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The Demonstration and Quantitation of Neuroendocrine Cells of the Rat Stomach

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The development of drugs used to block parietal cell stimulation, and therefore decrease gastric acid production, represented a major advance in the therapy of peptic ulcer disease. A second generation of more potent, longer-acting compounds has been investigated, and it has been shown that treatment with some of these agents results in an increase in neuroendocrine cell numbers and mucosal height in the rat oxyntic stomach.¹⁻³ These increases lend themselves to evaluation by morphometric methods. To ensure meaningful results, it is essential that representative areas of the stomach are sampled.

Neuroendocrine Cells of the Rat Stomach

The neuroendocrine cell system is a group of diffusely distributed endocrine cells that show biological similarities to neurons. These cells are found throughout the body, including the gastrointestinal tract. Many cell types have been described in humans and were last classified by Solcia et al.⁴ 1981. There are species variations with six cell types being described in the gastric mucosa of the rat (Table 1). The major cell types in the fundic oxyntic mucosa are the ECL (65% to 66%) and the A-like cells (24% to 25%).

These cells are classified by their characteristic ultrastructural appearance, based mainly on the morphology of their storage granules (Table 2) and on the cell product.⁵⁻⁹ The identification techniques available include argentaffin and argyrophil silver methods, electron microscopy, and immunocytochemical demonstration of cell products. It is the latter that provides an easily reproducible, light microscopical method. Table 3 shows antigens that have been demonstrated using the Peroxidase-

Anti-Peroxidase (PAP) technique on formalin-fixed paraffin sections.

Experimental Methods

The method employed in our laboratory involves standard sampling of rat gastric mucosa. The stomach is opened along the greater curvature, pinned out on cork board and fixed in neutral-buffered formalin. Strips of mucosa were selected from the fundic area, and this tissue was processed to paraffin wax and sectioned at 4 micrometers. Care was taken to ensure that a cross

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No reader should utilize or undertake procedures 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.

section of mucosa was obtained. Neuroendocrine cells were demonstrated using a primary antiserum against chromogranin A and the PAP immunocytochemistry method. Quantitation involved measuring the area of immunocytochemical staining within a known length of mucosa. These measurements were carried out using an image analysis system—Quantimet 920 (Cambridge Instruments, Cambridge, UK). The image was detected using a Plumbicon scanner and was converted to a binary image. A gray level threshold was set to detect only chromogranin A positive neuroendocrine area within a field of fixed width. The measurement frame was set extending from the line of muscularis mucosa up to the luminal mucosa surface. These measurements gave a representation of cell number, and, therefore, the magnitude of cell increase could be measured. The oxyntic mucosal height was measured on the same field.

This methodology has been employed in the evaluation of gastric hyperplasia following chronic administration of H₂-antagonist and proton pump inhibitors² and also in untreated rat stomach. By mapping the position of each measured field, a series of profiles has been produced. Figure 1 shows a plot of mucosal height and Figure 2 of neuroendocrine cell area. These figures represent a rat stomach opened along the greater curvature; the forestomach is shown as "a"; the fundic area, "b"; and the pyloric area leading to the duodenum, "c." This shows that care should be taken regarding which area of the tissue should be sampled to give a representative evaluation.

Conclusion

Six types of neuroendocrine cells have been described in the gastric mucosa of the rat. These may be identified using immunocytochemical demonstration of chromogranin A (Figs. 3 and 4), which gives a reliable, reproducible light microscopic method. Changes in the neuroendocrine cell population and oxyntic mucosal height may be evaluated concurrently on a single preparation, using image analysis.

TABLE 1
Neuroendocrine Cells
of the Gastric Mucosa in the Rat

| Cell Type | Mucosal Location | | Cell Product |
|--|------------------|--------|--------------------------|
| | Pyloric | Fundic | |
| EC Enterochromaffin | + | - | 5-hydroxytryptamine |
| G Gastrin | + | - | Gastrin |
| ECL Enterochromaffinlike | - | + | Histamine (rodents only) |
| A-like | - | + | Histamine (rodents only) |
| Endocrinelike cell of the lamina propria | - | - | Unknown |
| D-cell | + | + | Somatostatin |

TABLE 2
Ultrastructural Characterization
of Neuroendocrine Cell Granules in the Rat

| Cell Type | Shape | Size | Core | Halo |
|-----------|-------------|--------------------|---|----------------|
| D | Round | 100-200 nm | Poorly osmiophilic | No halo |
| EC | Pleomorphic | Small 50-150 nm | Highly osmiophilic | |
| ECL | Round | | Vesicular Occasional dense core | |
| G | Round | 150-400 nm | Loose, flocculous, clotted or filamentous | No halo |
| A-like | Round | 200-250 nm | Dense | Regular, clear |
| LP | Round | 200-500 nm | High electron dense 100-200 nm | Wide space |

TABLE 3
Immunocytochemical Marking
of Neuroendocrine Cells in the Rat

| Antigen | Neuroendocrine Cell(s) Demonstrated |
|-------------------------------|-------------------------------------|
| Chromogranin A | All gastric cells |
| Neuron Specific Enolase (NSE) | All gastric cells |
| PGP 9.5 | All gastric cells |
| Histidine decarboxylase (HDC) | ECL cells |
| Somatostatin | D cells |
| Gastrin | G cells |

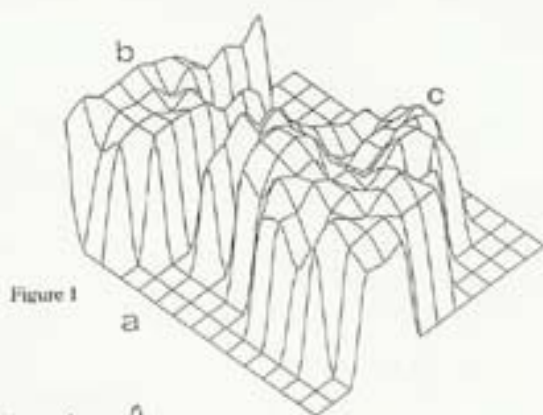


Figure 1

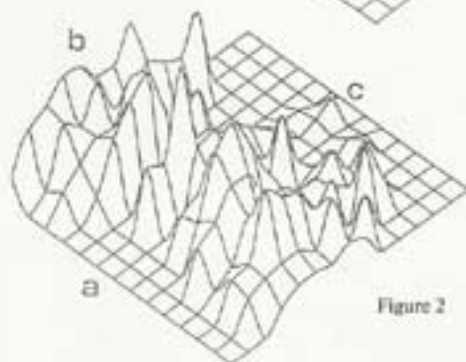


Figure 2



Figure 3: Low-power demonstration of chromogranin A in the fundic mucosa of the rat stomach.

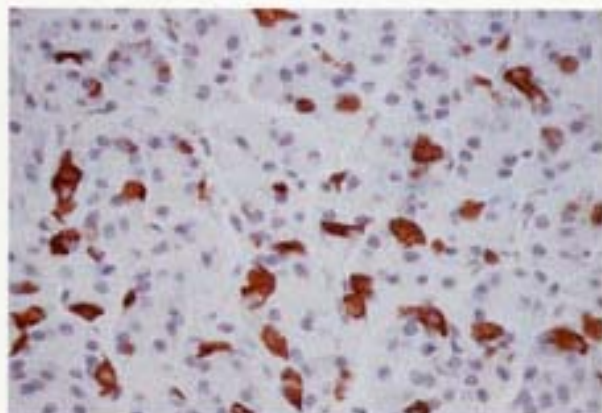


Figure 4: High power of Figure 3 to illustrate the well-demarcated chromogranin A positive cells.

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Implementing a Special Stain Program

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The purpose of this paper is to provide an overview in some areas and specific details in others of how to implement a special stain program. When I arrived at my present position, the only special stains that were being performed were Grocott methenamine silver, iron, and acid fast bacilli. Implementing a special stain program was both challenging and enjoyable. My first step was to determine which special stains our pathologists would need to assist them in providing a diagnosis. After completing a list, the next steps were to:

- Find the most useful procedure for the best results.
- Make a list of all chemicals needed (order if not in stock).
- Test solutions and control blocks.
- Train personnel in the special staining procedure we wished to implement.

I will list in order what was done at our hospital to implement this program. This is not to say that this is all inclusive or the best; it is an approach to organizing that I hope can be of help to someone who is having difficulty implementing a special stain program.

Procedures

We use 5×8 cards and type the procedures in numerical order with the results at the bottom. These cards are placed in a cardex for easy access. The cards may also be attached onto cabinets with the aid of a magnetic clip for quick reference. There is also an additional 5×8 card behind the procedure that provides information on how to make the solutions and maintains an ongoing inventory of expiration dates.

Chemicals/Dyes

The chemicals and dyes are separated into two areas, alphabetically and chronologically. There is an index of both the chemicals and dyes outside the cabinet for easy retrieval.

Labeling Solutions

Using a computer, we preprint labels for our solution bottles. The following information is on the label:

- Reagent:
- Stain:
- Date made:
- Expiration date:
- Location: (refrigerator, shelf, etc.)
- Comments: (pH tested, refilter, mixing, etc.)

Solution Storage

All solutions can be kept together in a final bin/drawer in an organized fashion or placed on a shelf in alphabetical order.

Control Slides

Tissue that is suspected of being positive for a specific entity should be stained with an existing known control to test its results. If the tissue is found positive, a series of sections should be cut at 50-slide increments labeling the first and last. If both the 1st and 50th slides are positive, it is safe to assume that all slides in between are also positive. Both slides should be kept with the unstained slides for verification and training. An additional 50 slides may be cut when needed and stained as mentioned above.

Tissues that are inherently normal for the substance being stained (basement membrane, elastic, reticulum, collagen, etc.) can be serially sectioned and labeled according to the entity being demonstrated.

Control slide boxes are labeled with the following information: tissue, stain, and substance being stained for, such as:

| | |
|----------------|------------------------|
| Skin | |
| Elastic fibers | Verhoeff's, Van Gieson |
| Mast cell | Luna's mast cell |
| RNA/DNA | MGP |
| Reticulum | Snook's |

Special Stains Quality Control

A quality stain control sheet should have the following:

- Date:
Stain: Stain performed
Case Numbers:
Control Number: (if applicable)
Result: Positive/negative
Correct Action: (if negative)

Note

If a control slide is negative, the following should be checked:

- Solutions improperly made, out of date, wrong solutions for stain, or contaminated.
- Control tissue not positive or positive entity no longer in section area cut through during microtomy.
- Procedure not properly followed.

A Rapid Method for Fungi and *Pneumocystis carinii*

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A recent increase in the number of specimens received from immunocompromised patients has drawn attention to the methods in use to demonstrate the presence of fungi and *Pneumocystis carinii* in tissue sections.

For many years, Grocott's modification¹ of Gomori's methenamine silver technique² has been the method of choice in many laboratories. This method, although very reliable, is rather time consuming, taking up to 2 hours to complete if one takes into account reagent preparation time.

As requests to demonstrate these organisms became more frequent, several modifications of the older method have been proposed. Most of these have attempted to shorten the time, and some have suggested the use of the microwave oven to achieve this aim.³⁻⁵

Another modification published recently⁶ suggested the use of an ammoniacal silver solution to replace the original methenamine silver solution with the object of reducing the background staining often seen in Grocott's method. The intensity of this background staining is unpredictable, sometimes being sufficiently intense to

(continued on page 190)



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mask small fungal structures. This problem can be particularly troublesome in sections of skin where large amounts of collagen or elastic fibers may be present.

The use of ammoniacal silver was also suggested by Churukian et al⁷ and again by Churukian and Schenk.⁹ In the latter paper, the addition of lithium hydroxide to the ammoniacal silver solution was recommended in order to extend its shelf life. However, it was found that although the addition of lithium hydroxide produced excellent results in unfixed specimens, i.e., bronchial washings, better results were obtained with paraffin sections if the lithium hydroxide was omitted and the ammoniacal silver solution was freshly made for each staining batch and then discarded.

The method presented here combines the elements of several previous modifications of Gromori's method to produce an exceptionally "clean" preparation free of significant background staining, which can be completed within 40 minutes including reagent preparation time.

Fixation, Processing, and Microtomy as Used in Our Laboratory

Tissues are fixed in 10% neutral buffered formalin, processed on an overnight schedule in a Tissue-Tek V.I.P. Tissue Processor using graded ethanols as a dehydrant and Shellex X3B as a clearing agent, then infiltrated with and embedded in Medim histological wax (M.P. 59°C). Sections were cut at 3 micrometers on a Leitz 1512 rotary microtome using a feather S35 disposable microtome blade, placed onto glass slides coated with Elmer's glue, and dried in a slide-drying cabinet at 50°C for a minimum of 30 minutes.

Solutions

5% Chromium Trioxide (Chromic Acid)

Chromium acid 5.0 gm
Distilled water 100.0 mL

2% Sodium Metabisulfite

Sodium metabisulfite 2.0 gm
Distilled water 100.0 mL

0.2% Sodium Gold Chloride

Sodium gold chloride 0.2 gm
Distilled water 100.0 mL

5% Sodium Thiosulfate

Sodium thiosulfate 5.0 gm
Distilled water 100.0 mL

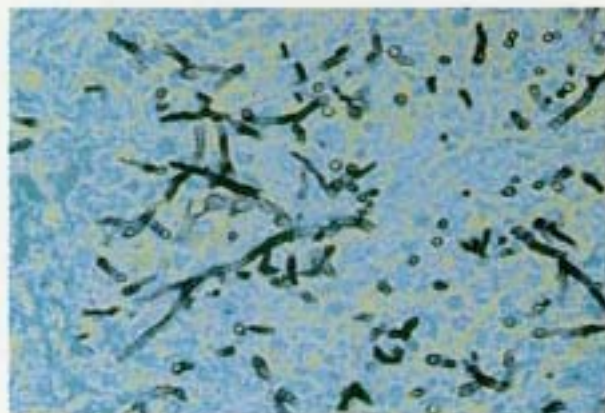


Figure 1: *Aspergillus* in lung. Microwave Grocott method. (Original magnification $\times 400$)

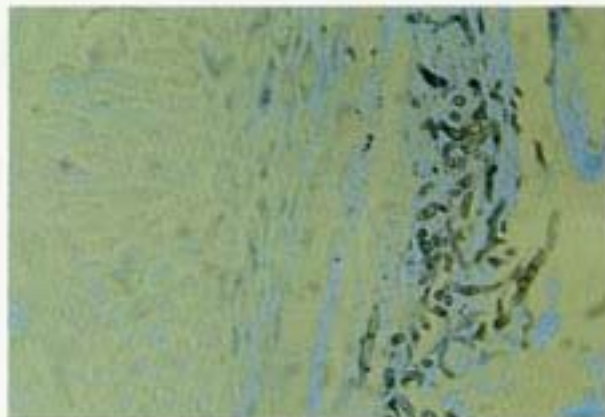


Figure 2: Fungal elements in the stratum corneum in a section of skin. Microwave Grocott method. (Original magnification $\times 400$)

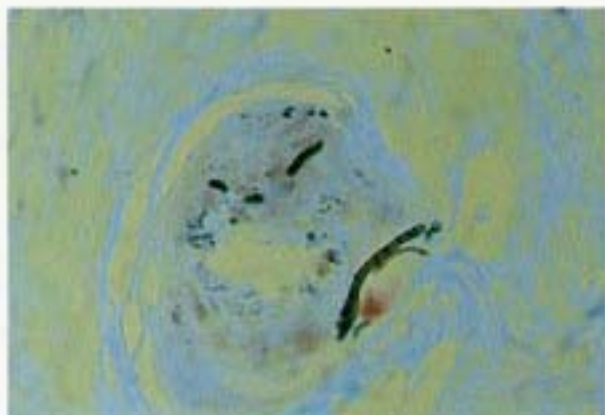


Figure 3: Fungal elements in hair follicle. Note minimal background staining. Microwave Grocott method. (Original magnification $\times 400$)

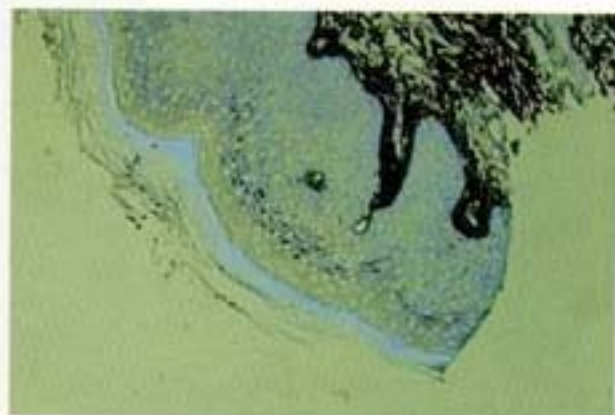


Figure 4a: Fungal elements in skin. Standard Grocott method. Note considerable staining of elastin and collagen. Compare with Figure 4b. (Original magnification $\times 225$)



Figure 4b: Same tissue as in Figure 4a. Microwave Grocott method. Note marked reduction in staining of elastin and collagen. (Original magnification $\times 225$)

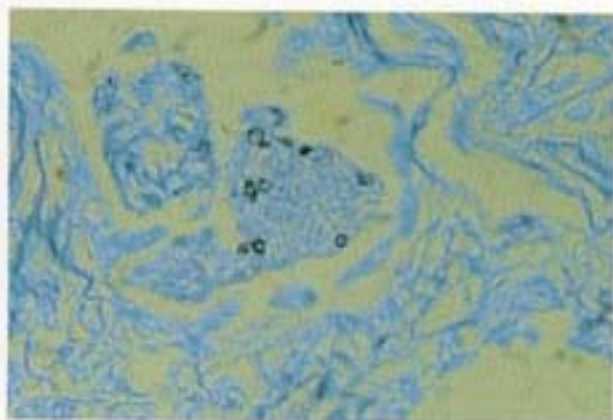


Figure 5: *Pneumocystis carinii* in lung. Microwave Grocott method. (Original magnification $\times 400$) Note the well-defined organisms in a light-green background.

Light Green-Acetic Acid

| | |
|-----------------|----------|
| Acetic acid | 2.0 mL |
| Distilled water | 100.0 mL |
| Light green | 2.0 gm |

Ammoniacal Silver Solution

Add strong ammonium hydroxide drop by drop to 5 mL of 10% silver nitrate until a precipitate forms, then just dissolves. Bring the volume to 50 mL with distilled water and mix. (Use acid-washed glassware.)

Staining Procedure

1. Decerate sections in selected agent and bring to distilled water.
2. Place sections in 5% chromic acid at room temperature for 20 minutes.
3. Wash sections in running water for 5 minutes.
4. Treat with 2% metabisulfite for 2 minutes.
5. Wash in running water, then rinse in distilled water.
6. Fill a plastic slide-mailer (previously acid washed and well rinsed in distilled water) with ammoniacal silver solution and place in the microwave oven.
7. Microwave ammoniacal silver at 450 watts for 35 seconds to bring the temperature of solution to approximately 70°C.
8. Place the sections into the hot silver solution and return the slide-mailer to the microwave oven.
9. Microwave at 80 watts for 2 minutes.
10. Remove the slide-mailer from the oven and allow it to stand at room temperature for 1 minute.
11. Remove the sections from the mailer and rinse well with distilled water.
12. At this stage the sections should be golden brown in color.
13. Check microscopically the staining intensity of the organisms in the control sections. These should be dark brown. If they are too light, return the sections to the hot silver solution until the desired staining intensity is achieved. Recheck slides at 20- to 30-second intervals.
14. Rinse the sections thoroughly in distilled water.
15. Tone in 0.2% gold chloride for 30 seconds.
16. Rinse in distilled water.
17. Treat with 5% sodium thiosulfate for 2 minutes.
18. Wash in water for 1 minute.
19. Counterstain in 2% light green for 1 minute.
20. Rinse in water for 20 seconds.
21. Dehydrate fairly rapidly, clear and mount coverglass with appropriate media (refractive index 1.48-1.56).

Results

| | |
|--|-----------------|
| Fungi, <i>P. carinii</i> , mucin, glycogen | brown to black |
| Other tissue elements | shades of green |

Remarks

In order to reduce the time to perform this method, some authors⁹ have advocated the use of chromic acid at 58°C for a shorter period of time. In my experience, however, this can result in sections becoming detached from the slides, especially when attempting to stain sections of skin. Also, Hinds⁸ and Churukian¹⁰ warn against the use of the microwave oven to heat chromic acid solutions, as damage may be caused to the oven by a phenomenon known as *arcing*.

As a control section for use with this method, I recommend a fungus such as aspergillus rather than a section containing *P. carinii*. The reason for this is that although *P. carinii* is clearly demonstrated in the finished stain, it is rather difficult to see at Step 13, as the foamy material typically found in the alveoli tends to obscure the *P. carinii* organisms. After gold chloride toning (Step 14), the staining of the background material is reduced markedly and the *P. carinii* organisms are well demarcated.

The microwave oven in use in this laboratory is a Miele, model M696, with four power settings from 80 to 700 watts. I suspect that other combinations of power and time settings would be just as effective as those given above, although those quoted give the operator some latitude in the handling of the sections without the risk of overheating and possibly boiling the silver solution. Removing the slides from the oven after 2 minutes and allowing the reaction to continue on the bench gives the operator better control over the final impregnation density than trying to reach an exact end point in the oven, with the risk of overimpregnation, which is difficult to correct.

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Twelfth Annual Practical Stain-Technology "Wet" Workshop and Seminar

April 7-12, 1991

Presented by: Lee G. Luna
The Center for Histotechnology Training

Workshop Content

This 5-day, extensive "wet" workshop and seminar will afford the registrants the opportunity to perform 25 special stains, 6 microwave, and 5 immunochemical staining procedures demonstrating more than 50 pathologic entities.

Some of the entities that will be stained are: human papilloma virus; insulin; glucogen; S-100 protein; hepatitis B surface antigen; helicobacter pylori; cat scratch disease bacilli; Lyme disease spirochetes; Legionnaire's disease bacilli; *Pneumocystis carinii*; all pathogenic fungi; gram-positive (*Staphylococcus aureus*) and gram-negative (*Pseudomonas*) bacteria; copper; iron; senile plaques and fibrillary tangles; leprosy; tubercle bacilli; malaria parasites; toxoplasma; amyloid; plasma cells (RNA and DNA); *Leptospira*; actinomycosis; elastin; calcium; granules from the neuroendocrine system; and myocardial infarctions. Staining of additional entities will be discussed. Also, the proper tinctorial qualities of a good hematoxylin and eosin (H&E) will be discussed in great detail. A problem slide, tissue, staining, problem-solving session will be conducted during the course. You are, therefore, encouraged to bring questions and/or problem slides to the course for this session. Microscopes will be available for slide study.

In addition to the practical special staining aspects, lectures will be presented daily (to include evenings) on Chemistry of H&E Staining; Silver Reactions; Fixation; Effects of Processing on Microtomy and Staining; Staining Mechanisms; Tissue Artifacts; Handling of Small Specimens; Tissue Identification for Histotechnologists (six lectures); Microtomy; Preferred Controls; Introduction to Immunochemistry Staining; Decalcification and various other subjects directly related to the identification of technical problems and the production of high

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Histo-Logic Salutes These Award-Winning Histotechnologists

The following awards were presented at the NSH Symposium Banquet, September 13th, 1990, at San Antonio, Texas:

Golden Forceps Award

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Leonard Noble
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Sponsored by Surgipath Medical Industries, Inc.
Lee G. Luna
Lanham, Maryland

Histotechnologist of the Year Award

Sponsored by Shandon, Inc.
Dorothy Cummings
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The Diamond Cover Award

Sponsored by Miles Inc., Diagnostics Division
Donna M. Simmons
Los Angeles, California

Diamond Cover Merit Award

Sponsored by Miles Inc., Diagnostics Division
James N. Turner
Albany, New York

Editor's Award

Sponsored by Miles Inc., Diagnostics Division
John Ryan
Missouri City, Texas

J.B. McCormick, M.D. Award

Sponsored by J.B. McCormick, M.D.
Phyllis I. Boris
Prescott, Arizona

President's Award

Richard Schroeder
Plainview, New York

N.S.H. Convention Award

Sponsored by Histology Control Systems, Inc.
Jimmy Stringer
Lubbock, Texas

The William J. Hacker Memorial Award

Sponsored by Hacker Instruments, Inc.
Andy Valls
Eagan, Minnesota

The Lee G. Luna Foreign Scholarship Award

Sponsored by Surgipath Medical Industries, Inc.
Patricia Turner
Winter Garden, Florida

Sakura Student Scholarship Award

Sponsored by Sakura Finetek, USA
Donna Kimsey
Little Rock, Arkansas

Lipshaw Educational Scholarship

Sponsored by Lipshaw Manufacturing Corporation
Anna Diliberto
Birmingham, Alabama

E.M. Diagnostics Systems, Inc., Educational Scholarship

Sponsored by E.M. Diagnostics Systems, Inc.
Katie Swihart
Toledo, Ohio

Dezna C. Sheehan Memorial Education Scholarship

Sponsored by NSH
Nancy J. Wood
Trussville, Alabama

Miles Educational Scholarship

Sponsored by Miles Inc., Diagnostics Division
Konstance Zeitner
Bellevue, Nebraska

Instrumentation Laboratories Educational Scholarship

Sponsored by Instrumentation Laboratories, Inc.
Rebecca Stamy
Iowa City, Iowa

Shandon Student Scholarship Award

Sponsored by Shandon, Inc.
Dick Walsh
Marshfield, Wisconsin

1990 NSH Symposium/ Convention—Nothing But Rave Reviews

Brent Riley
Managing Editor

"It was fantastic."

"I loved it."

"It was an outstanding meeting."

"It was the best meeting I've ever been to."

"The workshops were invaluable."

The 16th Annual NSH Symposium/Convention closed on September 14 to a string of compliments from those who attended and participated. The meeting, which began on September 8, broke the attendance record set in 1989 as more than 960 registrants converged on San Antonio, Texas, in addition to exhibitors, lecturers, and workshop leaders.

"Each convention is drawing a larger number of participants," remarked Marilyn Gamble, NSH president. "It just keeps getting bigger and better."

"I couldn't have been more pleased," responded Sue Judge, local coordinator for the symposium/convention. "It was really a great meeting."

The theme of the meeting was "Shining Advances in Technology" and, as always, education was the major thrust. Workshops began on Saturday and continued through Tuesday. An extra day of workshops was added to accommodate the strong demand, according to Judge. Workshops started on Saturday so those people who could come only for the weekend could still enjoy 2 full days of workshops.

"Workshops are a great learning experience," Judge said. "We had hoped that with more workshop opportunities employers would be more likely to see the symposium as a good learning experience for their people. And that's the way it worked out."

All the workshops with limited space were filled to capacity. Those that were not limited had to be presented theater style rather than classroom style in order to accommodate the high numbers. "Some of the higher attended ones were those which covered laboratory safety, hazardous chemicals, chemical wastes, estrogen receptors and techniques dealing with breast biopsies," Judge recalled.

"We attempt to stay current with technology," Gamble explained. "As technology becomes more sophisticated, we try to keep up through our workshops and lectures. There's really something for everybody. We have topics from the very basic to the very sophisticated. I think that's one thing that keeps people coming back."

Kim Simmons served as workshop coordinator on the national committee. This was the first year a special committee member was appointed to coordinate workshops. "She did a fantastic job," Judge said.

The Professor C.F.A. Culling Memorial lecture was given by Joseph H. Keffer, M.D., immediate past president, American Society of Clinical Pathologists. His topic was "The Laboratory in the Year 2000." "Dr. Keffer was a very dynamic speaker," Judge recalled. "It was nice to hear from someone like him. He felt that our role in the laboratory was of vast importance. And he was also very impressed with our meeting."

"I was very excited about our keynote speaker," Gamble added. "It was quite an honor to have him there. I think it helped bind the relationship between the ASCP and the NSH. More and more pathologists are beginning to realize what the NSH has to offer."

The Swiss Society Exchange Lecture was again very popular this year. Bert Jaspers, president of the Swiss society, spoke on "Practical and Technical Comments in the PAS Reaction and Worthwhile Information Concerning Hematoxylin." Another Swiss speaker, Salome Kiefer-Raez, of Searle SA, spoke on "Preparation of Undecalcified Bone for Histologic Evaluation."

"I think the meeting took on a different dimension this year because of our Swiss exchange and international speaker programs," Gamble noted.

Another addition to the 1990 agenda was a panel discussion held on Friday morning. The 3-hour session was moderated by Gamble and included a panel



of experts with wide-ranging expertise answering questions that were submitted by participants throughout the week as well as during the discussion.

"I received very good feedback about the panel discussion," Gamble said. "So it is something we will probably continue to place on the agenda."

Additional educational opportunities were presented by the technical exhibits, poster sessions, and clinical and veterinary, industrial, research scientific sessions.

The social functions also drew large crowds throughout the week. On Tuesday night, Miles Inc. sponsored a Saturday Night Live Party. More than a few coneheads, killer bees, land sharks, and other Saturday Night Live characters were seen mingling with the crowd. A good time was had by all.



The grand finale social event was the Thursday night Awards Banquet, attended by more than 600. "It was festive. It was fun. It was great—the highlight of the week," Judge said. The theme of the banquet was "Fiesta."

Jimmy Stringer was coordinator for the banquet. "He outdid himself," Judge said. "We had a room full of fresh flowers. He stayed up all night making fresh flower arrangements for every table—and there were sixty tables."

"I had never seen so many flowers in my life," Gamble exclaimed. "It was extraordinary!"

At least 17 awards and scholarships were presented at the banquet, including a special award, the Histotechnologist of the Decade Award, which was presented to Lee G. Luna for his outstanding contributions to the field of histotechnology during the 1980's.

The banquet was preceded by a cocktail reception sponsored by Miles Inc. A strolling mariachi trio provided music and entertainment during the hour.



There were more than 300 first-time attendees—another record. For the second year, a special reception was held for first-time attendees. Its purpose was to orient them to the symposium/convention, answer questions, and provide the new people with an opportunity to meet each other, as well as the NSH officers. "Sometimes coming into a national symposium/convention can be quite overwhelming," Gamble said.

(continued from page 192)

quality microscopic slides. National Society for Histotechnology Continuing Education Units will be awarded. Note: Registration limited to 60; therefore, request information early.

For program and related information, contact:

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Histotechnologists Provide Training in Central America

Brent Riley
Managing Editor

Recognition is due a group of histotechnologists who have been traveling to Central America presenting educational programs in order to aid in the progress of histology throughout those countries. Their objective is to establish a permanent group of pathologists and histotechnologists to exchange ideas, books, journals, equipment, and information. A November 1990 conference is scheduled for San José, Costa Rica. This will be followed by a histotechnology symposium in Nicaragua. This will be the third trip to Central America for our colleagues. Special recognition is due Dr. Ernesto Hoffman and Teresa Flores of the LSU Medical Center, New Orleans, Louisiana, for their time and effort in establishing and promoting these conferences. Other faculty participants include Cheryl Crowder, Rosemary Velasquez, Lynn Montgomery, and Lee G. Luna. Every-



Histology Horror Films

one in the discipline of Histology and Pathology is grateful to those who dedicate their talents to teach and train others to ensure better health care on a worldwide basis.

The reception included a scavenger hunt where participants had to find people who fit certain criteria, for example, a native of San Antonio, a visitor from another country, or someone who did flow cytometry in their lab. "This really got people talking to each other," Judge commented. "They had a lot of fun with it."

More than 50 scientific exhibit companies displayed the latest in laboratory equipment and supplies from Tuesday through Thursday. This gave attendees an opportunity to learn about new products and discuss their needs with company representatives. "Exhibitors were delighted with the meeting, the accommodations, and the city as a whole," Judge said.

The city of San Antonio was a hit with everyone who attended. The beautiful River Walk, which was just outside the hotel, provided a perfect respite after a busy day of workshops or lectures. "Everyone was particularly pleased with the hotel itself," Judge said. "The layout of the hotel gave everyone an opportunity to really see and talk with one another. The upper lobby where the workshops were held had plenty of seating for people to relax and talk between workshops. It was a very congenial atmosphere—a great place to be."

"The people who made up our committee were very enthusiastic, and it showed," Judge said. But she was also impressed by all those who attended. "Everybody pitched in and helped. People would come by registration, or the hospitality table, or the workshop room and offer to help whenever they had a few minutes." This type of support and helpfulness was apparent throughout the meeting, according to Judge.

"I'm sure that those people who took advantage of all the educational and social opportunities went home emotionally and intellectually changed," Gamble concluded. "Even though they are probably very tired, they will have a renewed enthusiasm for their profession."

Correction:

In the article entitled "Histologic Techniques for Analysis of Bone Implants (A Historic and Artistic Perspective)," published in the June/July 1990 issue of *Histo-Logic*, it was reported that vacuum infiltration can be accomplished "by drilling a hole in the side of a refrigerator and placing a vacuum desiccator inside, making a vacuum infiltration at 40°C possible." The temperature should have read "4°C."



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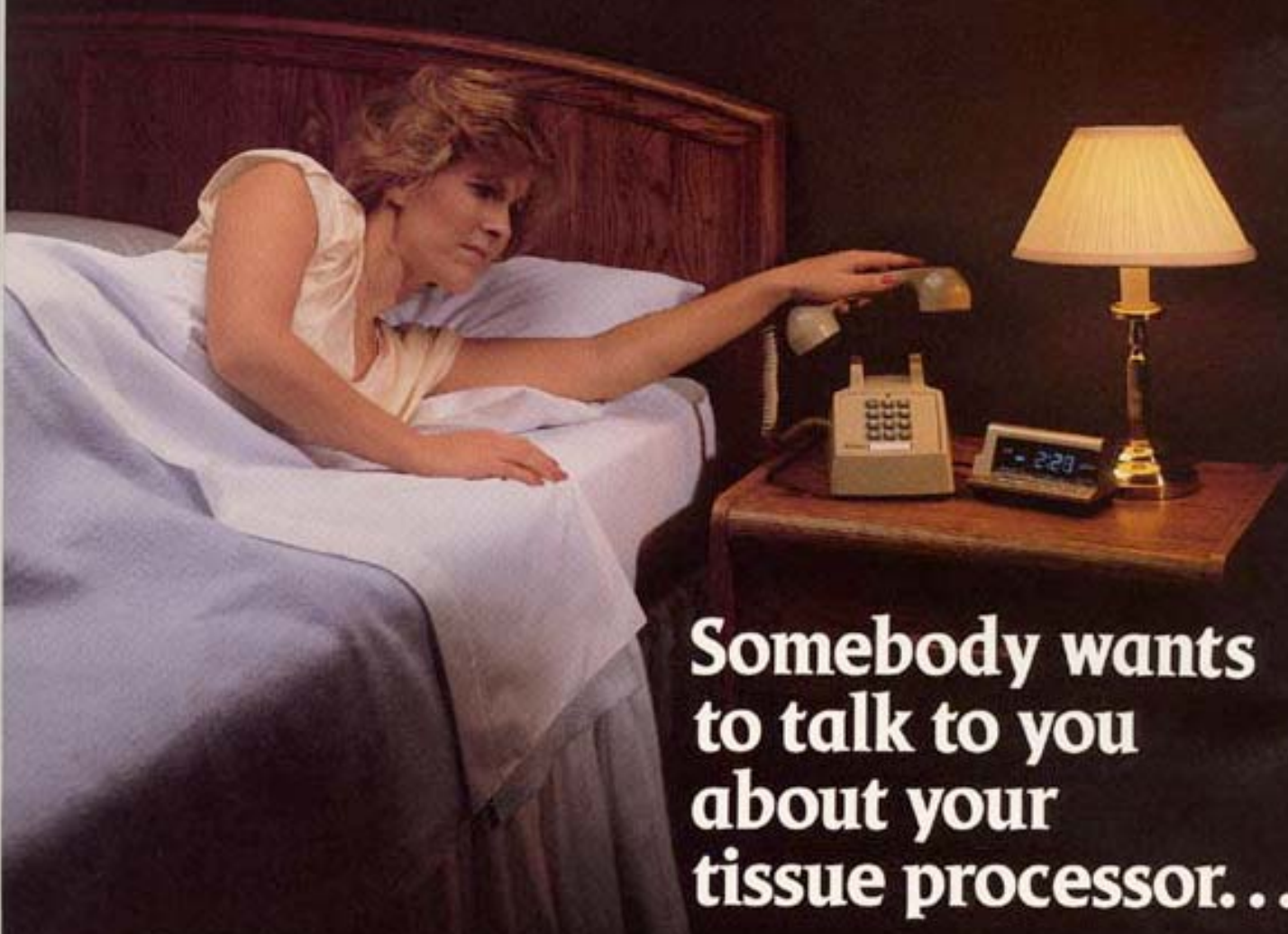
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A Modified Bielschowsky for Neurofibrillary Tangles and Senile Plaques

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In recent years there have been numerous articles written suggesting the use of a Bielschowsky stain for demonstrating neurofibrillary tangles and senile plaques in suspected cases of Alzheimer's disease. We have tried a few modifications of Bielschowsky's original procedure and found the one presented below to work best for us. It demonstrates many more senile plaques and neurofibrillary tangles than are seen on our Bodian stained slides (Fig. 1).

The procedure does require some experience. The technician will need to use the technique several times to adjust the time the slides are exposed to the developer. The staining end point should be controlled by checking frequently with a microscope in order to adhere to the precise tinctorial quality desired.

Fixation 10% buffered neutral formalin
Microtomy Cut paraffin section at 6 to 8 micrometers
Solutions

20% Silver Nitrate

Silver nitrate 20.0 gm
Distilled water 100.0 mL

Developer

Concentrated formalin 20.0 mL
Distilled water 100.0 mL
Concentrated nitric acid 1 drop
Citric acid 0.5 gm

Evaporated Ammonium Hydroxide

Place 200 mL of 28% ammonium hydroxide in an open dish for 20 minutes under a hood. This will allow some of the ammonia gas to evaporate, thereby making the ammonium hydroxide weaker.

5% Sodium Thiosulfate (Hypo)

Distilled water 100.0 mL
Sodium thiosulfate 5.0 gm

Staining Procedure

1. Decerate slides and hydrate to distilled water.
2. Place slides in 20% silver nitrate for 20 minutes. Remove slides and place in distilled water until silver nitrate solution below is ready.
3. To the 20% silver nitrate used at Step 2, add evaporated ammonium hydroxide, 1 drop at a time, while stirring vigorously until the precipitate that forms dissolves and the solution turns clear. Then add 2 more drops of ammonium hydroxide. Return slides to this solution for 15 minutes in the dark.
4. Add 3 drops evaporated ammonium hydroxide to a coplin jar of distilled water. Immerse slides in this solution.
5. Add 3 drops of the developer to the coplin jar containing the slides and ammonia-silver solution and stir. Place slides in this solution until the fibers are black with a tan background. Control development with the use of the microscope, which usually takes 3 to 5 minutes.
6. Wash in distilled water for 1 minute.
7. Place in hypo for 5 minutes.
8. Wash in distilled water for 2 minutes.
9. Dehydrate, clear, and mount coverglass with appropriate media (refractive index 1.48 to 1.56).

Results

Fibrillary tangles black
Senile plaques black
Background yellow

Remarks

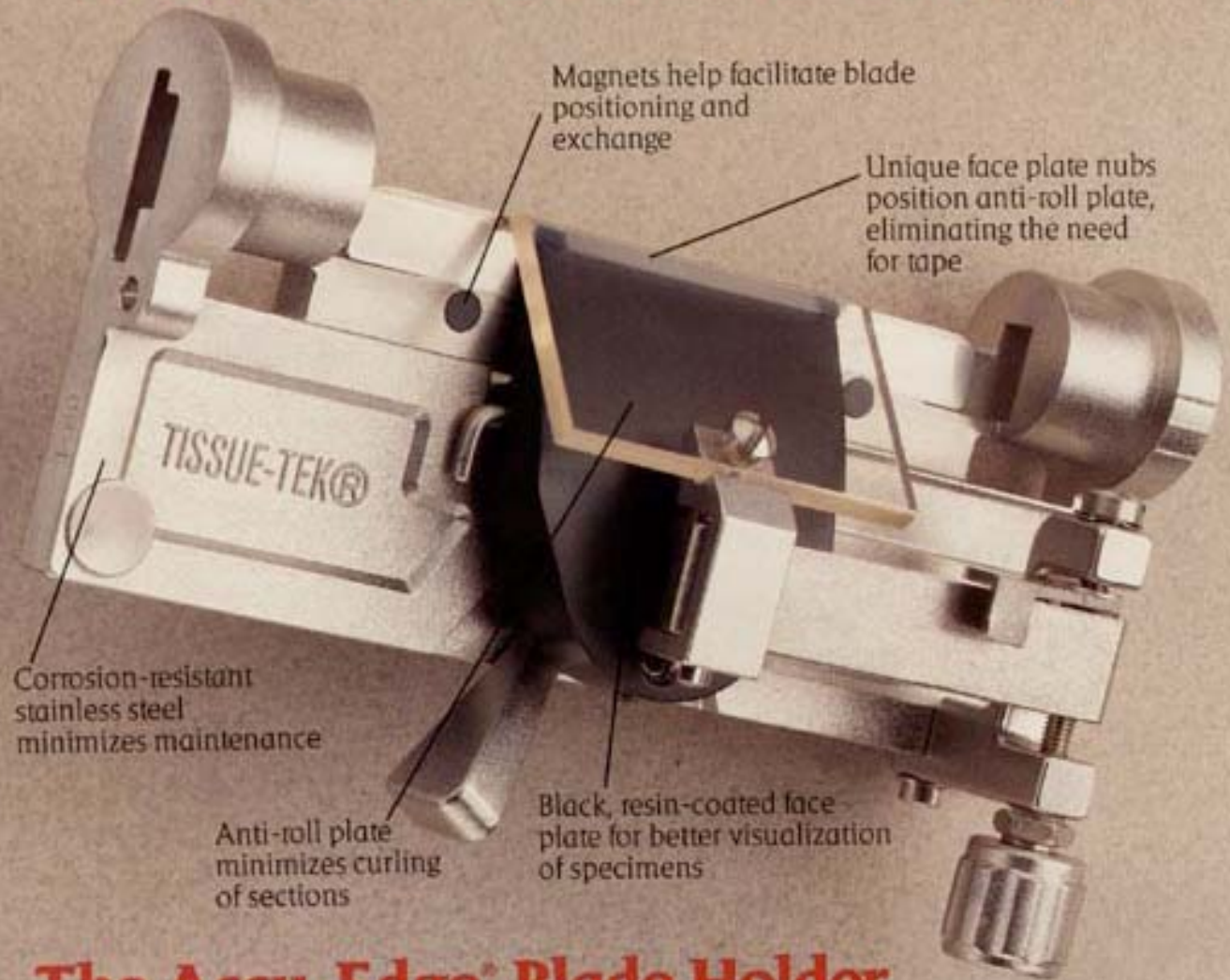
It is necessary to evaporate the ammonium hydroxide the full 20 minutes in an open dish.



Figure 1: Senile plaques and fibrillary tangles can be seen in the middle of this photograph. Notice that nerve fibers are demonstrated quite well, while the plaques are seen stained intensely black.

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Microwave Modification of Bielschowsky's Method for Nerve Fibers

Charles Churukian, HTL(ASCP)
University of Rochester Medical Center
Rochester, NY 14642

This staining method differs from Bielschowsky's method in that it requires much less silver nitrate. In Bielschowsky's method at least 8.0 gm of silver nitrate is used as compared to only 0.7 gm of silver nitrate in the method described below.

We have found that neurofibrillary tangles (Figs. 1 and 2) and plaques (Figs. 3 to 5) of Alzheimer's disease stain better with the described method than they do with Bielschowsky's method. This method is also less time-consuming, requiring only 30 to 35 minutes to perform.

Fixation 10% buffered neutral formalin
Microtomy Paraffin sections are cut at 8 micrometers
Solutions

0.5% Silver Nitrate

Silver nitrate 0.5 gm
Distilled water 100.0 mL
Prepare fresh.

5% Silver Nitrate

Silver nitrate 0.1 gm
Distilled water 2.0 mL
Prepare fresh.

1% Ammoniacal Silver

Silver nitrate 0.4 gm
Distilled water 40.0 mL
Add 28% ammonium hydroxide, drop by drop with constant shaking, until the initial precipitate disappears and the solution turns clear. Then add 5% silver nitrate, drop by drop, until the solution becomes slightly cloudy.
Prepare fresh.

Developer Solution

Formaldehyde, 37% to 40%, reagent grade 2.0 mL
Distilled water 10.0 mL
Citric acid 0.5 gm
Nitric acid, approximately 70% HNO₃ 1 drop
Prepare fresh.

1% Ammonium Hydroxide

Ammonium hydroxide, 28% 1.0 mL
Distilled water 99.0 mL

2% Sodium Thiosulfate

Sodium thiosulfate 2.0 gm
Distilled water 100.0 mL

Staining Procedure

1. Deparaffinize slides and hydrate to distilled water.
2. Place slides in 40 mL of 0.5% silver nitrate solution in a plastic coplin jar and microwave at power level 5 (300 watt) for 1 minute. Dip the slides up and down several times and allow them to remain in the hot solution for 5 minutes.
3. Place slides in distilled water.
4. Pour the ammoniacal silver solution in a plastic coplin jar and microwave at power level 5 (300 watt) for 45 seconds. Mix with a glass rod and place the slides in this hot solution for 15 minutes.
5. Place slides in 1% ammonium hydroxide solution for 1 minute.
6. Add 1 drop of developer to ammoniacal silver solution used in Step 4. Quickly mix with a glass rod and immediately place the slides in the solution for 2 minutes. The solution will rapidly turn a grayish color and a mirror of silver will form on the sides of the coplin jar and the slides but not on the tissue sections. The tissue sections should be brown.
7. Place slides in 1% ammonium hydroxide solution for 1 minute.
8. Rinse in 3 changes of distilled water.
9. Wipe off the mirror of silver from both sides of the slides, taking care not to damage the tissue sections.
10. Place slides in 2% sodium thiosulfate solution for 1 minute.
11. Rinse in 4 changes of distilled water.
12. Dehydrate in graded alcohols.
13. Clear in 3 or 4 changes of xylene.
14. Mount coverglass with appropriate media (refractive index 1.48-1.56).

Results

Nerve fibers brown to black
Cytoplasmic neurofibrils brown to black
Neurofibrillary tangles and
plaques of Alzheimer's disease dark brown or black
Granulovacuolar bodies black
Neurons and background varying shades of brown
Neuromelanin black
Lipofuscin brown to black

Remarks

Plastic coplin jars are used instead of glass ones in Steps 2 and 4 of the staining procedure because glass coplin jars will sometimes crack when the solutions in them are rapidly heated with microwave irradiation.

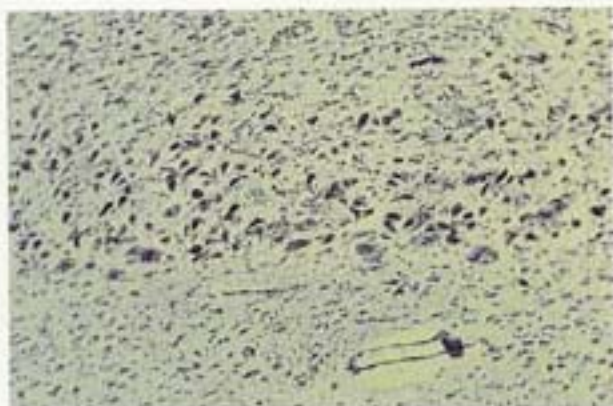


Figure 1: This demonstrates fibrillary tangles across the middle of the photograph. (Original magnification $\times 40$)

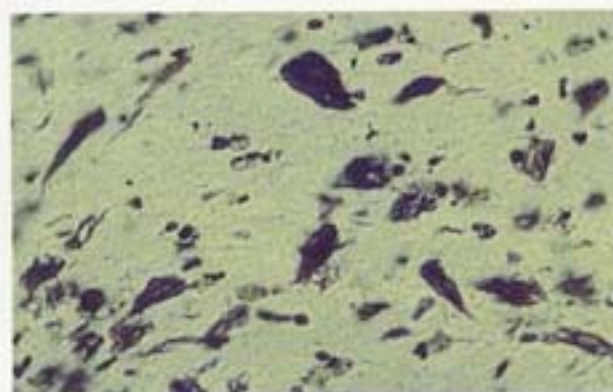


Figure 2: A high-power photograph of fibrillary tangles and a clear background. (Original magnification $\times 100$)

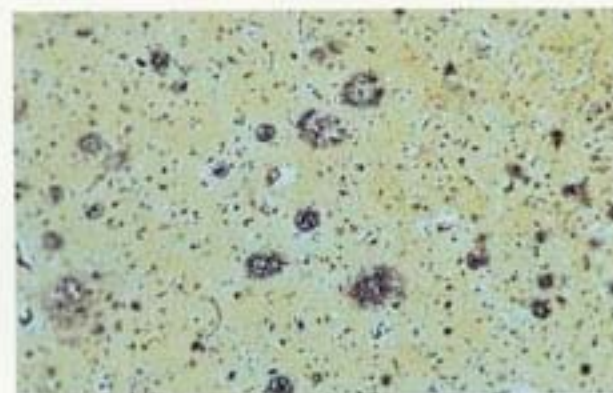


Figure 3: Senile plaques can be seen in this low-power throughout the section. Notice the lack of background staining. (Original magnification $\times 40$)

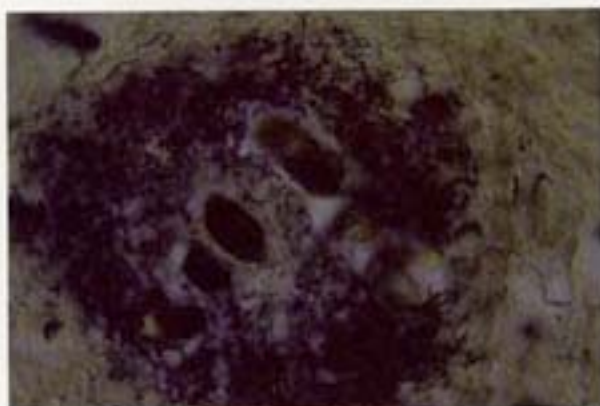


Figure 4: Three senile plaques can be seen in this photograph, which also points out the well-demonstration of these entities. (Original magnification $\times 200$)



Figure 5: This photograph demonstrates the massive amount of nerve fibers in a senile plaque. Notice that individual fibers are easily observed. (Original magnification $\times 400$)

The times that the solutions are heated with microwave irradiation in Steps 2 and 4 of the staining procedure are used when staining five slides at a time. When staining fewer than five slides, the time that the solutions are heated should be reduced by 5 to 10 seconds, depending on the number of slides being stained. The temperature of the 0.5% silver nitrate should be about 85°C and that of the 1% ammoniacal silver about 75°C after the solutions have been heated.

When solutions are heated with microwave irradiation, there can be up to a 15°C difference in temperature between the top and bottom portions of the solutions. Therefore, in order to equalize the temperature of the solutions, the slides are dipped up and down in Step 2, and the solution is mixed in Step 4 of the staining procedure. This assures uniformity of staining results.

It is essential to use acid-cleaned glassware, rinsed in double distilled water. The shelflife of silver nitrate can be greatly increased by storing it in a refrigerator at 3° to 6°C .

Acknowledgment

The advice of Lowell W. Lapham, MD, chief of the Neuropathology Unit of the University of Rochester, is gratefully acknowledged. This investigation was supported by grant #AG03644 from the National Institutes of Health, Bethesda, Maryland.

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Writing, Teaching, and Managing Head List of Priorities for Leonard Noble

Brent Riley
Managing Editor



Leonard Noble was afraid that his statement would be misinterpreted. "I've got it made in the shade," he declared. "But please don't print that."

When you fully realize everything Noble contributes to his profession, however, there is only one way to interpret his statement: He loves what he does. He loves histotechnology. He loves management. He loves teaching, lecturing and writing for publication. These are the things that make him happy, so he's got it "made in the shade."

Noble is the 1990 Golden Forceps Award winner. The prestigious award, presented at the recent National Soci-

ety for Histotechnology Symposium/Convention, is given annually for the best article published in *Histo-Logic*.

The winning article was the fifth authored by Noble for *Histo-Logic*. The Durham, North Carolina native's first published article appeared in *Laboratory Management* in 1977. It was titled "Histochemical Staining Gives Muscle Biopsies New Utility."

"I'd been writing for a long time before I had my first article published," Noble explained. "I had done the technical research on a number of published articles authored by others. So I had been mentioned a lot, but never as an author."

Noble is the chief technologist of the anatomic labs for North Carolina Baptist Hospitals, Inc., part of Wake Forest University. He supervises 40 people in the cytology, surgical pathology, autopsy, and molecular diagnostics labs. He is also a charter member and vice president of the National Society for Histotechnology.

It was somewhat inadvertently that Noble entered the histotechnology profession 21 years ago. He finished school in the midst of the Vietnam crisis. After reporting for his physical, he was told that he would be drafted within 60 days. He decided to put those last few weeks to good use, so he applied for a job—any job—at Duke University. Two weeks later Noble was working in the histology lab. He was never called up for the service and has been a histotechnologist ever since, spending 8 years at Duke University Medical Center before moving to his current position.

"It was a fascinating experience being thrown right into a discipline like that," Noble said. "With my background in biology and chemistry, it was nice to be able to do something practical."

Noble's first day on the job was his first exposure to histotechnology. But within 6 months he was training others in the laboratory.

He has made 37 formal presentations at various meetings and conventions, including one this year at the national symposium of the Swiss Society for Histotechnology. Noble still remembers his first presentation. It was in 1973 to the American Society for Medical Technology. His topic was "Histochemical Techniques in the Histology Lab." He is currently planning a trip to Pakistan where he will also lecture.

Noble's winning article was titled "A Simplified and Reliable Technique for the Demonstration of *Pneumocystis carinii* in Cytosmear Smears." "It isn't new technology," Noble explained. But it does solve a problem that most histotechnologists have with silver stain.

"We modified and modified and modified until we came up with this technique," Noble continued. "And it proved very successful. We have fantastic results. Cells do not wash away. It gives us a better chance of identifying some types of organisms such as *Pneumocystis carinii*."

"We've also tried it with other stains and it works better than just putting the jar with the fluid in the microwave. We actually take the jar with the staining solution and set it down inside another container of a liquid such as water. Then the microwaves do not all congregate in one small area."

Noble encourages other histotechnologists to write. "I think there are a lot of people out there who have the desire to be published, but they don't have the time or the backing to do it," Noble remarked. "With more encouragement from the institutions where they work, I think you'd see a lot more articles coming from histotechs."

"Of course, you have to get the work out first. And if there is time remaining, and something the histotechnologists feel would be enlightening to others,

they should go to their director and say 'I would like to pursue this.' I think the administration or the pathologist would support them."

"I work in an excellent environment—a medical school environment. There are plenty of doctors here who would just love to sit down and get another article published. And that's what we're doing now. We're working on two more articles that will be submitted to the *Journal of Histotechnology*."

Not all articles take months of extensive research. According to Noble, some can be written in a matter of hours. "It doesn't have to be a drawn-out, lengthy, researched item," he said. "It can be something that you have worked on and simplified in the lab. You should make sure your article is cleaned up as much as possible. And make sure your pictures look good."

Noble is dedicated to his responsibilities as a manager. "The lab is only as good as the work that's being organized," he said. But he also feels he should be publishing more. "There are a lot of things I want to do," he said. "I have this need—this urge—to write. I love to write."

If Leonard Noble isn't doing something to advance the field of histotechnology, he just isn't happy. So after 21 years as a histotechnologist, he is still making significant contributions to his profession.

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