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GENERAL SECTION

SHORTENED HISTOTECHNIQUE

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ABSTRACT :

An improvisation of the traditional long method of tissue processing and slide making is presented, allowing slides to be made in an average of 4 hours as compared to 1-2 days by the long method. It is hoped that this new method will facilitate routine and research work in the histology and histopathology laboratories.

INTRODUCTION:

The routine method of histological slide making is a fairly long method requiring 1-2 days for slide preparation as a routine.[1,2,3] This causes delays in diagnosis and backlogs of laboratory work. It was thus decided to undertake research on the method and try to evolve out a shorter and simpler method for making tissues slides. A few short methods are listed in the books,[1] but they are not in routine use for unknown reasons.

MATERIALS AND METHODS:

Essentially all materials and methods were used as in the routine preparation of tissue slides.[1-3] Attention was given to improvisations and innovations that would allow the long method to be shortened without sacrificing slide quality. Simple laboratory tests were devised to assess the effectiveness of any modification, and comparisons were made between the old and new methods. Important areas researched were: penetration times of processing chemicals; effects of heat, acids and alkalies on processing; use of different combinations of processing chemicals; synthesis of stronger and quicker adhesives; and the developing of quicker staining methods. Equipment used was that routinely used in histology and histopathology for tissues processing and slide making.[1-3] Chemicals used were of analytic reagent grade, supplied by Merck, BDH or from China.

After relevant research, it became possible to devise a new short method that could be used in routine to give good quality slides in an average time of four hours using essentially manual techniques, i.e. without the use of automatic tissue processing, sectioning or staining machines. The protocols are given in table 1.

Tissues may be fresh or received in 10% formalin. Small samples are cut from the tissue (1-2 cm2 x 2-4 mm) and processed as per protocol. Processing is done in airtight jars of convenient sizes and tissue samples put in tissue cassette,s labelled and put in a tissue basket as usual. Multiple jars may be used, one for each step or a single jar may be used by draining out the processing fluids after each step. The processing fluids may be

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stored in their respective containers and used repeatedly. Protocol I performs better for normal tissues, while protocol II gives better results with certain difficult pathological tissues, although for routine purpose both can be used without any serious problems for normal or pathological tissues.

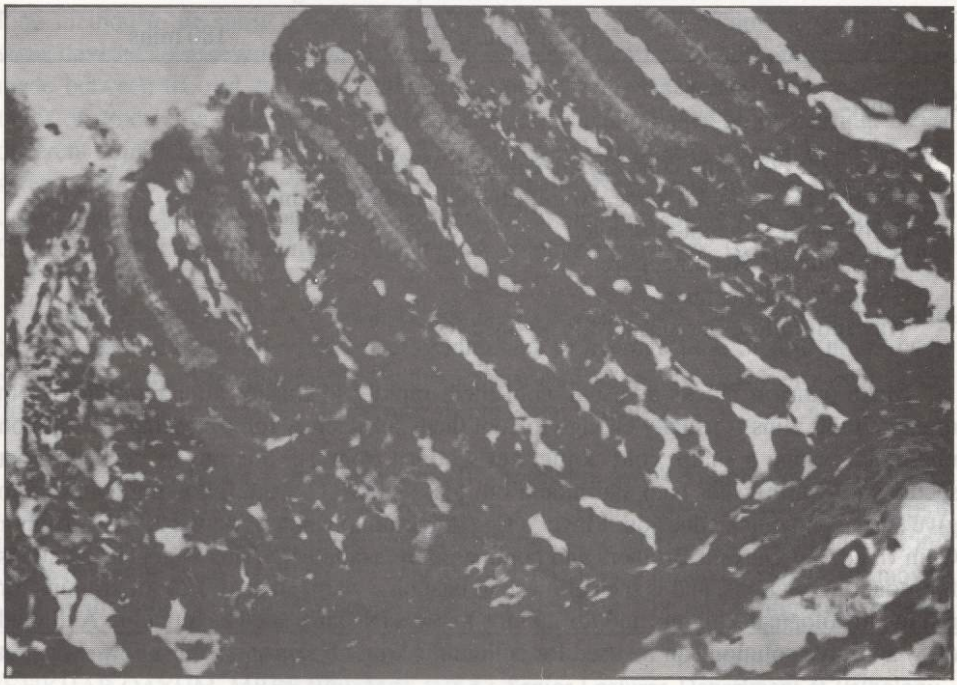
TABLE I

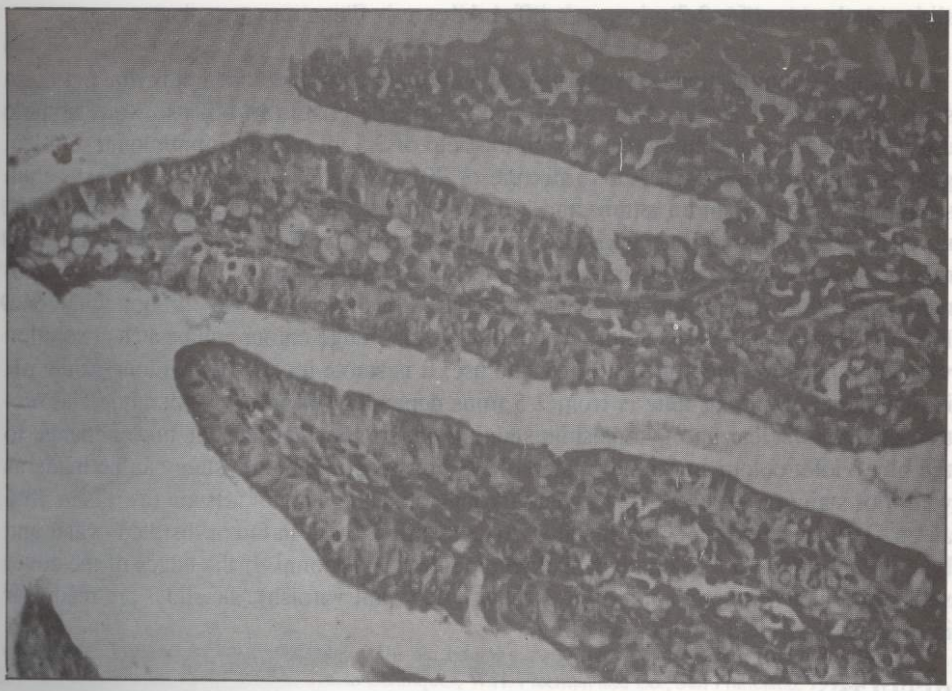
STEP	PROTOCOL I	PROTOCOL II
1. Fixative I	20 mins at 45°c	15 mins at 45°c
Fixative II	20 mins at 45°c	15 mins at 45°c
2. Dehydrant I	20 mins at 45°c	15 mins at 45°c
1st change	20 mins at 45°c	15 mins at 45°c
2nd change	Optional (for some pathological tissues)	15 mins at 45°c
3. Dehydrant II	20 mins at 45°c	15 mins at 45°c
1st change	Not needed	15 mins at 45°c
4. Clearant	120 mins at 45°c	15 mins at 45°c
1st change	Usually not needed	15 mins at 45°c
5. Wax Infiltration	20 mins at 65°c	15 mins at 65°c
1st change	20 mins at 65°c	15 mins at 65°c
2nd change	Not needed	15 mins at 65°c
Total Time	160 mins usually	180 mins

Solutions:

Fixative I: Formalin, conc, 20 ml; Ethanol, absolute 80 ml
Fixative II : formalin, conc. 10 ml; Ethanol, absolute 90 ml.
Dehydrant I: Ethanol Absolute.
Dehydrant II: Acetone, pure.
Clearant: Xylene, pure.
Wax Infiltration: Paraplast, M.P. 56-58°c.

Make paraffin blocks as usual, cool them rapidly in freezer after removing from mould; 20-30 mins is usually enough to cool them sufficiently for sectioning. Section on microtome, using a sharp, cold knife; cut sections from 1-5 microns as needed. Float sections on warm water bath and mount on glass slides. Use the following adhesive to give strong and quick adhesion: Mayer's Egg Albumin to which is added 20-25% Author Gum (made by Qureshi Brothers and commercially available). This is coated on slides for mounting sections, and is then allowed to heat on a warm stage (60°c) for 10 mins for routine histological staining, and for 20 mns for special stains such as reticulin. A diluted (1:5) adhesive solution can be used for celloidinization of sections. Pour a few drops on





slide, gently warm for 2-3 mins, pour off and dry over flame or warm plate.

A rapid staining method is possible by making a combined haematoxylin and eosin stain as follows: Dissolve 500 mg of alcohol-soluble eosin (do not use water soluble eosin) in 50 ml of absolute ethanol with the help of 2.5 ml of conc. ammmonia solution, gentle heating and stirring. Add a mixture of 20 ml of glycerol and 20 ml of 1% alum solution (K or ammonium alum), and mix thoroughly. Then add 2.0 ml of conc. HCL, mix, and add 500 mg of haematoxylin stain powder. Lastly add 125 mg of KMnO4 to 5.0 ml of distilled water and add to the mixture. Heat gently and mix to dissolve the components, and allow the mixture to stand overnight. Adjust pH to 3.0 by titration with conc. HC1 or Ammonia; the final solution should have an orange colour and be a clear solution with perhaps a few precipitates. Filter and use. It is stable indefinitely as long as the pH is maintained. Staining time is from 2-5 mins depending on room temperature and section quality. Nuclear and cytoplasmic staining occurs together and the nuclei change to the blue-violet colour in the next step of blueing. A stronger 1% stain can be made as well. For staining, proceed from xylene through absolute alcohol to stain (as it is in 50% alcohol), or to stain after 75% alcohol. After staining, wash in tap or distilled water and blue in tap water for 2-3 minutes. Dehydrate, clear and mount. If the edges of the cover slips are sealed with any appropriate sealant (clear nail varnish), the slides are ready for immediate examination, after labelling.

RESULTS :

Despite serious reservations, it became possible to devise a short method that would give acceptable slides in 4 hours average time. The method outlined is just about the shortest possible without causing major changes in the long method. It will be noted that the changes are merely related to real short-cuts by using combinations of processing fluids, use of higher temperatures, and shortened section adhesion and staining times. The processing is easy to do, can be done in a water bath or hot air incubator, and gives results on the same day. preliminary testing for tissue preservation gave good results for PAS and mucin stains; perhaps other enzymes and cell components are also well preserved. When making the H&E stain, it is important to use fresh and strong haematoxylin, as weak haematoxylin tends to be inhibited by the eosin. Two technicians working together can easily master the technique and further reduce the time. It is recommended that a few initial test-processing of samples be done to get a feel of the method.

DISCUSSION:

One is surprised at the paucity of short tissue processing methods in the literature,[1-3] even though these are possible and give results comparable or better than the long method. The benefits are obvious in terms of patient care, better laboratory function, and quick results for tissue researchers. The method can be adapted for use with automatic temperature-controlled tissue processors and other automatic histological laboratory equipment. It is hoped that the present short method and others like it will contribute to filling some of the gaps in our present system of histological techniques. Further

research is needed to try and make the method still shorter, as hopefully this goal is possible.

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CAPTIONS FOR MICROPHOTOGRAPHS:

1. Gerebral Cortex, Dog x 100
2. Gastric Mucosa, Dog, x 100
3. Jejunal Villi, Dog, x 200
4. Tuberculous Lymph Node, Dog, x 200

REFERENCES:

1. Brown, Geoffrey G. An introduction to Histotechnology; Appleton-Century-Crofts, New York, 1979.
2. Humason, Gretchen L. Animal Tissue techniques; W.H. Freeman and Co., San Francisco, 1972.
3. Baker, F.J. and Silverton, R.E. Introduction to medical laboratory technology, 6the ed., Butter worths and Co., U.K. 1985.