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CLEARING AGENTS FOR HISTOLOGICAL
TISSUE

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This invention relates to the preparation of histological tissue specimens for microscope examination, and more particularly to clearing agents employed in treating the tissue in the course of such preparation.

The preparation of tissue to enable the microscopic examination thereof involves a series 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 series 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, usually by immersion of the tissue successively in alcohols or other 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. Such preparation of the tissue has been carried out in automatic immersion apparatus sold under the trade-mark "Auto-technicon" and disclosed in United States Patents 2,157,875 and 2,341,198. 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, after which the sections are stained and mounted on the slides.

As pathologists are well aware, the purpose of treating tissue with a clearing agent before infiltrating the tissue with paraffin is to eliminate from the tissue all liquid which, if not removed, would decrease the refractive index of the tissue and would interfere with the thorough infiltration of the paraffin into the tissue resulting in poorly cut tissue sections. Among pathologists it is recognized that the higher the refractive index, i. e., the transparency, of the tissue the more easily and accurately it can be examined microscopically. It is of the utmost importance, therefore, that a tissue-clearing agent be capable of removing all such liquid from the tissue and have a high degree of solvency for, or miscibility with, paraffin so as to facilitate the infiltration of the paraffin into the tissue. It is also very important that the clearing agent be of such character that it does not harden the tissue, does not impair the cytological structures of the tissue, is volatile at temperatures of 120° to 135° F., and is substantially non-inflammable under the conditions of use. Substantial volatility at temperatures about 120° to 135° F. is important from the standpoint of the efficient operation of the automatic immersion apparatus. The use of a clearing agent which is non-volatile at temperatures about 120° to 135°

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F. results in the accumulation of clearing agent in the paraffin bath in which the tissue is immersed after treatment with the clearing agent solution. Such accumulation detrimentally affects the homogeneity of the paraffin bath which in turn results in unsatisfactory infiltration of the tissue with the paraffin. The use of a clearing agent which is substantially non-inflammable under the conditions of use is important from the standpoint of eliminating a fire hazard.

The primary object of the present invention is to provide a clearing agent which has the following properties:

1. It has a high solvent power for liquid present in the tissue.
2. It does not harden the tissue and does not impair the cytological structure thereof.
3. It has a high solvent power for or miscibility with paraffin and thus facilitates the infiltration of the paraffin into the tissue.
4. It does not decrease the refractive index of the tissue.
5. It is substantially non-inflammable under the conditions of use.
6. It has substantial volatility at temperatures about 120° to 135° F.

Other objects and advantages of this invention will be apparent from the following detailed description thereof.

In accordance with this invention a clearing agent is provided consisting essentially of a chlorinated naphthalene, liquid at atmospheric temperature (25° C.), a chlorinated aliphatic hydrocarbon, preferably a chlorinated aliphatic hydrocarbon having from 2 to 4 carbon atoms, liquid at atmospheric temperature, and carbon tetrachloride. These constituents are mixed in any desired order at room temperature in the proportions of 10% to 40%, preferably 20% to 30%, liquid chlorinated naphthalene, 20%–80%, preferably 60%–40%, liquid chlorinated aliphatic hydrocarbon and 10% to 40%, preferably 20% to 30% carbon tetrachloride. All proportions in this specification are by volume.

The chlorinated naphthalene used is an oil, liquid at atmospheric temperature, having a white to pale straw color, a specific gravity at 77° F. of 1.19 to 1.25, a viscosity (Saybolt Universal seconds at 77° F.) of 33 to 37, a distillation range (ASTM °F.) 480 to 590, a flash point (ASTM Cleveland open cup °F.) 203, and a fire point (ASTM Cleveland open cup °F.) 338. The preferred chlorinated naphthalene is that sold under the trade name "Hallowax 1000" by the Hallowax Products Division of Union Carbide and Carbon Corporation.

The chlorinated aliphatic hydrocarbon used

is ethylene dichloride, ethylene trichloride, trichloropropane or other chlorinated aliphatic hydrocarbon having good solvent properties for fats and waxes and compatible with liquid chlorinated naphthalene. Ethylene dichloride is preferred because of its ready availability, its high solvent power for paraffin and liquids present in the tissue and its excellent compatibility with liquid chlorinated naphthalene. The use of ethylene dichloride in the proportions indicated results in an efficient clearing agent of reduced cost.

The carbon tetrachloride raises the flash point of the mixture and this without detrimentally affecting the solvent properties of the liquid chlorinated naphthalene and chlorinated aliphatic hydrocarbon for paraffin or for the liquids present in the tissue.

The liquid chlorinated aliphatic hydrocarbon when mixed with the liquid chlorinated naphthalene results in a mixture having a lower viscosity than that of the chlorinated naphthalene. Hence, the mixture penetrates the tissue much more readily than would the chlorinated naphthalene alone. Further, the mixture has exceptionally high solvent power for the liquids present in the tissue and for paraffin which is due chiefly to the chlorinated naphthalene component.

A preferred example of the clearing agent embodying this invention is given below. It will be understood this invention is not limited to this example.

	Percent
Ethylene dichloride	60
Carbon tetrachloride	20
Liquid chlorinated naphthalene (Halowax 1000)	20

This clearing agent when subjected to a flash point test (ASTM Cleveland open cup) gave off sufficient chlorine at 80° C. to extinguish the flame. It has a distillation range as follows:

Initial boiling point	77° C.
50% off	81.5° C.
Dry point	297.0° C.

Its refractive index at 25° C. is 1.4886 and its specific gravity 20/20° C. is 1.319.

It will be understood, that in using the clearing agent of the present invention, the tissue is immersed therein after the dehydration treatment and before the paraffin infiltration process. The clearing agent of this invention involving the three constituents above enumerated has been found to combine to a surprising and exceptional extent the six properties hereinabove enumerated and has been found to be an exceptionally efficient clearing agent. It has a pleasant odor. Further, it is a materially better solvent for paraffin than butyl acetate which has heretofore been used as a clearing agent. In these respects it is far superior to butyl acetate.

Tissue blocks comprising tissue which has been treated with the clearing agent of this invention have greatly improved cutting qualities, the cytological structures of the tissues are effectively preserved, and the appearance of the tissue sections is considerably improved.

It will be understood that various changes may be made in the clearing agent of the present invention within the skill of the art, without departing from the underlying idea or principles of my 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. A clearing agent for use in the preparation of histological tissue for microscopic examination, consisting essentially of from 10% to 40% by volume of a chlorinated naphthalene liquid at atmospheric temperature, having a specific gravity at 77° F. of 1.19 to 1.25, a viscosity (Saybolt Universal seconds at 77° F.) of 33 to 37 and a distillation range (ASTM) of 480° F. to 590° F., from 10% to 40% by volume of carbon tetrachloride and from 80% to 20% by volume of a chlorinated aliphatic hydrocarbon having from 2 to 4 carbon atoms and liquid at atmospheric temperature.

2. A clearing agent for use in the preparation of histological tissue for microscopic examination, consisting essentially of from 10% to 40% by volume of a chlorinated naphthalene liquid at atmospheric temperature, from 10% to 40% by volume of carbon tetrachloride and from 80% to 20% by volume of a chlorinated aliphatic hydrocarbon having from two to four carbon atoms and liquid at atmospheric temperature.

3. A clearing agent for use in the preparation of histological tissue for microscopic examination, consisting essentially of from 20% to 30% by volume of a chlorinated naphthalene liquid at atmospheric temperature, having a specific gravity at 77° F. of 1.19 to 1.25, a viscosity (Saybolt Universal seconds at 77° F.) of 33 to 37 and a distillation range (ASTM) of 480° F. to 590° F., from 60% to 40% of a chlorinated aliphatic hydrocarbon from the group consisting of ethylene dichloride, ethylene trichloride and trichloropropane, and from 20% to 30% of carbon tetrachloride.

4. A clearing agent for use in the preparation of histological tissue for microscopic examination, consisting essentially of from 20% to 30% by volume of a chlorinated naphthalene liquid at atmospheric temperature, having a specific gravity at 77° F. of 1.19 to 1.25, a viscosity (Saybolt Universal seconds at 77° F.) of 33 to 37 and a distillation range (ASTM) of 480° F. and 590° F., from 60% to 40% ethylene dichloride and from 20% to 30% carbon tetrachloride.

5. A clearing agent for use in the preparation of histological tissue for microscopic examination consisting essentially of about 60% ethylene dichloride, about 20% carbon tetrachloride and about 20% of a chlorinated naphthalene liquid at atmospheric temperature, having a specific gravity at 77° F. of 1.19 to 1.25, a viscosity (Saybolt Universal seconds at 77° F.) of 33 to 37 and a distillation range (ASTM) of 480° F. to 590° F.

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