المحاضرات العملي

المادة: التحضيرات المجهرية microscopic technique

المحاضرة : الثالثة

المرحلة : الأولى. **الدراسة : الصباحية**

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Tissue Preparing

3. Washing:

The sample must be washed after fixation in order to remove the remaining effect of the fixative on the sample.

_ Samples fixed in Boun's fixative with 70% alcohol until the yellow color is gone.

_ The samples fixed in Zinger fixative are washed with alcohol 96% saturated with iodine, and the washing time ranges from 5-8 hours.

_Formalin-fixed samples were washed in running tap water for 24 hours.

4_Dehydration:

Dehydration is the removing of the water from the tissue by replacing it by dehydrating agents, like alcohol, propanol. The dehydration was manifested by the immersion of the tissue in ascending strength of alcohol. (usually from 5%). The length of time spends in each strength of alcohol and total time of dehydration depends on the tissue thickness and tissue density and fixative used.

Solutions used as dehydrant agents:-

1. Alcohol

2. Acetone: (dehydrate quickly).

3. Diaxone: it can use sheaf of a high strength at alcohol. Caused little shrinkage it is a poison material.

4. Tetranydrofuran:- like dioxane.

5_Clearing:

The process by which a substance is replaced by a dewatering substance, as this process allows a substance paraffin wax by entering the tissues in the next step because the alcohol used in dewatering does not mix with paraffin wax so a thinner is used that dissolves in alcohol and paraffin wax as well as makes the tissue transparent.

Examples of liquids (xylol - chloroform - toluene - benzene - cedarwood oil).

And when using xylol and toluene, the color of the softened solution sometimes becomes dark, which is evidence of incomplete dehydration of the sample tissues. In this case, the sample should be returned to the alcohol series to ensure that the sample is completely dehydrated. As for how long to leave the sample in the clarified solution, it depends on the type and size of the sample. The greater the sample size removal process, the longer the clarification period.

Alcohol does not mix with paraffin wax, so the xylol solution is considered one of the most suitable solutions for softening because it is easy to mix with paraffin and alcohol. There are substances that can be used as liquids such as toluene, benzene and chloroform, but they are volatile.

6_Infiltration or Impregnation:

It is a complete substitution of the material used in burial in place of the laid material. Paraffin wax is one of the most popular materials used in impregnating the tissue as it permeates the sample quickly without causing damage to its tissue structure. It also gives it a strong support to prepare it for microtome cutting, and helps to preserve it under normal conditions for a long time. without any harm..

The process is done by passing the sample in an equal mixture of wax and the thinner (1:1), then the sample is transferred to the molten absolute paraffin wax inside the oven, and this process is repeated several times (2-3 times) each time for half an hour, and the number of times the wax is changed also depends. According to the type of sample, so that it decreases whenever the sample is soft and increases whenever the sample is solid.

7_Embedding:

It is a process whose purpose is to make a mold from the sample that is surrounded and supported by the visible material. The materials used are of two types:

1_ Some of them dissolve in aqueous media at high temperatures, such as agar, gelatin, and carbonic wax

2_ Others do not dissolve in aqueous media, such as paraffin wax, celluloid, methacrylate, and ester wax.

8_Trimming:

After preparing the wax molds, it is advisable to trim them with a sharp blade so that the sample is in a position suitable for cutting, so that its edges become parallel and can fit on the edge of the microtome knife.