HISTO-LOGIC™

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A Silver Technique for the Study of Cellular Injuries

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Introduction

Hematoxylin and eosin (H&E) have been adopted as routine stains in most of the histopathology laboratories. It has been well recognized that in the event of cellular degeneration, biochemical changes usually precede morphological changes.[1] Since H&E are non-specific stains involving merely basophilic and acidophilic reactions,[2] early detection of cellular degeneration by means of the H&E staining method becomes difficult.

Degenerating or degenerated cellular components, at least in the nervous system, are found to acquire an argyrophilic character. Silver nitrate has been employed in staining methods such as Cajal, Nauta, Fink-Heimer and Guillery, to localize specific areas and cellular structures of degeneration.[3] Despite the successful use of silver techniques in studying cellular degeneration of the nervous system, none of these histochemical principles have been adopted for use in studying other organ systems.

In the present report, we would like to introduce a silver staining method through which early degeneration of cells may be localized and detected.

Method

Tissue samples may be fixed with 0.5% glutaraldehyde, 4% paraformaldehyde or neutral buffered formalin and embedded in paraffin. The staining method is basically the same as that of Wilder's reticulum method.

Solutions

10% Phosphomolybdic Acid

Phosphomolybdic acid	10.0 gm
Distilled water	100.0 ml

1% Uranium Nitrate

Uranium nitrate	1.0 gm
Distilled water	100.0 ml

Ammoniacal Silver Solution

Add 28% ammonium hydroxide, drop by drop, to 5 ml of 10.2% aqueous solution of silver nitrate until the precipitate disappears and then add 5 ml of 3% sodium hydroxide. Bring the solution up to 50 ml with distilled water.

10.2% Silver Nitrate

Silver nitrate	10.2 gm
Distilled water	100.0 ml

3% Sodium Hydroxide

Sodium hydroxide	3.0 gm
Distilled water	100.0 ml

40% Neutral Formalin

Concentrated formaldehyde solution (37-40%)	50.0 ml
Calcium carbonate	in excess

Reducing Solution

40% neutral formalin	0.5 ml
1% uranium nitrate	1.5 ml
Distilled water	50.0 ml

0.2% Gold Chloride

Gold chloride	0.2 gm
Distilled water	100.0 ml

5% Sodium Thiosulfate (HYPO)

Sodium thiosulfate (hypo)	5.0 gm
Distilled water	100.0 ml

Staining Procedure

1. Decerate and hydrate sections in distilled water.
2. Oxidize sections in 10% phosphomolybdic acid solution for 1 minute.
3. Rinse slides in distilled water.
4. Sensitize sections in 1% uranium nitrate solution for 1 minute.
5. Rinse slides in distilled water.
6. Place slides in ammoniacal silver solution for 1 to 2 minutes.
7. Dip slides in 95% alcohol.
8. Place slides in reducing solution for 1 minute.
9. Rinse slides in distilled water.
10. Tone slides in 0.2% gold chloride solution for 1/2 to 1 minute.
11. Rinse slide in distilled water.
12. Treat slides with 5% sodium thiosulfate (hypo) solution for 1/2 to 1 minute.
13. Rinse in distilled water.
14. Dehydrate in 95% absolute alcohol and clear in xylene, 3 changes each.
15. Mount cover glass with resinous mounting media.

Results

This staining method has been tested on various tissues obtained from various experimental conditions such as mercury intoxication, halothane intoxication and ethylamine poisoning. In each case specific localization of silver was found in known areas of cellular injury while no significant silver deposits were formed in the tissues of the control animals.

For demonstrative purposes, two illustrations are shown to demonstrate the selectivity and sensitivity of the present technique. Figures 1A and 2A represent kidney and liver sections from rats intoxicated with mercuric bichloride ($HgCl_2$) and methylmercuric chloride (CH_3HgCl) respectively. Injured renal tubular cells (mainly proximal convoluted tubules) and hepatic cells (mainly periportal) were selectively impregnated with silver. Such lesions were not demonstrable with H&E staining. No significant silver deposit was observed in the corresponding cells of the normal tissues (Figures 1B, 2B).

The amounts of silver deposit may be correlated with the extent of the cellular injury.

Discussion

The mechanism of action of silver impregnation techniques is not completely understood. However, certain aspects of these techniques have been reported in the literature and these reports give some insight into the factors which influence such techniques. The widest application of silver techniques is for the demonstration of neurofibrils and reticulum fibers. These techniques produce essentially similar results insofar as visual observations are concerned, however, considerable evidence exists which would suggest that the mechanism of the staining of these two tissue components are dissimilar.[4,5,6,7]

Another application of the reticulum technique involves the oxidation of the tissue sections with an aldehyde producing oxidant and a sensitizer (secondary oxidizing agents) prior to staining. On the basis of various reports which have appeared in the literature it is obvious that aldehydes liberated by oxidation, as well as pre-existent aldehydes, are involved in the staining of reticulum by the various silver staining techniques. It is known that aldehydes *per se* are capable of reducing ammoniacal silver or silver nitrate to the metallic state.[8] It is obvious that not only the reticulum fibers were stained in the present study. The denaturation may be responsible for the sudden availability of aldehydes for such reaction rendering stainability of such cells. Electron microscopic examination of tissues stained by this method also revealed specific localization of silver granules in the cytoplasmic matrix of the injured cells (unpublished observation).

Nevertheless, we found that the silver technique described in the present report to be most useful and sensitive in the detection of cellular injuries which may otherwise be missed in routine H&E stainings. We are sure that other histopathology laboratories will also find this technique beneficial.

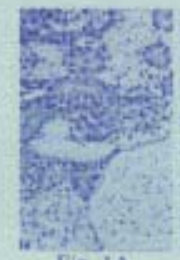

Fig. 1A

Fig. 1B

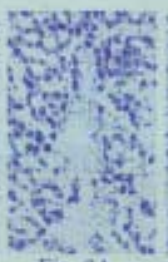

Fig. 2A

Fig. 2B

Legends

Figure 1A - Rat kidney. $HgCl_2$ intoxication (1mg/dg b.w., 2 wks). Note selective and heavy silver deposit in the proximal convoluted tubules marking cellular injuries in these tubules. No significant silver deposit was found in the glomerulus and other renal tubular cells indicating the selectivity of the staining technique.

Figure 1B - Rat kidney. Normal. No significant silver deposit was found in the renal tubular cells and glomeruli.

Figure 2A - Rat liver. CH_3HgCl intoxication (1mg/kg b.w. 3 wks). Dilatation of the hepatic sinusoid and heavy silver deposit in the periportal hepatic cells.

Figure 2B - Rat liver. Normal. No significant silver deposit was observed in the hepatocytes.

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Histotechnology Handout Material Available

The following is included as it is felt that the handout material mentioned may be beneficial, particularly to those histotechnologists who seldom have an opportunity to attend scientific meetings. The Editor

The Southwestern Pennsylvania Histology Society has accumulated about 120 printed handouts; some are from our own seminars, some are from the AFIP Symposium, and the balance are from a variety of sources. We, as a Society, would like to make these articles available to anyone who can use them.

Unfortunately, printing is expensive. Therefore, a "postage and handling" charge must be made. This charge will be noted in a brochure which will be sent out via national and state histology societies.

We realize that not all the authors will be pleased with this idea, but there is no feasible way to ask permission from all these individuals. So, we ask that this letter serve as a notice.

If you have written any articles, been a speaker at seminars or workshops, and will not permit us to include your work, please write to me immediately at the address provided below.

If available, the author's name, address, and source of the handout will appear on each article. We do not plan to take any credit for the articles; we only want to make them available to others. Please direct inquiries to: Elaine Kurtz, 4757 Delma Drive, Pittsburgh, Pennsylvania 15236.

National Histotechnology Convention and Histopathology Symposium

The First Annual Convention of the National Society for Histotechnology and the Tenth Annual Symposium on Histopathologic Techniques will be held simultaneously during the week of October 7-11, 1974 at the Sheraton, Silver Spring, Maryland. Additional information will be forthcoming in the July issue of HISTO-LOGIC. Due to an anticipated increase in attendance and limited available space, it is strongly suggested that early plans be made if you anticipate attending these meetings.

In order that we may guarantee your reservation please send your name and address to the symposium registrar as soon as possible if you plan to attend this National Convention/Symposium. A tentative program of the meeting and hotel room reservation card will be sent to you when you have preregistered. THIS IS NOT A COMMITMENT! It is, however, important information which will guide the symposium staff in making necessary plans and arrangements for the convention.

Registration information is to be sent to: Roberta Mosedale, Registrar, P. O. Box 36, Lanham, Maryland 20801. Thank you for your cooperation.

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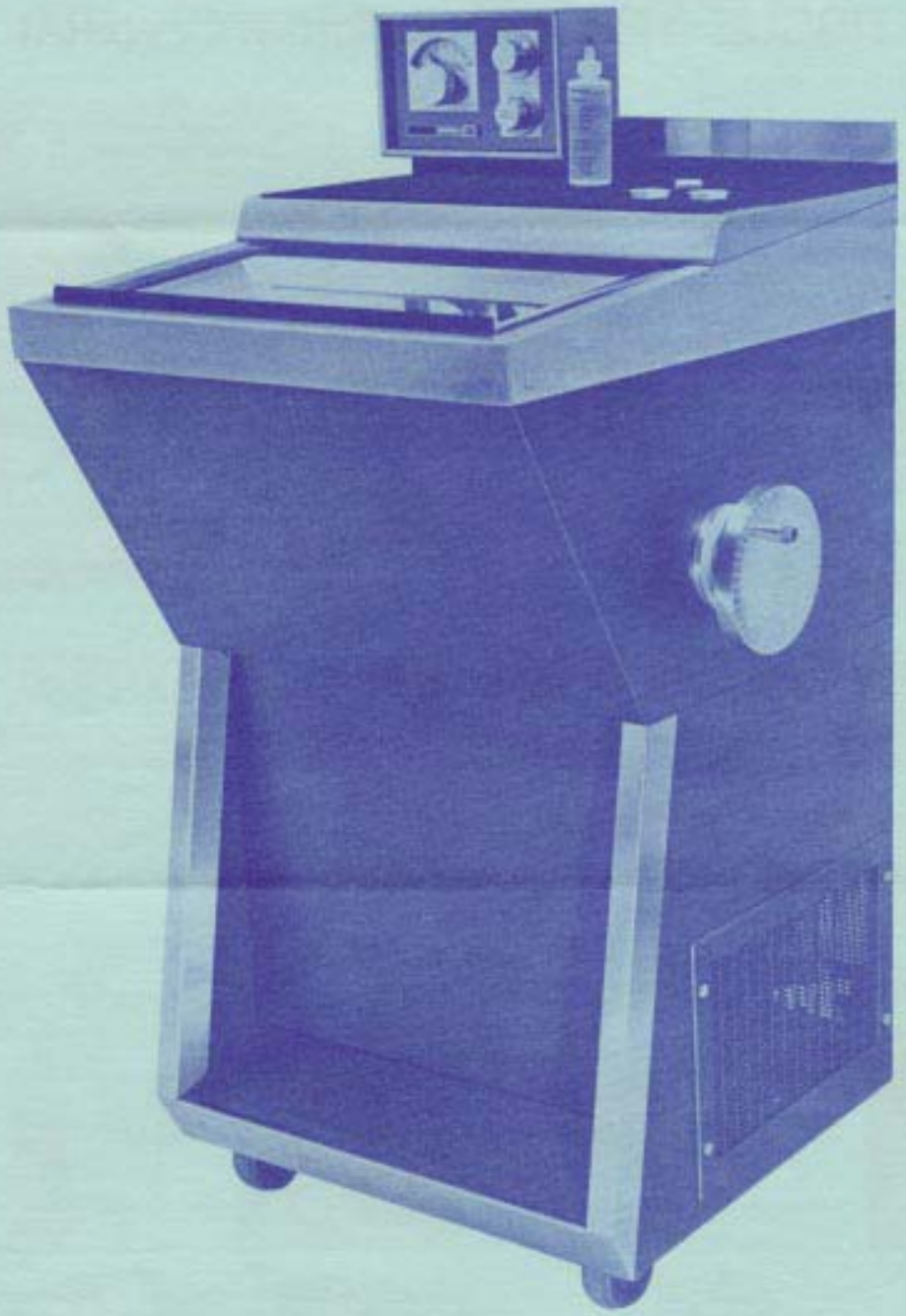

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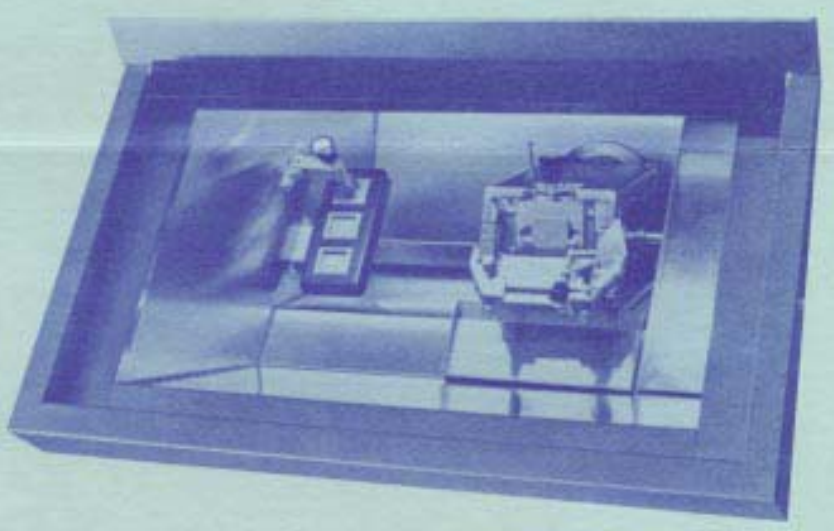

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Editor's Corner

Did You Know

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Virginia Histology Workshop

The Virginia Society of Histology Technicians will present their spring histology workshop in Northern Virginia on April 26-27, 1974. For information contact: Mrs. Shirley Pulley, 2200 Shiver Drive, Alexandria, Virginia 22307.

Southwestern Pennsylvania Seminar

The Southwestern Pennsylvania Histology Society Spring Seminar will be held in Pittsburgh, Pennsylvania, May 22-24, 1974. For information contact: Mrs. Adeline Qualtrough, Registration Chairman, 146 W. Riverview Avenue, Pittsburgh, Pennsylvania 15202.

Renewed Availability of "Selected Histochemical and Histopathological Methods" Text

Normally, announcements of this type are not included in HISTO-LOGIC. However, it is felt the information provided below is extremely important.

The excellent book *Selected Histochemical and Histopathological Methods*, by Samuel W. Thompson, D.V.M., is being reprinted because of continuous demand. Since only a limited number of copies are to be reprinted, it is suggested that all interested parties contact the following source as to availability: C. C. Thomas, Publisher, 301-327 East Lawrence Avenue, Springfield, Illinois 62717.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20801. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

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