

A RAPID TECHNIC FOR DEMONSTRATING MELANIN IN FRESH TISSUE\*

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To know rapidly if pigmentation is caused by melanin or some other material is a convenience to the clinician. Because the usual procedures are time-consuming, a technic which demonstrates melanin in fresh tissue in less than five minutes has been developed. Alcohol-fixed tissues can be used, but technical difficulties may be encountered.

MATERIALS

Sections are cut from the frozen specimen and floated on a container of tap water. The reagents are prepared in wide-mouthed containers in the following manner.

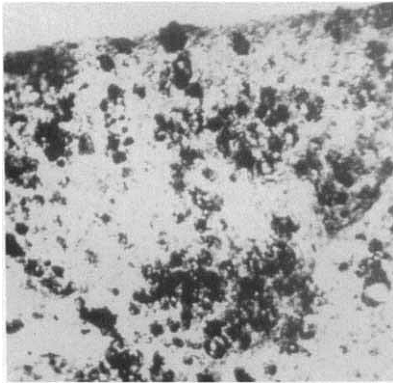


FIG. 1. Metastatic melanoepithelioma in a lymph node stained for melanin.

*Reagent 1.* Add 10 to 12 drops of concentrated ammonia to 50 cc. of tap water. This solution must not be made too strong because it could disintegrate the tissue.

*Reagent 2.* Dissolve approximately 1 Gm. of silver nitrate in 50 cc. of tap water.

METHOD

By using the Ward "rolling rod" technic (1) a section of tissue is lifted from the container of water onto a glass rod and floated on and off the surface of the ammonia hydroxide several times.

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It then is transferred to the silver nitrate solution and floated on and off the surface several times. The sections then are rinsed with tap water, mounted while wet, and examined under a microscope. Melanin is demonstrated by the reduced silver (Fig. 1).

If a counterstain is desired to aid in the localization of melanin (Fig. 2), Terry's polychrome methylene blue may be used (2). The tissues to be counterstained are floated on and off the Terry's stain, rinsed in tap water, and mounted for study.

Permanent slides can be prepared by allowing

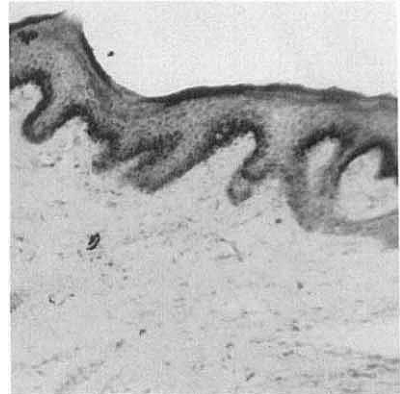


FIG. 2. Skin stained for melanin and counterstained with Terry's polychrome methylene blue stain.

the stained specimens to dry and then applying coverslips with the usual cement.

DISCUSSION

The reactions in this procedure are dependent upon the characteristic ability of melanin to reduce a solution of ammoniacal silver nitrate. This differs from the standard, more time-consuming procedures in that the preparation of diamine silver compounds prior to staining is unnecessary. The Ward "rolling rod" apparently massages the chemicals into the tissues. A diamine silver compound is formed, and the silver is reduced rapidly if melanin is present.

Under certain conditions, glycogen, desoxyribonucleic acid, uric acid, and some granules of

enterochromaffin cells may reduce the silver solution. In most instances, however, the presence of these substances will be suspected.

#### SUMMARY

A rapid method for the demonstration of melanin in fresh or alcohol-fixed tissues has been described. This procedure which employs the Ward "rolling rod" technic is dependent upon the ability of melanin to reduce the silver in a solution of ammoniacal silver nitrate. The tissue sections can be

counterstained with Terry's polychrome methylene blue and mounted permanently.

#### REFERENCES

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