MILES

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Microwave Modification of Pascual's[1] Argyrophil Method

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Introduction

A widely used method for demonstrating argyrophil cells is the Churukian-Schenk.[2] The method gained acceptance after a study by Smith and Haggitt[3] showed it to be superior to some other argyrophil methods. Smith and Haggitt tried six argyrophil methods on 73 carcinoid tumors and found that the Churukian-Schenk gave the highest percentage (97%) of positive staining results. The superior results obtained by their method is achieved by reducing background staining, which is accomplished by buffering the silver nitrate solution used in the method to pH 4.2 with dilute citric acid. The reduced background staining makes it possible to identify small, faintly stained argyrophil granules, which is especially important in identifying the granules in rectal carcinoids.

We have found that the Churukian-Schenk method can be performed much faster in a microwave oven than in a water bath. It takes only about 15 minutes to perform in a microwave oven as compared to about 2½ hours in a water bath. The staining results obtained are at least as good if not slightly better than those obtained using a water bath. A new, easy to prepare buffer solution is utilized with the microwave oven method that does not require the use of a pH meter to achieve the required pH of 4.2.

Fixation

10% buffered neutral formalin

Technique

Paraffin sections cut at 5 microns

Solutions

Citric Acid-Glycine (Stock)

Citric acid	0.06	gm
Glycine	1.2	gm
Distilled water	100.0	ml

The pH of this solution is approximately 3.8. Store in a refrigerator at 3-6°C.

Citric Acid-Glycine (Working)

Citric acid-glycine (stock)	5.0	ml
Distilled water	500.0	ml

The pH of this solution is approximately 4.2.

(continued on page 122)

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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.

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0.5% Silver Nitrate Solution

Silver nitrate	0.5	gm
Citric acid-glycine, working solution	100.0	ml

Bodian's Developer

Sodium sulfite (anhydrous)	5.0	gm
Hydroquinone	1.0	gm
Distilled water	100.0	ml

Prepare fresh.

0.1% Nuclear Fast Red (Kernechtrot) Solution

Dissolve 0.1 gm nuclear fast red in 100 ml of 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add a few grains of thymol as a preservative.

Staining Procedure (use control slide)

1. Deparaffinize and hydrate in citric acid-glycine working solution.
2. Place in 40 ml of 0.5% silver nitrate solution in a plastic Coplin jar and microwave at power level 8 (400W) for 1 minute (approximately 92°C).
3. Dip the slides up and down several times and allow them to remain in the hot solution for 2 minutes.
4. Rinse in four changes of distilled water.
5. Place 40 ml of Bodian's developer in a plastic Coplin jar and microwave at power level 8 for 40 seconds (approximately 72°C). Immediately place the slides in this hot solution and allow them to remain in the solution for 1 minute.
6. Rinse in four changes of distilled water.
7. Place the slides in the same 0.5% silver nitrate solution for 1 minute.
8. Rinse in four changes of distilled water.
9. Place the slides in the same Bodian's developer for 1 minute.
10. Rinse in four changes of distilled water.
11. Place the slides in the same 0.5% silver nitrate solution and microwave at power level 8 for 15 seconds.
12. Dip the slides up and down several times and allow them to remain in the hot solution for 1 minute.
13. Rinse in four changes of distilled water.
14. Microwave the same Bodian's developer at power level 8 for 15 seconds. Immediately place the slides in the solution and allow them to remain in the solution for 1 minute.
15. Rinse in four changes of distilled water.
16. Counterstain with 0.1% nuclear fast red for 1 minute.
17. Rinse in three changes of distilled water.
18. Dehydrate in graded alcohols.
19. Clear in three or four changes of xylene.
20. Mount with Permount.

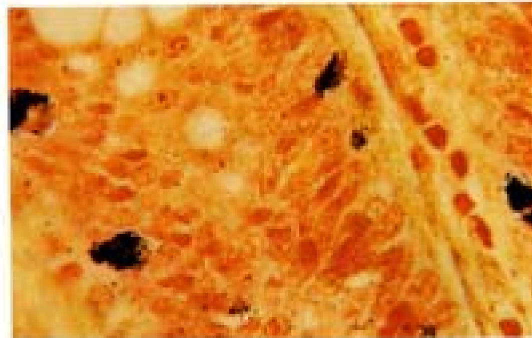

Figure 1: A section of jejunum that demonstrates argentaffin (Kulchitsky) cells stained in black. X1000, modified Pascual.

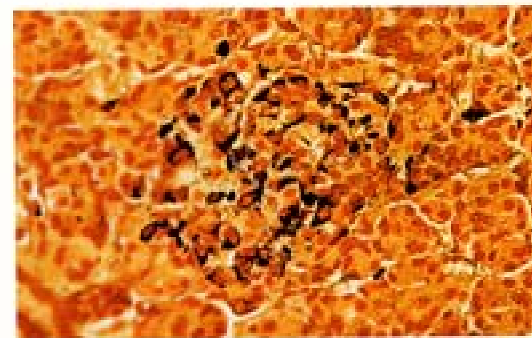

Figure 2: A section of pancreatic islet demonstrating beta cell. X400, modified Pascual.

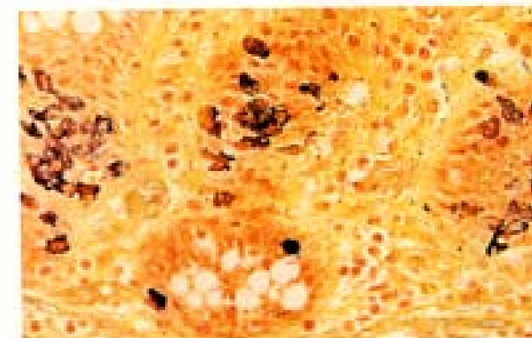

Figure 3: A section of jejunum that demonstrates carcinoid tumor cells. This tumor was found in the same section of jejunum seen in Figure 1. X400, modified Pascual.

Results

Neuroendocrine cell granules (argentaffin and argyrophil) in various organs stain dark brown to black. Nuclei are orange to red and the background a light yellow-orange (Figs. 1-3).

Comments

Argyrophil cells are found in the gastrointestinal mucosa (Fig. 1), pancreas (Fig. 2), trachea, bronchi, prostate, and ovary. Tumors derived from these cells are called carcinoids (Fig. 3), apudomas, or neuroendocrine tumors. Argyrophil cells, as their name indicates, have an affinity for silver ions but lack the ability to convert the silver ions to the metallic state. This is why a reducing agent is needed in the staining procedure.

In Pascual's method and our modification of Pascual's method, tissue sections are double impregnated with silver nitrate followed by treatment with a reducing solution. In the described staining method, a triple treatment with silver nitrate and the reducing solution is necessary in order to obtain optimal staining results.

In our modification of Pascual's argyrophil method, the pH of the distilled water is adjusted to 4.2 with dilute aqueous citric acid. This requires the use of a pH meter and is somewhat time-consuming. The described method utilizes a citric acid-glycine buffer with a pH of 4.2 that is easy to prepare. Like most buffer solutions, ours is stable when stored in a refrigerator at 3-6°C.

When solutions are heated with microwave irradiation, there can be up to a 15°C difference in temperature between the top and bottom portion of the solutions. Therefore, in order to equalize the temperature of the solutions, the slides are dipped up and down in Steps 3 and 12 of the staining procedure. The microwave oven that we use is a Litton Model 1450 with touch pad variable power supply up to a maximum of 500W. Undoubtedly, other makes of microwave ovens can be used in performing this method but it may be necessary to make changes in the selection of the power setting and times used in Steps 2, 5, 11, and 14 of the staining procedure.

The shelf-life of silver nitrate and hydroquinone can be greatly increased by storing the reagents in a refrigerator at 3-6°C.

References

1. Pascual JS: A new method for easy demonstration of argyrophil cells. *Stain Technol* 1976;51:231-235.
2. Churukian CJ, Schenk EA: A modification of Pascual's argyrophil method. *J Histotechnol* 1979;2:102-103.
3. Smith DM, Haggitt RC: A comparative study of generic stains for carcinoid secretory granules. *Amer J Surg Pathol* 1983;7:61-68.

Do You Know?

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- that tissue-processing schedules used today in most laboratories are longer than necessary to produce good impregnation of tissue specimens. This generally results in overhardening of the specimens, primarily due to the excessive removal of water. This means that water must be reintroduced into the specimen just prior to microtomy to ensure the cutting of good sections. The amount of water that is necessary for a given specimen is determined by the density and type of specimen, reaction of specimen to processing solutions, and extent of overprocessing. For example, a lung specimen may not require more than 5 seconds of soaking. On the other hand, a similar size piece of liver exposed to the same processing schedule may require 10 to 20 seconds. A thyroid specimen may require 30 seconds to 1 minute of soaking; a decalcified cortical bone, up to 3 minutes of soaking; however, bone marrow spicules will require no more than 1 minute of water soaking. Even more significant is the soaking required for a well-decalcified tooth. In this case, one may have to soak the tooth for as long as 30 minutes before obtaining 12-15 well-cut sections of the tooth. The important message here is that all tissue specimens require reintroduction of water to ensure the cutting of good sections. Please note that the references made to soaking or reintroduction of water in above comments refer to placing a piece of cotton soaked with lukewarm water against the surface of the specimen that has been faced (rough cut) prior to microtomy.

- that potassium permanganate and oxalic acid bleach of melanin cannot be performed on slides destined for the Fontana-Masson silver reaction. This is because the oxidative effects of these chemicals prevent good, specific staining of silver reactive entities. In addition, the adhesive properties of many section adhesives are lost, resulting in sections falling off the slides.

- that light-green solutions containing acid should not be used as a counterstain for procedures staining calcium, iron, or copper. The presence of acid results in removal of these entities from the tissue section during the counterstaining exposure period. One can and should use a light green without acid for counterstaining slides containing above-mentioned entities.
- that hematoxylin solutions vary in staining vigor according to the amount of dye used, stage of oxidation or over-oxidation, and acidity.

Techniques for Examining Ventriculo-Peritoneal Shunts

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Recently, neurosurgeons have become increasingly interested in having old shunts examined more thoroughly at the time of shunt revisions.

Occasionally, shunts that have been in place for many years will have a granular gray-white material adhering to the exterior of the valve and shunt catheter (Fig. 1). This material can be identified as containing calcium by immersing the shunt in alizarin red solution. The coating on the shunt will be stained red if it contains calcium (Fig. 2).

To examine the contents of the shunt, it is better to "fix" it in 10% buffered formalin so the cells contained in the valve and catheter will be preserved. Then express the liquid from the valve and catheter onto a millipore filter (size: 13 mm, 0.45 μm pore size). The filter is supported by a 13-mm Swinny Filter Adapter. Next, assemble the adapter and express a small amount of air through the filter with a 3-ml syringe. This helps push the cerebrospinal fluid through the filter. The filter is then stained with the following hematoxylin and eosin procedure:

1. Fix filter in 95% ethyl alcohol for 10 minutes.
2. Running water, 5 minutes.
3. Gill's hematoxylin, 30 seconds.
4. Running water, 5 minutes.
5. Bluing solution, 12 dips.
6. Running water, 5 minutes.
7. Eosin, 30 seconds.
8. 95% ethyl alcohol, 10 dips.
9. 100% isopropyl alcohol, 10 minutes.
10. Xylene, 5 minutes.

Mount coverslip with resinous media and seal edges wit clear nail polish.

Although the shunt contains only a small amount of flui the millipore preparations often contain inflammatory cell macrophages, and even giant cells (Fig. 3).

In some cases, no fluid can be squeezed out of the valv Its contents can still be examined by flushing the shur with 95% ethyl alcohol and collecting the fluid on th millipore filter.

Figure 1: This shunt shows a whitish-gray material adhering to surface of the val and catheter.

Figure 2: Red staining calcium coating can be seen on this shunt stained in alizari red S solution.

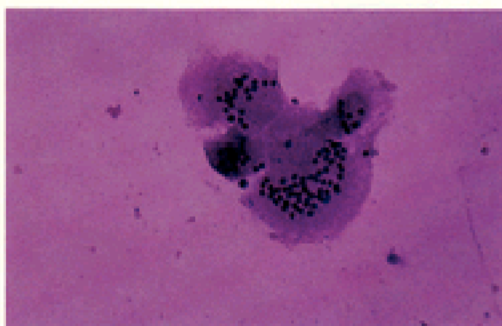

Figure 3: Results of millipore filter processing can be seen clearly.

Education Gains More Ground at NSH Symposium Convention

Brent Rilley
Managing Editor

It can't be overemphasized. It can't be overvalued. There simply is no such thing as "too much" education for histotechnologists. And nothing proved that better than the 15th annual symposium/convention of the National Society for Histotechnology held September 23-28 in Las Vegas.

The theme of this year's convention was "Cash In On NSH," and, based on attendance, plenty of people did. Even with the sights and attractions of the entertainment capital of the world, the main draws were the workshops, lectures, scientific displays, and social events of the convention. And what a draw it was as a record number of histotechnologists — an estimated 1,200 in all — from throughout the country descended upon the Riviera Hotel for the six-day event. That's a 20% increase over last year's record-breaking crowd in Louisville.

"I think part of the reason attendance was so high is that pathologists are beginning to take a closer look at the educational opportunities that the NSH is offering," explained Marilyn Gamble, NSH president. "With the more sophisticated techniques being incorporated into the histopathology lab, pathologists are becoming more supportive of their histotechnicians attending avocational training courses."

"Histotechs go for the education," Gamble continued. "We're keeping up with a lot of the more sophisticated procedures. I think that's enticing a lot of people to attend, and their pathologists to send them. They certainly enjoy the entertainment and social events, but they always show up for the workshops and lectures. Even on the last day of the meeting, we had to put in extra chairs for the lecture series because so many people attended."

The workshops this year were more technical, according to Susan McCoy, program coordinator for the symposium/convention. Workshops and lectures concentrated on more advanced topics to keep up with the more "routine" use of specialized procedures, a trend that is becoming more common in labs across the United States.

Most of the workshops were filled to capacity. And the new workshops proved to be very popular: "Comparative Silver Staining Methods Used in the Diagnosis of Alzheimer's Disease," "An Overview of Cytology," "Medical Terminology," "Human Cytogenetic Analysis," "Plant Histology," and others. New workshops were also conducted on starting a school of histotechnology, the laws of motivation, quality control, and the "right-to-know" law.

Another popular new workshop was conducted by Dr. J. B. McCormick on the study and preparation of antique prepared microslides. Forty-five workshops were held in all from Sunday through Tuesday.

Even though the symposium/convention centered around education, there was plenty of time to "Cash In" on the fun as well. "I was surprised at the number of people who attended the banquet," Gamble said. "We had over 600 people at the banquet, and we had to stop selling tickets because we ran out of space."

The traditional awards banquet was held on Wednesday. During the banquet, awards were presented for outstanding service to the histotechnology profession. Among the awards were two presented by Miles for authorship of journal or newsletter articles. The Golden Forceps Award was presented to Terri Staples, HT, from Baptist Medical Center, Birmingham, Alabama, for an article published in *Histo-Logic*. The Diamond Cover Award recipient was Shakti Unny, PhD, from the biology department of the University of Texas in El Paso. Her article was judged the best work published in the *Journal of Histotechnology*.

Before the banquet, a champagne reception sponsored by Miles Inc., Diagnostics Division, also drew record numbers. A strolling string trio entertained at the party. In addition, etched champagne glasses were given to all those who attended.

Another Miles-hosted event also proved to be very popular. A "Crazy Hat Party" was held Monday night in the "Top of the Riv" room. Music, dancing, and a variety of finger foods attracted virtually everyone who attended the symposium/convention. Prizes were awarded for the "Funniest," "Naughtiest," "Craziest," "Most Feminine," "Most Masculine," "Smallest," "Most-Apropos-for-a-Histologist," and "Most Creative" hats worn. Our hats are off to the winners!

Clinical and nonclinical scientific sessions began on Tuesday and continued through Thursday. Included were two lectures conducted by representatives from the Swiss Society of Histotechnology.

Technical exhibits and posters prepared by histotechnologists were displayed Monday through Thursday demonstrating a variety of specialized techniques. This gave registrants an opportunity to learn more about what other technologists are doing in their laboratories and research centers.

Attendees were also attracted to the scientific exhibits from a number of manufacturers and suppliers. Histotechnologists got a good review of state-of-the-art laboratory equipment, supplies and services.

"It was an excellent meeting—very well organized," concluded Gamble. "The committee did an excellent job and is to be commended. I also think the encouragement and support of the membership has been outstanding during the past year. They deserve the credit. They did the work."

Now that the '89 event is history, plans are already well underway for the 1990 NSH Symposium/Convention. The sixteenth annual event will be held in San Antonio. Plan now to attend. You won't want to miss the many opportunities it presents.

Toasting a successful NSH Convention/Symposium.

One of the many outstanding scientific displays at this year's convention.

Finalists at the Miles Inc. "Crazy Hat Party."

1989 NSH Award Winners

Each year, certain individuals are singled out for their achievements in the histotechnology profession. Miles Inc. and *Histo-Logic* extend their congratulations to those who were recognized for their outstanding contributions in the field of histotechnology at the 1989 NSH Symposium/Convention.

President's Award
Lee G. Luna
Lanham, Maryland

J. B. McCormick, M.D. Award
Leonard W. Noble
Winston-Salem, North Carolina

Miles Golden Forceps Award
Terri Staples
Alabaster, Alabama

Histotechnologist of the Year
Sumiko Sumida
Langley, Washington

Convention Scholarship Award
Herbert Skip Brown
Van Nuys, California

Dezna C. Sheehan Memorial Educational Scholarship Award
Mary-Ione Jackman
Storrs, Connecticut

Lipshaw Educational Scholarship Award
Christina A. Genaw
Flat Rock, Michigan

E.M. Diagnostic Systems, Inc., Educational Scholarship Award
Lynn D. Sachs
Helena, Alabama

Instrumentation Laboratory Educational Scholarship Award
Sandra J. Sendor
Hamburg, New York

Miles Educational Scholarship Award
Janet L. Kliethermes
Raytown, Missouri

Shandon Student Scholarship Award
JoAnn Harris
Little Rock, Arkansas

Sakura Student Scholarship Award
Annie Lynn McCray
Mount Pleasant, South Carolina

William J. Hacker Memorial Award
Diane L. Sterchi
Champaign, Illinois

Newsletter of the Year Award
"SLICE OF LIFE"
The State of Kentucky

Newsletter Merit Award
"MIKRO-GRAF"
The State of Michigan

Miles Diamond Cover Award
Shakti Unny, Ph.D.
El Paso, Texas

Miles Diamond Cover Editor Award
A.R. Villanneva
West Bloomfield, Michigan

Miles Diamond Cover Merit Award
David F. Brigati, M.D.
Oklahoma City, Oklahoma

Just A Reminder

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Something for the HT Student "A Question and Answer Session"

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Editor's Note:
The following questions on histological techniques are intended for students in a program of study dealing with the specialty of histotechnology. This material is being provided in the hope that it can be a learning experience to many of those in training. This is the first time that *Histo-Logic* has presented this type of information, and we trust it will be helpful to many students. It is also being included since many, if not all, publications in this specialty have a tendency to forget those in training. It is suggested that you copy these questions and answer them on the copy. This will keep your issue of *Histo-Logic* clean and intact.

Answers and comments to the following questions can be found on page 135.

1. **T F** A fixative mixture is composed of several ingredients each of which is a single fixative but when combined may compensate for the disadvantage of another, thus making a good fixative.
2. **T F** Upon completion of dehydration, the tissue is put immediately into paraffin.
3. **T F** Toxicity to personnel and the drying effect on the tissue should be a consideration in choice of a clearing agent used in tissue processing.
4. **T F** The purpose of clearing is to remove the dehydrant and to introduce a medium that is acceptable to the embedding compound.
5. **T F** A paraffin block with edges not parallel to the knife edge makes it difficult to obtain a ribbon.
6. **T F** Sections of varying thicknesses are a result of rhythmic operation of the microtome.
7. **T F** Melanin pigment is removed with iodine and hypo after staining has been completed.
8. **T F** The purpose of fixation is to kill and harden the tissue quickly, thus keeping the cells as near to their normal state as possible.
9. **T F** After the use of mercuric fixatives, the tissues must be heated before processing.
10. **T F** Following fixation by alcoholic fixatives, the tissues must be subjected to excessive washing.
11. **T F** A counterstain is used as background upon which is displayed the "principles of the picture," that is, the nuclei, the bacteria or spirochetes, or the fungi.
12. **T F** The paraffin is removed with xylene before slides are stained by the Fite-Faraco technique.
13. **T F** Tissue fixed with Carnoy's does not require washing before processing.
14. **T F** Fixing mixtures are best when they contain six or more ingredients.
15. **T F** Graded alcohols are the only solutions that can be used for dehydration.
16. **T F** All tissue specimens that have been decalcified, no matter by what method, should be washed well before processing.
17. **T F** Tissue that is to be used for fat stains should be washed and dehydrated before cutting.
18. **T F** Mercury crystals are removed from tissue sections with the use of iodine and thiosulfate (hypo).
19. **T F** The MacCallum-Goodpasture and the Brown and Brenn Stains are used for demonstrating Gram-positive and Gram-negative organisms.
20. **T F** Kinyoun's technique is used to demonstrate Donovan bodies and spirochetes.
21. **T F** All glassware should be acid cleaned for any pigment stain.
22. **T F** Potassium permanganate and oxalic acid are used in removing melanin pigment from tissue sections.
23. **T F** Acetic acid in a fixative mixture increases the penetration and prevents excessive hardening of the tissue.
24. **T F** The Movat stain is known as an all-purpose stain because it demonstrates nuclei, cytoplasm, elastic fibers, collagen, muscle fibers, ground substances, and fibrinoid very clearly.

25. **T F** At any point where heat is used in the paraffin technique, it is necessary that the temperature be carefully controlled so as not to burn the tissue.

26. **T F** Formalin crystals (pigment) can be removed by use of an alcoholic solution of picric acid.

27. **T F** Natural ripening of hematoxylin comes by aging at room temperature.

28. **T F** The danger points in making hematoxylin are the additions of hematoxylin and mercuric oxide (red).

29. **T F** When cutting frozen sections, the temperature of the block cannot get too cold.

30. **T F** The use of nitric acid for decalcification may cause poor nuclear staining.

31. **T F** "Zenker crystals" cannot be removed from the tissue once they have been formed in the tissue.

32. **T F** A special stain demonstrates one or more elements or an organism that is not clearly defined with nuclear and cytoplasmic stains.

33. **T F** Bouin's solution is not used in the Masson stain.

34. **T F** Malarial parasites are demonstrated by the phloxine methylene blue procedure.

35. **T F** Parlodion is the trade name for nitro cellulose.

36. **T F** Formalin, to which an excess of calcium or magnesium carbonate has been added, is a good neutralizing solution for decalcified specimens.

37. **T F** Decalcification is aided by heat and vacuum.

38. **T F** Potassium permanganate is used to blue nuclei.

39. **T F** Alizarin red S stains calcium.

40. **T F** The approximate temperature for the water (flotation) bath for routine paraffin ribbons is 45°C.

41. **T F** Fungi is black, inner parts and mycelian rose with a green background in the Grocott's Methenamine Silver nitrate method.

42. **T F** Fontana silver method stains argentaffin granules.

43. **T F** Chemical testing is one of the better ways to determine decalcification end point.

44. **T F** Three of the most probable methods of decalcification in a small laboratory include nitric acid, formic acid-sodium citrate, and commercially available decalcifying fluid.

45. **T F** Following fixation by alcoholic fixatives, the tissues must be subjected to excessive washing with water.

46. **T F** Acid fast bacteria are demonstrated by the GMS and the PAS string.

47. **T F** Hyaluronidase is the enzyme used to digest hyaluronic and sialic acid.

48. **T F** Hematein is the staining element in hematoxylin.

49. **T F** Tissue will not stain if it is not rinsed in water before and after the use of kernechtrot.

50. **T F** The primary fixative agent controls the results of most stains.

Method for Removing Wrinkles from Tissue Sections

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Wrinkles in tissue sections are extremely disturbing to both pathologists and histotechnologists. Wrinkles are produced by different factors related to tissue processing and/or microtomy. Very often the pattern of wrinkle formation provides one with clues to their origin. Wrinkles that run in various directions (Fig. 1) are produced by underprocessing of tissue specimens. Characteristically, this wrinkle pattern is produced by a tissue specimen that lacks sufficient paraffin matrix within its interstices and therefore is squeezed in all directions by the paraffin contraction/expansion factor. In this case, 12-15% contraction of the paraffin takes place around the tissue when it goes from its molten to solid state. This contraction produces a shrinking effect on tissue (Fig. 2). This compression (shrinking) effect may not allow the paraffin to expand sufficiently in a 45°C flotation bath, resulting in the section being contained within the paraffin framework with little possibility of expanding to its original size. This, in turn, results in the development of wrinkles that microscopically go in various directions (Fig. 1).

Conversely, wrinkles that appear to be parallel to each other are produced by several factors – paraffin is too soft, excessively dull microtome blade, or a blade that has too much of a positive angle (angle leaning excessively toward the microtome).

Methods for Removal of Wrinkles in Tissue Sections

Regardless of the reason for wrinkle formation in paraffin sections, there are simple ways to remove most of these wrinkles from tissue sections.

The most effective method is to use a 60°C oven and follow the method outlined below:

1. Pick up sections from flotation bath on an adhesive prepared slide. (Use adhesive of choice.)
2. Allow slide to drain in a vertical position for approximately 30 seconds to 1 minute. (Do not allow section to become dry since wrinkles will "set fast" and will not come out with overexposure).
3. Place slide in a 60°C oven for approximately 20 minutes.
4. Remove slide from oven and allow to come to room temperature before staining with desired stain.

This simple procedure will ensure a wrinkle-free section (Figs. 3 and 4).

Figure 2: This is an unstained section that is being observed under polarized microscope. The white material is paraffin crystals. Note the aggregation of paraffin against the tissue (horizontal line across middle of picture). This aggregation of paraffin produces an inward pressure contraction on tissue, causing it to shrink. X100.

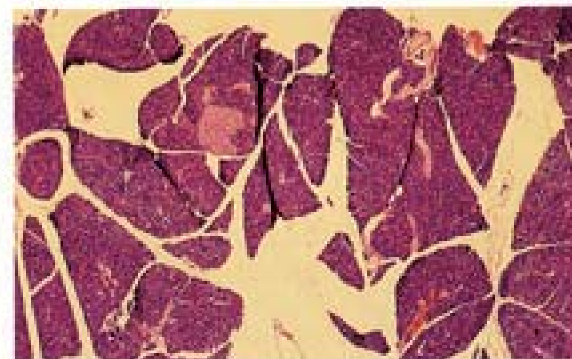

Figure 3: This section was cut, drained, and placed on a slide warmer to dry. Notice presence of wrinkles. X100.

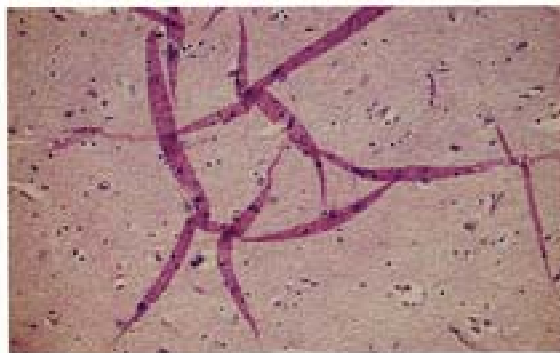

Figure 1: This section demonstrates wrinkle formation in different directions. This pattern usually suggests improperly processed tissue. X100. Brain H&E.

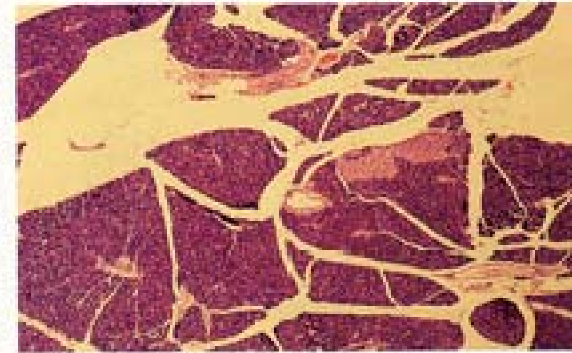

Figure 4: This section, obtained from the same blocks as Figure 3, was cut and drained in a vertical position for 1 minute. This was followed by 20-minute exposure to 60°C oven. Notice absence of wrinkles. X100.

NSH Symposium/Convention—Another Point of View

Brent Riley
Managing Editor

"It was great," recalled Anton Schoepfer. "It was a most enjoyable and valuable experience. We had a great time in Las Vegas."

Schoepfer, immediate past president of the Swiss Society for Histotechnology, had high praise for the Fifteenth Annual Symposium/Convention of the National Society for Histotechnology. He attended the Las Vegas meeting in conjunction with a speaker-exchange program between the NSH and the Swiss society.

On Tuesday, Schoepfer made a presentation on the Swiss Roll technique, based on a paper published in the Swiss histology journal. Schoepfer, who is Supervisor of the Pathology Laboratory for Ciba-Geigy's Pharmaceutical Division in Basel, Switzerland, was accompanied by another Swiss society member, Peter Ossent, D.V.M., University of Zurich. Ossent conducted a workshop on special stains in the routine pathology laboratory.

The exchange program, sponsored by Miles Inc., was conceived by Schoepfer about five years ago. "It took time," he explained, "but now, fortunately, it has become reality. We hope it will become a yearly exchange. We already have three or four candidates to send to next year's NSH convention."

In April, John Ryan, immediate past president of the NSH, traveled to Switzerland to speak at that country's annual symposium. The Swiss symposium is a one-day meeting modeled after the NSH scientific sessions. "We combine five to eight presentations with a trade show and some demonstrations. Our topics are very similar to yours," Schoepfer said. "But the biggest difference is the size. We will have about 150 attendees at our meeting."

"We also have a workshop every year in the fall where our people can practice new techniques," he said. The 1989 two-day workshop, which took place in mid-November, was in Neuchâtel, Switzerland.

While this is the first year for the exchange program, it is not the first time Schoepfer has attended an NSH Symposium/Convention. "I attended the first time in 1981 at Salt Lake City," he said. "I also became an NSH member in 1981."

"The organization of your meetings is improving," Schoepfer continued. "I think it's a matter of age—as you gain more experience, the meetings get better."

"Our society is young," Schoepfer said. It was founded in May, 1982, with objectives very similar to those of the NSH—education and communication. There were about 100 members at the founding meeting. Today the Swiss society has about 350 members, representing a very high percentage of histotechnologists in Switzerland.

"I was very impressed with the Americans," Schoepfer concluded. "They were so kind to us, and it was very easy to get acquainted with new people. It was a fantastic experience."

Anton Schoepfer (left) and Peter Ossent

Answers and Comments to "A Question and Answer Session"

Provided below are the answers and comments to the questions on pages **129** and **130**. Readers are encouraged to send comments to the editors if they have an opinion that is not consistent with that provided on any of the answers or comments. The answer to each question is provided after the number of the question as either true, false, or true and false (for those questions that have both answers). Comments have been provided for questions that require them or if it was felt comments would add to the learning experience.

1-True; **2**-False; **3**-True; **4**-True; **5**-True; **6**-False, sections of varying thickness are produced by varying the speed of the microtone advance wheel. **7**-False, melanin pigment is removed with potassium permanganate and oxalic acid prior to staining. **8**-True; **9**-False; **10**-False; **11**-True; **12**-False, paraffin is removed with a mixture of xylene and peanut oil before the Fite-Foraco stain for lepra bacilli. The use of peanut oil preserves the fatty coating of the lepra bacilli that then appears much thicker microscopically. **13**-True; **14**-False; **15**-False; **16**-True; **17**-False; **18**-True; **19**-True; **20**-False, Donovan bodies and spirochets are demonstrated with silver reactions, such as the Warthin-Starry. **21**-True; **22**-True; **23**-True; **24**-True; **25**-True; **26**-True; **27**-True; **28**-True; **29**-False, if the block is too cold, shattering will be produced and the specimen will not cut well. **30**-True, but only if tissue is allowed to remain in the nitric acid after complete decalcification has been reached. **31**-False; **32**-True; **33**-False; **34**-True; **35**-True; **36**-True; **37**-True, anything that moves the liquid molecules will assist the decalcification process. Heat should not exceed 37°C if used. **38**-False; **39**-True; **40**-True; **41**-True, only if sections are stained properly. Overstaining is what causes the entire organism to stain black throughout. **42**-True; **43**-False, x-ray of decalcified specimens is the only way to be absolutely sure no calcium remains in tissue specimens. Chemical testing provides only a good guideline. **44**-True; **45**-False, washing of tissue after alcohol fixative may cause some swelling of tissue resulting in cracking at microscopic levels. **46**-True and False, acid fast bacteria are demonstrated with the GMS stain but not the PAS. **47**-True and False, hyaluronidase digests hyaluronic acid but not sialic acid. Sialidase (neurominidase) is used to digest sialic acid. **48**-True; **49**-True; **50**-True.

Practical Stain-Technology "Wet" Workshop and Seminar

Presented by: Lee G. Luna **March 25-30, 1990**

This five-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; *Campylobacter pyloris;* cat-scratch disease bacilli; Lime disease spirochetes; Legionnaires' 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. Problem slide, tissue, staining problem-solving session will be conducted during the course. Therefore, you are 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 (6 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 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:

Registrar
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An Open Invitation

Thanks to you, your technical knowledge, and your willingness to share ideas, *Histo-Logic®* has become a valuable resource.

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Send your technical subject matter to: Lee G. Luna, Scientific Editor, *Histo-Logic*, 7605-F, Airpark Rd., Gaithersburg, MD 20879.

Send your nontechnical subject matter to: Brent Riley, Managing Editor, Miles Inc., Diagnostics Division, P.O. Box 70, Elkhart, IN 46515.

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, *Histo-Logic* Editor, 7605-F, Airpark Rd., Gaithersburg, MD 20879. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.

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