Serous Membrane Processing Technology Using Microwave Irradiation with Silver Impregnation

Andrey Bossin, Dina Rybalkina, and Vitaly Fomin

***Abstract*—**Investigated use of microwave processing on stages of histological technique in processing of serous membranes with impregnation in silver. The purpose was to reduce the time spent on fixing, degreasing, sensitization and impregnation. Microwave treatment of morphological material was carried out in a microwave oven (Samsung DE68-03673D). As a result of investigation was produced sugnificant decrease in time in 10 times faster for preparation of material. Morphological properties were evaluated at flat-field microscopy of non concluded slides and with morphodensitometry of relief forming structures with the program PhotoM1.21 and in comparison with the preparations made by the classical method of histological techniques. Acceleration of processing of morphologic material at different stages of histologic technique have huge significance in medical diagnostic.

***Keywords*—**histologic technique, impregnation in silver, microwave acceleration of process, microwave radiation.

I. Introduction

At the study of serous membranes, particularly in study of relief forming structures in normal and pathological processes in the histological technique used impregnation with silver. Stages of current histological methods require too much time. It is known that microwave field applied to processing morphology of substance in order to reduce time waste and increase quality, in particular for the presentation of material in the electron microscopy, decalcification, in staining with hematoxilin and eosin and acetoarcein, in immunocytochemistry, etc [1-7]. In known to us sources the use of microwave in silver impregnation has not been investigated.

The purpose and objectives - obtain optimal microwave parameters in processing of serous membranes with silver impregnation in stages of fixation, deffating, sensitization and impregnation of morphologic material.

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II. Materials and methods

To collect material from different parts of visceral and parietal peritoneal and pleural layers in autopsy. Part of separated membranes processed by modification of classical method by Minigazimov R.S and his colleagues in compliance with the time on his gradual processing [8]. It included desquamation of epithelial cells in 30% aqueous spirit solution (6 hrs), fixation in 10% formalin (10 days), deffating in 96% aqueous spirit solution (30 min), sensitization in 10% silver nitrate solution(10 min), impregnation by Gomori in ammoniac solution of silver nitrate(30 min). Each step interspersed by washing of material in tap water (total time 6 hours 30 minutes) , in several portions of distilled water (total time 40 minutes). Background silver removed in the wash solution of 10 ml of ammonia, 40 ml alcohol, 50 ml of distilled water and 0.5 g of detergent (powder "Lotus" without brighteners and dyes) for 30 minutes. The amount of solution for handling each step of separated membrane was 30 ml, the solution was in plastic cups, material movement held with plastic tweezers.

Another part of biological membranes were processed using a microwave oven (Samsung DE68-03673D) with a power of 100-180 W from 1-2 to 3-5 minutes at each step under the control of flat-field microscopy (lenses 10 and 40, the eyepiece 10) and serial photographing of results with digital PC micro eyelid (Bresser 5913500). He micro photos were studied by method of computed morpho-densitometry using the program PhotoM1.21 after prior calibration. In each slice was determined the thickness of the collagen fibers and trabeculae, and their optical density. Analysis of the data was carried out by methods of variational statistics (t-test).

III. Results, discussion

The duration of each step in the processing of material in a microwave field is the reduced by 10 times compared to the classic protocol, and was 1-2 minutes. While reducing the processing time does not degrade the quality of the material. It is confirmed by the results of other methods of histological research techniques of several authors [1-7].

The first stage in which was used microwave irradiation-rinsing after 10 days of fixation in formalin. According to protocol duration of washing in flowing water is 6 hours. If to

consider the high solubility of formaldehyde in water, fairly long washing may seem unreasonable, but its necessity is confirmed experimentally by the authors. The low speed of removal of formaldehyde from the slide is due to its chemical properties. When stored in an aqueous solution formaldehyde is polymerized to form paraformaldehyde HO-(CH2O) n-H. The same process occurs on the surface of biologic preparations. With increasing degree of polymerization of paraformaldehyde its solubility in water is reduced , it precipitates. The solubility of paraformaldehyde in fats with rising of molecular weight passes through a minimum (Table I).

TABLE I
CALCULATED DISTRIBUTION COEFFICIENTS OF FORMALDEHYDE AND ITS POLYMERS IN THE SYSTEM N-OCTANOL/WATER

n	**1**	**2**	**3**	**4**	
LogPO/W	-0,686	-2,062	-2,6	-3,138	
n	**5**	**6**	**8**	**12**	**24**
LogPO/W	-0,031	0,089	0,33	0,813	2,26

Note: in the calculation of distribution coefficients in octanol-water system used software package ChemOffice 2012.

Since n is typically in the range 8-100, we believe that paraformaldehyde firmly retained by fatty inclusions of biological tissues and is removed by washing with water to form formaldehyde, which is formed as a result of depolymerization. It should also be noted that paraformaldehyde may be covalently bonded to the functional groups of proteins and polysaccharides of biological tissue. In this case, its removal by water will occur slower. The role of microwave irradiation at the stage of washing of preparation after fixation in formalin is likely acceleration of depolymerization of paraformaldehyde. Since in paraformaldehyde all of the C-O-C bonds are equivalent, there is possibility the formation of fragments of different lengths, including that having a high hydrophilic moiety with n = 2-4, which are easily removed by water. The chemistry of the preparation process of sensitization of serous membrane with silver nitrate solution is to form the donor-acceptor bonds and other silver ion bonds with functional groups on the biological tissue surface. Electron donating hydroxyl and amino groups of amino acids, glycosides and amino glycoside are capable to form a complex (see Fig. One).

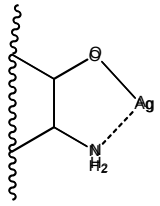

Fig. 1 Silver ion bonds

In the solution thus accumulates free nitric acid which must bind ammonium and other basic substances of biological origin. Effect of microwave irradiation at this stage accelerates the chemical binding of silver ions, with this even at stage of sensitization the preparation acquiring a significant differentiated coloring. During the process there is sensitization of type IV collagen present in the basal membrane. Processing of thus-prepared preparation by ammoniac silver nitrate solution is reduced to chemical adsorption on the surface of the tissue. Definitely can not be excluded processes of complex formation and salt formation, similar to those described for step of sensitization. Impregnated collagen of I, III, V, VI and VIII types. During assembly of fibrils between tropocollagen molecules remain gaps width of 35 nm, and each molecule in the chain is shifted relative to their connections in the neighboring chain molecules by a quarter of its length (Fig. 2), which makes it possible to virtually free penetration cations of diamminesilver Microwave field accelerates the chemisorption of silver on the surface of fibrils, activating ligand exchange processes in the complex. In turn, collagen fiber formed from many parallel connected glycoproteins of collagen fibrils and bundles of collagenous fibers - is immersed in the collection of the extracellular matrix of collagen fibers.

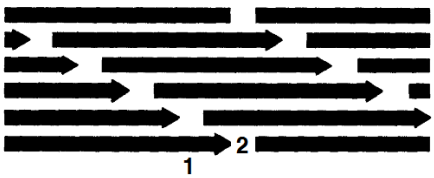

Fig. 2 Packaging of polarized tropocollagen molecules (1) during the formation of collagen fiber with gaps (2). [from Johnson K.E., 1991]

Subsequent rinsing in distilled water is used to remove any excess silver. Elimination of this step results in a dark-colored, little contrast slides that are not suitable for visual observation. Step of "manifestations"- Thirty second treatment in 1% formaldehyde .The chemistry of this stage, is analogous with the processes of "silver photography." Silver is restored by to metal by formaldehyde. Since the process occurs faster on the surface of silver grains, their growth occurs. In bulk of solution and on tissues which do not contain sensitizing silver atoms hardly occurs restoration. Formaldehyde is oxidized to formic acid (1):

$$[Ag(NH_3)_2]NO_3 + CH_2O + H_2O \xrightarrow{Ag} Ag + NH_4NO_3 + NH_4COO \quad (1)$$

Through this process increase the intensity of staining of the target tissues (Fig. 3), whereas the surrounding cells are not noticeably stained and the contrast increased.

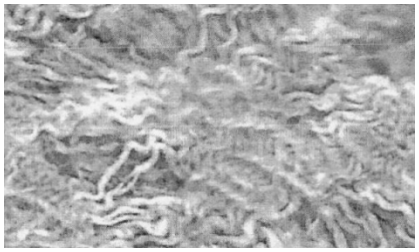

Fig. 3 Relief-forming structure of the peritoneum (x200)

Ammonia water flushing of the preparation aims to decrease the restoration activity of formaldehyde and deletion of non restored silver which are capable for further reduction of slide contrast during the storage.

The next stage is the removal of metallic silver formed in minor amounts on "non-target" tissues.

The main active ingredients of lightening solution are ammonia part of the detergent bleach (usually peroxide nature) or oxygen in the air.

Included in the composition surfactants promote the subsequent washing. Visually by microscopy of wet not concluded under the cover glass film preparations under all material handling methods prevailed collagen fibers, which were synchronized with respect to the amplitude and wavelength. During the processing of material in a microwave field of 180 W and 100 watts over 2 minutes there was a substantial increase in the temperature of the treatment solution and the compaction of membranes with the appearance of surface roughness. Significant differences between the mean thickness of the fibers, their optical density when compared with the traditional method of processing and treatment in a microwave field at 100 W for 1-2 minutes have not been identified. At the microwave field at 180 W and the increase residence time of material in a microwave oven for more than 2 minutes observed decrease in the thickness of fibers, vacuole formation, and the deformation of waves of collagen fibers, indicating the need for optimal microwave activation parameters of chemical reactions.

IV. Conclusions

In that way processing of serous membranes with use of microwave field of 100 W during 1-2 min at each step of fixation, degreasing, sensitization, silver impregnation and mid step washing of material significantly reduces the time of the standard-classical method of processing of the material without altering its morphological properties.

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