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PRINCIPLES OF MANUFACTURING MACROPREPARATIONS

There are many ways to preserve organs and tissues, but the most accessible and most commonly used method is that of Melnikov-Razvedenkov. After fixation in 5% or 10% formalin and subsequent washing with water, the macropreparation is mounted in a jar in the desired position. The following three solutions are used sequentially:

I.	Formalin 10%	100 mL
	Potash chloride	5 g
	Acetic acid potassium (Potassium acetate)	30 g
	Water	1000 mL
II.	Pure ethyl 96% rectified alcohol	600 mL
III.	Glycerin	600 mL
	Acetic acid potassium	400 g
	Water	1000 mL

The macropreparation is fixed in solution I until it stops releasing red blood, during which time the solution acquires a dirty brown color. The process takes from one to several days. The disadvantage of preservation in formalin is a color change of the tissues, since the hemoglobin in the blood is converted to methemoglobin, which is dirty brown, almost black color. To restore the natural color of the organs, a second solution (solution II) is used, which converts methemoglobin to stable cathemoglobin. Since the latter has the color of oxyhemoglobin, the preparations return close to their natural color. For permanent storage, preparations are placed in solution III, in which they are stored indefinitely and, in addition, remain suitable for microscopic examination (*Fig. 1*). The jars are not sealed hermetically, so that when the temperature rises, warm air in the vessel exits through small holes; when the temperature drops, cooler air enters the vessel in the same way. If the jar were to be sealed hermetically, when heated, not only could the lid peel off, but the jar itself could also burst.

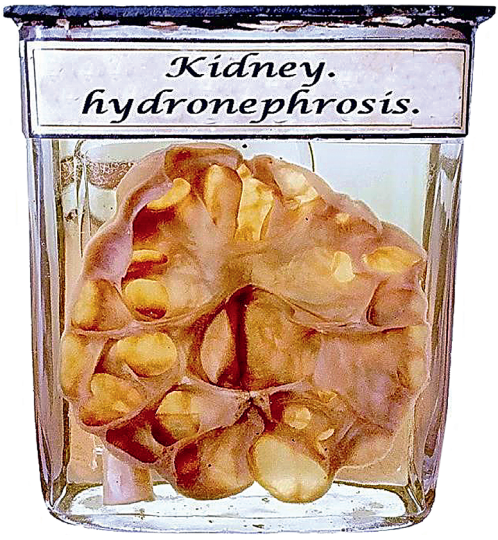

Fig. 1. Finished macropreparation

MICROSCOPIC STUDY

Fundamentals of histological technique

Stages of manufacturing histological micropreparations.

1. Fixation (10% formalin, 70% ethyl alcohol, acetone or others).
2. Flushing (running tap water).
3. Dehydration (a battery of alcohols of increasing concentration).
4. Removal of alcohol in an intermediate medium (chloroform, xylene, toluene).
5. Impregnation and filling (paraffin, celloidin, gelatin).
6. Making paraffin blocks with a tissue sample.
7. Preparation of paraffin sections using a microtome and placement on a glass slide.
8. Dewaxing (battery: xylene-alcohols-distilled water).
9. Staining.
10. Dehydration and clearing of sections (xylene or toluene).
11. Encapsulation in resins under a coverslip (Canada balsam, etc.).

Fixation, as the term itself indicates, preserves in the processed piece of organ the structure that it had during life. Fixatives are divided into two main groups: (1) fixing substances (*simple fixatives*) and (2) fixing mixtures (*complex fixatives*).

Examples of simple fixatives.

1. *Formalin* is the most common fixative, used as a 10–20% aqueous solution. Pieces are fixed in formalin for a period of 24 to 48 hours.
2. *Ethyl alcohol* (*ethanol*). The fixing action occurs due to displacement of water from the tissues, and coagulation of proteins. Ethyl alcohol provides quick fixation and is used in 70% concentration.
3. *Methyl alcohol* (*methanol*). Methanol is used in its absolute form, free of alcohol impurities, for fixing cytological smears. It is important to note that methanol, being a strong poison, requires compliance with the rules for use and storage provided for toxic substances of Group A.

Examples of complex fixatives.

1. *Zenker's fluid* (sublimate mixture). This is one of the best fixatives for the preparation of survey preparations and preparations for a number of special histological studies. It is also a good mordant for use in dyeing fabrics. Zenker's fluid consists of Müller's fluid (10 g sodium sulfite, 25 g potassium dichromate, 100 mL water); sublimate (mercuric chloride), 5 g; and glacial acetic acid, 5 mL. Fixation time ranges from 1 to 24 hours, depending on the thickness and properties of the object being fixed.
2. *Bouin solution*. This is the best and most versatile of fixatives. Relatively quick to penetrate into tissues, it causes a slight (2.5%) compression, and is used both for review micropreparations and for special staining methods. The fixing mixture consists of saturated solution of picric acid, 75 mL; 40% formalin (formaldehyde), 25 mL; and glacial acetic acid, 5 mL. The material being fixed remains in the mixture from 2 to 24 hours, depending on the size of the object.
3. *Carnoy's fluid*. This serves as a good fixative both for histological studies and for many histochemical techniques. The fixative mixture consists of absolute

(100%) ethyl alcohol, 60 mL; chloroform, 30 mL; and glacial acetic acid, 10 mL. Pieces up to 5 mm thick are fixed for 1–5 hours, depending on the thickness of the object.

Flushing. This procedure removes any excess fixative from the object under study. The method of washing depends on the method of fixation. In the vast majority of cases, tissue samples are washed under running tap water.

Dehydration. Compaction of the material by dehydration is carried out in alcohols of increasing concentration: 50, 60, 70, 80, and 96% (two portions) and 100% (absolute alcohol). This successive row of vessels with increasing concentrations of alcohol is called histological battery (*Fig. 2*).

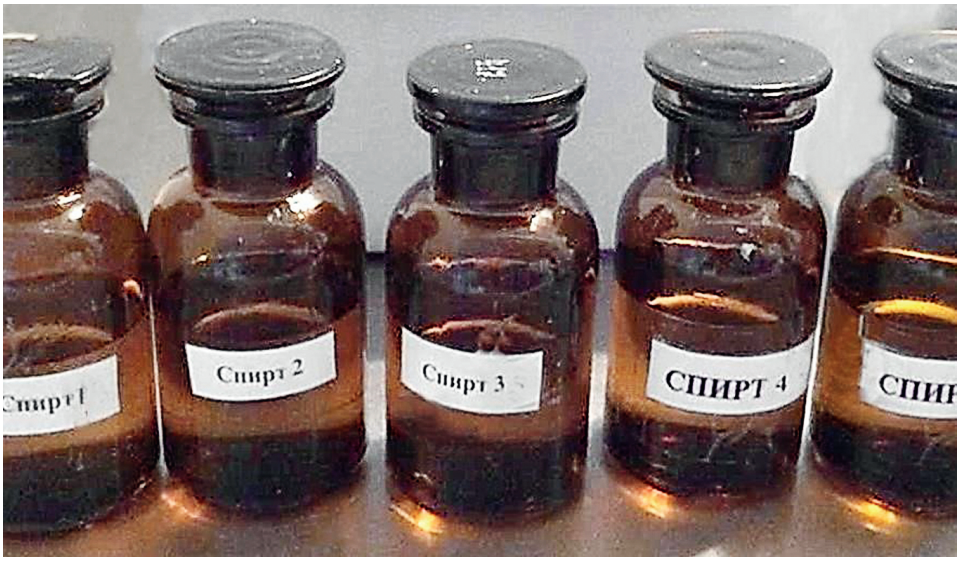

Fig. 2. Histological battery of alcohols

Removal of alcohol. To remove alcohol and prepare for impregnation with paraffin, the material is treated with one of several paraffin solvents, which displaces the alcohol and additionally compacts tissues. These solvents are chloroform, benzene, toluene, xylene, carbon disulfide, etc. These solvents are also referred to as intermediate media.

Impregnation. Paraffin is used for this purpose. However, before exposure to paraffin, the dehydrated tissue pieces are transferred to a mixture of absolute alcohol and chloroform (1:1) for 2–3 hours, and then to pure chloroform for 30 minutes. After that, to take advantage of the gradual replacement of chloroform by paraffin, the object is placed in a mixture of equal parts of chloroform and paraffin at 37 °C for 3–6 hours, followed by 30 minutes to 1 hour at 56 °C. To completely remove chloroform, this is carried out sequentially using 2–3 applications of molten paraffin. In modern histological laboratories, a machine with automatic wiring is used (*Fig. 3*).

Fill. After the final impregnation of the object, it is poured with molten paraffin (58–60 °C) into special molds, which are then cooled with water (+10–18 °C) or on a special cooling board.

Fig. 3. Carousel wiring machine

Making paraffin blocks. After being removed from the molds, the solid paraffin samples are attached to a wooden (preferably beech) or plastic base (block) (*Fig. 4*).

Preparation of paraffin sections using a microtome. A microtome is a special mechanical device designed to cut histological sections of a specific thickness. There are three types of microtomes: sledge, rotary (wheel), and freezing. The latter has a special device for freezing the object, allowing unfixed fresh material to be cut.

Fig. 4. Paraffin block with tissue sample on a wooden block

Sledge microtome (*Fig. 5*). This device is named so because the knife and the feed mechanism with a clamp for the block move on a special sled.

A rotary (*wheeled*) *microtome* is equipped with a fixed knife, relative to which the object moves (*Fig. 6*). On modern microtomes of this type, the slice thickness parameters are adjusted automatically.

The freezing microtome is designed, like the sledge model, according to the sled principle with the addition of a freezing table.