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Tracking Malaria in Mosquitoes A Direct Immunohistochemical Assay

Claudia F. Golenda, Ph.D
Research Entomologist
Department of Entomology
Walter Reed Army Institute of Research
Washington, DC 20306

Yearly, more than 1 million people die from malaria, while over 100 million people suffer from the illness caused by the disease.[1] The resurgence of malaria in many parts of the world[2] is making it necessary to develop new methods of control. A better understanding of the life cycle of the parasite on the mosquito and more accurate estimates of infected mosquitoes are necessary if the disease is to be effectively controlled.

The malaria cycle involves human beings and anopheline mosquitoes. When a mosquito bites a malaria-infected individual, the blood meal contains the male and female sexual stages (gametocytes) of the parasite. The gametocytes mate in the stomach of the mosquito, forming a fertilized cell (the ookinete) that invades the gut wall. The ookinete develops into a cyst (the oocyst) and matures on the gut wall. The mature oocyst ruptures, releasing malaria sporozoites that invade the salivary gland of the mosquito. When an infected female mosquito bites an individual to take a blood meal, it can transfer sporozoites and initiate the disease.

Detection of sporozoites in mosquitoes is often based on viewing crushed salivary glands with a phase microscope. Sporozoites may be missed amongst a background of pulverized tissue, especially when they are present in low numbers.

Visualization of sporozoites is greatly enhanced by using a rapid one-stage immunohistologic assay. Sporozoites are selectively stained red using alkaline phosphatase-labeled monoclonal antibodies directed to the surface coat (the circumsporozoite [CS] protein) (Fig. 1).

A one-stage immunoassay permitted a study of the distribution of the CS protein of the human malaria parasite *Plasmodium falciparum* in tissue sections of *Anopheles stephensi* mosquitoes. Alkaline phosphatase was directly conjugated to a monoclonal antibody directed against the CS protein of *P. falciparum*.[3] The CS proteins, which bound the antibody-enzyme conjugate, were permanently stained red using the HistoMark® RED system.[4] The cell structure was defined by hematoxylin.

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IN THIS ISSUE

In studies with serial sections of whole mosquitoes, the CS protein was first detected during the oocyst stage 2 to 3 days before sporozoites were observed (Fig. 2). As oocysts matured, detectable CS protein increased (Fig. 3a-c). Sporozoites released from mature oocysts were easily visualized in insect blood, even when low levels of infections were present (Fig. 4). CS protein, not associated with the surface of the parasite, was detected as small circular regions (1 micron diameter) on the surface of the salivary glands. These regions may mark the entrance path of sporozoites into the gland. Subsequent studies on whole glands and midguts dissected from mosquitoes indicated that sporozoites deposited CS protein on these tissues (Fig. 5).

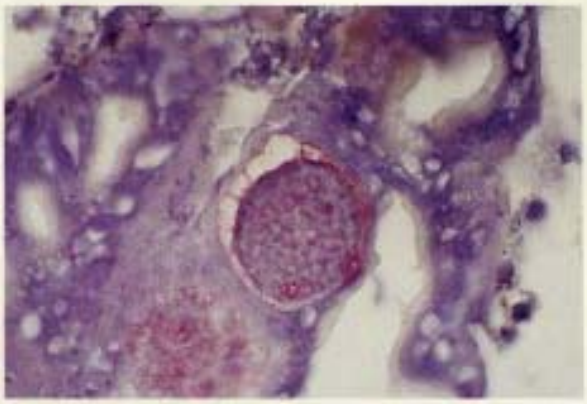


Figure 3a: Oil magnification (1000×) demonstrating the details of an immature oocyst in which CS protein is synthesized in membranous pockets before the appearance of sporozoites.

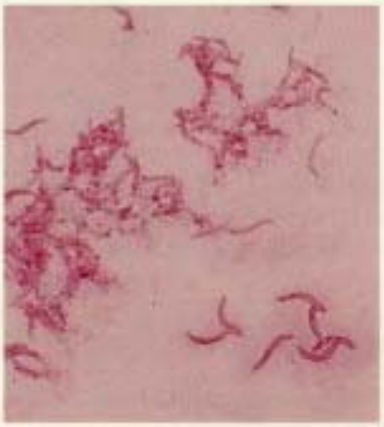


Figure 1: Plasmodia sporozoites stained red using an alkaline phosphatase-labeled monoclonal antibody directed to the surface coat (the circumsporozoite [CS] protein. (Magnification 500×)

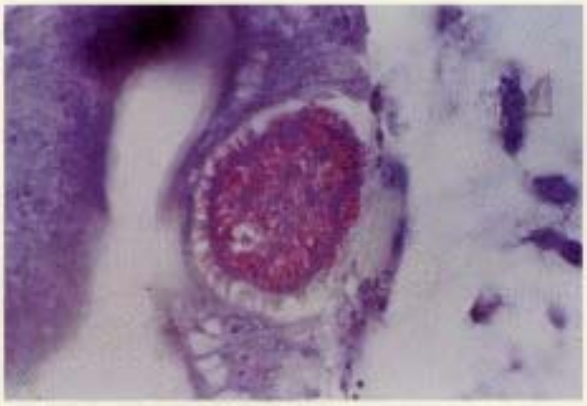


Figure 3b: A mature oocyst containing sporozoites.

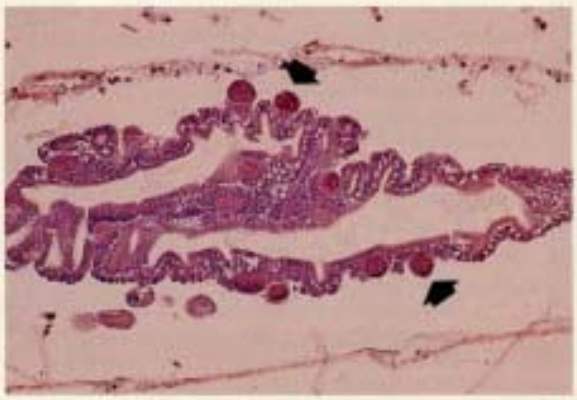


Figure 2: Section through midgut of the mosquito with several red-stained oocysts (arrows) demonstrating the presence of the CD protein. Hematoxylin counterstain. (Magnification 200×)

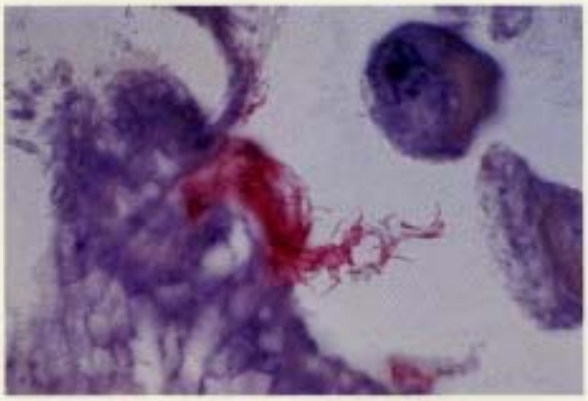


Figure 3c: A ruptured oocyst releasing sporozoites.

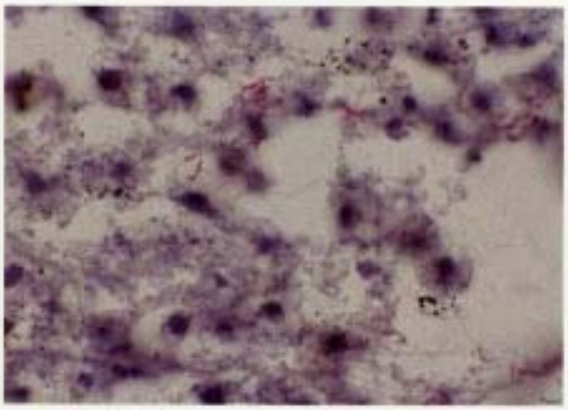


Figure 4: Several red-colored sporozoites present in the insect blood (hemolymph) in a low-level infection. Hemotoxylin counterstain shows blood cells. (Magnification 1000×)

Figure 5: Section of mosquito salivary gland showing both sporozoites on the gland and CS protein associated with the gland where sporozoites are not evident. Two small circular regions are also evident between secretory cells of the gland. These may mark the entrance path of the sporozoite into the gland. (Magnification 1000×)

The deposits of CS protein on mosquito tissues by sporozoites were a significant finding. The immunohistochemical procedure detected not only CS protein, but also differentiated between CS protein on the surface of the sporozoite and that deposited by the parasite on insect tissue. This ability gives the immunohistochemical assay an advantage over microwell Enzyme-Linked ImmunoSorbent Assay (ELISA) techniques that cannot quantitate numbers of sporozoites. In the ELISA, shed CS protein is measured along with CS protein on the surface of the sporozoite.

The immunostaining technology has been extended to quantify and speciate double malaria infections in mosquito salivary glands. Monoclonal antibodies to the CS protein of the human malaria parasites *P. falciparum* and *Plasmodium vivax* were conjugated to horseradish peroxidase and alkaline phosphatase, respectively, and co-incubated with salivary gland tissue. The enzymes were assayed separately using HistoMark® Blue for alkaline phosphatase and HistoMark® Orange for horseradish peroxidase.[4] In salivary glands containing double infections, *P. falciparum* sporozoites were stained orange while *P. vivax* sporozoites were stained blue. The orange and blue stains gave good contrast and the different sporozoites were easily distinguished by light microscopy.

The enhancement of conventional histological procedures with enzyme immunoassay technology permits high resolution analysis of the malarial infection process and provides a more accurate method to determine the number of sporozoites in mosquito salivary glands.

Detection of Malaria Sporozoites in Mosquito Salivary Glands

Fixing Tissue

Salivary glands, dissected from mosquitoes, are transferred to the well of a microwell slide containing 20 µL of Medium 199. Once the gland is centrally aligned in the well, excess medium is removed with a fine-tipped pipette and the gland is pressed onto the slide using a blunt-tipped probe. Immediately after air drying, the gland is fixed in Bouin's reagent for 5 minutes, then washed in running tap water for 15 minutes.

Immunoassay

The slide is transferred to a moisture chamber and 25 µL of blocking buffer (BB, Dulbecco's phosphate buffer saline [PBS] containing 1.0% bovine serum albumin, 0.5% casein, 0.01% thimerosal, and 0.002% phenol red) is added to the well(s) containing gland tissue. After incubation for 10 minutes at room temperature (RT), the BB is removed, and 25 µL of alkaline phosphatase-labeled monoclonal antibody at a concentration of 5 µg/mL is added. After incubation in a moisture chamber for 30 minutes at RT, the slides are washed in a coplin jar with 3 changes of either PBS, pH 7.4, or tris-HCl, pH 7.4, for 10 minutes. After drying around outer surface of wells, the slide is put into a moisture chamber and 30 µL of substrate-dye mixture from the HistoMark® RED kit is applied for the detection of alkaline phosphatase. After reacting for 10 minutes at RT, the slide is rinsed in running tap water for 2 minutes and counterstained for 20 seconds with Contrast Blue stain supplied in the HistoMark® RED kit. After rinsing in running tap water for 2 minutes, the slide is mounted for viewing.

(continued on page 208)

References
1. *Science*, 247: 399, 1990.
2. *Science*, ibid., p. 400.
3. Golenda CF, Starkweather WH, and Wirtz, RA. The distribution of the circumsporozoite (CS) protein in *Anopheles stephensi* mosquitoes infected with *Plasmodium falciparum* malaria. *J Histochem Cytochem*. 1990;38:475-481.
4. Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD 20879

Reprinted with permission from KPL's *Antibody at Work*, Vol. 3, Summer, 1990, published by Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD 20879.

Luna's Method for Argentaffin Cell Granules and Goblet Cell Mucins

Lee G. Luna
American HistoLabs, Inc.
Gaithersburg, MD 20879

Fixation	10% buffered neutral formalin.
Process	Paraffin.
Microtomy	Cut paraffin sections at 6 micrometers.

This method utilizes the reducing functions of potassium ferricyanide to demonstrate argentaffin cell granules and other reducing substances like melanin granules. Additionally, the mucicarmine stain demonstrates the mucin in the goblet cells. This makes for a useful demonstration of reducing substances and mucins.

Solutions

0.1% Potassium Ferricyanide (Stock)

Potassium ferricyanide ..... 0.1 gm
Distilled water ..... 100.0 mL

1% Ferric Chloride (Stock)

Ferric chloride ..... 1.0 gm
Distilled water ..... 100.0 mL

Ferric-Ferricyanide (Working)

1% ferric chloride (stock) ..... 150.0 mL
0.1% potassium ferricyanide (stock) ..... 50.0 mL
Adjust the pH to 2.4 with a few drops of diluted hydrochloric acid if necessary.

Mucicarmine

Carmine ..... 1.0 gm
Aluminum chloride, anhydrous ..... 0.5 gm
Distilled water ..... 2.0 mL
Carefully mix stain in evaporating dish. Heat on electric hot plate for 2 minutes. Liquid becomes almost black and syrupy. Dilute with 100 mL of 50% alcohol and let stand for 24 hours. Filter. Dilute 1 part mucicarmine with 4 parts water.

Metanil Yellow

Metanil yellow ..... 0.25 gm
Distilled water ..... 100.0 mL
Glacial acetic acid ..... 0.25 mL

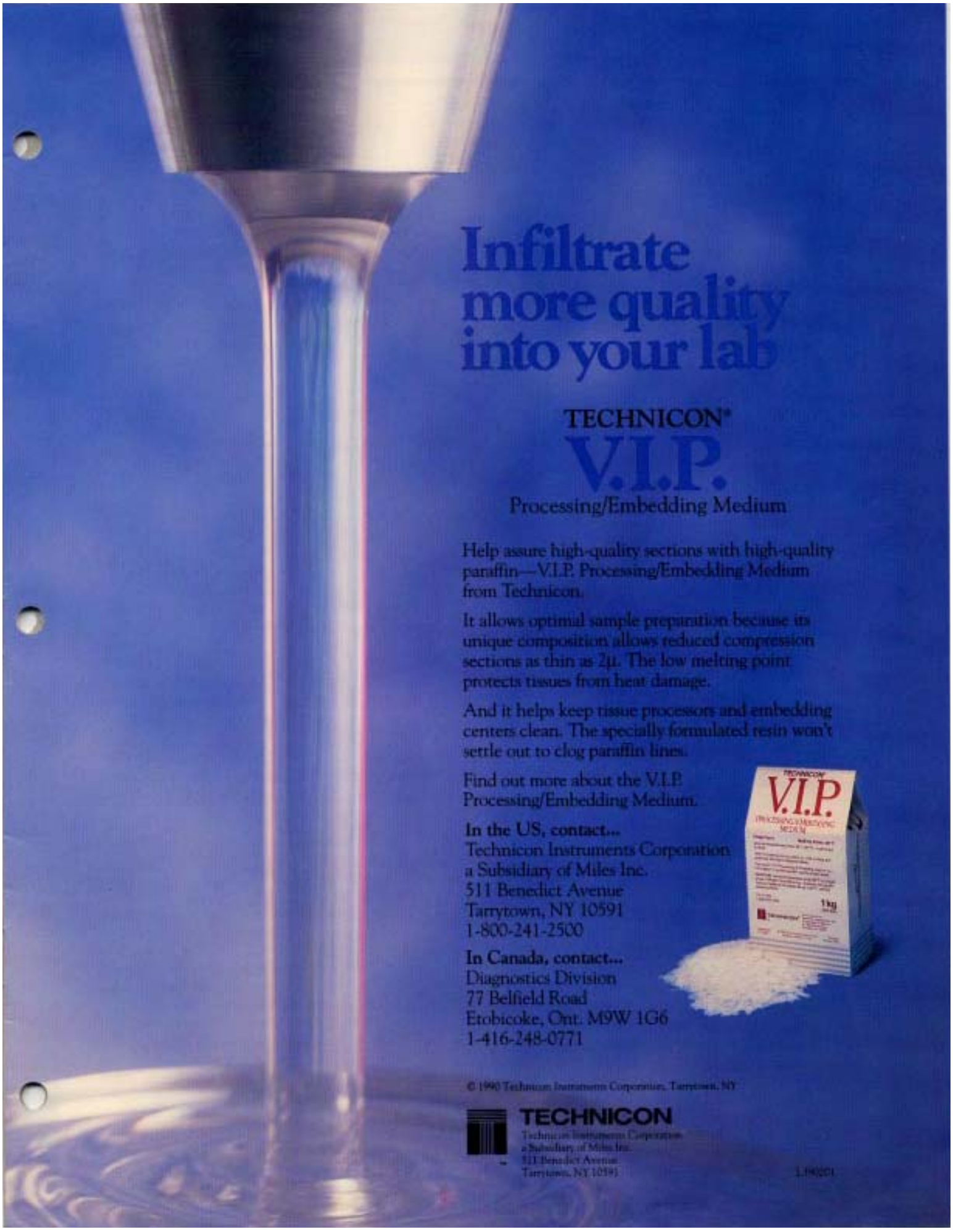
Staining Procedure

1. Decerate in the usual manner.
2. Wash in running water.
3. Rinse in 3 changes of distilled water.
4. Immerse slides in 3 changes of ferric ferricyanide solution for 15 minutes (5 minutes for each change).
5. Rinse well in distilled water.
6. Place in Mayer's mucicarmine for 30 minutes.
7. Rinse quickly in distilled water.
8. Counterstain with metanil yellow for a few seconds.
9. Rinse quickly in distilled water.
10. Dehydrate, clear and mount with appropriate media.

Results

Argentaffin cell granules ..... blue
Goblet cell mucin ..... reddish pink
Cytoplasm ..... yellowish green
Melanin ..... blue
Other reducing substances ..... blue

You'd think Pathologists would stop insisting on doing a Histologist's job.



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Answers to "Questions in Search of an Answer"

The following are responses to "Questions in Search of an Answer," which appeared in *Histo-Logic*, Vol. XX, No. 2, Mar./Apr. 1990.

- In response to "Questions in Search of an Answer" submitted by Fred Argilan concerning an artifact seen on H&E stained slides, I believe it to be a fairly commonly seen artifact in cytology preparations. This artifact, which Cytotechnologists refer to as "cornflake appearance," is caused by air trapped on the surface of cells when xylene is allowed to evaporate. It can be avoided by applying the mounting media and coverslip more rapidly.

  To remove it, soak the slide in xylene, absolute alcohol, 95% alcohol, running tap water, and then restain in eosin. In stubborn cases the slide can be placed in glycerin for 30 minutes, rinsed again in water, and then recounterstained in eosin. (Editor's note: See page 160 of above referenced *Histo-Logic* for question and photograph.)

  Judy McConnell, CT, HT(ASCP)
  JFK Medical Center
  P.O. Box 1489
  Lake Worth, FL 33460

- Dear Mr. Luna, the answer for the problem of Mr. Fred Argilan on page 160 of *Histo-Logic* Vol. XX, No. 2, Mar./Apr. 1990, is probably as follows:
  1. Filtering on the conventional hematoxylin before use. After the step eosin-staining: dehydrate slowly.
  2. Alcohol 80% 2 minutes, twice.
  3. Alcohol 96% 2 minutes, twice.
  4. Alcohol 100% 2 minutes, twice.
  5. Acetone 2 minutes, twice.
  6. Xylene 2 minutes, twice.
  7. Mount with "Coverslipping Resin" art. nr: 4495. This is the Tissue Tek II product of Miles Inc., Diagnostics Division.

  (Editor's note: We thank Rob Bosma for his suggestions. However, the correct answer can be found on page 160 of above-referenced *Histo-Logic*.)

  Rob Bosma
  Central Lab for National Health
  in Friesland, Department of Pathology
  Jelsumerstraat 6,
  8917 EN Leeuwarden
  The Netherlands

- This is in response to "Questions in Search of an Answer" submitted by Fred Argilan. Assuming mounting medium is not contaminated, it seems that the tissue sections of both figures become dry before mounting medium and coverglass are applied. Air becomes entrapped beneath the mounting medium, producing a glassine stippling effect. To avoid this problem, the slides should be at least moist with xylene before mounting medium is applied. (Editor's note: See page 160 of above-referenced *Histo-Logic* for question and photograph.)

  Peter Vitelli
  New Rochelle Hospital Medical Center
  New Rochelle, NY 10802

- This is in regards to "Questions in Search of an Answer" on ATPase fading, by Cathy Houseman.

  I worked for 3 years in the Neuromuscular Lab of the Scott-Ritchie Research Laboratory, Auburn University, Alabama. I performed the "calcium" ATPase method on numerous muscle specimens and found the same problem fading after one year. I did some research and found that cobalt sulfide, the end product, prefers a "reducing" resin. Resins such as Permount, Polystyrene and Euparal-Diaphane mixtures tend to cause fading, whereas Natural Canada balsam, B-Pinene and piccolyte resins such as HSR (Harleco Synthetic Resin) or Bioloid and certain esther gums tend to preserve the coloring best.

  Additionally, mountants, such as glycerol gelatin, apathys and those types, tend to hasten fading.

A cure for already faded slides is simple. Just remove coverglass, hydrate, and reimmerse in ammonium sulfide solution. This should fully restore your slides to their once pristine beauty. (Editor's note: See page 161 of above referenced *Histo-Logic* for original question and photograph).

Clint E. Lincoln, HT(ASCP)
JFK Medical Center
P.O. Box 1489
Lake Worth, FL 33460

- This is in response to "Questions in Search of an Answer" submitted by Lee G. Luna in regards to an artifact found in tissue sections. Our laboratory experienced a similar looking "organism" several years ago, and through a lengthy process of elimination, we traced the problem to the 50-gallon drum of 95% ethanol that we obtained from the hospital pharmacy. The barrel had a large pump attached on the edge for easy distribution and could have contaminated the contents within. We started ordering our alcohol in smaller 5-gallon containers that can be emptied in less time, eliminating the need for a pump.

  Although this may not be the solution to this particular problem, it is another area of concern. (Editor's note: See page 161 of above-referenced *Histo-Logic* for original question and photograph.)

(Editor's Note: The answer above is not consistent with the correct answer submitted by Lee G. Luna (see below). However, the point is well made by Ms. Qualin, that the information presented above is another area of concern.)

Alice Qualin
Pathology Medical Labs
San Diego, CA 92121

- This is an answer to a question presented by Lee G. Luna that appeared in *Histo-Logic*, Vol. XX, No. 2, Mar./April 1990, regarding a Brown funguslike growth. The funguslike growth illustrated in the original two photographs was the result of extensive washing (in this case 24 hours) of tissue specimens through a contaminated rubber hose. The rubber hose had been attached to the specimen washing faucets for some time without replacement. This extended use on a daily basis resulted in the production of funguslike growth in the inner surface of the hose. This material will break loose on occasion, resulting in its deposition into the container being washed. This viable funguslike growth will grow in tissue specimens that are washed for extended periods of time. The growth can be seen in Figs. 1 and 2 included in the above-stated issue of *Histo-Logic*. Because of this potential problem, it is advisable that any washing system such as pans, rubber, or plastic hoses be changed, cleaned, or replaced frequently. It is suggested that washing hoses be replaced at a minimum of once every 3 months. Pans or similar containers being used to wash specimens and/or slides should be cleaned with an appropriate soap at least every 2 weeks.

Lee G. Luna
American HistoLabs
Gaithersburg, MD 20879

Immunoassays: Valuable Signals Lurk in the Background Noise*

Tim Johnson, Ph.D.
Colorado State University
Fort Collins, CO 80524

From the top of a remote mountain, the night sky is a miraculous show for those who usually view the upper atmosphere while they are surrounded by city lights. Fireworks attract large crowds on the night of the Fourth of July, but few would be interested if the exhibition were held at noon. Without a dark background, these light displays would draw little attention.

One frequently contrives to extend immunoassays beyond their present use. When pushing the technique, one's attention is frequently focused on more sensitive signaling capability. This issue discusses membrane substrate systems for horseradish peroxidase. It also reviews avidin- and strept-avidin-based signaling systems. Today, when one designs or remodels an immunoassay, it is good fortune that there are many sensitive signaling systems readily available. The most powerful signaling system is one that can detect the fewest molecules. Indeed, the

detection of a single antigen may be a heady goal for an immunoassay. But can that goal be a source of greater mischief than help?

Let us look for evidence of a virus infection by immunoassay. A short list of things needed includes: 1) viral antigens, 2) a surface for irreversibly binding viral antigens, 3) blocking molecules that are used to minimize the nonspecific accumulation of subsequent labeling molecules on the assay surface, 4) antibodies that recognize unique viral antigens, 5) a second antibody-signal molecular conjugate that recognizes only the first antibody in a way that allows the signal molecule to function, and 6) a clean work area and a clean technical performance that minimizes background.

Even when one fulfills this short list imperfectly, the molecular "needle in the haystack" is routinely detected and quantified. New questions push us to extend the results further, and one looks to more sensitive signaling capabilities. However, the importance of time spent to reduce background cannot be overemphasized. Make careful observations of background color or fluorescence signals. How do these signals arise? How can these signals be suppressed? Is the background diffuse? Are all strong signals accounted for?

Even partial answers to these questions allow refinements of the assay. Can the antigen source be "cleaned up" to give better specificity? Does the blocking molecule displace or sterically mask antigens? Does the blocking molecule preparation bind weakly to either the primary antibody or the secondary antibody-signal molecule conjugate? Try different blocking molecules. Be innovative. Dilute antibodies in blocking molecule preparations so that if there is weak interaction between the blocking molecules, the soluble blocking molecules provide competition for those bound on the surface.

There are many variables and thus many approaches to improving an immunoassay, but the path is self-correcting if positive controls and negative controls are always included. Controls are your compass. Use them to improve the signal-to-noise ratio. Work on lowering the background signal. Improvements in signal-to-noise ratios can yield impressive results akin to a view of the night sky from the mountaintop. Or, perhaps to see the glow from individual fireflies.

*Reprinted with permission from KPL's *Antibody at Work*, Vol. 3, Summer 1990, published by Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD 20879.

European Society of Histotechnology Formed

Andre Bexter
Nestec Ltd.
Lausanne, Switzerland

Early in 1989, a group of Histotechnologists from a number of European countries met for the first time in Ghent, Belgium, to discuss the possibility of organizing a European Histotechnology Society.

Since that date, a constitution has been written and Society officers selected, two members each, from the founding countries. These countries are Great Britain, France, Germany, Belgium, Holland, and Switzerland.

The Society that has been formed addresses histotechnology from a completely new concept, is independent of existing societies, and allows membership to anyone who is interested in the field of histotechnology.

The Society's main interest is based on the following aims:

- To standardize educational qualifications in order that they become accepted throughout Europe.
- To exchange personnel between laboratories and countries.
- To standardize histological techniques throughout Europe.
- To organize European seminars, conferences, and workshops.
- To establish a reference library of tissues, slides, and stains.

Individuals interested in the Society should write to the following address:

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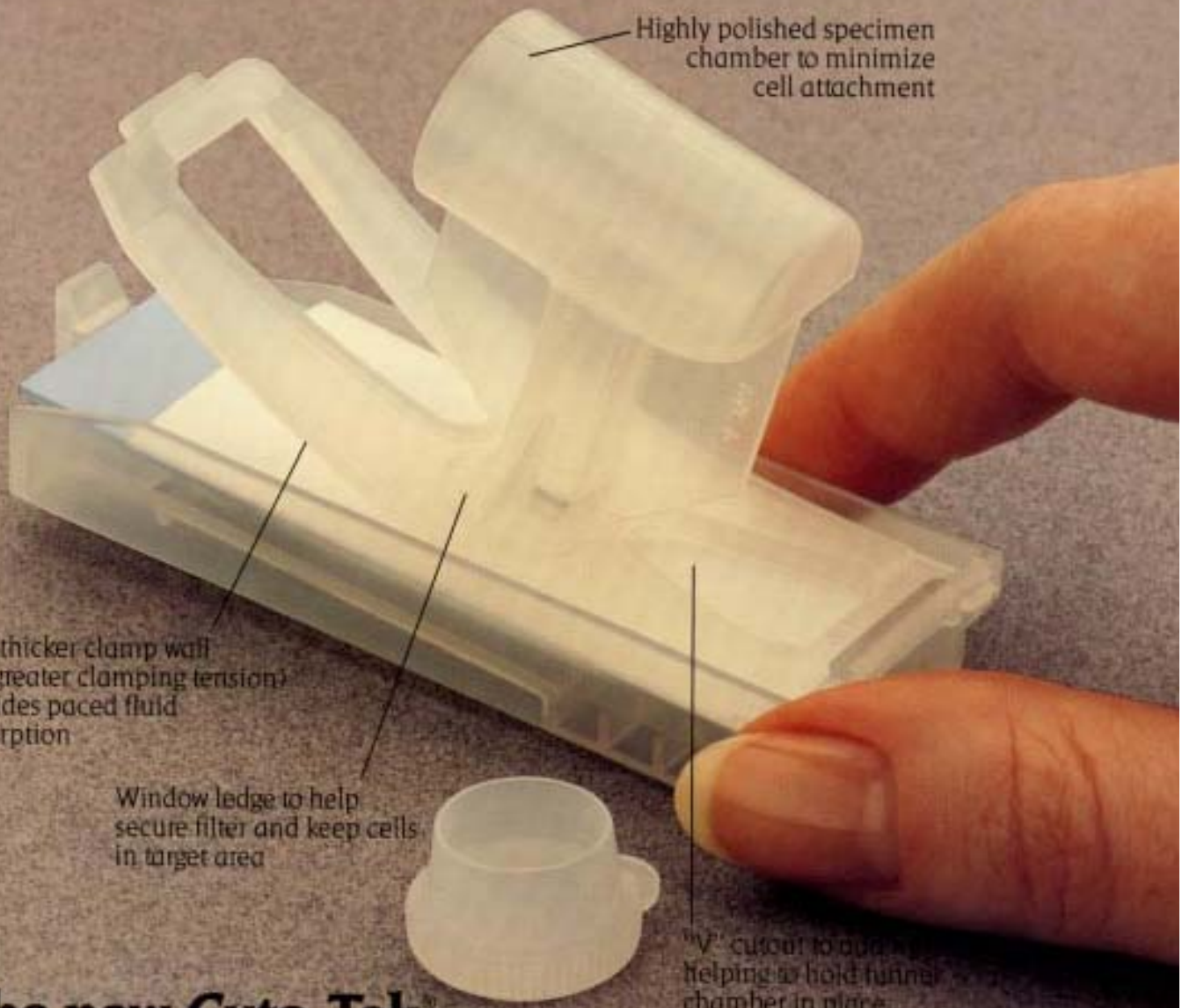

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A Warthin-Starry Silver Technique for the Demonstration of Bacillus Piliformis

Frank Razzaboni, HT(ASCP)
Charles River Laboratories
Wilmington, MA 01887

The Warthin-Starry silver technique is modified for use in demonstrating Bacillus piliformis in paraffin sections. Microwave heating is used, which reduces staining time, but more importantly, when used in conjunction with Walpole's buffer, it dramatically improves the reliability of the technique.

Bacillus piliformis (Fig. 1), the pathogenic bacterium that causes Tyzzer's disease, is a major concern for all rodent-breeding laboratories as well as research facilities that use rodents. An outbreak of this disease often means that an entire colony must be broken down, equipment disinfected and the animals destroyed. The devastating effect of Tyzzer's disease both financially and on long-term research is immeasurable. Thus, the Warthin-Starry silver technique is a valuable tool in prevention/detection and eradication of this pathogen.

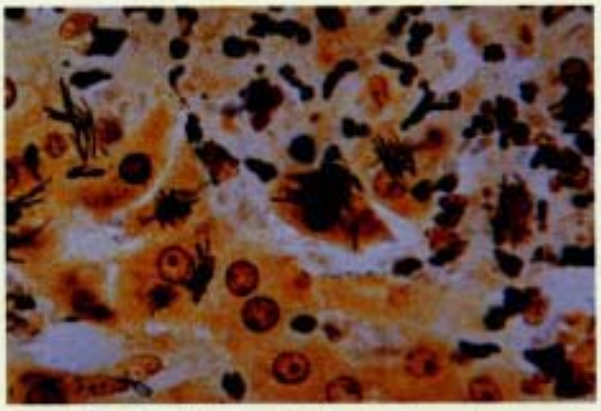

Figure 1: The pathogenic bacterium, Bacillus piliformis, can be seen across the middle of this photograph. Note that the long, rod-shaped bacterium is well delineated on a fairly clear background.

The modification of this procedure further refines an excellent diagnostic tool, which I hope many veterinary histologists will find helpful.

Solutions

5M Acetic Acid (Stock)

Glacial acetic acid	11.8 mL
Deionized water	1000.0 mL

5M Sodium Acetate (Stock)

Sodium acetate	16.4 gm
Deionized water	1000.0 mL

Walpole's Buffer

5M acetic acid (stock)	17.6 mL
5M sodium acetate (stock)	2.4 mL
Deionized water	480.0 mL

2% Silver Nitrate

Silver nitrate	2.0 gm
Walpole's buffer	100.0 mL

1% Silver Nitrate

Silver nitrate	1.0 gm
Walpole's buffer	100.0 mL

5% Gelatin

Gelatin	5.0 gm
Walpole's buffer	100.0 mL

3% Hydroquinone

Hydroquinone	3.0 gm
Walpole's buffer	100.0 mL

Developer

2% silver nitrate	1.5 mL
Gelatin	7.5 mL
3% hydroquinone	0.5 mL

Staining Procedure

1. Deparaffinize and hydrate to deionized water.
2. Rinse in Walpole's buffer.
3. Place slides in a plastic coplin jar, cover with 1% silver nitrate, and microwave for 1.5 minutes at a medium setting (see number 4 in remarks). Let slides stand for 5 minutes in this solution.
4. Rinse in Walpole's buffer.
5. Flood slides with developer that has been freshly prepared during the preceding 5 minute standing time.
6. Wash in warm tap water.

7. Dehydrate in two changes of 95% ethanol, followed by two changes of absolute ethanol.
8. Clear in xylene.
9. Mount with permount.

Results

Bacillus piliformis stains black and the background is light brown to golden brown.

Remarks

1. In our laboratory, we make up a large volume of the gelatin solution in advance and then aliquot 7.5 mL into 15 mL screw-top vials that are then stored in the refrigerator until needed. We can then take one vial of gelatin, heat it in warm (56°F) tap water, add the solutions, and then discard the vial after the procedure has been finished. Using a disposable vial greatly cuts down on the chance of contamination.
2. Most Warthin-Starry variations that have been used in the past have required triple distilled water; this modification works fine with deionized water.
3. Plastic coplin jars used in this procedure should be acid cleaned.
4. The microwave used in this procedure was a 600-watt Sharp Carousel-II.
5. As with most silver stains, the slides should never be touched with metal instruments.

References

Luna LG. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York, NY: McGraw-Hill; 1968:238-240.

Sheehan DC, Hrapchak BB. *The Theory and Practice of Histotechnology*. 2nd ed. Columbus, Ohio: Battelle Press; 1980:151.

Acknowledgments

Photography and procedure reviewed by Elias T. Gaillard, DVM, MS. Technical assistance by J. Paul Cowley.

The Histotechnology Group of N.S.W.: Alive, Well, and Active

Lee G. Luna
American HistoLabs, Inc.
Gaithersburg, MD 20879

The histotechnology group of New South Wales (NSW), Australia, continues its active role in the promotion of histotechnology. This group publishes a multipage, interesting, and informative newsletter (Fig. 1); organizes scientific conferences; and, in general, actively serves the histotechnology community of New South Wales, Australia. This group recently honored the Scientific Editor of *Histo-Logic* by making him an Honorary Life Member of the New South Wales Histotechnology Group. The Scientific Editor has been honored in the past by this and other histotechnology groups in Australia. These honors have come in the form of invitations to speak throughout Australia.

Figure 1: This photograph is from the front cover of the *Newsletter of the Histotechnology Group of N.S.W.*, Australian newsletter.

The University of Texas Health Science Center Teleconference Program

The readers of *Histo-Logic* will be interested in the teleconference programs available for Histotechnologists, Cytotechnologists, Pathologists, and Medical Technologists presented by the Teleconference Network of Texas at The University of Texas Health Science Center at San Antonio. By using a speaker or conference phone, laboratory personnel can participate and earn Continuing Education Units. The program titles, dates, and related information are provided below.

HISTOTECHNOLOGY - ISSUES - SOLUTIONS - TOPICS - OPPORTUNITIES
Fridays, February 1, 1991-August 2, 1991
1:00-2:00 PM, ET; 12:00-1:00 PM, CT;
11:00 AM-12:00 PM, MT; 10:00-11:00 AM, PT

February 1
"Unlocking the Door to Immunohistochemistry: Troubleshooting and Applications" Part I
B. Lynn Caron, BA, HT, HTL(ASCP)

March 1
"Unlocking the Door to Immunohistochemistry: Troubleshooting and Applications" Part II
B. Lynn Caron, BA, HT, HTL(ASCP)

April 5
"Infectious Diseases Associated with AIDS"
Charles Churukian, HT, HTL

May 3
"In Situ Hybridization in Utilizing DNA Probes"
Dorothy Clark, MT (ASCP)

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Leo Kelly: The Making of a Pathologist's Assistant

Brent Riley
Managing Editor

Finding his niche in the medical profession was a struggle for Leo Kelly. He graduated from Central Connecticut State University with a Bachelor of Arts degree in biology; still he felt like he had no profession.

"I applied to hospitals for work in their laboratories," he explained, "but quickly found out I was over-educated, yet under-qualified." Without specific training or practical experience, work was hard to find.

"Finally, I did get a job typing and grouping blood for one of the mobile laboratories of the American Red Cross," he continued. "This was a start — a job — but certainly not a profession I'd want to do for the rest of my life."

With four years of college, he was qualified to take the one-year, hospital-based Medical Technology (MT) training program to be eligible for ASCP certification. Such a program was offered through Yale University and coordinated by Quinnipiac College in Hamden, Connecticut.

"During my interview," Kelly recalled, "the coordinator must have sensed that I was not overly enthusiastic about mass production and computerization. He suggested I look into a brand-new, government-sponsored educational endeavor between Quinnipiac College and the VA Medical Center in West Haven. The program focused on anatomic pathology.

"This was my career break," Kelly said. "After interviewing with the Medical Director of the newly established Pathologists' Assistant Training Program, I was convinced that the hands-on of anatomic pathology was what I really wanted."

Kelly became one of four students in the first class of Pathologists' Assistants in 1971 at Quinnipiac College. Two years later, he received a Bachelor of Science degree as a Pathologists' Assistant — and a profession.

After graduation, the West Haven VA offered him a position as Coordinator of their Pathologists' Assistant Training Program. Kelly jumped at the opportunity. By that time, Quinnipiac College was offering a Master's degree for the Pathologists' Assistant. Working in the college area, he was able to continue his education on a part-time basis. He received his Masters of Health Science (MHS) degree as a Pathologists' Assistant in 1976.

Pathologists' Assistants perform tasks that were once reserved exclusively for Pathologists. In addition, they are qualified to perform some Histotechnologist duties, such as cutting and staining frozen sections.

The Pathologists' Assistant is trained to perform autopsies, from summarizing the clinical history, to evisceration and dissection of organs and tissues. The Assistant presents the gross findings to Pathologists and submits the sections to the Histology Laboratory, where the microscopic sections are made. The Pathologist then makes the final anatomic diagnosis.

In surgical pathology, the Pathologists' Assistant is trained to describe and dissect all specimens. The tissues are then submitted to the Histology Lab, where the microscopic sections will be made.

"Since the three professionals—Pathologist, Pathologists' Assistant, and Histotechnologist — have so much in common, I have tried to become involved with each group," Kelly said. He has worked with the American Association of Pathologists' Assistants (AAPA), the Fairfield County Pathologists Society (FCPS), the Connecticut Society of Histotechnologists (CSH), and the National Society for Histotechnology (NSH). "I feel that the combined educational benefits of these organizations have immensely helped my role as an educator in anatomic pathology," he explained.

Kelly is not just a member but an active participant in all four organizations. He is currently Treasurer of CSH and has served as the Financial Coordinator for three NSH Region I meetings. Since 1983, Kelly has been responsible for the NSH Educational Resource Booth at the national convention. He has updated the Society's education resource booklet and has helped secure more than 75 books for review at the national meetings.

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In addition, Kelly has served as Secretary and President for the AAPA and has served on a number of AAPA committees. His papers have been published in a number of publications, including *Laboratory Medicine*, the *Journal of Clinical Gastroenterology, Human Pathology,* and the *Medical Laboratory Observer.*

"I view anatomic pathology as a single discipline with many essential components," Kelly explained. "All participants are professionals and their work must reflect it. As long as people continue to have illnesses, anatomic pathology will continue to play an important role in quality health care."

Jules M. Elias, Ph.D.: Always a Step Ahead

Brent Riley
Managing Editor

When it comes to talking about the *Journal of Histotechnology,* Jules Elias is never at a loss for words. For the past five years, Elias has been Editor of what has perhaps become the most prestigious histotechnology publication in the world.

His passion for biology began at Brooklyn College. Although he was a musician and fully intended to be a music major, his first biology course kindled an interest that would change his life forever. At the time, biology was merely a required course for his degree.

"I began to go the route of biology, because there wasn't anything that didn't interest me," Elias explained. "Anatomy intrigued me. Embryology intrigued me. I had a chemistry minor.

"My first love was botany," he continued. "I took every course they had in botany but, as a city boy, I didn't have a wide enough frame of reference to know what I could do as a botanist."

One teacher, in particular, influenced his ultimate career decision. "I was fascinated by my botany teacher, Carl Withner, who was a world authority on orchids. His method of teaching got to me. He always said plants were more sophisticated than animals because they can't talk or walk, yet they function and survive in ways that are unbelievable.

"Carl Withner had a great skill of drawing," he continued. "He did the most beautiful drawings of orchids, the kind you would see in a *National Geographic.*"

The son of immigrant parents, Elias grew up in Brooklyn, New York. He was the first in his family to attend college. His two older brothers went to trade school and, as a child, he had the same intentions. "In those days, trade school was expected of children from immigrant families," he said, but his academic record while in junior high school prompted him to go to an academic high school.

"I had two sets of friends: street friends and school friends. My street friends were the ones who quit school at 16 and went into business and became very successful and very wealthy, and my school friends went into accounting and teaching. They were the ones who influenced me."

After his first year at Brooklyn College, Elias enlisted in the Army for two years. After serving in Germany during the Korean War, he returned to college and finished his baccalaureate degree while his wife, Renee, worked to support the family.

Most of Elias' undergraduate classmates went on to medical school or dental school, but Elias wanted to teach. After graduation, he enrolled in a masters program in biology at C.W. Post College. He attended classes at night and, during the day, worked at Helena Rubenstein Laboratories. "I was doing their FDA testing work on rats and rabbits to see the effects of hormone creams and other cosmetics," Elias explained.

He then went into research at Brookhaven National Laboratories. "At Brookhaven, I was fortunate to have the opportunity to work with some very cutting-edge people," he said. "I learned a lot of good biology."

Later, Elias left Brookhaven and became a "freelance" Histotechnologist, working for hospitals at night. "I would leave the house at about 11 o'clock at night and cut 100 to 150 blocks every night," he said.

"I was making a lot of money, but getting very bored," he recalled. "So I cut my salary almost in half to go back to Brookhaven Labs and spent four more years doing research in the Medical Department. That's where I published my first paper."

After his second stint at Brookhaven, Elias went to Stony Brook University, part of the State University of New York (SUNY) system. They had just opened a new medical school, and Elias helped organize their Department of Pathology. This year, he celebrated his 20th anniversary at Stony Brook.

Elias' clinical expertise is in leukemia. "I actually do the leukemia workups for patients—with M.D. support," he explained. "Over the years I had a keen interest in blood, so I had a good insight into the leukemic process. So I do function at the M.D. level in that one small area."

In 1980, an incident occurred that led to his formal title of Professor Elias. "I had always been called Professor Elias because I had a master's degree and my own laboratory, and I was teaching a few courses. I did pretty well considering the fact that I did not have a Ph.D. One day I was introduced by a Pathologist friend as "Mister" Elias, and it sort of rang a bell in my head. I liked the idea of being called Professor Elias because it distinguishes what I am and what I do. So after the other person left, I confronted my friend and asked him why he did not call me by my title. He said it was something he was not used to doing because in Europe, professor has much more prestige than it does in this country.

"Then he said something that really stuck in my mind. He said that when you're working in a clinical area, and you're surrounded by M.D.s, the only title that counts is "doctor," even if it is with a Ph.D. He then said, 'At some time in your life, you're going to have to come to terms with getting your doctorate.' And I said, 'If I'm staying in this business, maybe you're right.'

"The opportunity presented itself because I had already taken my master's degree and I already had the 30 doctoral credits above the masters, but I could never finish my thesis because the person I was working with had left his position and there was no way anybody else was going to pick up on that thesis project.

"I was able to get my Ph.D. through the Union Institute, which was a spinoff of Antioch College. This was a college that administers programs. It's up to the individual to form a credible committee to validate that his level of knowledge in a field reflects a conventional experience. I got the biggest names on campus to be on my committee." That's when it became official. He received his Ph.D.

Elias had already published his first text book, as well as more than 50 other publications in the field. These are things most people don't accomplish until after they get a Ph.D. "The Ph.D. enabled me to get grants and to function at levels where the people were more comfortable in dealing with me," Elias said. "I'm the same person. I'm not any smarter, but it does prove that titles do count."

Elias' second textbook has just been published. It is a second edition of his first book, *Diagnostic Immunohistopathology—A Practical Approach*. The 550-page book was published by the American Society of Clinical Pathologists. He has also co-authored chapters in four other books.

"I could not have done it without my wife's support," he said. "She does all the word processing. I do everything in longhand, and she takes my scraps of paper and turns them into cohesive copy."

Elias is the fifth editor of the *Journal of Histotechnology*. He has been editing the publication for the past five years. His wife, a former English teacher, serves as managing editor.

"This is a mom and pop operation," he said. "We work out of the house. Renee is the secretary. She's the managing editor. She's the copy editor. She's the long distance caller. And she's the typist.

"If you talk to professionals, they would never believe how we're running this journal, especially when they see the copy and the quality that's coming out. In most cases, these people have offices and three or four people. We find it very comfortable operating on this level.

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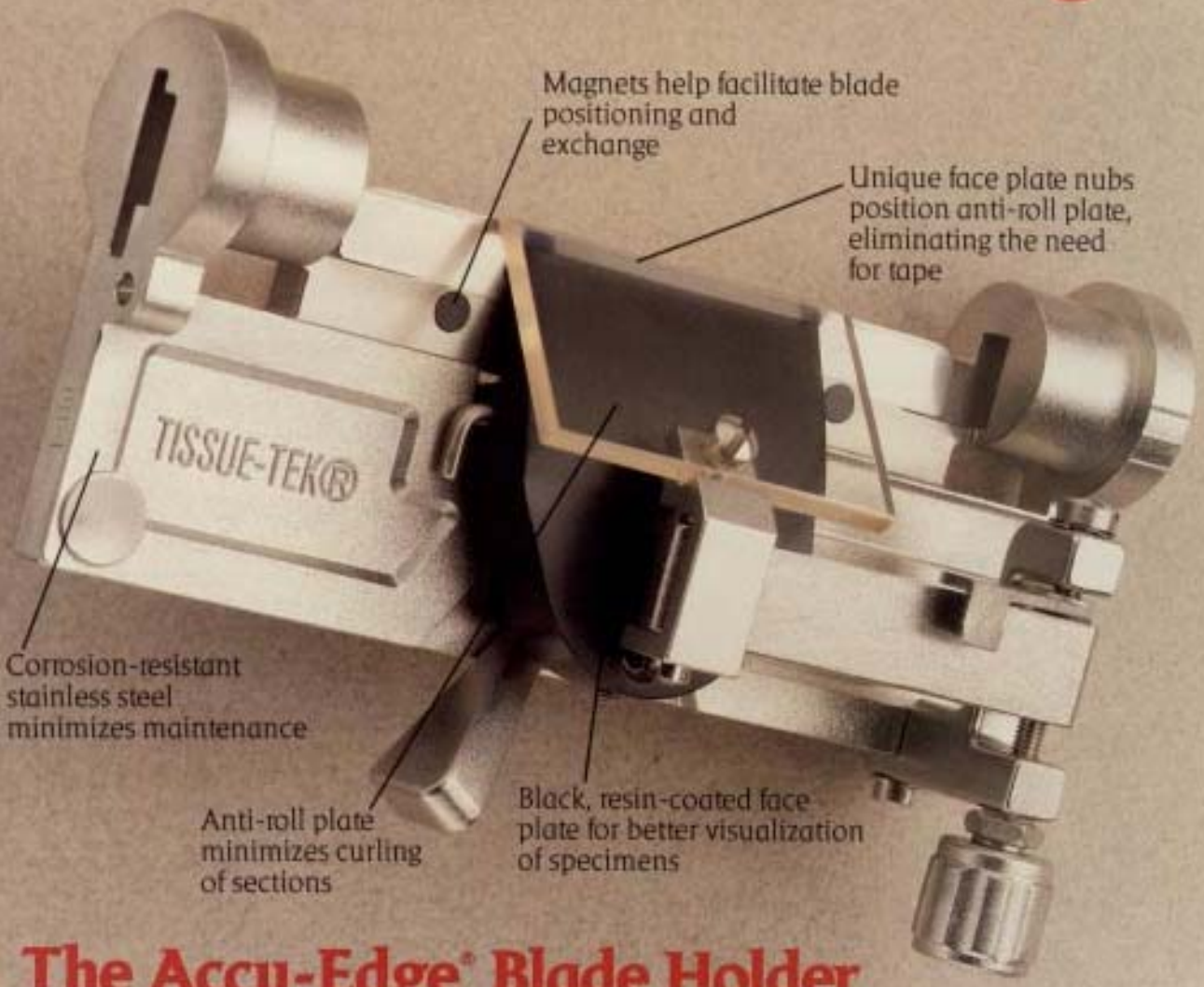

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"The only major anxiety I have about running this journal is not the work involved, but the anxiety of not having enough copy to turn out the kind of full issue that we've grown to expect." Fortunately, this has never happened. "In fact, we're now two full issues ahead," Elias said.

Elias' journal responsibilities might seem like a full-time position, but he also heads the university hospital histology unit. That includes 15 or 16 Histotechnologists in three separate labs. He also is involved in research to bring on new technologies for use in the University's Laboratories.

He also teaches a number of classes. "My teaching is just extra duty," he claims. "I teach a course called 'Current Concepts in Diagnostic Pathology.' I'm also helping to teach a course in the medical school called 'Medicine in Contemporary Society.'" In addition, Elias teaches a course to high school science teachers called "Understanding Immunology." The course is funded by the National Science Foundation and is designed to address the current AIDS crisis.

Elias had discovered an important side benefit to his journal responsibilities. "I nurture a very strong writing relationship with people all over the world," he explained. "And even though I've never met some of these people in person, we know each other through the mail. This kind of rapport has established alliances that would not have come that easily. That's one reason we receive so many papers from foreign authors. They also feel honored to be published in an American journal. At any rate, I have a large writing obligation that will sustain itself even when the journal is no longer in my tenure. That will certainly be a fringe benefit when it's all over."

Today, Elias lives on Long Island. He has three adult children. In his spare time (if, indeed, he has spare time), he enjoys gardening, yoga, and his two grandchildren. He and his wife also enjoy the opera and theater.

Still, one of his greatest sources of pride is the *Journal of Histotechnology.*

The *Journal of Histotechnology*— Evolution, Revolution, and Revelation

Brent Riley
Managing Editor

Flip through a copy of the *Journal of Histotechnology* from 1985. You'll see a very different journal compared with the one published today. Go back to 1979, and you'll see another different approach to the journal.

Through the years, each version of the journal has served a need very well. But, according to the journal's current editor, Jules Elias, Ph.D., the journal must be a dynamic publication in order to reflect the dynamic nature of histotechnology. Change, therefore, is inevitable. The journal has evolved to reflect the changing needs of its readers, the progress of the profession it represents, and the philosophies of its editor.

Originally, the journal served as a combination of journal and newsletter for members of the National Society for Histotechnology (NSH). It included both scientific papers and news articles about the NSH. Today the journal is more... and less. There is more science, and the news has been eliminated altogether.

Elias has certainly left his own mark on the publication. "Essentially, the journal served the need as a major communication vehicle for the society," he explained. "But it was, in a way, incestuous. The opportunity to get outsiders to read the journal and contribute to it was limited. A great deal of the information in the journal was of interest only to members.

"That was a good idea in the beginning," he continued. "But I felt that a journal should bring forward information that keeps our members up to date as to what is the cutting edge of new technologies."

The concept works. The journal is now part of a well-rounded trio of publications, which includes *Histo-Logic* and *NSH in Action*, serving all the needs of the society and the profession in general.

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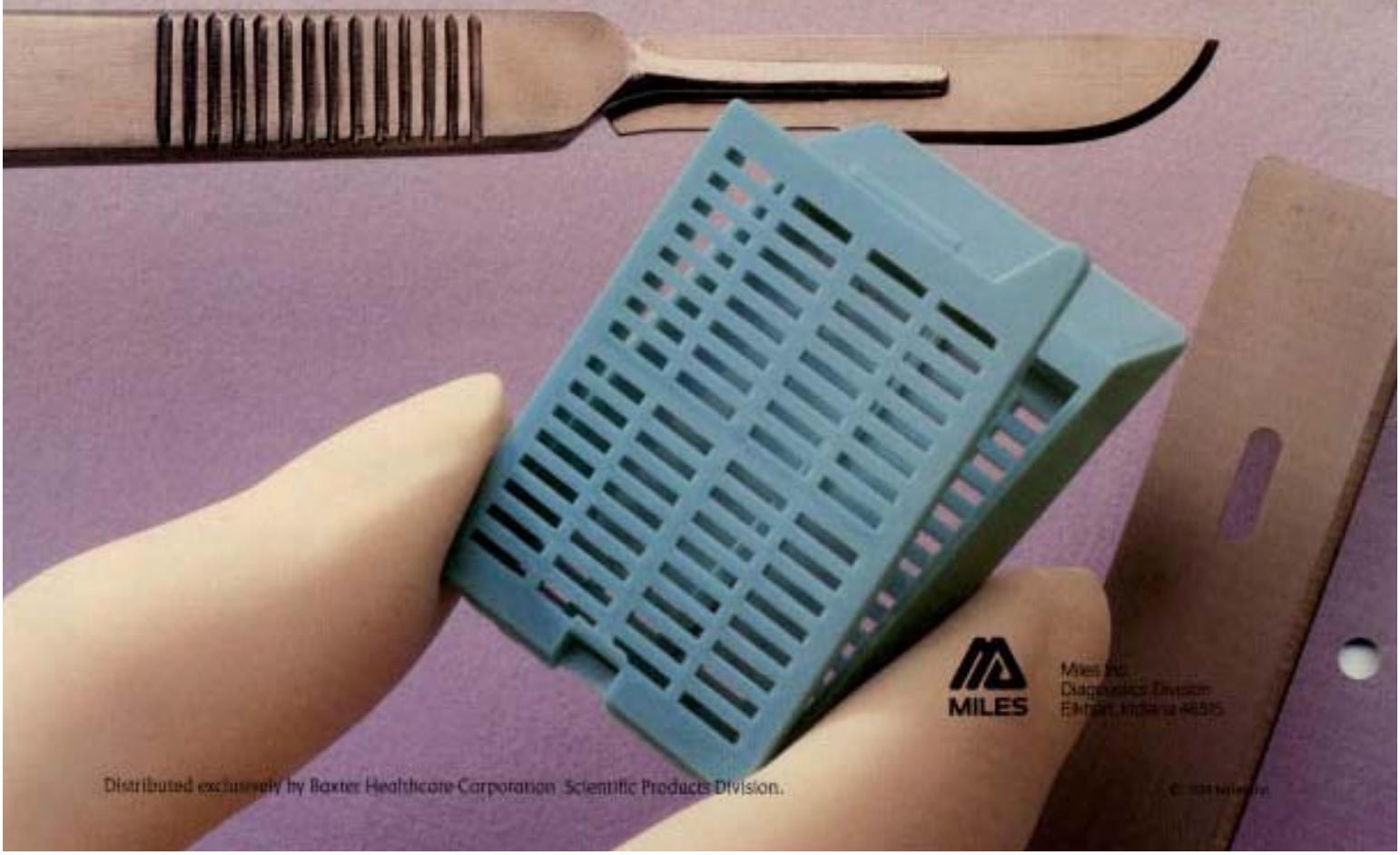

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"It turns out that the journal has taken root," Elias said. "The journal is now the major vehicle in this country—and maybe the world—for the histotechnology profession as far as disseminating the broadest information possible, including clinical, basic science, and new technologies."

An additional source of pride for Elias and the NSH is the fact that the journal is now abstracted in Current Contents—Life Sciences, as well as Excerpta Medica, Subis and Biosis. "Current Contents is a major abstracting service," Elias explained. "So I feel rather proud that we've gotten to that level."

One goal Elias would like to reach is to be included in Index Medicus, which he considers to be the most prestigious medical abstracting service and the highest level of recognition possible. He admits, however, that it will be tough to pull off for a quarterly publication because inclusion in Index Medicus is based on the numbers of outsiders who read the journal and cite its articles in their published papers.

Attaining such a goal would probably mean increasing the size and/or the frequency of the publication. The journal now consists of an average of 92 pages with between 8 and 11 papers per issue. The average issue contains about 60% editorial material and 40% advertising.

"I would like to see the journal become thick enough so it can be perfect bound," Elias said. "That would mean about 120 pages with 14 or 15 papers per issue." Currently the journal is stitched (stapled). *Perfect bound* refers to a machine binding operation where the pages are held together with a backbone adhesive.

Elias would also like to see a fifth issue about every 18 months. This would be a special issue dedicated to a single topic matter. "In essence, it would become a mini textbook of sorts," he added.

Elias is pleased with the progress the journal has made. The changes have resulted in positive feedback from all ranks. "We're getting a good reputation for knowing what we're doing and having a reasonably good threshold of acceptability," he said. "I recently learned that the Library of Congress is getting a lot of inquiries about our journal," Elias continued. "This is another indication that we are a growing entity."

The content of the journal is evolving. "The editor is only a conduit," Elias explained. "We don't pick topic matter. We encourage a broad array of papers."

One of the changes Elias made to the journal was to add four words to the masthead—"anatomy, histochemistry, microscopy, pathology." "Those are the four subtopic areas that deal with everything we do," he stated. "We have a section called "Clinical Case Studies" that deals with pathology. We have occasional papers on microscopy. (In fact, one of our recent microscopy papers received an award. It was a magnificent paper showing how graphics can be used in microscopy.) We have a lot of technique papers. We have basic anatomy papers. So in every way, we're trying to fulfill our charge as being a broad vehicle for the major topic areas that interest our own people.

"If you look at the credentials, the bulk of the articles is coming from combinations of histotechnologists and MD/PhDs who work in the same institutions," Elias said. "I would say that a good 25% of our papers come from out of the country—Europe, eastern block nations, and Japan. Another 15% to 20% come from Canada."

Every published paper is first reviewed by an associate editor or a member of the editorial board. The editorial board consists of a balance of MDs, PhDs, and HT/HTLs. "With this general spectrum of credentials," Elias explained, "if I'm going to consider a clinical paper, probably an MD will review it. If I'm going to consider a basic science paper, a PhD will review it. And if I'm going to consider a technique paper, an HT/HTL will review it.

"In many instances, members are finding that the topic matter is beyond their interest," Elias observed. "This may be true. But, as a member of the histochemical society, I find myself in the same predicament. Their journal is one I used to read from cover to cover. I'm now reading about a third of what's in that journal. What the histochemical journal has done is a reflection of what's going on in that particular field. What we have done is a reflection of what's going on in our field. So the reality is that we all have to grab on and take what we want out of it.

"If you get one good read out of one single paper in a particular issue, you should feel satisfied," Elias continued. "It may not be necessary for you to read the whole journal. The other papers might still be a reference to you later on.

"Some of our papers are of greater depth than others," Elias explained. "But half of our membership have baccalaureate degrees, which, 15 years ago, was unheard of. And a great many of our members have masters degrees and some have PhDs. And we're attracting all types of readers—research people from drug companies as well as clinical people from hospitals. So we have to satisfy a wider need."

The total circulation of the journal is now about 4,000. While most recipients are NSH members, Elias estimates that about 500 are not members.

Evolution is a slow process. But any publication that keeps up with changing needs is destined to survive. The *Journal of Histotechnology* is certainly no exception. It keeps growing, and it keeps getting better. "I couldn't have done it without the cooperation of the national," Elias said. "They have never said no to anything we've asked for."

The bottom line is this: each time you read the latest issue of the *Journal of Histotechnology*, you're seeing a changing publication—one that serves a variety of needs. And one that keeps up with a dynamic profession. It truly is an evolutionary process—with just the right balance of revolution and revelation.

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