Histo-Logic®

Miles Scientific
Vo. XIV, No. 1, 1984
Technical Bulletin for Histotechnology • Editor, Lee G. Luna, D. Lit., H.T. (ASCP)

Plastic Section Trichrome Staining

Dr. D. Juan S. Fernandez Pascual
Servicio de Anatomia Patologica, Residencia Santaria de la S.S., Manuel Lois Garcia, Huelva, Spain

The July 1981 (Vol. XI, No. 3) issue of *Histo-Logic*, contains an article dealing with the difficulties of staining different tissue components embedded in plastic (methacrylates) with trichrome methods.

As pointed out by Dr. Gerrits in that article, "besides the enormous advantages of plastic embedments, there are many problems associated with its use." One of the most striking problems is related to the poor results that we currently obtain with some methods such as Masson's or Mallory's trichromes. Some pathologists use Van Gieson's, Lendrum's and other methods in efforts to obtain similar results, but none of these are as good. The high contrast possible between smooth muscle and connective tissue, the colorful picture obtained and the great ease in identifying different cell types make the Masson and/or Mallory techniques insubstitutable in the study of organs such as skin, kidney and liver.

With this problem in mind, we have been searching for a long time for a possible substitute stain which could be used on plastic sections. The new technique provided below fulfills this need.

Solutions:

1% Orange G Solution (stock)

Orange G	1.0 gm
Distilled water	100.0 ml

Ponceau-Acid Fuchsin Solution (stock)

Ponceau	2.0 gm
Acid fuchsin	1.0 gm
Distilled water	100.0 ml

1% Aniline Blue Solution (stock)

Aniline blue	1.0 gm
Distilled water	100.0 ml

Staining Solution (washing)

1% Orange G (stock)	1.0 ml
Ponceau-acid fuchsin (stock)	5.0 ml
1% aniline blue (stock)	4.0 ml
Hydrochloric acid 0.1 N (normal)	50.0 ml

Staining Procedure:

1. Fix in Bouin's solution. Cut methacrylate sections at 2 microns. Do not remove plastic nor hydrate.
2. Stain in working solution (basic formula: 1-5-4-50) for 15 minutes at 60°C in a coplin jar.
3. Rinse in tap water.
4. Briefly differentiate with 95% alcohol.
5. Dry the sections and mount as usual, or dehydrate in absolute alcohol, clear in xylene and mount.

INSIDE THIS ISSUE

No reader should utilize materials and/or undertake procedures discussed in HISTO-LOGIC articles unless the reader, by reason of education, training and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished.

COPYRIGHT ©1983, **MILES SCIENTIFIC**, DIVISION OF MILES LABORATORIES, INC., NAPERVILLE, ILLINOIS 60566

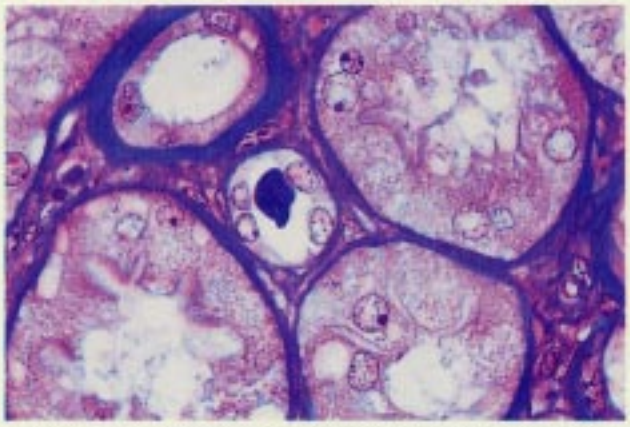

Figure 1. Basement membrane can be seen clearly in this well stained section of kidney tubules.

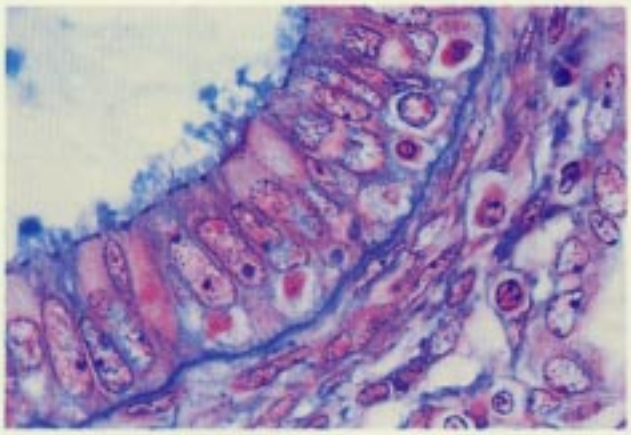

Figure 2. Note the fine, delicate demonstration of the well stained basement membrane.

Discussion:
This method is not only very easy to perform, but it offers:

1. A striking spectrum of colors that aid in the differentiation of various cells and tissue constituents.
2. Good resolution, enabling the use of extremely thin sections.
3. An extremely versatile method that allows one to vary the amount of the basic working solutions used (1-5-4-50), in order to obtain the best results. If tissue is very rich in connective tissue, one can use the formula (1-5-2-50). On the contrary, if tissue is of parenquimatous type, the formula (1-5-7-50) can be used.

References:

1. McManus, J.F.A. and Mowry, R.W., *Tecnico Histologico*. Atika S.A., Madrid, pp. 75, 293, 1968.

Organizing Your Special Stain Solutions

Chris Manor
Seaway Hospital
Trenton, Michigan 48183

Organization is the key to doing a job well—and rapidly. In applying this policy to the special stain set-up in our laboratory, we have found the following steps to be most helpful:

1. Group together dyes and chemicals to be used for each stain.
2. List the stains alphabetically.
3. List the solution used under each stain.
4. Number each bottle accordingly and mark the assigned number on the staining procedure.

Below is an example of how the special stain set up can be organized using these steps. The following list shows the grouping and numbering of solutions under stain categories.

Acid Fast
Kinyoun's:
Carbol fuchsin A-1
Acid alcohol, 1% A-2
Methylene blue, working A-3
Methylene blue, stock A-4

A & R Fluorescent:
Auramine and rhodamine A-5
Acid alcohol, 1% A-2
Potassium permanganate A-6

Acid Mucopolysaccharides
Alcian blue:
Acetic acid, 3% aqueous B-1
Alcian blue, 0.1% B-2

The solutions are grouped on the shelves alphabetically, according to stain, with a procedure manual always nearby. The solutions can also be placed in a different box for each stain, and then stored alphabetically. In that case, a card which describes the stain procedure is inserted inside each box.

Some of the solutions used in our laboratory are not used specifically for any special stain. These solutions are listed alphabetically as "Miscellaneous Solutions" and assigned consecutive numbers, as illustrated in the example below:

Fixative for frozens 1
Hematoxylin, Harris' 2
Iodine, 0.5% alcoholic 3
Toluidine blue, 1% aqueous 4
Zenker's solution 5

These solutions are stored in a place separate from the special stain solutions (e.g., on a different shelf) in numerical order. It is important that the bottles are numbered clearly.

Next, a master list is composed, listing all ready-made solutions in stock. Their assigned number follows so that any solution can be found rapidly. You can also check the master list to see where any given solution is located or to see if that solution is in stock.

The sample list shown below represents solutions listed in the above stains:

Acetic acid, 3% aqueous B-1
Acid alcohol, 1% A-2
Alcian blue, 0.1% B-2
Auramine-rhodamine A-5
Carbol fuchsin, Kinyoun's A-1
Fixative for frozens 1
Hematoxylin, Harris' 2
Iodine, 0.5% alcoholic 3
Methylene blue, working A-3
Methylene blue, stock A-4
Toluidine blue, 1% aqueous 4
Potassium permanganate A-6
Zenker's solution 5

We post the lists inside the staining shelf doors, next to where the solutions are kept. A copy of the list is also kept in the histology staining manual. Lists are updated continuously as new procedures and/or solutions are added.

This system has worked quite well in our laboratory during the past ten years. It has simplified both the performance and the teaching of special stains.

SURGICAL PATHOLOGY SYMPOSIUM 1984

September 21–22, 1984

"Immunohistopathology Update" and "Immunoperoxidase Workshop" sponsored by Lutheran General Hospital, Park Ridge, Illinois, and Miles Scientific.

Program Coordinators include: N.K. Bharani, M.D.; E.V. Pellettiere, M.D.; and M. Costello, Ph.D.

Faculty will include: Nancy Harris, M.D., Massachusetts General Hospital; Joseph Corson, M.D., Brigham and Women's Hospital in Massachusetts; Prabodh Gupta, M.D., Johns Hopkins Hospital in Maryland; Victor Gould, M.D., Rush-Presbyterian-St. Luke's Medical Center in Illinois; Edmund Pellettiere, M.D., Lutheran General Hospital; N.K. Bharani, M.D., Lutheran General Hospital; Michael Costello, Ph.D., Lutheran General Hospital; Imad Almanaseer, M.D., Lutheran General Hospital; Irene Grigaitis, M.T. (A.S.C.P.), H.T.L., Lutheran General Hospital; Nic Luna H.T.L.(A.S.C.P.), Lutheran General Hospital; and Diane Burica, H.T.L. (A.S.C.P.), Lutheran General Hospital.

Workshop fees are $40 and $50 respectively (includes lunch).

For a free brochure/application write to:
Susan Diederich
Clinical Pathology Office
Lutheran General Hospital
1775 Dempster Street
Park Ridge, IL 60068

Late registration at the reception desk is subject to availability. Call 312-696-6178 for more information.

An Improved Heidenhain's Azan Technique Using "Susa" Fixation

William Barlow, HT (ASCP)
Riverside Hospital, Wilmington, Delaware 19899

There are numerous connective tissue stains listed in histotechnology procedural manuals, but many of these stains are time-consuming and the results are often capricious. Described below is a simple three-step method which demonstrates connective tissue structures in sharp contrast to muscle and cells.

Fixation:
"Susa" fixative is preferred; however, most fixatives with an acid pH will also work. Microscopic slides of tissues previously fixed in formalin may be placed in "Susa" fixative after sectioning. Alternatively, if the tissue has been fixed (e.g., overnight) in formalin, it may be post-fixed in "Susa" after a 10-minute wash in running tap water. Four hours fixation is adequate for most conventionally-sized tissues. After fixation, tissue should be transferred to 95% alcohol to prevent excess swelling of the connective tissue.

Microtomy:
Cut sections at 6 microns.

Solutions:

Heidenhain's "Susa" Fixative

Mercuric chloride*	45.0 gm
Sodium chloride	5.0 gm
Formaldehyde (concentrated 37 to 40%)	200.0 ml
Distilled water	800.0 ml
Glacial acetic acid	40.0 ml
Trichloroacetic acid	20.0 gm

*Zinc chloride may be used in place of mercuric chloride.

1% Azocarmine B Solution

Azocarmine B	1.0 gm
Distilled water	100.0 ml
Acetic acid	1.0 ml

Filter solution before use. Solution has a long shelf life.

5% Phosphotungstic Acid Solution

Phosphotungstic acid	5.0 ml
Distilled water	100.0 ml

Aniline Blue-Orange G Solution

Aniline blue	0.5 gm
Orange G	2.0 gm
Acetic acid	8.0 ml
Distilled water	300.0 ml

Solution has a long shelf life.

Procedure:

1. Prewarm the azocarmine B in a 56°C oven.
2. Deparaffinize slides and hydrate to distilled water.
3. Remove mercury precipitate, if necessary.
4. Place slides in 56°C prewarmed azocarmine B solution for 15 minutes.
5. Rinse off excess stain in distilled water.
6. Place slides in 5% phosphotungstic acid solution for 15 minutes.
7. Rinse slides in distilled water.
8. Place slides in aniline blue-orange G solution for 15 minutes.
9. Rinse in distilled water.
10. Dehydrate slides in alcohol, clear in xylene, and mount coverglass with resinous media.

Results:

Chromatin, osteocytes, neuroglia	red
Collagen reticulum	blue
Muscle	red to yellow

References:

1. Culling, C.F.A., *Lynch's Medical Laboratory Technology*, Vol. II, W.B. Saunders Co., Philadelphia, Toronto & London, p. 876, 1976.
2. Everett, M.M., and Miller, W.A., The Role of Phosphotungstic and Phosphomolybdic Acids in Connective Tissue Staining—A Histochemical Study. *Histochemical Journal* 6:25, 1974.
3. Wheater, P.R., et al. *Functional Histology; A Text and Color Atlas*. First Edition, Churchill Livingstone, Edinburgh, London & New York, 1969.
4. Luna, G., *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition, McGraw-Hill Book Co., New York, 1968.
5. Conn, H.J., *Biological Stains*, edited by Lillie, R.D., Ninth Edition, The Williams and Wilkins Co., Baltimore, 1977.

Schiff's Stained Hands and Clothing—A Possible Solution

Nancy Cameron
Pomona Valley Community Hospital
Pomona, California 91767

Periodic acid Schiff's stain is a procedure commonly used in the histology laboratory, but it does pose a problem: it is generally considered a permanent stain on hands and clothing. I have recently discovered, however, that two forms of Ivory soap can remedy the problem.

Use of Ivory hand soap will readily remove the feulgen or Schiff's stain from your skin and laboratory coats. A paste made by adding a small amount of water to Ivory laundry soap, applied directly to the stain on laboratory clothing, can also be used to remove the stain immediately. Although Ivory liquid dishwashing soap has not been tried, it will likely work as well.

Power Processing

Tissue-Tek® V.I.P.
Vacuum Infiltration Processor Series

- Microprocessor controlled — retains 10 user-defined processing programs and monitors the processing sequence.
- Fail-safe system protects specimens from drying if processing is interrupted.
- 3 processing variables — pressure, vacuum and heat.
- "Sealed System" fume control.
- 10 reagent and 4 paraffin reservoirs.
- Operator training at Miles Scientific.
- Choose from 100 or 200 specimen capacity units in floor or bench-top configuration.

Miles Scientific, Division of Miles Laboratories, 30W475 North Aurora Road, Naperville, IL 60566.

Miles Scientific

The Use of Agar for Orienting Small Biopsies and Tissue Fragments

Thomas Rogers
Regional Hospital, Galway, Ireland

A method using agar and the Tissue-Tek® III Cassette System for handling small biopsies and tissue fragments is described below. The problems associated with agar embedding are also discussed.

The number of biopsies and small fragments of tissue being handled by the busy histopathology laboratory has increased greatly over the past few years. Because of the small size and nature of the tissues, it is very important to orient the specimen properly to prevent loss of tissue, carry over, or contamination of the processed block. Traditionally, small biopsies and friable tissue are wrapped in tissue paper and oriented at the blocking-out stage. During this stage it can be difficult to orientate correctly. In inexperienced hands, the results can be poor, with the danger of contamination much more likely.

On reviewing the literature on agar embedding, we have found two references to the subject. Elliot and Moores[1] described a method for handling bone marrow aspirates. Cook and Hotchkiss[2] described a way of treating biopsies. Both of these methods have been tried, used and modified.

Materials and Methods:
Our findings suggest that a 3% solution of oxoid DST agar prepared in 10% neutral buffer formalin (pH 7.0) is suitable. A stock solution is prepared each week, then divided into 5 x 30 ml aliquots and stored at 4°C. We have found that one aliquot is sufficient for each day's work. The aliquot is melted at the beginning of the grossing session and stored in a 56°C oven. The tissues are subsequently handled as follows:

Procedure for Bone Marrow Aspirates:

1. Fix marrow aspirates for 1 hour in Zenker fluid (fresh) in a conical universal container.
2. Wash in distilled water for 5 minutes.
3. Discard the free R.B.C. that is left floating in the supernatant (the heavier particles will sink to the bottom rapidly). In fatty specimens, skim the marrow globules off the surface into a clean glass vial.
4. Wash the marrow aspirates thus harvested into a 20 ml plain glass tube and spin at 2000 rpm for 30 seconds.
5. Discard supernatant fluid.
6. Remove agar from the oven and allow to cool to between 40° to 45°C.
7. Pour 1 to 2 mls of agar into the glass vial containing the aspirate.
8. Shake sample to mix well, then spin at 2000 rpm for 1 minute.
9. Allow the aspirate, which forms a button at the bottom of the vial, to cool for 5 minutes.
10. Wrap the vial in a facial tissue, then sharply tap the end of the vial furthest from the aspirate to crack the glass. The agar button will slide out. The specimen can then be grossed and placed in a Tissue-Tek® III Biopsy Uni-Cassette (Miles code no. 4172) for processing in the normal manner.

This method produces a clean specimen to block out after embedding in paraffin, with minimal danger of contamination.

Procedure for Biopsies and Friable Tissues:

1. Fill a 15 mm x 15 mm Tissue-Tek® II metal Base Mold (Miles code no. 4162) with agar solution that has been cooled to between 40° to 45°C (as above). For large specimens, use 24 mm x 24 mm molds (Miles code no. 4163).
2. Number the embedding cassette with the appropriate number which has been cross-checked with the request form and specimen bottle.
3. Place biopsy in the molten agar-filled mold and orientate.
4. Allow the agar to stand for 5 minutes to solidify. Place the cassette on top of the mold to identify the tissue.
5. Once the block is solid, place a scalpel down one side of the mold to detach the specimen. It can then be trimmed and the agar can be notched to show the surface to be embedded, if required. We usually leave a 5 mm strip of agar around the tissue.

With this method, we are able to process up to 20 biopsies per day without problems. This technique has also been used for friable tissue such as curettings. After casting, the agar block is placed in a cassette and fixed for 1 to 2 hours. The tissue processor is often used for this purpose.

Discussion
Some of the advantages of agar double embedding have been discussed by Bancroft[3], Elliot et al[1], and Cook et al[2]. The method is considered advantageous because 1) the amount of time the tissue is handled is reduced to a minimum; 2) tissue can be oriented at the grossing step before it shrinks due to processing; and 3) the dangers of contaminating the block with "wash

in" or "floaters" are easily reduced. By using the Tissue-Tek III Cassette system, we feel we have also reduced the danger of misnumbering specimens because the cassette can accompany the tissue.

In our four-year study, the problems we have encountered with agar fall into two areas. First, not all tissues are suitable for the double embedding techniques. Skin biopsies and all needle biopsies (i.e., liver, kidney, bone marrow biopsies) do not cut easily with agar strip surrounding the tissue. It is very difficult to get a thin section of renal tissue with agar embedding.

Second, if the agar blocks are not adequately fixed, the agar acts as a barrier to the processing fluids. Thus, the fixation of the agar block itself is very important. When a laboratory is running a 24-hour biopsy service, the size of the agar block must be one that allows adequate fixation of both block and tissue. We use 5 mm x 5 mm x 5 mm blocks for overnight processing, which is a 15-hour processing schedule. Rapid processing of agar embedded tissue can be detrimental, as we have found by trying to process agar embedded biopsies on a 3-hour schedule using a vacuum and heat processing schedule.

Through other observations, we have noted that in hematoxylin and eosin staining techniques there is a background halo around the tissue which corresponds to the agar block shape. This is also noticeable in other stains, i.e. periodic acid Schiff.

We have also found that the shelf life of molten agar stored at 56°C is very limited and varies from batch to batch. We prepare our stock solution at the beginning of each week, test it for solidity and divide it into 5 aliquots of approximately 30 mls each and then store them at 4°C. We melt a fresh bottle each day and discard the one used on the previous day.

Finally, we have observed that if the agar is too hot when tissue is placed in it, the exterior layer of cells may be damaged. It is therefore advisable to let biopsy specimens fix for 1 to 2 hours before embedding them in agar, and to keep the agar temperature just above the melting point before embedding the tissue.

Conclusion

We have found that the use of agar double embedding with paraffin has improved the handling of small biopsies in our laboratory, regardless of the problems associated with its use. In addition, we have found that staff with limited experience can use the method successfully. During our four-year project, agar double embedding has proved to be a very suitable method for orienting delicate tissues. We wish to thank the staff of the histopathology department for their assistance throughout this project.

References:

1. Elliot, M.D., and Moores, B.D., A Method for the Preparation of Histological Sections on Bone Marrow Aspirates, *Med. Lab. Tech.*, 32:105, 1975.
2. Cook, R.W., and Hotchkiss, G.R., A Method for Handling Small Tissue Fragments in Histopathology, *Med. Lat. Sci.*, 34:93, 1977.
3. Bancroft, J.D. and Stevens, A., *Theory and Practice of Histological Techniques*. Chapter 3, 58, 1982.

Schmorl's Method for Melanin

Mack Alexander, HTL (ASCP)
Saint Joseph Hospital, Omaha, Nebraska 68131

Editor's Note: Of general interest is the fact that the author was in the first (1958A) Military Course for Histotechnology given at the Armed Forces Institute of Pathology, and is the first certified HTL, with Board of Registry Certification Number 00001.

I was introduced to Schmorl's method for reducing substances through the HTL practical examination, which I completed in August 1980. Our laboratory had no previous experience with this method. However, after some trial we decided that this was the method of choice, over the Fontana-Masson method, for the demonstration of melanin. The next step was to convince the pathologist of the value of this procedure.

This was done by making a control paraffin block which contained the following: a piece of skin (for melanin); an iron positive piece of liver; and a piece of brain which was negative for both melanin and iron. When the laboratory receives a request for melanin, we automatically perform Gomori's modified iron procedure and the Schmorl's method[1] on both the control slide and the unknown slide. This provides the pathologist with both a negative and positive control for comparison with the unknown.

Both the iron[1] and Schmorl's[1] method are run in 10 minutes as outlined in the reference below. The short procedure time, as well as the elimination of expensive silver nitrate, are the reasons we decided to use the above procedure.

Reference:

1. Sheehan, D.C., *Theory and Practice of Histotechnology*, Second Edition, C.V. Mosby Co., St. Louis, MO, pp. 218-223, 1980.

in" or "floaters" are easily reduced. By using the Tissue-Tek III Cassette system, we feel we have also reduced the danger of misnumbering specimens because the cassette can accompany the tissue.

In our four-year study, the problems we have encountered with agar fall into two areas. First, not all tissues are suitable for the double embedding techniques. Skin biopsies and all needle biopsies (i.e., liver, kidney, bone marrow biopsies) do not cut easily with agar strip surrounding the tissue. It is very difficult to get a thin section of renal tissue with agar embedding.

Second, if the agar blocks are not adequately fixed, the agar acts as a barrier to the processing fluids. Thus, the fixation of the agar block itself is very important. When a laboratory is running a 24-hour biopsy service, the size of the agar block must be one that allows adequate fixation of both block and tissue. We use 5 mm x 5 mm x 5 mm blocks for overnight processing, which is a 15-hour processing schedule. Rapid processing of agar embedded tissue can be detrimental, as we have found by trying to process agar embedded biopsies on a 3-hour schedule using a vacuum and heat processing schedule.

Through other observations, we have noted that in hematoxylin and eosin staining techniques there is a background halo around the tissue which corresponds to the agar block shape. This is also noticeable in other stains, i.e. periodic acid Schiff.

We have also found that the shelf life of molten agar stored at 56°C is very limited and varies from batch to batch. We prepare our stock solution at the beginning of each week, test it for solidity and divide it into 5 aliquots of approximately 30 mls each and then store them at 4°C. We melt a fresh bottle each day and discard the one used on the previous day.

Finally, we have observed that if the agar is too hot when tissue is placed in it, the exterior layer of cells may be damaged. It is therefore advisable to let biopsy specimens fix for 1 to 2 hours before embedding them in agar, and to keep the agar temperature just above the melting point before embedding the tissue.

Conclusion

We have found that the use of agar double embedding with paraffin has improved the handling of small biopsies in our laboratory, regardless of the problems associated with its use. In addition, we have found that staff with limited experience can use the method successfully. During our four-year project, agar double embedding has proved to be a very suitable method for orienting delicate tissues. We wish to thank the staff of the histopathology department for their assistance throughout this project.

References:

1. Elliot, M.D., and Moores, B.D., A Method for the Preparation of Histological Sections on Bone Marrow Aspirates, *Med. Lab. Tech.*, 32:105, 1975.
2. Cook, R.W., and Hotchkiss, G.R., A Method for Handling Small Tissue Fragments in Histopathology, *Med. Lat. Sci.*, 34:93, 1977.
3. Bancroft, J.D. and Stevens, A., *Theory and Practice of Histological Techniques*. Chapter 3, 58, 1982.

Schmorl's Method for Melanin

Mack Alexander, HTL (ASCP)
Saint Joseph Hospital, Omaha, Nebraska 68131

Editor's Note: Of general interest is the fact that the author was in the first (1958A) Military Course for Histotechnology given at the Armed Forces Institute of Pathology, and is the first certified HTL, with Board of Registry Certification Number 00001.

I was introduced to Schmorl's method for reducing substances through the HTL practical examination, which I completed in August 1980. Our laboratory had no previous experience with this method. However, after some trial we decided that this was the method of choice, over the Fontana-Masson method, for the demonstration of melanin. The next step was to convince the pathologist of the value of this procedure.

This was done by making a control paraffin block which contained the following: a piece of skin (for melanin); an iron positive piece of liver; and a piece of brain which was negative for both melanin and iron. When the laboratory receives a request for melanin, we automatically perform Gomori's modified iron procedure and the Schmorl's method[1] on both the control slide and the unknown slide. This provides the pathologist with both a negative and positive control for comparison with the unknown.

Both the iron[1] and Schmorl's[1] method are run in 10 minutes as outlined in the reference below. The short procedure time, as well as the elimination of expensive silver nitrate, are the reasons we decided to use the above procedure.

Reference:

1. Sheehan, D.C., *Theory and Practice of Histotechnology*, Second Edition, C.V. Mosby Co., St. Louis, MO, pp. 218-223, 1980.

This presentation will elaborate on the role veterinary diagnostic laboratories play in modern veterinary practice and animal production with emphasis on the contribution of histopathology.

1:30 - 2:00 PM

FORENSIC ODONTOLOGY

(Robert M. Howell, D.D.S.)

The identification of persons by dental tissues remains one of the least disputed methods available to legal authorities when dealing with human remains unidentifiable by visual examination or in pointing out the perpetrator of a crime where dental evidence is found (i.e., bite marks).

2:00 - 2:45 PM

AGING AND THE ONSET OF NEOPLASIA IN THE RAT PROSTATE

(Michael Wilson, Ph.D.)

Aging in males is associated with a generalized decline in reproductive function and associated degenerative changes in the sex accessory glands. However, in aged humans the prostate gland also has a propensity of localized renewed growth which leads to benign hyperplasia and/or cancer. The study of aging in the rat has been undertaken as a model for some aspect of these pathologies.

3:15 - 4:00 PM

MICROWAVE OVENS FOR METALLIC HISTOLOGIC STAINING: A NEW CONCEPT

(Nathan Brinn, B.S., HT [ASCP])

Many of the metallic impregnations currently employed in the histology laboratories may be modified so that they may be performed in a microwave oven. This heat source has reduced impregnation time in many of the silver stains to one-sixtieth the normal time in convection ovens. For a Fontana or Methenamine Silver the impregnation time in the microwave oven is seventy seconds.

4:00 - 4:30 PM

SPECIALIZED SECTIONING TECHNIQUES IN BIOMEDICAL MATERIALS

(Gwen S. Nelson, HTL [ASCP])

A biomedical material is any material used to fabricate a prosthesis or implantable device which comes in contact with living tissue. In order to evaluate tissue response to the material it is necessary to prepare quality histologic sections with the tissue—material interface intact. This is achieved either by sectioning with the material in place or by removing the material so as to leave the interface undisturbed.

This presentation reviews 3 such techniques used for biomaterial evaluation and includes slides of stained sections to illustrate the results.

THURSDAY, OCTOBER 4, 1984

8:30 - 9:15 AM

TERATOLOGY — THE STUDY OF BIRTH DEFECTS: TECHNIQUES IN THE VETERINARY HISTOLOGY LABORATORY

(N. Lucille Rossi, M.S., HT [ASCP])

Long-term, multigeneration effects of dietary intake are measured in generation reproduction and teratology studies. The project investigator is usually responsible for the feeding and breeding schedules, then the veterinary histologist, in many instances, is called to become an active part of the continuing procedures by performing caesarean sections at the proper period of gestation, processing of the fetuses to detect the birth defects ad result of dietary intake.

Methods and procedures are well developed, have proven efficient and effective. They will be discussed at this meeting, and comparative anatomical results will be pointed out.

9:15 - 10:00 AM

HISTOLOGY AND HISTOLOGICAL TECHNIQUES IN DENTISTRY

(Theodore J. Urban, Ph.D.)

It is important for Histotechnicians to realize that the methods for studying the tissues of the oral cavity are in many ways unique. The tooth must be studied in ground or decalcified sections; both techniques presenting unusual technical problems. The soft tissues are often surrounded by calcified ones so careful techniques are needed to interpret physiological activity. The epithelial reaction of the connective tissue immediately below is essential for diagnosing normal and abnormal situations in the dental field. Methods of handling dental tissues such as dry ground and decalification procedures will be discussed. Useful staining and preparation techniques will also be presented.

10:30 - 11:00 AM

THE USE OF HISTOCHEMICAL METHODS TO IDENTIFY ALTERED FOCI IN RAT LIVERS

(Otis Lyght, HT [ASCP])

Foci of altered liver cells, also known as preneoplastic loci, are believed to be precursors of liver tumors in rats. Some foci can be identified in an H&E stained section as an area of altered staining. Histochemical stains assist the recognition and quantitation of these foci in livers of carcinogen exposed animals.

11:00 - Noon

ANCIENT MUMMIFIED TISSUE: HISTOLOGIC TECHNIQUES AND PATHOLOGIC FINDINGS

(Theodore A. Reyman, M.D.)

Egypt and other cultures have produced mummified remains. Details of processing such mummified tissue for histologic examination beginning with selection of specimen, rehydration, double embedding, sectioning, and finally, the use of several histologic techniques will be discussed.

Highlights of pathologic findings and a discussion on a variety of other techniques as they apply to mummified tissue will be presented.

1:00 - 1:30 PM

PREPARATION, EMBEDDING, AND STAINING OF BIOLOGICAL TISSUES IN PURIFIED GLYCOL METHACRYLATE

(John H. McNeil, Jr., B.A.)

Glycol methacrylate (GMA) plastic embedding of biological material preserves tissue structure more faithfully than does paraffin embedding. The water solubility of the GMA monomer and the hydrophilic properties of the GMA polymer allow for convenience in dehydration and versatility in staining of sections. A method for the purification of commercial GMA will be presented. Procedures for fixation, dehydration, embedding, polymerization, sectioning and staining will be discussed. When compared with paraffin, GMA offers opportunities for simpler and quicker procedures and yields thinner sections (0.5-2 µm) of superior quality, less distortion and more cytological detail.

1:30 - 2:15 PM

THE INTERRELATED ROLES OF THE PHARMACOLOGIST, TOXICOLOGIST, HISTOLOGIST AND PATHOLOGIST IN A DRUG DEVELOPMENT PROGRAM

(Miles P. Hacker, Ph.D.)

2:15 - 2:45 PM

PLANT HISTOLOGY: TECHNIQUES AND APPLICATIONS

(Maud A.W. Hinchee, Ph.D.)

Knowledge of plant structure has proved useful in a variety of disciplines. The study of plant anatomy requires the use of basic histological techniques such as free-hand sectioning, paraffin and lastic microtomy, quantitative and qualitative histochemistry, immunocytochemistry, autoradiography, and transmission and scanning electron microscopy. Examples and details of the techniques will be discussed.

3:15 - 4:00 PM

GENETIC COUNSELING

(Michael L. Begleiter, M.S.)

Genetic counseling is a communication process which deals with the human problems associated with the occurrence, or the risk of a genetic disorder in a family. The process involves an attempt to help the family comprehend the medical facts, hereditary factors, reproductive options and choose a course of action and subsequent adjustment. Through the use of appropriate clinical examples, this lecture will address these issues, as well as review the basic principles of human heredity.

No. 22 Repeat No. 33 LIMIT: 40 1:30 PM - 5:00 PM

AVIDIN/BIOTIN AFFINITY METHODS IN HISTOPATHOLOGY

(Robert Weimer, Jr.)

Lectures and wet laboratory to introduce and explain some of the newer and more sensitive methods being utilized in immunohistology. The workshop will discuss and perform the Avidin/Biotin Affinity technique of immunostaining, demonstrate its usefulness, advantages and disadvantages over the more familiar PAP technique. Open to experienced and inexperienced persons.

No. 23 1:30 PM - 5:00 PM

BASIC TECHNIQUES IN DIAGNOSTIC HISTOPATHOLOGY

(Janet Maass, HTL, CT [ASCP])

Workshop will emphasize proper handling of specimens through choice of fixative, record keeping, identification and sampling. Applications of special techniques will be discussed. Participants will be given a self assessment test and handout materials. Recommended for students in the NSH External Degree Program.

No. 24 1:30 PM - 5:00 PM

SUPERVISORY SKILLS

(Jerry Hamilton, M.A.)

Brief introduction to basic skills necessary for effective supervision. A limited introduction to the topics of Selection/Interviewing, Performance Appraisals, Problem Employees, Meeting Management and Group Problem Solving will be presented. Exercises, lecturettes and simulations. Handouts will be provided.

No. 25 Repeat—See No. 19 LIMIT: 30 1:30 PM - 5:00 PM

No. 26 1:30 PM - 5:00 PM

RECOGNITION AND MANAGEMENT OF STRESS IN THE WORKPLACE

(Johnnie W. McClinton, Ph.D.)

This workshop will focus on helping the participants to become aware of what stress is; of what the major stressors are in their lives; of ways to reduce and/or manage their personal stress.

Workshop will include lecture, discussion, video, film, and participant activities.

TUESDAY, OCTOBER 2, 1984

No. 27 8:30 AM - 5:00 PM

HISTOCHEMISTRY

(John P. Koski, B.S., HT [ASCP])

Introduction to histochemistry for those who plan to take the full NSH External Degree course. The workshop will serve as an introduction to histochemical methods by reviewing the biochemical principles of carbohydrates, proteins, nucleic acids, lipids, enzyme mechanisms and immunochemical complexes. The workshop defines reactive groups in terms easily understandable to those who have not had biochemistry. A course study guide is provided. Recommended for students in the NSH External Degree Program.

No. 28 Repeat—See No. 2 LIMIT: 25 8:30 AM - 5:00 PM

No. 29 LIMIT: 30 8:30 AM - 5:00 PM

TECHNIQUES IN PLASTIC FOR LIGHT MICROSCOPY

(Alexandra N. Brady, M.S., MT/HTL [ASCP],
Mary McDonnell, HT [ASCP] & Robert Schoohoven, HTL [ASCP])

35mm slide presentations and "wet" workshops on types of plastics available, advantages and disadvantages of glycol methacrylate, the equipment available, four processing protocols, sectioning, and staining possibilities. Stained slides, blank slides, tissue blocks, glass knives, and manual provided.

No. 30 8:30 AM - 5:00 PM

TROUBLE SHOOTING SPECIAL TECHNIQUES

(Coranelle Lambert, HTL [ASCP])

Outlines problems encountered during certain special staining procedures. Identification and resolution of these problems will be discussed and illustrated. Participants will be able to recognize microscopically technical problems that occur in specific techniques. Procedures to be discussed include: Gomoris methenamine silver nitrate technique, periodic acid-Schiff's reaction, Snook's reticulum technique, and Masson's trichrome stain.

No. 31 8:30 AM - 5:00 PM

HUMAN MICROSCOPIC ANATOMY

(John Ryan, MBA, HTL [ASCP])

Workshop will emphasize basic tissues and their organization into the different organs. Visual identification, function and some special staining correlation will be included. A self assessment test and handout material will be in a workbook format. Recommended for students in the NSH External Degree Program.

No. 32 LIMIT: 40 8:30 AM - 5:00 PM

LABORATORY SAFETY FOR A HEALTHIER WORKPLACE

(Janet I. Minshew, HTL [ASCP] & Judy McKinney, HTL [ASCP])

Workshop will familiarize participants with the safe handling, use, storage and disposal of hazardous chemicals; how to avoid accidents; what to do if accidents occur; what safety equipment should be available; where the safety equipment should be placed, and how to use it correctly. Regulatory agencies and their effect on the laboratory will be discussed.

In the afternoon session a panel will be available for questions and answers regarding waste disposal, ventilation, chemical hazards, federal regulations, and other laboratory safety related problems. At 3:00 p.m. the participants will be bused to the Labconco Corporation facilities for a tour and information regarding ventilation systems.

No. 33 Repeat — See No. 22 LIMIT: 40 8:30 AM - Noon

No. 34 LIMIT: 35 8:30 AM - Noon

KNIFE SHARPENING WITH APPLICATION TO MICROTOMY

(Ernestene Sims, HTL [ASCP])

Basics of sharpening microtome knives and application to the art of microtomy. Emphasis on safe handling and use of knives while sharpening and performing microtomy. Self evaluation of the sharpness of knives will be reviewed along with troubleshooting cutting problems. Demonstration of proper use of the Tissue-Tek, Shandon Mark V and other knife sharpeners will be presented.

No. 35 LIMIT: 30 8:30 AM - Noon

QUALITY SECTIONS FROM WHOLE EYE SPECIMENS

(Mary Knight, HTL [ASCP] & John Tarpley, B.A., HT [ASCP])

Workshop will teach registrants how to produce quality sections from whole eye specimens — human and animal. Emphasis will be on paraffin-embedded material. Applications for glycolmethacrylate embedding of small animal eyes will also be considered. Anatomy, histology, and pathology of human and animal eyes will be reviewed and proper grossing, fixation, embedding, microtomy and staining techniques will be demonstrated.

No. 36 8:30 AM - Noon

ESSENTIALS OF QUALITY EMBEDDING AND THEIR IMPACT ON MICROTOMY

(Billie L. Swisher, HTL [ASCP])

Workshop defines and describes desirable embedding practices. Various types of tissue routinely submitted for paraffin embedding and microscopy will be discussed, emphasizing the specific anatomic areas where disease most frequencty occurs. Tissue grossing section selection and basic tissue identification will be discussed. Color transparencies will be used to correlate gross tissue sections with their microscopic counterparts.

No. 37 1:30 PM - 5:00 PM

HOW TO DEVELOP, ORGANIZE, AND WRITE PROCEDURE MANUALS FOR THE HISTOPATHOLOGY LABORATORY

(Doris A. LePage, B.S., HTL [ASCP] & Joyce D. Eaton, B.S., HTL [ASCP])

Assists the participants in the development and organization of procedure manuals for the histopathology laboratory required by inspection agencies. The participants will be given guidelines for the content of the following manuals: 1. administrative; 2. standard technical histology; 3. special stains; 4. quality control; 5. equipment maintenance; and 6. safety and infection control.

Workshop attendees should bring a histotechnology textbook to aid in writing various procedures.

No. 38 LIMIT 20 1:30 PM - 5:00 PM

MOUSE WHOLE BODY CRYOTOMY

(Jimmy Wesley, HT [ASCP] & Robert Schoonhoven, HTL [ASCP])

Participants in this workshop will be taken via 35mm slide presentation from the injection of an animal with a fluorescent compound to the mounting, freezing and cutting of the specimen to the actual demonstration of the fluorescence. Participants will cut whole body sections on a cryostat set up at the workshop.

No. 39 1:30 PM - 5:00 PM

BASIC CHEMISTRY FOR HISTOTECHNOLOGISTS

(Caryle J. Carr, Ph.D.)

Presents some basic chemical concepts including atomic structure, chemical bonding, molecular structure, chemical reactivities and the classification of compounds, functional groups present in organic compounds, with emphasis on reactive groups found in proteins, lipids, carbohydrates and nucleic acids. Reactions that allow visualization by light microscopy will be discussed. Designed for those with little or no prior knowledge of chemistry.

No. 40 1:30 PM - 5:00 PM

LABORATORY CALCULATIONS

(Dale L. Largent, HTL, MT [ASCP])

Basic knowledge in preparation of molar and normal solutions; correct calculations for weighing and diluting solutions. Bring calculator.

SYMPOSIUM/CONVENTION SCIENTIFIC SESSIONS

WEDNESDAY, OCTOBER 3, 1984

PROFESSOR C.F.A. CULLING MEMORIAL LECTURE

8:30 - 9:30 AM

9:30 - 10:00 AM

STEROID RECEPTOR IN BREAST CANCER

(Lowell Tilzer, M.D., Ph.D.)

Estrogen and Progesterone Receptors are proteins located in the cells of some breast cancer tissue. Presence of these proteins will effect the type of treatment a patient will receive and the prognosis of the patient. The proper handling of the tissue prior to assay and the methods used for the assay will be discussed in addition to the interpretation and usefulness of results.

10:30 - 11:30 AM

ESSENTIALS OF CYTOPREPARATION FOR HISTOTECHNOLOGISTS

(Gary W. Gill, B.A., CT [ASCP])

Cytopreparation is the science of controlling cytomorphology for diagnostic applications. This lecture deals with the laws of biology, chemistry, physics, and optics that act during cytopreparation and can be identified and used to guide the selection of materials and methods. To be presented: (1) the concepts of controlling cytomorphological artifacts, cell flattening, and technically satisfactory results vs. functionally satisfactory results, (2) fresh vs. preserved cell suspensions, (3) using saponin to convert bloody cell suspensions in positive cell spreads, (4) slide preparation, membrane filtration, and cytocentrifugation, (5) immediate wet-fixation vs. air-drying, (6) standard Papanicolaou modified Papanicolaou stains, (7) the proper use of mounting media, (8) cover glasses, (9) controlling image quality by Kohler illumination.

11:30 - Noon

METABOLIC BONE DISEASES DIAGNOSED BY UNDECALCIFIED METHODS

(H. Clarke Anderson M.D., Ph.D.)

Preparation and processing of undecalcified human bone for microscopic histomorphometric analysis. A method of quantitating dynamics of tetracycline labelled bone. Useful in the diagnosis of metabolic bone diseases.

1:00 - 1:30 PM

HT/HTL PRACTICAL EXAMS — HOW ARE THEY CONSTRUCTED?

(Thomas J. Fritzlen, M.D.)

Historical background, means used to establish relative difficulties of different stains and tissue sections, and the present format for constructing new practical exams. A question and answer session will follow the presentation.

1:30 - 2:00 PM

DERMATOPATHOLOGY — GROSS ROOM AND LABORATORY PROCEDURES

(Daniel J. Santa Cruz, M.D.)

The role of the histotechnicians as pathologist assistants. The different procedures involved in processing skin specimens are reviewed. Tips on trimming, specimen orientation and cutting will be given.

2:00 - 2:15 PM

A TIME SAVING MOVAT STAIN

(Konstance Zeitner, HTL [ASCP])

A discussion of the Movat stain in which a number of different stain timing methods are compared. Each yields the same staining result but the most time-efficient method reduces the total staining time by one hour.

2:15 - 3:00 PM

PATHOLOGY AS SEEN BY A MEDICAL EXAMINER

(Harlan L. Papenfuss, M.D.) *Continued 3:30 - 4:15*

Presentation of case histories from the coroner's service to include the pertinent pathology observed. The first series of cases is concerned with infants and children. This is followed by a series of adults dying from natural causes; adults dying accident; adults dying suicide; finally, adults that died as homicides.

4:15 - 4:30 PM

EVALUATING THE QUALITY OF BONE MARROW DECALS

(Theresa R. Chenoweth, B.S., HTL [ASCP])

The lecture will coincide with 35mm slides of bone marrows and examples of overdecalcification with and without proper fixation, cell types, and different H&E staining results.

4:30 - 6:00 PM

NSH MEMBERSHIP MEETING

Everyone Welcome

THURSDAY, OCTOBER 4, 1984

8:30 - 9:30 AM

BASIC HISTOLOGY — "DO YOU MEASURE UP"

(Connie Lummis, B.A., HTL [ASCP])

A slide self assessment presentation covering basic artifacts of fixation, processing, improper embedding, medical settings, staining, and coverslipping.

9:30 - 10:00 AM

PROCESSING RENAL BIOPSIES

(Morton Berlin, MA)

Lecture about the processing of renal biopsies for immunofluorescence and for embedding in glycol methacrylate. Various pit-falls which can occur as well as some helpful hints are discussed.

10:30 - 11:00 AM

CARDIOVASCULAR PATHOLOGY: HOW TO MAKE THE DIAGNOSES

(Bruce M. McManus, M.D., Ph.D.)

Gross morphologic and microscopic diagnoses in cardiac and vascular conditions including pulmonary vascular disease will be presented. Use of special reactions and stains including immunoperoxidase for specific diagnostic situations will be discussed.

11:00 - Noon

CURRENT TRENDS IN DERMAL PATHOLOGY

(Janet M. Chavez, HT [ASCP] & Connie Micko, B.A., HT [ASCP])

Part I: MODIFIED MOHS' CHEMOSURGERY TECHNIQUE: A lecture and slide presentation following a Mohs' surgery candidate from Stage 1 to the complete healing of the wound. Includes current theories on cause of basal cell and squamous cell carcinoma, staining techniques and map drawing. Also, psychological aspects of this surgery, follow-up procedures and the availability of plastic surgery as a method of wound treatment.

Join NSH now and take advantage of special "Member" rates for Symposium Workshops

Check the Symposium Fee Schedule and you will find that NSH members save $10 to $30 on listed Workshops and Scientific Sessions. If you are planning to attend the Symposium, and you aren't already a member of NSH, apply now and take advantage of the reduced rates.

Membership also includes a subscription to the Journal of Histotechnology. In addition, you'll be participating in a professional group that supports histotechs nationwide. Don't delay! Share the learning opportunities NSH provides and join a dynamic and growing group. . . today. Fill out the application below and mail it with your check to: NSH, 5900 Princess Garden Pkwy., #805, Lanham, MD 20706.

National Society for Histotechnology, Inc.

APPLICATION FOR MEMBERSHIP *(Open to Any Person With an Interest in Histotechnology)*

NAME _____

ADDRESS _____

_____

_____

TELEPHONE # _____ Social Security # _____

Place of Employment _____

Address _____

_____

Telephone # _____

Signature _____ Date _____

$10 of dues applied to Journal of Histotechnology subscription
PROFESSIONAL SOCIETY DUES ARE TAX DEDUCTIBLE

Date of Birth _____

PLEASE CHECK ALL APPLICABLE BOXES:

- ☐ HT (ASCP)
- ☐ HTL (ASCP)
- ☐ MT (ASCP)
- ☐ CT (ASCP)
- ☐ RT (CSLT)
- ☐ ART (CSLT)
- ☐ Other _____
- ☐ Not Certified

- ☐ AA
- ☐ BA/BS
- ☐ MA/MS
- ☐ PhD
- ☐ MD
- ☐ DVM
- ☐ Other _____

- ☐ University
- ☐ Hospital
- ☐ Private Lab
- ☐ Veterinary
- ☐ Marine
- ☐ Botany
- ☐ EM
- ☐ Research
- ☐ Industrial

Dues January–May, $15

Annual dues $30.00 United States funds
Please remit to 5900 Princess Garden Pkwy., Suite 805, Lanham, MD 20706

Symposium Registration Form/Fee Schedule

Name: _____
(last) (first) (initial)

Home Address: _____
(street)

_____
(city) (state) (zip)

Employer: _____

Address: _____
(street)

_____
(city) (state) (zip)

Work Telephone No. (_____) _____
area code

DO NOT USE THIS SPACE

Are you an NSH Member? Yes_____ No_____

Is this your first attendance to an NSH Symposium/Convention? Yes_____ No_____

_____ Yes, desire room sharing information.

_____ Hearing impaired; require interpreter for attendance.

On fee schedule please indicate first and second choice for limited workshops, marked with an asterisk.

Check to ensure registration is for ONE all-day workshop, or an AM and PM combination.

PRE-REGISTRATION DEADLINE, September 15th
Please remit fees in U.S. currency.
Return form with check/money order to: National Society for Histotechnology, 5900 Princess Garden Pkwy, #805, Lanham, Maryland 20706.

SUNDAY

		Member Fee	Non-Member Fee
All Day	#1*	_____$50	_____$65
All Day	#2*	_____$50	_____$65
AM	#3*	_____$25	_____$35
AM	#4*	_____$25	_____$35
AM	#5	_____$25	_____$35
AM	#6	_____$25	_____$35
PM	#7	_____$25	_____$35
PM	#8*	_____$25	_____$35
PM	#9	_____$25	_____$35
PM	#10	_____$25	_____$35

MONDAY

		Member Fee	Non-Member Fee
All Day	#11*	_____$50	_____$65
All Day	#12*	_____$50	_____$65
All Day	#13	_____$50	_____$65
All Day	#14	_____$50	_____$65
AM	#15*	_____$25	_____$35
AM	#16*	_____$25	_____$35
AM	#17	_____$25	_____$35
AM	#18	_____$25	_____$35
AM	#19*	_____$25	_____$35
AM	#20	_____$25	_____$35
PM	#21*	_____$25	_____$35
PM	#22*	_____$25	_____$35
PM	#23	_____$25	_____$35
PM	#24	_____$25	_____$35
PM	#25*	_____$25	_____$35
PM	#26	_____$25	_____$35

Totals: _____

TUESDAY

		Member Fees	Non-Member Fees
All Day	#27	_____$50	_____$65
All Day	#28*	_____$50	_____$65
All Day	#29*	_____$50	_____$65
All Day	#30	_____$50	_____$65
All Day	#31	_____$50	_____$65
All Day	#32*	_____$50	_____$65
AM	#33*	_____$25	_____$35
AM	#34*	_____$25	_____$35
AM	#35*	_____$25	_____$35
AM	#36	_____$25	_____$35
PM	#37	_____$25	_____$35
PM	#38*	_____$25	_____$35
PM	#39	_____$25	_____$35
PM	#40	_____$25	_____$35

_____

WEDNESDAY - THURSDAY - FRIDAY

	Member Fee	Non-Member Fee
Scientific Sessions (Wed.-Fri.)	_____$60	_____$95
Single Day Fee (Circle: Wed., Thur., Fri.)	_____$30	
BANQUET (Thursday)	_____$20	

TOTAL REGISTRATION

Sunday Workshops	$_____
Monday Workshops	_____
Tuesday Workshops	_____
Scientific Sessions	_____
Banquet	_____
Late Fee $10 (After Sept. 15)	_____
TOTAL FEES:	_____

NATIONAL SOCIETY FOR HISTOTECHNOLOGY
SYMPOSIUM/CONVENTION
SEPTEMBER 30–OCTOBER 6, 1984

Type of Room	No. of Rooms	Convention Rates	*Regency Club*
Single (1 person)		$56-$68-$80	$85
Double (2 people)		$73-$83-$95	$100
1 Bed. Rm. Suite		$125-$400	
2 Bed. Rm. Suite		$185-$500	

If all rooms in the requested rate category are already reserved, the next available rate will be assigned.
*Regency Club accommodations include special guest room amenities and continental breakfast served in our exclusive Regency Club Lounge.

Reservations must be received by Sunday September 9, 1984

Date of Arrival _____
I will arrive via _____
Time of Arrival _____
Date of Departure _____
Check in Time: 3 p.m.
Check Out Time: 12 Noon
Name _____
_____
Address _____
_____
_____
Telephone No. _____
Sharing room with _____
_____

Your reservation will be held until 6 p.m. unless one night's deposit is received or guaranteed by credit card below. Failure to cancel 24 hours prior to arrival will result in 1 night's charges billed to your credit card.
☐ Hold until 6 p.m. only.
☐ Guaranteed by one of the following.
Deposit of $ _____
American Express # _____
Diners Club # _____
Carte Blanche # _____
MasterCard # _____
Visa # _____
Expiration Date _____

Signature _____

An Elastin Stain

P.J. Miller
King's Mill Hospital, Sutton-in-Ashfield, Nottinghamshire, England

A method is described to demonstrate elastin fibers in tissue using crystal violet, new fuchsin and Victoria blue 4R. The method fulfills the following requirements:

1. Precise demonstration of both coarse and fine fibers with high selectivity, eliminating the need for differentiation.
2. Intense staining, preferably black, to provide maximum contrast with the usual counterstains especially Van Gieson.
3. A stable staining reagent.
4. Fixation of tissues in 10% buffered formol saline that does not preclude good results.
5. Good staining results possible within a short time.

Fixation:
Satisfactory results are obtained from tissues fixed in 10% buffered formol saline, formol sublimate, Heidenhain's Susa, Helly, Zenker, Clarke's and Bouin. Cryostat sections fixed in Wolman's fixation also give good results.

Solutions:

Elastin Staining Solution

Victoria blue 4R (C.I. 42563)	1.0 gm
New fuchsin (C.I. 42520)	1.0 gm
Crystal violet (C.I. 42555)	1.0 gm

Dissolve above dyes in 200 ml of hot distilled water, then add in the following order:

Resorcin	4.0 gm
Dextrin	1.0 gm
30% ferric chloride (freshly prepared)	50.0 ml

Boil solution for 5 minutes, then filter while hot. Transfer precipitate plus filter paper to original beaker and redissolve in 200 ml of 95% alcohol. Boil on a hot plate, or in a water bath for 15-20 minutes. Filter and make up to 200 ml with 95% alcohol. Finally, add 2 ml of concentrated hydrochloric acid.

0.5% Potassium Permanganate

Potassium permanganate	0.5 gm
Distilled water	100.0 ml

5% Oxalic Acid Solution

Oxalic acid	5.0 gm
Distilled water	100.0 ml

Van Gieson Solution

Acid fuchsin	0.1 gm
Picric acid, saturated solution	100.0 ml

Staining Procedure:
1. Deparaffinize slide and hydrate to water.
2. Place slides in 0.5% potassium permanganate for 5 minutes.
3. Rinse slides in distilled water.
4. Decolorize slides in 5% oxalic acid for 2 minutes.
5. Rinse slides in distilled water.
6. Rinse slides in 95% alcohol.
7. Place slides in elastin staining solution for 3 hours. Or, place in 50% elastin solution and 50% of 95% alcohol for overnight staining.
8. Wash slides in 95% alcohol to remove excess stain.
9. Wash slides in distilled water.
10. Counterstain in Van Gieson for 1½ to 2 minutes.
11. Dehydrate in 95%, absolute alcohol and clear in xylene, 2 changes each.
12. Mount coverglass with resinous mounting media.

Results:

Elastic fibers	—Black
Mast cell granules	—Black
Cytoplasm	—Depends on counterstain used.

Discussion:
The iron resorcin dye lake of Weigert[1] using basic fuchsin was modified by French[2], with the addition of crystal violet and dextrin. Fullmer and Lillie[3] substituted orcinol for the resorcin and new fuchsin for the basic fuchsin. Humberstone and Humberstone[4] modified the Weigert-French formula by using Victoria blue 4R and ethyl violet instead of basic fuchsin and crystal violet.

The original method of Weigert was further modified by the amalgamation of crystal violet, new fuchsin and Victoria blue 4R.

Treatment of the section with potassium permanganate and oxalic acid improves the staining quality especially after fixation in an acetic acid containing fixative.

Conclusion:
The method described stains elastin selectively and precisely, and no differentiation is required. The staining reagent does not deteriorate and may be used repeat-

The Cutting Properties of Wide Edge "Histo" (Ralph) Knives

Stewart Chew
USC, Orthopaedic Hospital
Los Angeles, California 90007
and
WAIT Bentley
Western Australia 6102

The use of "Histo" or Ralph knives for histology is well documented,[1,2] and a comparison of these wide edge glass knives to the narrow edge standard EM knives can be useful to histologists. Ralph knives differ in principle from standard EM knives through the manner in which they are fractured. It is the width of the strip of glass rather than its thickness that determines the length of the resulting Ralph knife edge. Based on this principle, a much wider edge is thus possible.

It is well known that the standard EM knife edge varies in sharpness. The first third of the edge that is tangential to the stress line is the area that is suitable for sectioning. Does this similarly apply to Ralph knives? Within the width of one knife edge, are there also variable zones of cutting excellence? The answer to these two questions is "no."

A Ralph knife, based on the principle by which it is fractured, should be uniform—be it uniformly good or bad. During the manufacturing of a Ralph knife, the score with the cutting wheel is scribed on the *opposite* plan *parallel* to the future knife edge. However, in the case of the standard EM knife, this weakness on the glass surface is made at right angles to the future edge. Whereas the edge of the Ralph knife is instantly created when a suitable fracturing force is applied, the edge of the standard EM knife trails the line of fracture. The direction of travel of the fracture which runs along the knife edge is responsible for the variable cutting zones in the standard EM knife.

References:

1. Bennett, H.S., Wyrick, A.D., Lee, S.W. and McNeil, J.H., *Stain Technology* 51: 71, 1976.
2. Lindner, M. and Richards, P., *Sci. Tools*, 25:61, 1978.

WINNERS!

Our 10 great Tissue-Tek® Cassette colors now have 10 great names! Miles Scientific sends its congratulations and a Canon 35mm camera to each of the 10 winning "name-droppers" listed below!

1 MARIGOLD•Donna Singular
2 BAMBOO•Debra Watters
3 BUTTERCUP•Brett Southard
4 TANGERINE•Katherine Wills
5 RASPBERRY•Karen Flessland
6 PINK CARNATION•Lynn Orlowitz
7 BLUEBERRY•Diane Hawkins
8 SHAMROCK•Tom Peterson
9 EDELWEISS•Holly VanWegen
10 KELP•Anthony Noto

edly. Reliable results are obtained on tissues fixed in a wide variety of fixatives. Counterstaining with Van Gieson or Masson trichrome yields excellent results.

The author wishes to thank Mr. P. Hatfield for his assistance and encouragement in the production of this manuscript.

References:

1. Weigert, C., Ueber eine methode zur farbung elastischer fasern, *Zentralblatt fur Allgemeine Pathologie und Pathologische Anatomie*, 9:289, 1898.
2. French, R.W., Elastic Tissue Staining, *Stain Technology*, 4:11, 1929.
3. Fullmer, H.M. and Lillie, R.D., A Selective Stain for Elastic Tissue, *Stain Technology*, 31:27, 1956.
4. Humberstone, G.C.W. and Humberstone, F.D., An Elastic Tissue Stain, *J. Medical Laboratory Technology*, 26:99, 1969.

A Rapid Trichrome Stain

Osvaldo Jose Vilar
Naval Hospital of Buenos Aires
Buenos Aires, 1405
Argentina

I have found the trichrome stain presented below to be quite satisfactory in several regards. It is a rapid procedure, it produces consistent results, and it is economical, since it employs some of the most common dyes found in the pathology laboratory. The time varies according to the nuclear stain used. For example: Mayer's hematoxylin requires 15 to 20 minutes, Weigert's iron hematoxylin requires 10 minutes, while Murray's hematoxylin needs only 2 minutes. The staining results vary, as well: black nuclei results when Weigert's iron hematoxylin is used, red when Kernechtrot is used, and blue when Mayer's alum hematoxylin is used.

Fixation:
10% buffered neutral formalin

Microtomy:
Cut paraffin sections at 6 microns

Solutions:

Acid Fuchsin-Orange G Solution

Acid fuchsin (C.I. 42685)	1.0 gm
Orange G (C.I. 16320)	0.4 gm
Glacial acetic acid	3.0 ml
Distilled water	100.00 ml

Light Green Solution

Light green (C.I. 42095*)	2.0 gm
Glacial acetic acid	2.0 ml
Distilled water	100.0 ml

*Aniline blue (C.I. 42755), 0.1 gm may be substituted.

Staining Procedure:

1. Deparaffinize slides through 2 changes of xylene, absolute and 95% alcohols to distilled water as usual.
2. Stain nuclei with nuclear fast red, 10 minutes; Weigert's iron hematoxylin, 10 minutes; or Mayer's hematoxylin, 10 minutes.
3. Wash in tap water (the time depends on the nuclear stain used).
4. Stain in acid fuchsin-orange G solution for 30 to 40 seconds.
5. Wash in distilled water for a few seconds.
6. Stain in light green solution for 30 to 40 seconds.
7. Wash in distilled water.
8. Differentiate in absolute alcohol, 2 changes.
9. Clear with 2 changes of xylene.
10. Mount coverslip with resinous mounting media.

Results:

Nuclei	according to the nuclear stain used
Muscle fibers	taupe or black
Connective tissue	pale green if light green solution is used; pale blue if Aniline blue solution is employed.

Fig. 1 illustrates some of these results.

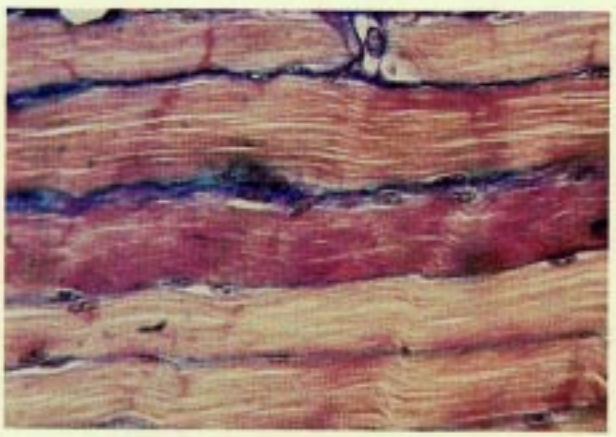

Figure 1. Section of heart muscle is demonstrated in its characteristic taupe color reaction with green connective tissue seen surrounding the muscle fiber.

(The author wishes to thank Mr. Osvaldo Delgado for the photomicrograph.)

References:

1. Luna, L.G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition, McGraw-Hill, New York, The Blakiston Div., 1968.
2. Lillie, R.D. *Histopathologic Technic and Practical Histochemistry*, McGraw-Hill, New York, The Blakiston Div., 1954.
3. Conn, H.J., *Biological Stain*, Sixth Edition, Biotech Publications, Geneva, N.Y., 1953.

Tissue-Tek® Immunohistology Kits

- 38 PAP kits for enzymes, hormones, immunoglobulins, viral antigens, tissue specific markers and other cellular antigens.
- Demonstrate tissue antigens or disease status to supplement routine morphologic analysis.
- Visualize the antigen-antibody complex with a standard light microscope.
- Each kit contains reagents for 30 tests.
- Reliable results with paraffin sections. Useful with frozen sections, smears, imprints or cytospins.

Stains You Can Identify With *Routinely!*

Miles Scientific
Division of Miles Laboratories
30W475 North Aurora Road
Naperville, IL 60566

Miles Scientific

MILES

Miles Scientific
Division of Miles Laboratories, Inc.
30W475 North Aurora Rd.
Naperville, IL 60566

Address Correction Requested

Histo-Logic

A Technical Bulletin for Histotechnology

BULK RATE
U.S. POSTAGE
PAID
PERMIT NO. 4954
Chicago, Illinois

To receive your own copy of HISTO-LOGIC, or to have someone added to the mailing list, submit home address to: **Miles Scientific,** Division of Miles Laboratories, Inc. 30W475 No. Aurora Rd., Naperville, IL 60566.

Printed in U.S.A.

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 20706. Articles, photographs, etc., will not be returned unless requested in writing then they are submitted.