear and mount.

Results:

Positive Control	shows pink bacilli
Negative control	no bacteria on blue background
Test slide	may or may not


ThankYou

Advances in Cytology

Mr. Ashish Duhan
Lecturer in DMLT

Automation in cytology:

- In the field of clinical cytology laboratory and in increasing number of diagnostic markers being discovered in human genome have been required automation in laboratory technique. Cytological and cytopathological tests are time consuming, and when performed in high numbers, are susceptible to human error. In recent years the development of automated microscopy has been refined. Automated technique in cytology laboratory are as follows:

1. Cytospin centrifugation
2. Automated microscopy
3. Flow-cytometery

Cytospin:

- Cytospin is the first device to use carefully controlled centrifugation for separation of deposit in a thin layer of cells on slides while maintaining cellular integrity. It produces better cell imprison and good representation of all cell types present in liquid samples.

Features of cytospin centrifuge:

- It processes 12 specimens at one time.
- Lid-release mechanism allows for one handed opening and closing of the outer lid for easy access.
- The lid remains locked at all times during rotation.
- Excellent safety design prevents accidental fluid spillage from damaging the mechanical or electronic components and allows for easy disinfecting.

Principle:

- Cytospin instrument works on centrifugal principles. Cytospin deposits cells onto a clearly-defined area of a glass slide and allows for the absorption of the residual fluid into the sample chamber's filter card. Cytocentrifugation also constructively flattens cells for excellent nuclear presentation. During operation, the instrument's spinning action tilts the Cytofunnels upright and centrifuges cells onto the deposition area of the slide, giving all cell types equal opportunity for presentation. In the Load or Stop position, this unique tilting feature reduces cell loss by preventing residual fluid from coming into contact with the prepared slide.

Importance of Cytospin:

- This technique is used for non-Gynecological Cytology, especially hypocellular fluids.
- This technique is used thin-layer technologies.
- Prepares both “air-dried” and “fixed” specimens.
- Cytocentrifugation effectively spins for micro-organisms onto the slide.
- Only a small sample volume is needed so multiple samples may be run for a variety of special staining and support testing.
- It also concentrates samples for Gram stains.
- Excellent technique for fluids like bone marrow and peripheral blood.
- Cytospin for the rapid detection of respiratory viruses, CMV Detection, ISH, FISH, etc.
- Excellent for molecular studies, specialized staining applications, and EM.