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Editor, Lee G. Luna, D. Lit., H.T. (ASCP) | Technical Bulletin for Histotechnology Published: January, April, July, October | Vol. VIII, No. 3 - July, 1978

Golden Forceps Award Winner

We are pleased to announce that Anna Marie England has been selected as the recipient of the Golden Forceps Award for 1978. Her paper, "Gram-Positive and Gram-Negative Bacteria Control," was selected from articles submitted to *Histo-Logic* during the past year. Criteria for selection are clarity, originality and scientific contribution. The Golden Forceps Award will be presented at the Symposium/Convention of the National Society for Histotechnology to be held in Oklahoma City, Oklahoma, September 25-29, 1978. Reprints of the winning article which appeared in the January, 1978 issue of *Histo-Logic* are available from Lab-Tek Division, Miles Laboratories Inc., 30W475 North Aurora Road, Naperville, Illinois 60540.

Gelatin-Chrome Alum: A Better Section Adhesive

Cathy Adler*
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Mayer's egg albumen is the traditional section adhesive which has been used extensively for many years. Its value as a section adhesive is well recognized, particularly if one sections brain, keratinized material and decalcified bone. These and other similar materials have a tendency to fall off the slides during the staining process and therefore require an effective adhesive.

According to Lillie,[1] the effectiveness of this solution is probably due to its action as a surface tension depressant, since the constituents are readily soluble in water. Egg albumen residue often stains and this produces an objectionable artifact on completed slides and tissue sections. In addition, slides on which egg albumen has been used must remain in the drying oven somewhat longer to insure effective adhesion.

A comparative study of four section adhesives was conducted to determine which of the four proved the most effective and eliminated the artifacts produced on slides by the use of egg albumen. The test sections were obtained from large brain specimens and decalcified dense bone specimens. Duplicate sections were obtained from each block and mounted on slides which contained the different adhesives. Test slides were dried in a 58° C oven for 45 minutes, 2 hours, and 24 hours.

ADHESIVES USED FOR STUDY

Mayer's Egg Albumen

White portion of an egg
Glycerin
} Equal Parts

Loosen egg white with a fork before mixing with glycerin.

Mix both ingredients well and filter solution through several layers of gauze. Add a crystal of thymol to the solution to prevent bacteria and fungal growth.

Starch Paste[2]

Powdered starch	1.0 gm
Cold water	10.0 ml

Mix well and pour this solution into 30.0 ml of boiling water. Add 2 drops of 1N hydrochloric acid. Boil solution for 5 minutes. Add a crystal of thymol.

5% Gelatin

Gelatin	5.0 gm
Cold water	15.0 ml

Allow this solution to stand for a short period until the gelatin becomes soft. Add 85.0 ml boiling water to above solution. Continue gentle heat with bunsen burner or hot plate if gelatin is not completely dissolved.

Gelatin-Chrome Alum[2]

Gelatin	1.0 gm
Cold water	15.0 ml

Allow this solution to stand for a short period until the gelatin becomes soft. Add 85.0 ml boiling water to above solution. Continue gentle heat with bunsen burner or hot plate if gelatin is not completely dissolved. Add 0.1 gm of chrome alum to the solution and mix well.

MODE OF ADHESIVE APPLICATION TO SLIDES

Mayer's Egg Albumen: Apply evenly on clean glass slide with a finger or small sponge.

Starch Paste: Same as egg albumen solution.

NOTE: This adhesive should not be used if periodic acid Schiff type procedures are to be performed on sections since the starch will react with this procedure.[2]

5% Gelatin: Add 10.0 ml to flotation bath and stir well.

Gelatin-Chrome Alum: Same as 5% gelatin solution.

Results:

The brain sections remained attached to the slides with all adhesives during the staining process. However, some stain residue (in varying degrees) was observed with all adhesives with the exception of the gelatin-chrome alum. Bone sections which were dried in a 58° C oven for 45 minutes fell off the slide during the staining process with all adhesives except the gelatin-chrome alum. The only bone sections remaining attached to the slides with 5% gelatin were those which were dried in a 58° C oven for 24 hours. All other sections using this adhesive and various drying periods fell off the slides. Bone sections placed on the starch paste adhesive and the Mayer's egg albumen remained on the slide only when exposed for 24 hours drying in the 58° C oven.

Conclusion:

The evaluation of these four section adhesives has convinced us that the gelatin-chrome alum solution is less likely to allow sections to detach from the glass slide during the staining process. In addition, many special stains, including silver reactions, can be performed without any chemical interference or accumulated debris.

*Ms. Adler was a histology student at St. Vincent Charity Hospital when she submitted this article for publication.

References:

1. Lillie, R.D.: Histopathologic Technic and Practical Histochemistry, 3rd ed., p. 77, McGraw-Hill, New York, 1965.
2. Culling, C.F.A.: Handbook of Histopathological and Histochemical Techniques, 3rd ed., p. 131, Butterworths, Reading, Mass., 1974.

A Modified Thiosulfation-Aldehyde Fuchsin Method for Pancreatic Beta Cell Granules

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Washington, D.C. 20306

Introduction:
In 1974 Bussolati and Bassa[1] reported the use of a solution of ammonium hydroxide and cupric sulfate as a pretreatment to the aldehyde fuchsin method for the demonstration of pancreatic islet B cells. They found this treatment, which they referred to as "thiosulphation," provided more specific and darker staining of beta cell granules. We have modified the procedure by substituting ammonium sulfate [$(NH_4)_2\ SO_4$] for ammonium hydroxide and dividing the pretreatment solutions into individual stock solutions. These are stable solutions, while the ammonium hydroxide-cupric sulfate-sodium metabisulfite solution used by Bussolati and Bassa is somewhat unstable.

In addition, we have substituted potassium dichromate for paraldehyde in the aldehyde fuchsin solution. This was done for two reasons:

1. Paraldehyde is a narcotic and therefore a controlled item.
2. This chemical is often difficult to obtain.

This procedure has been used in our laboratories for over a year with excellent results. It has provided us with darker and more specific demonstration of pancreatic B cell granules.

Fixation: Buffered neutral formalin

Microtomy: Cut paraffin sections at 6 micra.

Solutions:

Thiosulfation Solution A (Stock)

Ammonium sulfate $[(NH_4)_2\ SO_4]$	6.0 gm
Copper sulfate, hydrate ($Cu\ SO_4\ 5H_2O$)	2.5 gm
Distilled water	400.0 ml

Thiosulfation Solution B (Stock)

Sodium hydroxide	2.0 gm
Distilled water	400.0 ml

Thiosulfation Solution C (Stock)

Sodium meta-bisulfite	4.0 gm
Distilled water	100.0 ml

Solutions A and B keep well, provided evaporation is minimized. Solution C remains stable for about one week.

Thiosulfation Solution (Working)

Mix in the following order immediately before use:

Solution A	20.0 ml
Solution B	20.0 ml

Solution is now dark blue due to the complexing of the ammonia and copper (pH is approximately 9).

ADD:

Solution C	5.0 ml

Solution turns bluish-green and may have a light precipitate (solution will become pale and clear as the reaction progresses).

Wenger's Aldehyde Fuchsin

Basic fuchsin	2.5 gm
Ethyl alcohol, 70%*	500.0 ml
Hydrochloric acid	5.0 ml
Potassium dichromate	5.0 ml

Combine the above ingredients and cap the container tightly. Allow solution to set at room temperature for five days. Check daily for the formation of acetaldehyde by smelling solution.** Acetaldehyde can be detected by its characteristic pungent fruity odor. If acetaldehyde is not detected within 24 hours, ethyl alcohol and/or potassium dichromate was omitted, or possibly an insufficient amount of hydrochloric acid was used. At the end of the five day period, check the quality of the solution by staining a known elastic control. If the elastic tissue is well demonstrated, the solution is good and should be placed in a refrigerator.

*Ethyl alcohol is necessary due to its reactivity with the potassium dichromate to form the acetaldehyde.

**Use *care* in smelling solution.

0.25% Metanil Yellow

Metanil yellow	0.25 gm
Distilled water	100.0 ml
Glacial acetic acid	0.25 gm

Staining Procedure:

1. Decerate and dehydrate slides to distilled water.
2. Place slides in fresh working thiosulfation solution for 2 hours.
3. Wash slides in tap water for 1 minute and rinse in distilled water.
4. Rinse slides in 95% alcohol
5. Place slides in Wenger's aldehyde fuchsin solution for 2 minutes.
6. Rinse slides in 95% alcohol until no more stain comes off the slides.

CHECK CONTROL SLIDE MICROSCOPICALLY. IF ISLET CELL GRANULES ARE PALE OR UNSTAINED, REPEAT STEPS 2 THROUGH 6 WITH FRESH SOLUTIONS.

7. Counterstain slides in metanil yellow for 1 minute.
8. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, 2 changes each.
9. Mount coverglass with synthetic resin.

Results:

Beta cell granules	- purple
Background	- tan to yellow

Reference:

1. Bussolati, G. & Bassa, T.: Thiosulfation Aldehyde Fuchsin (TAF) Procedure for the Staining of Pancreatic B Cells. Stain Tech., Vol. 49, No. 5, pp. 313-315, 1974.

Patron Program

National Society for Histotechnology

The National Society for Histotechnology has instituted a Patron Program to allow those individuals who are interested in the Society's activities to support its many programs with monetary contributions. **These contributions are tax deductible.**

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Examination of the Central Nervous System (Spinal Cord) in Situ

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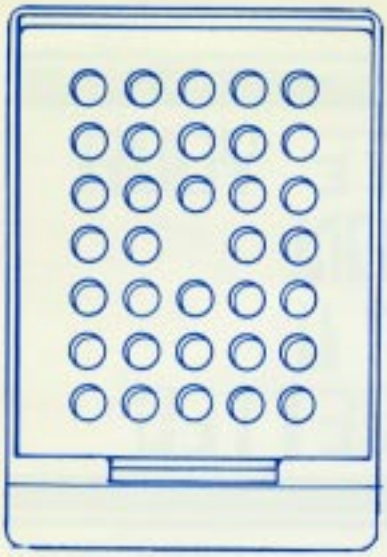
The use of a 10-15% (saturated) solution of disodium EDTA* for decalcification has been well recognized.[1,2,3] An advantage to its use as a decalcifying agent is that the stainability of bone and



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associated soft tissue is not adversely affected following immersion in the decalcifying solution for several weeks or months. Two disadvantages to its use in histological preparations are predominant. Because of its slowness of action, decalcification may not be complete for several weeks. Material decalcified for too long (over 5 months) may exhibit diluted staining reactions.

In the study of the immature spinal cord many problems are encountered. One problem is the poor fixation, or more specifically, the softness of the tissue after perfusion and fixation in 10% buffered neutral formalin (BNF). The inability to prepare this soft tissue for embedding without gross artifact made a new way for examination imperative. After perfusion through the carotid with 10% BNF, the vertebral column was removed with the spinal cord and dorsal root ganglion in situ. The column was bisected through the twelfth intervertebral disc and placed in fresh 10% BNF. After allowing time for proper fixation, the tissue was cut between vertebral bodies for paraffin embedding. The tissues were placed in individual polyethylene plastic tissue capsules.** (This is important in that the stainless steel capsules seem to deteriorate after much use in this solution.) Complete vertebral columns from adult rodents were immersed and cut for blocking after decalcification. After two to five months in the EDTA solution, the tissue cubes were embedded and sectioned at 6 and 10 microns. The solution was changed every two months. H & E and cresyl echt violet stains were used. Stainability of bone and soft tissues was excellent. Adult rodents, decalcified for 5 months, and young dogs which were less than eight weeks of age, decalcified for two months, have been processed this way (Figures 1-5).

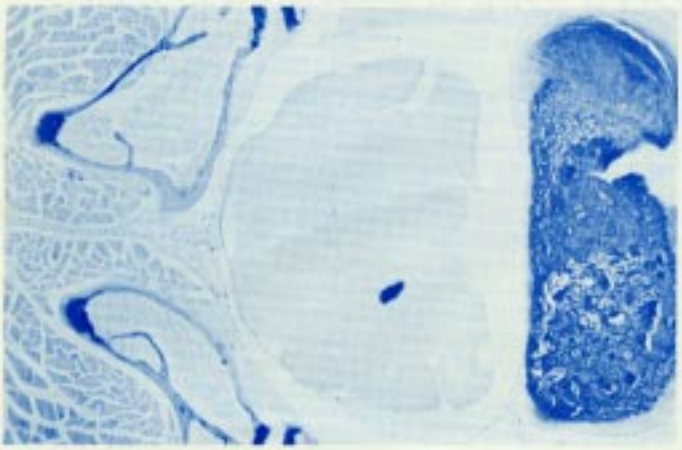

FIGURE 1:
Adult mouse spinal cord at low lumbar level. Whole vertebral column fixed in 10% BNF, decalcified in 10% (saturated) sodium EDTA for 5 months. Animal had spina bifida. (Cresyl echt violet, x 10, 10 microns)

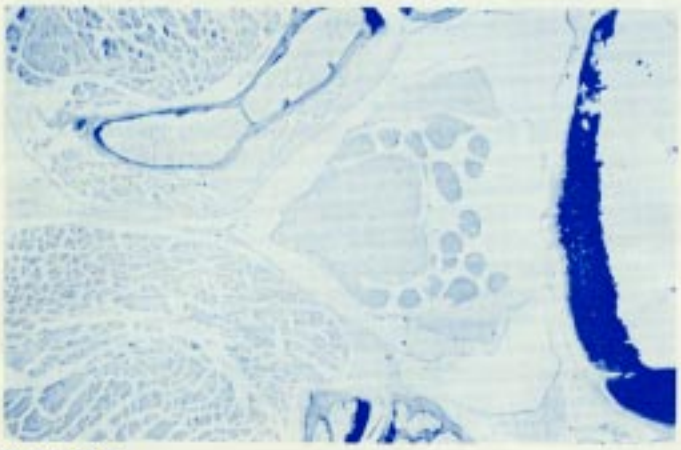

FIGURE 2:
Upper sacral level of same vertebral column as Figure 1. (Cresyl echt violet, x 10, 10 microns)

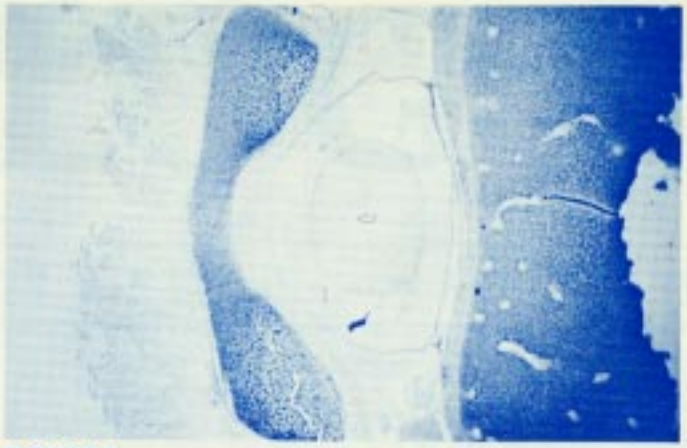

FIGURE 3:
First lumbar section from 2 day old canine. Animal was perfused with 10% BNF. Decalcification proceeded for 2 months in 10% sodium EDTA. (Cresyl echt violet, x 4, 10 microns)

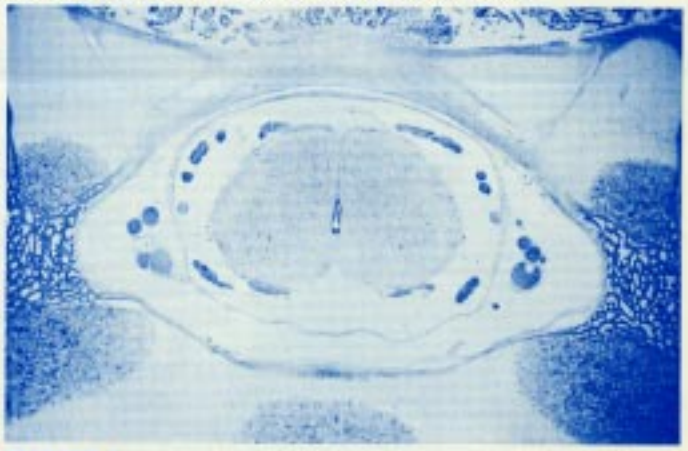

FIGURE 4:
Section from sixth lumbar level from same animal as Figure 3. (H & E, x 4, 10 microns)

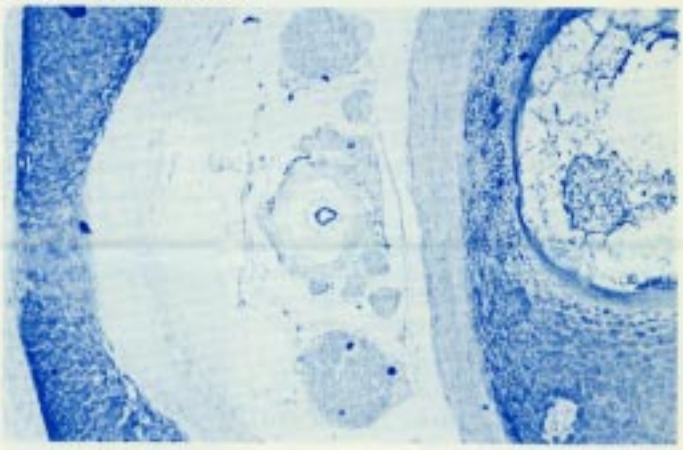

FIGURE 5:
Section from upper sacral level. Same as Figure 3. (Cresyl echt violet, x 10, 10 microns)

References:

1. Wallington, E.A.: Histological Methods for Bone. Butterworth and Co., Reading, Mass., 1972.
2. Brain, E.G.: The Preparation of Decalcified Sections. Charles C Thomas, Springfield, Ill., 1966.
3. Graham, D.: Personal communication.

*Ethylenediaminetetra-acetic acid, disodium salt: $C_{10}H_{14}O_8N_2Na_2 \cdot 2\,H_2O$

**TC-3 Plastic tissue capsules, Lipshaw Manufacturing Corp., Detroit, Michigan 48210.

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Technically Speaking

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Lab-Tek has received numerous inquiries regarding the installation and use of the exhaust deflector used on Tissue-Tek II Tissue Embedding Center.

Like any true refrigeration system, the compressor will operate more efficiently if the warm air generated by the compressor is diverted away from the unit.

The exhaust deflector provided with the embedding center has several functions. It directs warm air generated by the compressor away from the unit and prevents the unit from being placed directly against a wall.

If your deflector has been lost or misplaced, it can be ordered from any authorized Lab-Tek distributor. The part number is 50090021.

To install the exhaust deflector, simply follow these steps:

1. Turn unit around so that rear of unit faces you.
2. Loosen screw which affixes paraffin reservoir to base unit.
3. Examine foil vent for accumulation of lint and dust. If necessary, remove vent, wash in hot, soapy water, rinse, dry and replace.
4. Locate round opening in foil vent.
5. Position exhaust deflector so that large scoop faces up and is centered over round opening. Make sure that left side of exhaust deflector extends approximately 1/8" over trim panel of base unit.
6. Holding exhaust deflector against rear of embedding center, compress both sides so that left lip of deflector slips under trim of base unit. Release pressure.
7. Lift rear portion of paraffin reservoir so that slot (located top right) engages under lip of paraffin reservoir.
8. Move paraffin reservoir so that it is properly positioned on base unit. Replace screw removed in step #2.

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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.