Date - 19/12/2013

Microtomy

For detail study of tissues under microscopes then section are necessary. For such purpose and object (i.e. an organ to be studied) is fixed, dehydrated and then embed in molten paraffin. The paraffin with object is made into a block and then the block is cut into sections of definite thickness by an instrument called microtome. Paraffin section containing tissue section are then fixed on glass slides for the purpose of staining and mounting. The entire operation is called microtechnique.

A. **Fixation**:- Any organ or tissue to be studied under microscope should be fixed properly. After killing the animal the organ to be fix remove quickly, carefully, subdevided, rinse in saline and placed in fixative. The object should be kept in the fixative for 24 hrs. (The usual fixative is Bouin's fluid.).

B. **Dehydration**:- Because of the aqueous fixative, before embedding in paraffin perfect dehydration is necessary. Dehydration is done through graded ethyl alcohol, passed through 30% → 50% → 70% → 90% → absolute alchohol, keeping 20-30 mins in each grade.

C. **Dealcoholization**:- Alcohol is not mixable with paraffin, so it must be removed. The object in absolute alcohol are placed in xylol and kept for 30 mins with 2-3 changes for the purpose. After dealcoholization the objects are left in clearing agent such as cedar-wood oil after removal of xylol. In this condition tissue object can be kept for sufficient long time.

D. **Infiltration**:- It is a replacement of xylol with paraffin. Paraffin with melting point 58°C - 60°C is best for cutting 6-10µ sections. In a paraffin oven set at 60°C, xylol saturated with paraffin is kept in a clean and dry porcelin cup. Tissue object kept in cedarwood oil are washed in xylol and then with the help of a forcep placed inside that porcelin cup containing xylol + paraffin mixture and placed inside the oven for 30 mins.

Then tissue blocks are processed through 2 changes of pure molten paraffin keeping 30 mins in the first phase and 1 hr in the 2nd phase.

E. **Embedding**:- Glass petridish or brass 'L' mould or paper boat are used for embedding. Inside the container then layer glycerine may be coated. Pure paraffin already molten in the oven is taken out to fill the container. Bottom layer of the paraffin in the container is allowed to solidify but upper part is kept molten with help of a hot plate.

With a pair of warm forceps the tissue blocks are taken out from the infiltration container and placed in the embedding container and placed in the definite orientation. Any air bubble is removed and the container is allowed to cool naturally or by adding cold water.

F. **Trimming**:- The paraffin block containing the tissue object is carefully trimmed with a sharp scalpe to give it a trapizium shape in transverse section. There should be enough paraffin on all slides of the tissue at least 3-4 mm at the base.

After trimming paraffin block is attached to a block holder is heated and the base just pressed on the holder. After cooling the two will attach to each other.

G. **Sectioning**:- After attachment of trimmed block to the holder it is moulted on a microtome in the front part. A sharp razor is fitted to razor holder and it is brought near to the block. Angle of the razor is adjusted to 45°, all parts of microtome checked, micron adjuster is set at whatever thickness of section is desired.

Now, rotation of driving wheel is started. As soon as the paraffin block makes contact with razor-section of paraffin started to cut. If every thing is perfect subsequent sections will adhere to the earlier ones and a continuous ribbon will be produced. The ribbon should be supported by a brass or forceps and any 5 inch

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ribbon should be removed from the razor. Ribbon should be kept on a clean paper or paper tray according to their serial. While cutting and removing the ribbons from the razor. No wind flow, even deep breadth is not advisable.

H. Storage of sections:- Long ribbon placed on the paper tray serially should be covered by proper lid and can be kept for stretching on the slides. Some problem in section cutting can be faced to attain straight ribbon. Their probable causes and remidies are as bellow.

Problem faced	Probable cause	Remidies
1. Successive sections do not adhere and a ribbon not formed.	(i) Opposite sides of the block is not parallel to each other. (ii) Block is too cold. (iii) Room temperature is too low.	(i) Retrimm the block. (ii) Block should be warm. (iii) A lighted spirit lamp should be kept near the razor.
2. Section curl into tube as they are cut, ribbon is not formed as a result.	(i) Section do tend to curl in the begining. (ii) knife angle inclined too much. (iii) Room temperature are too low for the paraffin used for embedding. (iv) knife is not sufficiently sharp.	(i) Straighten the section with the brush gently tracing it downwards and cut more section. The weight of the ribbon might prevent further curling and a ribbon may be formed. (ii) Reduce the knife angle. (iii) Increase the temperature of the working area with a lightened spirit lamp or a electric table lamp. (iv) Sharpen the edge of the knife.
3. The ribbon curve sharply to one side.	(i) Block not properly trimmed. Opposite sides not parallel and unequal in length. (ii) The edge of the knife is not uniformly sharp.	(i) Trimmed the block properly. (ii) Try another part of knife.
4. Ribbon splits longitudinally or shows scratches on the surface.	(i) Some dust particles or paraffin wax left on the knife edges. (ii) knife has nicks. (iii) Block contain some hard particle.	(i) Clean the knife edge with xylol using the index finger with upward strokes on both the faces. Never make a downward stroke while cleaning the knife. Apart from the danger of the finger being cut it may produce such rinks edge. (ii) Select another portion of the knife. (iii) Melt the block in an oven and re-embed it.
5. Section greatly compressed. Some compression is normal particularly in thin sections but excessive compression has to remedied.	(i) Room temperature too high. (ii) Low melting point paraffin used. (iii) knife inclined too forward.	(i) Cool the block by immersing for a shortwhile in a beaker containing ice water. (ii) Melt the block in an oven and re-embed it. Material for very thin section ($< 5\mu$) should be embedded in the high melting point wax. (iii) Reduce the angle slightly.
6. Section compressed or shattered or mashed. The face of the block appears white especially at the edge.	(i) The knife is not inclined sufficiently. The block is hitting the face of knife.	(i) Increase the angle of the knife.

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Problem Faced	Probable cause	Remedies
7. Sections not available and the block feels soft to touch.	(i) Methyl benzoide left in the tissue.	(i) Melt the block into oven. Passed through benzene before reembedding.
8. Alternate section thick and thin	(i) Something may be loose. (ii) knife not inclined sufficiently.	(i) Check and tighten all the screws. (ii) Reset the angle of knife.
9. Section adhere to the paraffin block on the upstroke instead to the edge of knife.	(i) knife edge may be dirty. (ii) knife angle is not enough. (iii) Static electricity being generated.	(i) Clean the knife edge. (ii) Slightly increase the angle of knife. (iii) keep a lighted spirit lamp near the razor or boiled water in an open container near the microtome.
10. Individual section shows thick and clear areas.	(i) knife may be out too far. (ii) knife angle excessive. (iii) One of the screws holding the knife or the paraffin block holder may be loose.	(i) Use more central part of knife. (ii) Set the knife angle correctly. (iii) Check and tightens the screws.
11. Tissues shatters or fall out of the paraffin block or has the chalky appearance.	(i) Infiltration of the tissue incomplete.	(i) Melt the block in the oven, take back to absolute alcohol via benzene and methyl benzoate. Dehydrate again in absolute 3 changes of absolute alcohol then reprocess and prepare the block.
12. Block shatters but not chalky in appearance.	(i) Material not suitable for paraffin embedding.	(i) Alternate method should be used.
13. Tissue falls out of the paraffin although it doesn't shattered and not chalky in appearance.	(i) Infiltration and embedding incomplete.	(i) Melt and re-embed it.

I. Affixing the tissue into glass slide:- Clean greese free glass slide need to be taken. A drop of affixing solution (Mayer's albumin) is put on the edge of the slide. With one finger the solution is spread uniformly on the slide. The slide is kept inclined with the affixive applied face downward. From the storage box of ribbon already cut a small piece about one inch length taken on the slide on the affixive applied side. Put a few drops of distilled water on the side to float the piece of ribbon but water should not flow off.

Next step is the spreading of section on the slide. It is done on the thermostatically controlled hot plate. Before placing the slide on the hot plate a few drops of water should be placed due to heat of the hot plate. Still any wrinkle present should be streached with the help of 2 needles. After complete spreading the piece of ribbon arranged on the slide and kept inclined against a raised object with the ribbon underside. During spreading over heating should be checked. With spreaded section the slide are labelled by glass marker and processed for staining.

Teacher's Signature

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Staining and Mounting of Histological Slides

Sections of tissues are usually stained with 2 dyes to bring contrast between different histological structures. This makes detailed study easier. Staining with 2 dyes is known as double staining. The most common double staining practised in class work is with haematoxylin and eosin. The haematoxylin being basic dye imparts blue colour to acidic materials, viz., nucleic acids, which are concentrated in the nucleus and only scattered in the cytoplasm. The eosin is an acid dye and the cytoplasmic materials being basic in nature are stained by it. The result is, the nucleus and only a small fraction of the cytoplasm appear blue, while the rest of the cells make red colour. After staining slides are mounted for making it ready to observe under microscope in future.

Technique:-

A. **Staining:-** (i) De-wax the section by immersing the slides in xylol for 10-15 minutes. Change to xylol again for 5 minutes.

(ii) Next the wax free slide is passed through different grade of alcohol as bellow—
- (a) Absolute alcohol - 5-10 mins.
- (b) 90% alcohol - 5 mins
- (c) 70% alcohol - 5 mins.
- (d) 50% alcohol - 5 mins.
- (e) 30% alcohol - 5 mins.
- (f) Distilled water - 5 mins.

(iii) Stain the slide in delafied haematoxylin for 1 minute. Wash the slide in distilled water. Examine undermicroscope. If overstained, destain it by 1% HCl solution. If understained, restain it.

(iv) Dehydrate the tissue through—
- (a) 30% alcohol - 5 mins
- (b) 50% alcohol - 5 mins.
- (c) 70% alcohol - 5 mins.
- (d) 90% alcohol - 5 mins.

(v) Counterstain the slide in eosin for 10-15 second. Remove excess stain in 90% alcohol and pass the slide to absolute alcohol.

(vi) After keeping the slide in absolute alcohol for 5 mins, quickly place it in xylol and leave it for 5 mins.

B. **Mounting:-** Canada balsam or DPX is used generally as mountant. One or two drops of mountant placed on the stained section. Take a clear cover slip and place it over the mountant to cover the sections. Precaution should be taken so that no air bubble be get trapped under the cover slip. Keep the slide on hot plate for drying the mountant over night.

The slide is now ready for microscopic examination.

Teacher's Signature

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