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LIQUID FOR TREATING TISSUE IN HISTOLOGIC PROCESSING

Andres Ferrari, Jr., Uniondale, N. Y., assignor, by mesne assignments, to Technicon Chemical Company, Inc., New York, N. Y., a corporation of New York

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This invention relates to the preparation of histological tissue specimens for microscopic examination.

The preparation of tissue to enable the microscopic examination thereof involves a plurality of treatments of the tissue prior to the cutting of the sections from the tissue specimens for the staining and mounting of the sections on the microscope slides. More particularly, in the preparation of the tissue it is necessary to immerse the tissue successively in a plurality of liquid agents for certain lengths of time, first to fix the tissue, then to wash the same for removing the fixative, then to dehydrate the tissue, by immersion of the tissue successively in a plurality of dehydration agents, then to immerse the tissue in a clearing agent, and thereafter to infiltrate the tissue with an infiltration agent such as, for example, paraffin, celloidin, etc. After the tissue is thus treated, it is cut into sections of the desired thickness; then the paraffin or other infiltration medium is removed from said sections, usually by a solvent for the paraffin, after which the sections are stained while mounted on the slides.

The present invention is concerned with the dehydration of the tissue after it is fixed, and the primary object in this respect is to obviate certain disadvantages associated with the prior methods of dehydration and substances heretofore employed for dehydrating tissue.

This invention is also concerned with the treatment of the tissue in respect to the removal of paraffin or other infiltrating agent from the sections and in respect to the staining of the deparaffined sections or slides, as they are usually called, and the primary object of the invention in these respects is to obviate various disadvantages associated with prior methods and substances heretofore used in removing paraffin from the tissue sections and in the course of the staining treatment.

Alcohol, ethyl or methyl, has been the time honored solvent and dehydrant in histological technique. It has been employed singly and in combination with various other reagents, such as acetone, chloroform and various hydrocarbons. In no instance has its employment been entirely satisfactory. Physically, it is highly inflammable, extremely volatile, and it possesses a degree of hygroscopic action which causes it to dilute itself. As a dehydrant and in terms of its effects on the tissues, it has always caused an undue amount of shrinkage and distortion and hardening of the tissues should they be exposed to it for too long a time. In staining, its defects are those outlined under physical characteristics but with the addition that in many cases it detracts the dye which has become attached to the tissues, i. e., it serves as a de-staining or differentiating medium.

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In dehydrating tissue it was the practice, in an attempt to lessen shrinkage and distortion of the tissue, to immerse the tissue first in diluted alcohol and then to immerse the tissue successively in alcohol of progressively increased strength, the final immersion being in pure or absolute alcohol. Notwithstanding these precautions, however, shrinkage and distortion of the tissue are not prevented to the extent necessary for desirable results in the preparation of the slides.

The above mentioned and other objections and disadvantages attending the use of the alcohols and other reagents which have been used as dehydrants and solvents in histological technique are obviated by the present invention pursuant to the primary objects thereof.

More specifically, my invention has for its purpose to provide a dehydrant and solvent which (1) eliminates or greatly minimizes distortion of tissue, (2) does not readily evaporate, (3) is not appreciably hygroscopic, (4) is non-inflammable, and (5) is non-toxic. The non-toxicity of the dehydrant and solvent of the present invention is an additional advantage which obviates the objections and disadvantages involved in the use of such substances as methyl alcohol and dioxane in the treatment of tissue.

The liquid agent utilized in accordance with my present invention for dehydrating the gross tissue specimens and also in the treatment of the sections on the slides comprises diethylene glycol monoethyl ether acetate

$$CH_3COOCH_2CH_2OCH_2CH_2OC_2H_5$$

hereinafter referred for convenience as Solvent F. It is a known and commercially available product. It has a specific gravity of 1.0114, a boiling point of 217.7° C., and is completely soluble in water. It is non-inflammable, substantially non-hygroscopic and non-toxic. Because of its complete miscibility with water, it dehydrates the tissue by displacing the water therein, as contrasted with the water-extraction action of alcohol, and therefore does not shrink and distort the tissue as when alcohol is used as the dehydrant.

When used for dehydration of tissue, the solvent includes a substance which promotes the

penetration of the dehydrating liquid into the tissue, such substance being preferably isopropanol, although the other alcohols (methyl or ethyl) may be used, isopropanol being preferred because it is less hygroscopic than said other alcohols. The relative proportions of Solvent F and the isopropanol or other diluent may vary within relatively wide limits, for example but without limitation from 2 parts of isopropanol to 6 parts of Solvent F to equal parts of isopropanol and Solvent F. The presently preferred composition according to my invention consists of 6 parts of Solvent F and 4 parts of isopropanol. For convenience in reference said composition will hereinafter be referred to as Solvent FS.

In using the above described liquid agent, Solbent FS, for dehydration of gross tissue specimens after the fixation and washing thereof, said specimens are immersed in said solvent successively in a series of separate beakers or containers provided with the solvent, so that the tissue is repeatedly immersed in fresh solvent during the dehydration process. The preferred period of immersion in each beaker of Solvent FS is about one hour, there being preferably five of such immersions of one hour each in immediate succession. After the fifth period, the specimens are immersed in a liquid consisting of equal parts of Solvent FS and butyl acetate, said immersion being preferably in two separate containers and the period of immersion being one hour in each container in immediate succession. Following the second immersion period last mentioned, the tissue is immersed for one hour in a beaker containing butyl acetate only. Then the tissue is immersed in paraffin to enable the tissue to be cut into sections for mounting them on slides in accordance with known practice. These successive immersions are preferably accomplished automatically by utilizing an automatic immersion apparatus such as that described in the U. S. patent to Edwin C. Weiskopf No. 2,341,198.

The treatment of the tissue with butyl acetate, first in the liquid containing Solvent FS and then in butyl acetate alone, is for the purpose of clearing the tissue, i. e., removing Solvent FS therefrom so that it may be infiltrated with paraffin, as paraffin is more readily miscible with butyl acetate than with Solvent FS and hence can more readily displace butyl acetate. Also, I have found that the clearing action of butyl acetate is improved if it is first added to Solvent FS in the last two immersions, as described above. Butyl acetate is preferred as the clearing agent because of its excellent clearing action and also because the tissue is not hardened thereby.

After the gross sections are dehydrated and infiltrated with paraffin, the tissue is cut into thin sections, a microtome being usually used for this purpose, and said thin sections are mounted on glass slides. Then these mounted sections called "slides" are treated to remove the paraffin therefrom and then are stained. The paraffin is ordinarily removed by immersing the slides in xylene and the staining is usually accomplished by hematoxylin and eosin stains for the basofilic and acidofilic materials respectively, of the tissue. These stains are preferably those specifically described in my co-pending application Ser. No. 58,161.

The process of removing the paraffin and staining the slides is desirably accomplished by using the automatic immersion apparatus referred to above. This process starts with the slides having the paraffin therein. The beakers are arranged in the positions designated in the following table and contain the liquid specified, and the slides are immersed in said liquids for the indicated periods of time, in minutes, as follows:

Position on Machine	Tissue Treating Agent	Time
1	Xylene	2 minutes.
2	0.25% Iodine in Solvent FS	Do.
3	Solvent FS	Do.
4	Distilled Water	Do.
5	Hematoxylin	10 minutes.
6	Distilled water	4 minutes.
7	Lithium Carbonate 0.01 Normal	2 minutes.
8	Distilled Water	Do.
9	Eosin solution	5 minutes.
10	Solvent FS	Do.
11	do	Do.
12	do	Do.

After treatment in position 12, the slides are removed and placed in a beaker of xylene for about 3 to 5 minutes, when the slides should be completed in the usual way.

In the above table, iodine is specified as included in the beaker of Solvent FS at position 2. It may be noted, therefore, that the use of iodine is preferred when the fixative employed is of the Zenkers type.

When used for removing paraffin from the sections and in the course of the staining process, the isopropanol or other alcohol may be omitted, and in such case the liquid agent would consist of Solvent F, namely diethylene glycol monoethyl ether acetate, which would be provided in the beakers instead of Solvent FS in the positions specified in the above table.

This application is a continuation in part of my application Ser. No. 6822, filed February 6, 1948, now abandoned.

Various changes may be made in the invention, in view of the above disclosure, without departing from the underlying idea or principles of the invention within the scope of the appended claims.

Having thus described my invention, what I claim and desire to secure by Letters Patent, is:

1. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid dehydrating agent comprising as the essential ingredients thereof a major proportion of diethylene glycol monoethyl ether acetate and a minor proportion of isopropanol.

2. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid dehydrating agent comprising diethylene glycol monoethyl ether acetate and isopropanol as the essential ingredients thereof in the relative proportions of from about two to six parts of isopropanol to about six parts of diethylene glycol monoethyl ether acetate.

3. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid dehydrating agent comprising diethylene glycol monoethyl ether acetate and isopropanol as the essential ingredients thereof in the relative proportions of about four parts of isopropanol to about six parts of diethylene glycol monoethyl ether acetate.

4. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid de-

hydrating agent comprising as the essential ingredients thereof a major proportion of diethylene glycol monoethyl ether acetate and a minor proportion of an alcohol selected from the group consisting of ethyl alcohol, methyl alcohol and isopropanol.

5. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid dehydrating agent comprising diethylene glycol monoethyl ether acetate and an alcohol selected from the group consisting of ethyl alcohol, methyl alcohol and isopropanol in the relative proportions of from about two to six parts of alcohol to about six parts of diethylene glycol monoethyl ether acetate.

6. A liquid for treating histological tissue in the processing thereof for microscopic examination, consisting essentially of diethylene glycol monoethyl ether acetate and isopropanol, the amount of the diethylene glycol monoethyl ether acetate being substantially in excess of the amount of isopropanol.

7. A liquid for treating histological tissue in the processing thereof for microscopic examination consisting essentially of diethylene glycol monoethyl ether acetate and isopropanol in the relative proportions of from about two to six parts of isopropanol to about six parts of diethylene glycol monoethyl ether acetate.

8. A liquid for treating histological tissue in the processing thereof for microscopic examination consisting essentially of diethylene glycol monoethyl ether acetate and isopropanol in the relative proportions of about four parts of isopropanol to about six parts of diethylene glycol monoethyl ether acetate.

9. A liquid for treating histological tissue in the processing thereof for microscopic examination consisting essentially of diethylene glycol monoethyl ether acetate and an alcohol selected from the group consisting of ethyl alcohol, methyl alcohol and isopropanol, the amount of diethylene glycol monoethyl ether acetate being substantially in excess of the amount of said alcohol.

10. A liquid for treating histological tissue in the processing thereof for microscopic examination, consisting essentially of diethylene glycol monoethyl ether acetate and an alcohol selected from the group consisting of ethyl alcohol, methyl alcohol and isopropanol in the relative proportions of from about two to six parts of alcohol to about six parts of diethylene glycol monoethyl ether acetate.

11. The method of treating histological tissue to dehydrate the same after fixation thereof, comprising immersing the tissue in a liquid dehydrating agent comprising diethylene glycol monoethyl ether acetate as the essential ingredient thereof.

12. The method of treating histological tissue in the processing thereof for microscopic examination, comprising dehydrating the tissue after fixation by immersing the fixed tissue in a liquid comprising as the essential ingredients thereof a major proportion of diethylene glycol monoethyl ether acetate and a minor proportion of isopropanol, infiltrating the dehydrated tissue with an infiltrating agent removable by an aromatic hydrocarbon solvent, cutting the infiltrated tissue into sections for slides, removing said infiltrating agent from said sections by immersing them in said solvent, and removing said solvent from said sections by immersing them in said first mentioned liquid.

13. The method of treating histological tissue in the processing thereof for microscopic examination, comprising dehydrating the tissue after fixation by immersing the fixed tissue in a liquid comprising as the essential ingredients thereof a major proportion of diethylene glycol monoethyl ether acetate and a minor proportion of isopropanol, infiltrating the dehydrated tissue with an infiltrating agent removable by an aromatic hydrocarbon solvent, cutting the infiltrated tissue into sections for slides, removing said infiltrating agent from said sections by immersing them in said solvent, and removing said solvent from said sections by immersing them in said first mentioned liquid, thereafter staining said sections, and removing excess stain therefrom by applying said first mentioned liquid thereto.

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