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MILES

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Miles Scientific: A New Division

On August 26, 1982, Miles Laboratories, Inc., announced the establishment of a new division called Miles Scientific within its Professional Products Group. This division comprises what was formerly the Lab-Tek Division and the Research Products Division. It is headed by V. M. Esposito, Ph.D., formerly President of the Lab-Tek Division. Headquarters for Miles Scientific will be located in Naperville, Illinois, a suburb of Chicago, with operations in nearby Lisle and Kankakee.

"This reorganization will enable us to combine the strengths of the two divisions and move aggressively forward in our established markets and in new product areas," T. H. Heinrichs, Chairman of Miles Laboratories, Inc., said in making the announcement.

Miles Scientific will continue the manufacture and marketing of more than 1,000 specialty biochemicals, specialty plastic labware and cellular diagnostic products, serving clinical laboratories, medical and academic research institutions as well as human and animal health care companies worldwide.

Specialty biochemicals include various blood proteins, enzymes and immunochemicals. These product lines will include the well-known Pentex®, Miles-Yeda and Seikagaku Kogyo Co., Ltd. (SKK) labels. Miles Scientific will continue the innovative traditions, which saw the company as the first supplier of restriction enzymes for genetic research and engineering and a leading supplier of monoclonal antibodies today.

Specialty plastic labware includes Lab-Tek® and LUX® products used for the collection, transport and testing of body-fluid and body-tissue specimens. Petri dishes, tissue culture flasks, test tubes and chamber/slides are among the most popular items in the line.

In addition, the new division's strategic mission will be to develop, manufacture and market cellular diagnostic products to both the research and clinical markets. Cellular diagnostics include instruments, devices and reagents for hematology, histology, cytology and the growing area of immunologically-based diagnostic products. Presently these products include Tissue-Tek® histology systems: Histo-Tek®, Cyto-Tek® and Hema-Tek® Automatic Slide Stainers; Tissue Processors; the Tissue-Tek® Microtome-Cryostat; and Tissue-Tek® Tissue Embedding Consoles.

The Lab-Tek and Research Products Divisions were well known for their high quality, reliable products, backed up with dependable customer and technical services. This proud tradition will be continued and enhanced by Miles Scientific. The integration of biological expertise and instrumentation know-how with plastics technology is expected to promote innovative developments throughout the product lines.

As we embark on our new business plans, we hope you, our customers, share with us the excitement and enthusiasm of Miles Scientific. Please note our address: **Miles Scientific**, Division of Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60566.

Inquiry Responses

Editor's Note: The following information was submitted in response to an inquiry by Brenda Collins, which appeared in *Histo-Logic*, Vol. X, No. 3, July 1980. In part, here is Ms. Collins' query: "I am having a problem obtaining reliable results with silver impregnation techniques for axons in the central nervous systems of large and small animals. I have experimented with both the Bodian and Holmes methods and obtained variable results. Axons may stain well in small animals and faintly with large animals with the same technique. Our tissues are perfused-fixed or immersion-fixed with 10% BNF, but the fixation method has not consistently been associated with either faint or deep staining. Is there any technique or modification of the above methods for axons which works well with large and small animal central nervous systems?"

James E. Rathke, Pathologist Assistant
Neurohistology, St. Anthony Hospital
Rockford, IL 61101

After seeing your inquiry about reliable results with silver stains for axons in the CNS, I thought that I could help with a possible solution. The Eager's Method gives good results on animal and human tissue. It is necessary to determine the correct impregnation time for the material being studied, using normal nervous tissue from the same animal as a control; this ensures that the time is not too prolonged with subsequent staining of normal axons.

Eager's Method for Degenerating Axons

Ammoniacal Silver Solution

1.5% silver nitrate	80.0 ml
95% alcohol	48.0 ml
Concentrated ammonia	8.0 ml
2.5% sodium hydroxide	7.2 ml

Reducer

Absolute alcohol	90.0 ml
10% formalin	27.0 ml
1% citric acid	32.0 ml
Distilled water	810.0 ml

Staining Procedure:

1. Cut 30 micron frozen sections from formalin-fixed tissue.
2. Rinse in distilled water for a few seconds.
3. Treat with 0.5% phosphomolybdic acid for 10 minutes.
4. Rinse in distilled water. Sections must be handled individually from this stage onwards.
5. Transfer section to ammoniacal silver solution for 2-5 minutes, agitating at 30-second intervals. Section should become brown at this step.
6. Without rinsing, transfer to reducer and agitate until section is golden brown. Leave section in reducer for an additional 1-2 minutes.
7. Rinse in distilled water.
8. Fix in 1% sodium thiosulphate for 2 minutes.
9. Rinse in distilled water.
10. Transfer sections to a mixture of equal parts of 1% gelatine and 80% alcohol at 37 °C before mounting section on slides. This mixture should be well stirred.
11. Allow sections to drain and dry completely at room temperature for 1-2 days before dehydrating, clearing and mounting.

Note: Sections may be counterstained with 1% cresyl violet, if desired, after step 9. In that case, the following bleaching step is necessary: Agitate the section in 1% potassium ferricyanide for 10-15 seconds until the background is clear, rinse in distilled water, mount section as in step 10, allow to dry at 37 °C for 1-2 days, then counterstain in 0.1% cresyl violet for 10 minutes before dehydrating, clearing and mounting.

Results:
Degenerating fibers — Black
Background — Pale brown, unless counterstained

Palmgren's Method for Nerve Fibers (Modified)

Solutions:

Acid Formalin

Formalin	25.0 ml
Distilled water	75.0 ml
1% nitric acid	0.2 ml

Silver Solution

Silver nitrate	15.0 g
Potassium nitrate	10.0 g
Distilled water	100.0 ml
5% Glycine	1.0 ml

Reducer

Pyrogallol	10.0 g
Distilled water	450.0 ml
Absolute alcohol	550.0 ml
1% nitric acid	2.0 ml

Staining Method:

1. Take 8 micron paraffin sections down to absolute alcohol, celloidinize.
2. Treat with acid formalin for at least 3 minutes; wash well.
3. Place in silver solution for 15 minutes at room temperature.
4. Drain slide, but do not rinse: Transfer to the preheated reducer for 1 minute at 40-45 °C, agitating gently. Use a fresh jar of reducer for each section.
5. Wash well in distilled water.
6. Fix in 5% sodium thiosulphate for 5 minutes.
7. Dehydrate, clear and mount.

Results:
Nerve fibers — Dark brown to black
Background — Golden brown
Note: Use fresh silver solution for best results. The stock reducer keeps well, but darkens after several months. Use reducer once and discard.

Other stains that may be of some help are Marsland, Glees and Erickson's method for nerve fibers; combined silver/luxol fast blue method for peripheral nerves; Bielschowsky's method for nerve endings (Schofield's modification); Hjorth-Simonsen's modification of Fink-Heimer's method.

An excellent text for neurohistology is "Techniques in Neurohistology" by H. M. Ralis, R. A. Beesley and A. Z. Ralis, Butterworths Publication, 1973.

Barbara McGuire, Department of Neuroscience
University of Florida College of Medicine
JHM Health Center
Gainesville, FL 32610

I read your dilemma in the July 1980 issue of *Histo-Logic*. In this laboratory we use the Bodian's technique. We add 5 gms of copper (shot) metal to the protargol which gives excellent results. Copper (shot) metal can be purchased commercially.

Jana Metheny
Syntex Research, 3401 Hillview Avenue
Palo Alto, CA 94304

I have modified the Sevier-Munger Method for Neural Tissues (*AFIP Manual*, pp. 215-216) for use in our laboratory and I believe it will be of help to you. This is a rapid and reliable method which I am pleased to share with you.

Metheny Modification of

Sevier-Munger Method for Neural Tissues

Fixation:
10% buffered neutral formalin. Avoid chromate fixatives.

Techniques:
Cut paraffin sections at 4-6 microns.
Use albuminized slides. Use acid clean glassware for silver nitrate and ammoniacal solution.

Recommendation:
Constant temperature water incubator is preferred. As with any histologic procedure safety precautions should be used. If oven is used, preheat 20% silver nitrate solution for 45 minutes, add slides and increase time in warm solution until a golden yellow color is attained.

Solutions:

20% Silver Nitrate
Aqueous, using distilled water.

10% Silver Nitrate
Aqueous, using distilled water.

Formalin Solution

Formalin, 37-40%	2.0 ml
Tap Water	98.0 ml

5% Sodium Thiosulfate Solution
Aqueous, using distilled water.

Sodium Carbonate Solution

Sodium carbonate (hydrated)	8.0 gm
Distilled water	30.0 ml

Ammoniacal Silver Solution (Working)
To 50 ml of 10% silver nitrate solution add ammonium hydroxide (28-30%). While swirling, slowly add 3 ml of ammonium hydroxide; a brown precipitate will form. Continue swirling and add drop by drop more ammonium hydroxide until the solution is slightly cloudy, avoiding complete discolorization. This may take 20 to 45 additional drops. Add to this solution 0.5 ml of sodium carbonate solution. Swirl vigorously. Add 25 additional drops of ammonium hydroxide and swirl vigorously. The solution should now be crystal clear. Filter into an adequate Erlenmeyer flask, cover and set aside. This solution can be prepared while the slides are heating.

Staining Procedure:
1. Deparaffinize and hydrate to distilled water.
2. Preheat 20% silver nitrate solution in a 60°C water incubator for 15 minutes. Add slides to warm solution and let stand for 15 minutes. The tissue will be the color of a manila envelope. An additional few minutes may be required.
3. Rinse one slide at a time in distilled water and place them in an acid clean coplin jar.
4. To working ammoniacal silver solution add 10 drops of formalin solution while swirling gently. Pour over slides and continually keep the coplin jar moving in a circular motion to avoid precipitate. Developing occurs in 4-5 minutes. After approximately 30 seconds, slowly add additional formalin solution, drop by drop, approximately 10 drops. The tissue will turn a dark, golden brown.
5. Examine microscopically. **Do Not Wash.** Axons should be black.
6. Rinse well, but gently, in 3 changes of tap water.
7. Sodium thiosulfate solution for 2 minutes.
8. Wash in gently running water for 5 minutes.
9. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene, 2 changes each.
10. Mount with resinous media.

Results:
Large and small peripheral neurites — Black
Axons — Black
Collagen and muscle — Brown
Myelin sheath — Light brown
Argentaffin granules — Black

Reference:
Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Ed., McGraw-Hill, 1968, pp. 215-216.

(To be continued, next issue)

A Modified Mayer's Mucicarmine Procedure

Mequita Praet
Susie Parker Stringfellow Memorial Hospital
Anniston, Alabama 36203

The compounding of mucicarmine solution from scratch is often messy and requires a long time to prepare. Because of this problem, I have been using a commercially* available mucicarmine stain kit. However, if the staining kit is used according to manufacturer's recommendation, one will not obtain proper staining results. This problem has been corrected by the following modification, made by the author.

The modification consists of using the mucicarmine staining solution full strength rather than at the percentage suggested by the manufacturer.

Solutions:

Weigert's Iron Hematoxylin
Solution A (Stock)

Hematoxylin crystals	1.0 gm
Alcohol, 95%	100.0 gm

Solution B (Stock)

Ferric chloride, 29% aqueous	4.0 ml
Distilled water	95.0 ml
Hydrochloric acid, concentrated	1.0 ml

Weigert's Iron Hematoxylin (Working)
Equal parts of Solution A and B

0.25% Metanil Yellow Solution

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

Procedure:
1. Deparaffinize and hydrate slides to water.
2. Stain slides in working Weigert's hematoxylin for 7 minutes.
3. Wash slides in running water for 10 minutes.
4. Stain slides in full strength mucicarmine solution for at least 30 minutes.
5. Rinse slides quickly in distilled water.
6. Counterstain in metanil yellow solution for 1/2 minute.
7. Rinse slides quickly in distilled water.
8. Dehydrate in 95% alcohol, absolute alcohol, and clear in 2 changes of xylene.
9. Mount coverglass with resinous media.

Steps 6 and 7 are optional.

Reference:
Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, Third Edition. McGraw-Hill, New York, 1968, pp. 161-162.

*A.J.P. Scientific, Clifton, NJ 07011
Editor's Note: The commercially available stain kit can be ordered from the above address.

Histotechnology Update — The State of the Art

The American Society of Clinical Pathologists is offering a one-day program entitled, "Histotechnology — The State of the Art" on Tuesday, April 12, at the Hyatt Regency-Chicago in Chicago, Illinois. This course is being presented as part of the ASCP/CAP Spring National Meeting to be held in Chicago, April 9-14, 1983. For more information, write Customer Services, American Society of Clinical Pathologists, 2100 West Harrison Street, Chicago, Illinois 60612; or telephone Michael Kelleher at 1-800-621-4152; in Illinois, call 1-312-738-4890.

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Practical Stain-Technology "Wet" Workshop and Seminar — March 6-11, 1983

Presented by Lee G. Luna and Staff
Rockville, Maryland

Editor's Note: A Reminder! This five-day extensive wet workshop and seminar will afford registrants the opportunity to perform 20 special stains, demonstrating more than 25 pathologic entities.

For program and related information, contact: Registrar, Center for Histotechnology Training, P.O. Box 2453, Rockville, MD 20852, 301/468-6552. This address was inadvertently omitted from the announcement which appeared in the October 1982 issue of *Histo-Logic*, p. 184.

NSH — 9th Symposium/Convention

For planning purposes, here is a list of prices one can expect to pay for attending the 1983 NSH Symposium/Convention.
Disneyland Hotel: Single — $76; Double — $84; 3-4 Occupants — $92; 1 Bedroom Suite — $140.
Full-Day Workshop: NSH Member — $40; Non-Member — $45.
Half-Day Workshop: NSH Member — $20; Non-Member — $25.
Scientific Sessions: (Includes Wed., Thurs. & Fri.) NSH Member — $50; Non-Member — $60.
Banquet: $20.

Workshops will be conducted Sunday, Monday and Tuesday, making it possible to attend 3 full-day or 6 half-day sessions. Plans are also in progress to conduct an Animal Histology Seminar concurrently with the Scientific Sessions. Complete program information and registration will appear in the April *Histo-Logic*.

To receive your own personal copy of HISTO-LOGIC, or to have an associate added to the mailing list, submit home address to: **Miles Scientific**, Division of Miles Laboratories, Inc., 30W475 North Aurora Rd., Naperville, Illinois 60566. Printed in U.S.A.

The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Lee G. Luna, Editor, Histo-Logic, P.O. Box 36, Lanham, Maryland 20706. Articles, photographs, etc., will not be returned unless requested in writing when they are submitted.