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Luna Modification of Shorr's Stain for Bone and Cartilage

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Shorr's staining method was originally used for the demonstration of inclusion bodies, but it stains other entities that can be used as an aid in the interpretation of other pathologic entities. Besides inclusion bodies, Shorr's stain demonstrated collagen light green; muscle-red; inclusion bodies-bright red; keratin-orange; erythrocytes orange-red; and nuclei-blue. The modification provided below (see Note) was made to differentiate bone from cartilage and other entities (Figs. 1-5).

Fixation
10% buffered formalin or Bouin's. If formalin is used, mordant slide as indicated in Step 2 below.

Process
Process in conventional manner and embed in paraffin.

Microtomy
Cut sections at 5 or 6 micrometers.

Solutions

Aldehyde Fuchsin Solution

Basic fuchsin	1.0	gm
Alcohol, 70%	200.0	ml
Hydrochloric acid, concentrated	2.0	ml
Paraldehyde	2.0	ml

Let stand at room temperature for 2 to 3 days or until stain is deep purple in color. Store in refrigerator.

1% Acid Alcohol

Hydrochloric acid, concentrated	1.0	ml
Alcohol, 70%	99.0	ml

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Miles Inc. Acquires Technicon Instrument Corporation

Kathleen Murphy
Manager, Marketing Communications
Miles Inc., Diagnostics Division

Miles Inc., the healthcare company that is a subsidiary of Bayer USA, enhanced its diversity and leadership in the marketplace by purchasing Technicon Instrument Corporation. Technicon will join the Diagnostics Division of Miles Inc.

Miles Executive Vice President and General Manager of the Diagnostics Business Group, Dr. Roger G. Stoll, said: "The acquisition of Technicon is a very important step for Miles and Bayer. Miles diagnostics products have traditionally been focused on the physician's office lab and home-testing markets. Technicon's business,

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Shorr's Stain

Shorr's Staining Solution

Alcohol, 50%	100.0	ml
Biebrich scarlet	0.5	gm
Orange G	0.25	gm
Fast green, FCF	0.075	gm
Phosphotungstic acid	0.5	gm
Phosphomolybdic	0.5	gm
Glacial acetic acid	1.0	ml

Harris' Hematoxylin

Hematoxylin crystals	5.0	gm
Alcohol, 95%	50.0	ml
Ammonium or potassium alum	100.0	gm
Distilled water	1000.0	ml
Mercuric oxide	2.5	gm

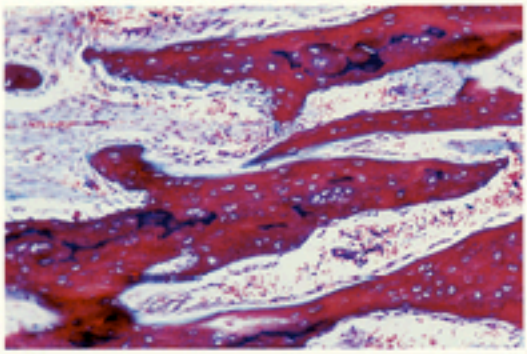

Figure 1: Bone trabeculae demonstrating cartilage staining (purple) with aldehyde fuchsin. The bone stains reddish-orange. Luna/Shorr. Original magnification X200.

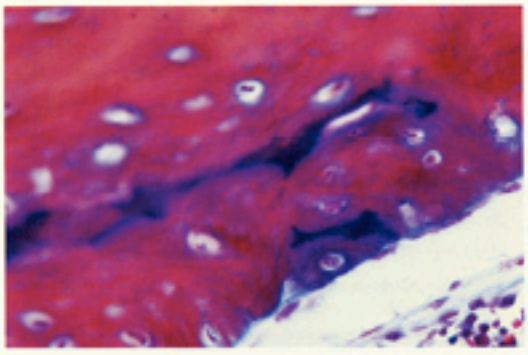

Figure 2: Higher power of section shown in Figure 1. Note the purple staining of cartilage. Also note the staining of the wall surrounding the osteocytes. These glycoprotein-rich walls stain green. Luna/Shorr. Original magnification X400.

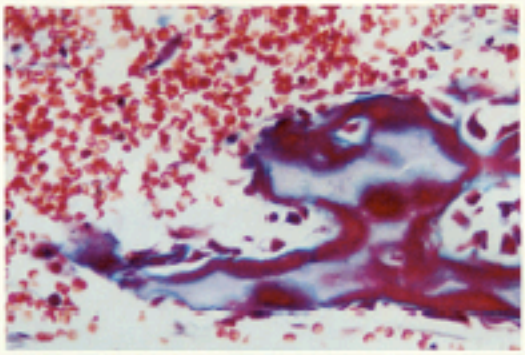

Figure 3: This figure differs from Figures 1 and 2 in that aldehyde fuchsin was not used. Note that cartilage is staining grayish-green, while bone is staining reddish-orange. Shorr. Original magnification X200.

Dissolve the hematoxylin in the alcohol, the alum in water by the aid of heat. Mix the two solutions. Bring the mixture to a boil as rapidly as possible and then remove from heat and add the mercuric oxide. Reheat the solution until it becomes a dark purple, about 1 minute, and promptly remove the container from the flame and plunge it into a basin of cold water. The solution is ready to use when cool. Add 2-4 ml of glacial acetic acid to every 100 ml of solution.

Ammonia Water

Tap water	1000.0	ml
Ammonium hydroxide	3.0	ml

Staining Procedures

1. Deparaffinize slides in 2 changes of xylene; run through absolute and 95% alcohols to distilled water.
2. Stain in Harris' hematoxylin for 3 minutes.
3. Rinse in tap water.
4. Differentiate in 1% acid alcohol until there is no hematoxylin in the cytoplasm of the cells. Check with microscope.
5. Dip in tap water 5 times.
6. Dip sections 5 times in ammonia water to blue. Check with microscope.
7. Wash slides for 10 minutes in running tap water. (See note below for differentiating cartilage from bone.)
8. Place slides in Shorr's staining solution for 1 minute.
9. Wash slides in 95% alcohol. Check with microscope. Connective tissue will be a clear light green when differentiation is complete.
10. Rinse several times in absolute alcohol; clear in 3 changes of xylene.
11. Mount coverslip with resinous media.

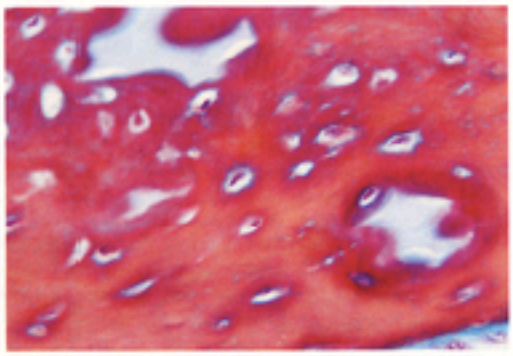

Figure 4: Higher power of section shown in Figure 3. Note that cartilage is staining grayish green. Glycoprotein rich wall surrounding the osteocytes stains green. Shorr. Original magnification X400.

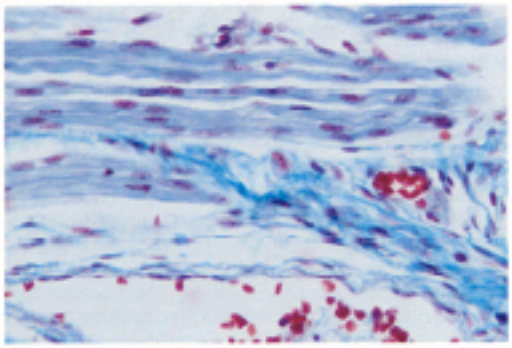

Figure 5: This photograph, stained with Shorr's stain, demonstrates collagen in green while skeletal muscle stains grayish-green. Nuclei stain gray-purple while red blood cells stain red. Shorr. Original magnification X200.

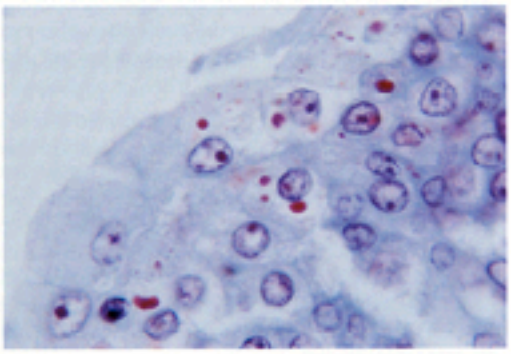

Figure 6: Red inclusion bodies of canine distemper virus is seen throughout this photograph. Note the clarity of the inclusion bodies and good differentiation between inclusion bodies and nucleoli. Also note the excellent staining of nuclei. Shorr. Original magnification X1000.

Results for bone and cartilage

Bone	reddish-purple
Cartilage	purple
Collagen	green
Muscle	red
Nuclei	purplish-black

Results for inclusion bodies

Inclusion bodies	orange-red
Collagen	green
Muscle	red
Keratin	orange-red
Erythrocytes	red
Nuclei	grayish-purple

Note: The staining procedure for differentiation of bone from cartilage requires the introduction of aldehyde fuchsin solution between Steps 7 and 8. Following Step 7, the slides are rinsed in 95% alcohol and placed in aldehyde fuchsin for 30 minutes. Rinse slides in 2 changes of 95% ethyl alcohol followed by a water rinse before placing slides in Shorr's solution.

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Technicon

which is oriented toward the hospital and large commercial laboratories, will give us a new breadth and diversity, and position us as a global leader in the diagnostics field. With the support of Bayer's research and development activities, I look forward to a promising future for our business."

Dr. Louis Foissac, Chairman and Chief Executive Officer of Technicon, stated: "This is a very good move for Technicon and its employees. The combination of Technicon and the diagnostics business of Miles, with the backing of the tremendous research capabilities of Bayer, will constitute a formidable force in the diagnostics industry."

Miles established its reputation nearly 50 years ago when it introduced the first solid-phase reagent for urine chemistry. Today, Miles researches, develops, and markets diagnostic systems in the fields of diabetes management, urine chemistry, blood chemistry, immunochemistry, cytology, and histology. Histology products are sold under the Tissue-Tek® brand name. Key products include the Tissue-Tek V.I.P.™ Tissue Processor, Tissue-Tek Tissue Embedding Console System, and the Cyto-Tek® Centrifuge.

Technicon Instrument Corporation was founded 30 years ago and has developed an excellent reputation for quality and innovation in its development of automated analytical systems and diagnostics for clinical laboratories.

Interestingly, Technicon's first product was the AUTOTECHNICON®, a tissue-processing instrument that introduced automation to the histology laboratory, accomplishing overnight a procedure that had previously taken three or four days of manual work.

Today, Technicon's product line includes blood chemistry and hematology analyzers and immunoassay systems.

With the addition of Technicon, Miles employs over 16,000 people worldwide. Miles and Technicon have regional branches in the United States and abroad to provide efficient worldwide sales, service, and distribution to their customers.

NSH Symposium Stresses Knowledge

Brent Riley
Managing Editor

Education. Education. Education. In this day and age, those are perhaps the three most important words to all histotechnologists as their desire and need for knowledge grows. So it isn't surprising that the NSH Symposium-Convention concentrates so much on education every year.

The 1989 convention is no exception. Susan McCoy, the program coordinator for the Las Vegas event, explained that many of the workshops and lectures scheduled will concentrate on more advanced topics. "Workshops will be more technical," she said. "And there won't be as many basic topics as in the past."

But even the "basic topics" are becoming more advanced. And the reason is simple. The specialized procedures that used to be done only in the research labs of large universities are now being done more routinely in clinical labs. At one time these procedures were considered "progressive." Now they're considered "basic."

"Pathologists and hospitals are expecting more out of the histotechnologist now," McCoy continued. "Since we've gotten into immunoperoxidase, they found out that there are a lot of other techniques that could be performed by the histotechnologist."

Consequently, histotechnologists who attend the NSH Symposium/Convention are more experienced and knowledgeable than ever because of the growing histology curricula offered by colleges and universities throughout the country.

In essence, the workshops will cover the new technical procedures that a histotechnologist is expected to do routinely now—DR Receptors, flow cytometry, DNA probes, and more.

The program for this year's symposium is also bigger than ever. There are 15 workshops each of the first three days—45 in all. In addition, the workshops will begin on Saturday and continue through Monday. Lectures will then be held Tuesday through Thursday.

Choosing workshops for the symposium/convention is a difficult task. Most workshops are developed from abstracts submitted by histotechnologists, medical technologists, pathologists, and PhD's. Many more proposals are made for workshops than can actually be presented in the three-day workshop period. "I was flooded with abstracts after the Louisville meeting," recalled McCoy. "We have to evaluate each one individually. It's very difficult to make those choices."

Among other things, the convention committee considers which workshops have filled up in the past and how many people were on the waiting list for each. They look at current issues, new technologies, and new procedures. And they try to balance the number of basic, intermediate, and advanced workshops with the experience level of the participants. Of course, the committee also evaluates the speaker. Has he or she spoken before? How was the reception?

A number of new workshops will be presented this year including "Comparative Silver Staining Methods Used in the Diagnosis of Alzheimer's Disease," "An Overview of Cytology," "Medical Terminology," "Human Cytogenetic Analysis," and "Plant Histology." New workshops will also be conducted on starting a school of histotechnology, the laws of motivation, quality control, and the right-to-know law. And Dr. J. B. McCormick will present two workshops on the study and preparation of antique prepared microslides.

Whether you're a novice or a veteran, if you're coming to the 1989 symposium/convention to improve your expertise in histotechnology or related areas, you'll be in the right place at the right time.

Dominic Europa: Contributions and Memories

A Personal Note from Lee G. Luna on the Passing of Mr. Dominic Europa

On the passing of Dominic Europa, I hope the things he stood for will never be lost by those of us who remain to carry on the torch: namely, complete and total dedication to his chosen profession of histotechnology. To this profession he brought an effervescent personality, strong dedication, hard work, solid commitment and, most of all, a genuine desire to help young histologists understand the values and rewards of getting involved in the profession. One of many fond memories I have of Dominic is the gleam in his eye and the smile on his face as he explained principles of histotechnology to many of the young histologists who often surrounded him, seeking those gems of knowledge that he freely imparted. We, the histotechnology community, can pay homage to our friend, Dominic, if we continue many of the fine attributes he exhibited during his many years as a histotechnologist, administrator, and leader.

When Dominic L. Europa performed his first section, there was no automation of any kind. He worked primarily in celloidin and frozen sections. Paraffin, a relatively new technique, was used only for surgicals in the neuropathology lab. Tissues had to be transferred from graded alcohols by hand. Knives were honed and stropped by hand. Refrigerator storage jars from the local five-and-ten-cent store were used as staining jars. There were no thermostatically controlled water baths—just a light bulb in a wooden box that held a pan of water. The temperature was adjusted by turning the bulb on and off.

In the span of his career, Dominic witnessed firsthand a remarkable evolution in histology. He saw it develop from a primitive science to a highly sophisticated, technical discipline. He saw the development of embedding units, the modern microtome, the automated tissue processor, and countless new stains and techniques. But just as important, he saw the histotechnology profession mature and gain respect in the medical and scientific community. He saw histotechnologists become better educated, more skilled, and a far more important part of the diagnostic team. In fact, Dominic Europa did much more than just observe these phenomena. He contributed significantly to the progress made by the profession.

In 1938, Dominic started working in the neuropathology department at Bellevue Hospital in New York. His salary was $960 a year. Although he had a college degree, many of Dominic's fellow staff members had no scientific background or training in histotechnology. Perhaps that explains why education was always a priority with him.

Ask anyone who knew or worked with Dominic and you'll hear the same flurry of compliments. He was an educator, an innovator, and a meticulous practitioner. He was gregarious but with a gentle manner. He was concerned about people as individuals and as histotechnologists—always interested, always willing to talk.

Lee G. Luna remembers the first time he ever met Dominic. "My introduction to Dominic Europa was in the middle sixties," Luna recalled. "I had heard of his dedication to the profession and asked him to come to the AFIP Symposium and give a lecture. As far as I know, it was the first time he ever gave a lecture in public. And the response was so good, I asked him to come back the following year. In fact, he came back every year at my invitation."

Dominic first became well known for the articles he published. He was a prolific writer. In fact, he wrote many articles for *Histo-Logic* beginning in the early days when the publication was trying to establish a foothold. "His contributions became very important to the establishment of a good foundation for the newsletter," Luna said.

He also played an important role in developing and perfecting various techniques. In 1976, he was awarded a tax-free, $5,000 grant for his service to the city of New York. Specifically, the city cited him for "perfecting techniques that have become standard in hospital laboratories throughout the world."

Dominic also was instrumental in the formation of the National Society for Histotechnology. He was an active member of the founding board of directors and then became the first NSH president.

Lee Luna was chairman of the board during Dominic's term as president. "All of our associations were very professional, very friendly. Potentially, our positions could have been very conflicting, but we always managed to work out any difficulties we encountered."

John Ryan, who has recently completed a term as NSH president, also recalled Dominic's professionalism: "The man was a perfectionist," Ryan said. "I remember his dedication to silver staining. It was absolutely critical to him. The silver stains had to be perfect, and the quality of his work showed it."

Dominic was eventually appointed supervising microbiologist at Bellevue Hospital, where he remained throughout his career. He also remained active in the NSH and, even after retirement, continued to write and lecture.

Dominic Europa was one of those extraordinary people who gave and gave...and then gave some more. And as a result, we have all felt the impact of his dedication to histotechnology.

1989 NSH Symposium Convention: A Preview

Brent Riley
Managing Editor

Here we are again. Only a few weeks away from the 1989 NSH Convention/Symposium. And this year's event promises to be the best ever. The program will be outstanding. And the city...well, you'll discover why they call it "The City That Never Sleeps."

The Symposium/Convention of the National Society for Histotechnology will be held September 23-28 at the Riviera Hotel in Las Vegas. Full- and half-day workshops will be held during the first three days of the symposium/convention. But the schedule is different this year as the program will begin on Saturday and workshops will continue through Monday. Scientific and veterinary sessions will begin Tuesday and run through Thursday. Scientific exhibits will open Monday afternoon and continue through the remainder of the convention.

There are plenty of activities planned for the evenings of the convention as well. Special hospitality functions are scheduled every evening. And one of the most popular social events, the annual awards banquet, is planned for Wednesday at the "Top of the Riv" ballroom. A number of awards will be presented at the banquet, including the prestigious Histotechnologist of the Year Award, the Golden Forceps Award, the Diamond Cover Award, and the J. B. McCormick Award.

If you've never been to Las Vegas, you're in for a real treat. Known for some of the world's greatest entertainment, Las Vegas showrooms offer spectacular shows and top-name headliners every night. Eating is also a Las Vegas delight—from a seven-course gourmet meal to the well-known "all you can eat" buffets.

If you arrive in Las Vegas before the convention begins, or plan to stay a few days after, don't miss the sights in and around the city. The Grand Canyon, Hoover Dam, Lake Mead, and many other natural attractions are all within easy driving distance from Las Vegas.

And then there are the casinos. Of course, you don't have to gamble to have a good time, but if you do want to "play the games," here are a few words of wisdom for the uninitiated. First of all, don't expect to come out ahead. Do it because you like to play the games. Do it because you want to tell your friends about the experience. But consider it an expense. Set your loss limit, and when you reach that limit, quit. With a little knowledge you can play a long time with only a few dollars. Sometimes you can play the slots for hours with a single roll of quarters.

Before you go, buy a book about Las Vegas games. There are many books available, and at least one can be found in most bookstores. These books will tell you game rules and game strategies. The large casinos and hotels also offer short courses on the various games and behind-the-scenes tours of the casinos.

Even if you don't want to gamble, be sure to visit the big casinos. Caesar's Palace is spectacular. And Circus Circus has live circus performances above the casino floor. You will also want to see downtown Las Vegas at night. The lights are dazzling.

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Las Vegas is a glamorous city, and the surrounding area is beautiful. But, as always, the symposium/convention will be the highlight of your trip. Its value in terms of education, self-improvement, and developing new friendships has been well documented over the years. And the 1989 symposium/convention will certainly continue that tradition.

Speaker Exchange Program Provides Insight into Other Histotechnology Societies

Brent Riley
Managing Editor

Did you ever wonder what it would be like to be a histotechnologist in another country? You might ask: Are their techniques similar to ours? Do they have the same educational opportunities? Are there other national societies? Are there other national symposia?

The answer is the same for all these questions—Absolutely! There are a number of histotechnology societies worldwide. John Ryan, immediate past president of the NSH, recently experienced a firsthand look at the symposium conducted by the Swiss Society for Histotechnology. Ryan attended the April 21 meeting through a special speaker-exchange program sponsored by Miles Inc.

The Swiss symposium is an annual, one-day event consisting of lectures and a business meeting. A banquet is held the night before where speakers and guests are welcomed.

Ryan's lecture consisted of two parts. In the first part, he provided some insights into our NSH. Then he discussed the diagnostic use of immunohistochemistry.

At the Swiss meeting, all of the lectures are translated into three or four languages. German is the dominant language in the northern part of Switzerland; French in the Geneva area; and Italian in the west. Therefore, simultaneous translations are made during all lectures. This year, the lectures were also translated into English.

Ryan arrived in Zurich on April 18. After a day-long tour of the city, he traveled to Lausanne where the meeting was held at the Nestle Research Center.

After the meeting, Ryan spent an extra day in Lausanne, touring the Nestle Research Laboratories. He then traveled to Basel, the home of the society's president, Anton Schoepfer. There, he toured Schoepfer's laboratory at Ciba-Geigy.

Ryan was in Switzerland for a week. "It's a very beautiful country," he said. "And the state of histotechnology is very similar to ours. Their equipment is very similar or the same as we have in the United States—except, of course, that instructions and labels are written in French or German."

The Swiss society has about 300-350 members. That represents a very high percentage—probably 30%-35%—of the histotechnologists in the country. About 150 people attended the meeting in all. "Lecture topics were very similar to ours," Ryan said. "But they concentrate more on animal research because a lot of the work they do there is on pharmaceutical and toxicology research."

Two representatives from the Swiss society will attend the NSH Symposium/Convention in Las Vegas. Peter Ossent, D.V.M., University of Zurich, will conduct a workshop on special stains in the routine pathology laboratory. And Anton Schoepfer will give a presentation on the Swiss Roll technique, based on a paper published in the Swiss histology journal.

"We would like to see the speaker exchange continue yearly," Ryan said. "In fact, the Swiss would like to make it an award for one of their top speakers."

Correction:

In the article entitled "Connective Tissue Staining in Glycol Methacrylate Sections," published in the Nov./Dec. 1988 issue of *Histo-Logic*, it was reported that Sirius supra blue GL (CI 23160) was used for collagen staining. The dye actually used was Sirius supra blue FLG-CF (CI 51300).

From Histotechnician to Research Technician: A Challenging Transition

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Editor's Note: This article was written at my request for several reasons: namely, to provide the histotechnology community worldwide with a perspective of a different aspect of histotechnology. Also, to challenge more of my colleagues to "test the waters" in new technologies. Third, to help promote the idea that one should never be afraid to take on new challenges related to job opportunities. The field of histotechnology is expanding at an interesting and rapid rate, and we must be prepared to professionally parallel its advancement. Last, we as histotechnologists should be excited about the opportunities available to us in this most rewarding field of histotechnology.

Orthopedic research is a challenging and stimulating career for a histotechnologist to consider. However, the path is neither risk-free nor without sacrifice.

After training and working 12 years in a clinical laboratory, I became frustrated with my work because of the emphasis on routine tissue processing. The only new techniques and challenges were new stains that the pathologists introduced into the laboratory.

In the clinical laboratory my duties consisted of rotations on sectioning, routine and special staining, cytology, and assisting with autopsies. The only writing experience was the preparation of a laboratory manual for the College of American Pathologists (CAP) inspections.

After years in the clinical environment, I began searching for a new position. The Harrington Arthritis Research Center (HARC) was being built on St. Luke's Medical Center campus in Phoenix, Arizona. The Chief Executive Officer (CEO) approached our chief pathologist for a histotechnician. Since it was known that I was eager for new challenges, I prepared my first résumé and applied for the research position. The first three months included part-time work for the hospital and HARC. A full-time research commitment began in February, 1984.

The very first day of the job I was instructed to fly to Los Angeles to meet and interview with Dr. Roy Bloebaum, who was to direct the Biological and Basic Science Department of the Research Center. What a frightening experience since I had never flown before, nor interviewed so far from home.

I took several histology slides to show the type of work I was capable of doing. I tried to impress how neat I was, never making a mess when cutting. Looking back now, and knowing Dr. Bloebaum over the past five years, I know he had his doubts whether I could do the job or not.

During the interim of the Director's arrival at HARC, the Chief Executive Office had started a project that required the specimens be embedded in polymethyl methacrylate (PMMA). I was handed the book *Bone Histomorphometry: Techniques and Interpretation* (Robert Recker, 1983) and told to embed all the specimens in PMMA. How? I didn't know anything about plastics. I was a paraffin technician!

Well, I read the chapter but found the techniques described lacked the essential information to achieve the goal. Now what was I to do? I called all over the country asking for help and finally received the assistance necessary to fill in the missing pieces.

The specimens that I processed were rabbit femoral condyles with a porous polyethylene transverse plug *in situ*. We didn't have an automatic processor, so all dehydration and clearing was done by hand (which I had never done before). Needless to say, these first generation PMMA blocks were nothing to brag about. Sections from these blocks were cut using a PolyCut S sliding microtome, with a tungsten-carbide knife, and stained with toluidine blue and basic fuchsin.

In 1984, I went to my first NSH symposium/convention and on my second round-trip plane ride. This was real exciting! I met many nice people and learned a lot just from the "shop talk" during breaks and in the evenings. The workshops were helpful and it was great fun viewing the scientific exhibits and "shopping" for equipment for

the center. I went home with new knowledge and techniques and better insight as to the type of equipment available to assist in our research efforts.

How was orthopedic research? It was getting very exciting! I found the work challenging, stimulating, and I had a great boss who encouraged our ideas and the performing of pilot studies.

Orthopedic research is a highly competitive field because there are so many investigators after the same research dollars. Being in research is high risk because when the funds are exhausted or improperly managed, the research staff is eliminated. I have felt the disappointments of such a situation. After being employed for over 15 years (not all in research), I found that I and six other research staff were being laid off. In order to get those precious research dollars, it is necessary to remain one step ahead of the other investigators; therefore, you must continue to work on the cutting edge of technology and forge new frontiers almost on a daily basis.

On my first major research project, I was privileged to work with a private veterinarian, Dr. Gary Yocham, from Southside Animal Hospital, who assisted Dr. Bloebaum with his research. In addition to the everyday house-pet owner problems, Dr. Yocham's practice was involved in treating the racing greyhound.

Although Dr. Yocham treated torn muscles, parvo virus, and broken toes, the most perplexing and frustrating injury was the central tarsal (Tc) fracture. Out of 29% of the racing greyhounds treated for severely fractured Tc bone, the routine screw technique worked on only 18% of the fractures, and the remaining 11% of the greyhounds were being euthanized at the peak of their racing career.

The fracture occurred when the dogs raced counterclockwise on the track, usually at the first turn as the dogs crowd near the rail to make the turn. The fracture always involved the right rear foot in the hock and the dog normally pulled up lame without completing the race. Dr. Yocham presented Dr. Bloebaum with this problem and asked whether a prosthesis could be developed to help the greyhounds resume their racing career.

I was involved with this project from the very beginning, first measuring the harvested normal Tc bones from former racers so that a prototype could be manufactured from high-density polyethylene. The prototype was placed in three greyhounds that were injured on the track to see if surgery was feasible. I assisted with the surgery and recovery of these animals. One dog did so well the owner requested the dog be returned to the kennel.

I went to the kennel many times to check on the dog's progress, walked him, filed reports, and kept the animal's chart up to date. Because the prototype was only polyethylene, the implant failed and the animal was euthanized. But we learned that surgery was feasible and that the dogs recovered quickly.

From this knowledge, a titanium alloy prosthesis (same material used in human orthopedic implants) was designed and ready for the next dog that came off the track with a Type IV or V fracture.

This is when Abby (a racing greyhound) came along. She sustained a severe Type IV fracture to her Tc bone and, with permission from her owner, was placed into the Tc prosthetic research project. Abby's surgery went well and she recovered quickly. Within three months (record time), Abby was back on the track running qualifying times. She started in Grade C races and moved up to more competitive Grade B races. Abby raced successfully for nine months. I went to the kennel and track to continually monitor her training and to the races to cheer her on. Abby's racing times began to drop off and she was retired from racing. She was the first greyhound to competitively race with a titanium implant in her foot. She became a television celebrity and a favorite in our hearts.

When she was retired, it was necessary to evaluate the implant, and, because Abby would not be used for breeding, she was donated for research. It was necessary to see how the implant withstood the tremendous pressure that Abby exerted on her right hock.

How did I like orthopedic research by that time? I loved it! I soon learned the heartaches that came with animal orthopedics. Abby will always be a memorable part of my life. This work has been published and it is the research team's hope that this treatment will help the other greyhounds return to the track or to breeding.

The many skills I have learned over the years, not common in clinical pathology laboratories, have been gross photography, contact radiography, photomicrography, organization of processing schedules (both

manual and automatic), microradiography, and ground section histology. Small pilot studies of new solvents and plastics are very important so that valuable information and dollars are not lost. I have assisted in sterile animal surgical procedures and animal care. I have learned tetracycline labeling in canines and rabbits and assisted in sacrifices. I enjoy working with the animals, although sacrificing makes the job difficult. My future goals are to learn the scanning electron microscope and backscattered imaging techniques and our advanced image analysis equipment.

Being a histology technician in orthopedic research is very rewarding. We are able to develop new processing techniques; and, in research, it is never mentioned that something cannot be done—it just takes time to develop a way. Our research team meets with one another to establish our 90-day objectives. This is beneficial as a team, because it gives the laboratory direction for that 90-day period, and if one of us is overburdened, we pull together to meet our objectives.

I have been privileged to be a co-member for two NSH workshops, author and co-author on several papers. It is all because I've been fortunate to have a boss who gives me the opportunity and encouragement to grow. I don't have a college degree, but I have grown professionally in my career and feel confident that I will continue to grow as the opportunities and challenges arise. NSH has been a tremendous asset to my career and, with continual nurturing from Dr. Bloebaum and NSH, I hope to continue to flourish in the realm of an orthopedic research laboratory.

My first paper, which I co-authored for the *Journal of Histotechnology*, felt like an endless task. The histology team was challenged to find the best paraffin for the large, decalcified bone sections. We did a study on three paraffins and wrote a comparison paper reporting our results. The research was fun, but the writing was painfully slow and agonizing. It seemed that the task would never end. The reward was seeing the article in print.

I also enjoyed helping other bone laboratories get started by assisting with the training of their technicians. We are a small minority in the larger field of histology. We feel fulfilled when we assist in helping others who are struggling to establish a new orthopedic research laboratory. There is no greater reward than to have technicians return to their own laboratory confident in the techniques they learned during their time with us. Another reward is the continued collaboration on the technical and scientific level. I have met and worked with people from China, Holland, Australia, Germany, and England. I have enjoyed working with these people, learning about their cultures, and exchanging ideas.

I realize that histotechnologists making their daily contributions in the clinical pathology laboratory are essential. These individuals will continue to be the backbone of histotechnology. But the challenge and opportunities of orthopedic research have been exciting for me.

Acknowledgment
I wish to thank Kent Bachus, Dr. Roy Bloebaum, and Margaret McGee for their critical review and to acknowledge the Veterans Administration Medical Center, Salt Lake City, Utah.

Answers to Questions in Search of an Answer

The following answers are in response to "Questions in Search of an Answer" that have been presented in past issues of *Histo-Logic*. The volume, number, month, and year of publication in which the question appeared is provided with each answer. Also provided is the name of the author of the original question and the name of the person providing the answer.

> This is in response to a "Question in Search of an Answer" concerning gray-staining H&E's. The question appeared in *Histo-Logic*, Vol. XVIII, No. 3, page 16, July/Aug. 1988, and was presented by Lee G. Luna.

One of the technical problems I encountered after coming to this laboratory in 1987 was "gray H&E's." Overall, hematoxylin- and eosin-stained slides were of similar appearance to those illustrated on page 16 of the issue of *Histo-Logic* cited above. Previous attempts at improvement were aimed at the staining mechanism and, as Mr. Dayman's experience confirms (*Histo-Logic*, Vol. XIX, No. 1, pages 56-57, Jan./Feb. 1989), efforts along these lines failed. A "back-to-basics" approach to the tissue-processing protocol appears to have solved the problem.

Realistic times were assigned to the solvent stations on our VIP 3000 tissue processor. Determination was made to adequately process the largest of specimens submitted. The routine overnight cycle is as follows:

Station	*Reagent*	*Time*	*Temp.*	*PV*
1	10% NBF	2:00	37°C	ON
2	10% NBF	1:30	37°C	ON
3	10% NBF	1:30	37°C	ON
4	80% ethyl alcohol	:30	—	ON
5	95% ethyl alcohol	:30	—	ON
6	95% ethyl alcohol	:30	—	ON
7	100% ethyl alcohol	:45	—	ON
8	100% ethyl alcohol	:45	—	ON
9	Xylene	:45	—	ON
10	Xylene	:45	—	ON
11	Paraplast X-tra	:45	56°C	ON
12	Paraplast X-tra	:45	56°C	ON
13	Paraplast X-tra	:45	56°C	ON

Back to basics: Improved clearing

A state-of-the-art laboratory doesn't always produce state-of-the-art results. Good people and excellent equipment were in place; however, one reagent used for tissue processing and staining had to be changed. Xylenes and xylene substitutes were eliminated and replaced with pure xylene, which in fact was less expensive than the blends and substitutes. Fumes are not a problem because of the "closed" system incorporated for processing, although we do use a nonaromatic xylene substitute only for holding slides while coverslipping. Staining is automated (Brigati's schedule*) and the improvement is evident in the illustrations provided.

Remarks

The switch to pure xylene from xylenes and xylene substitutes appears to have eliminated the overall "gray H&E's" in this laboratory. Sporadic inconsistencies in staining can often be traced to preprocessing mistreatment of specimens. Excellent staining is achieved with homemade, generic, and name-brand hematoxylins and eosins, but solvent purity can be the limiting factor in any system.

*Brigati DJ: Automation and the Future of Histotechnology, Advanced Immunocytochemistry, workshop presented for the Georgia Society for Histotechnology, Atlanta, Georgia, May 16, 1987.

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This is in response to a "Question in Search of an Answer" concerning staining of tissue background after hyaluronidase treatment. The question appeared in *Histo-Logic*, Vol. XVIII, No. 5, page 52, Nov./Dec. 1988, and was presented by Jennie Achstetter.

In the past we have also experienced such problems with our digestive treatment, staining the enzyme-treated slide darker. We tried in vain to order and make up fresh hyaluronidase enzyme until one day we decided to try some pharmaceutical-grade hyaluronidase. It is called "Wydase" in the pharmaceutical community. The results were fantastic. We found that the "commercial" reagent grade hyaluronidase is contaminated most of the time.

The "Wydase" comes in a 1-ml injection vial. You can get it from your pharmacy and remove it with a 1-cc tuberculin syringe and put it directly on your slide.

I hope this will help you in your digestive treatment. Thank you for the opportunity to be able to respond to your question.

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This is in response to a "Question in Search of an Answer" concerning staining of tissue background after hyaluronidase treatment. The question appeared in *Histo-Logic*, Vol. XVIII, No. 5, page 52, Nov./Dec. 1988, and was presented by Jennie Achstetter.

I, too, have experienced a similar staining increase when I perform the safranin O-fast green technique.[1] My experience lies solely with hyaluronidase, so if my postulation is correct, I must assume that Ms. Achstetter uses a similar buffer for both the hyaluronidase and sialidase techniques, or both buffers have a similar effect on the tissue.

Routinely, when performing the hyaluronidase digestion technique, I run a negative control slide at the same time. This consists of incubation of an adjacent slide, to that being treated with hyaluronidase, in just the acetate buffer.[2] An apparent increased staining was noted in both my treated and negative control slides. Subsequently I took two more adjacent slides, one treated with the acetate buffer without the hyaluronidase, and the other slide left untreated. Both slides were stained

simultaneously with safranin O-fast green. The slide treated with the acetate buffer stained noticeably more intense than the adjacent untreated slide. I, therefore, attributed this staining phenomenon to a mordant-like potential of the acetate buffer. Since my laboratory uses safranin O-fast green for qualitative evaluations and photomicrographs, I have added the acetate buffer incubation as a routine prestaining step for the intensification of this technique.

I would appreciate input into the feasibility of this theory or alternative theories for this staining phenomenon.

References

1. Rosenburg L: Chemical Basis for the Histological Use of Safranin O in the Study of Articular Cartilage. *J Bone and Joint Surg* 1971;53-A:69-82.
2. Thompsom SW: *Selected Histological and Histopathological Methods*. Springfield, Ill. C.C. Thomas, 1961, p 521.

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This is in response to a "Question in Search of an Answer" concerning microvibration. The question appeared in *Histo-Logic*, Vol. XIX, No. 2, page 80, March/April 1989, and was presented by Lee G. Luna.

The problem of microvibration was first noticed when processing renal biopsies using the VIP short processing schedule, with 15 minute stations. Tissue was cut using a new Accu-Edge knife; knife angle was not a factor as you (Lee G. Luna) have stated. Sections were cut at 3 micrometers.

We determined that microvibration resulted when the tissue was "over-processed." Processing was shortened and done by hand using a Wheaton O-Ring seal system, vacuum pump (15 PSI), at 10 minutes per station, and 2 paraffin changes, 30 minutes each. Temperature was kept at 58-59°C. Fresh paraffin was used for each change due to the minute size of the tissue. All alcohols were ethanol starting with 70% (1 change), 95% (2 changes), 100% (2 changes), and xylene (2 changes). These solutions were decanted at each change and *not* reused. All paraffin infiltration was done in a vacuum oven using Surgipath's infiltration media.

The problem of "microvibration" was eliminated with the above procedure. Temperature may contribute to the problem, as our low temperature paraffin is right at ±2° of the melting point. Our routine tissues processed on the VIP using vacuum/pressure and cut at 5 micrometers have not presented this problem.

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This is in response to a "Question in Search of an Answer" concerning microvibrations. The question appeared in *Histo-Logic*, Vol. XIX, No. 2, page 80, March/April 1989, presented by Lee G. Luna.

Although a dull knife or acute knife angle may cause the microvibration artifact, histotechnologists know that a cold, moist block will section better than a warm, dry block. In this laboratory, blocks to be sectioned are held either in a crushed-ice bath or on a partially melted ice block (an ordinary household ice cube tray will do). Blocks that require lengthy microtomy are periodically cooled and remoistened by application of an ice cube to the cut surface for a few seconds. More detail on methods and explanation can be found in the article: Luna LG: Amorphous bevel surface and moisture-important factors in microtomy. *Micro Views* Fall 1988;4:2:5-7:0:5-7.

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This is in response to a "Question in Search of an Answer" concerning microvibrations. The question appeared in *Histo-Logic*, Vol. XIX, No. 2, page 80, March/April 1989, presented by Lee G. Luna.

At Indiana University Hospital in Indianapolis, Indiana, we encounter horizontal lines (microvibrations) in many of the tissue specimens produced for microscopic slides (Figs. 1 and 3). A wide variety of tissue specimens, adult and pediatric, are processed daily in our laboratory. These include both small biopsies (myocardial, liver, kidney, and gastrointestinal) and larger specimens that are difficult to cut because of varying densities. We have

found the following procedure very effective in eliminating or minimizing the problem of microvibrations (Figs. 2 and 4):

1. Face the embedded block until tissue is completely exposed.
2. Immerse block in ice water thirty minutes or more.
3. Realign block on microtome and gently cut ribbons.
4. Allow ribbons to float on water bath at least thirty seconds before selecting representative sections for microscopic slides.

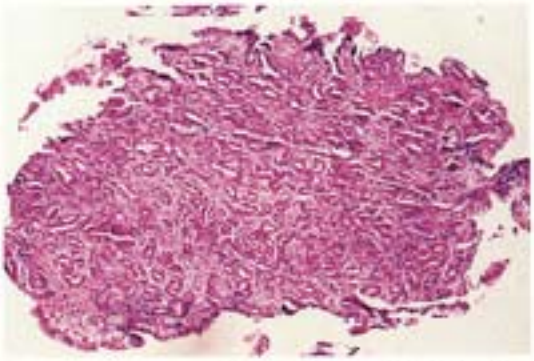

Figure 1: Section demonstrates microvibration. Specimen was not soaked in water before microtomy. H&E stain.

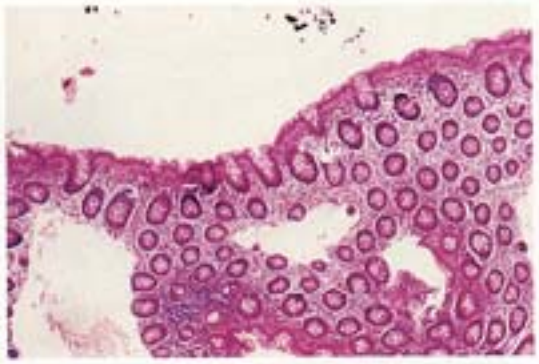

Figure 2: This section does not demonstrate microvibration after specimen was soaked in water for 30 minutes prior to microtomy. H&E stain.

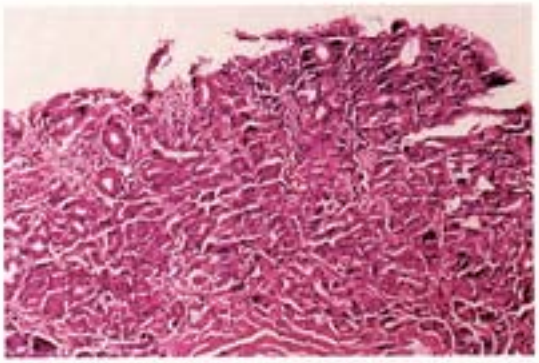

Figure 3: Section demonstrates extensive microvibration. Specimen was not soaked in water prior to microtomy. H&E stain.

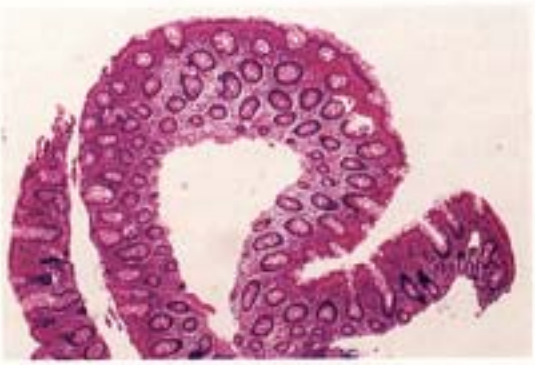

Figure 4: This section does not demonstrate microvibration after specimen was soaked in water for 30 minutes prior to microtomy. H&E stain.

If the tissue contains intensive areas of hemorrhage, the above procedure can be followed substituting cold tap water for the "soaking" or immersing medium.

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This is in response to a "Question in Search of an Answer" I posed in regards to microvibrations in tissue sections. The question appeared in *Histo-Logic*, Vol. XIX, No. 2, page 80, March/April 1989.

Microvibration such as that presented in above cited reference is produced as the results of overprocessing and subsequent lack of sufficient moisture in the tissue specimen. Over-tissue processing removes excessive water from specimens, resulting in dried tissues. This dried tissue becomes hard (similar to a dried sponge), which results in microvibration as the microtome knife travels through the hard tissue. This in a simplified way can be compared to taking a kitchen knife and cutting multiple dried, uncooked strands of spaghetti. In this instance the strands of spaghetti break at different lengths. Conversely, cutting cooked (water containing) spaghetti will result in easy-cutting strands of spaghetti. There is no doubt that reintroduction of moisture into tissue is one of the most important steps in preventing most artifacts (including microvibration) produced during microtomy. This fact can be proven easily if one performs the following steps: a. Process a piece of conventional-sized kidney in a routine manner making sure specimen is not underprocessed. b. Cut a section at 6 micrometers without exposing the specimen to ice, water, skin refrigerant, or other methods generally used to improve sectioning. (This section and all subsequently cut sections are picked up from the flotation water bath and dried in the normal manner.) c. Dip your thumb in the warm flotation water bath and rub your water-soaked thumb on the surface of the specimen one time for 2 seconds. Without icing, cut the section and dry in the normal manner. d. The third section is cut after the surface of the specimen has been exposed to the water-soaked thumb 3 times for 2 seconds each. e. The following 4 to 5 sections are cut after water-soaking treatment similar to that provided above (Step d) except that the steps (water-thumb soaking and seconds of exposure) are increased by one each time a section is cut. Sections are stained after the above steps have been completed. By performing these steps one can see microscopically a dramatic gradual improvement in each subsequent section as one increases the time of water-soaked thumb treatment and seconds of exposure. The section quality improvement seen microscopically includes all artifacts often seen including microvibration.

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Editor's Note: The National Society for Histotechnology is a professional society representing all of those involved in histology. We at Miles Inc. encourage all histotechnologists to consider joining this highly respected and worthwhile organization.

If you are interested in becoming a member of the NSH, please fill out this application and return it to:

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Heads Up
It's the 1989 NSH Convention

From September 23-28, the Riviera Hotel in Las Ve
Nevada, will host histotechnologists from all over
country.

The 15th annual NSH convention is where you'll learn
skill and fun. Workshops, scientific discussions,
exhibits are all meant to enhance your histotechno
skills. And, Miles will provide *the* fun.

Last year, you camped out and dressed up for the M
M*A*S*H party at the NSH convention.

This year, you can dress up or dress down, but, what
you do, don't leave home without a H.A.T. (Histolo
Are Tops!!)

Miles "Crazy Hat" party will be the perfect way to
off" the 15th annual NSH. To get there, follow the
or go to the top floor of:

The Riviera Hotel – "Top of the Riv"
Monday, September 25, 1989
9:00 P.M.-1:00 A.M.

Be prepared for the fun and festivities. You may w
prize in one of eight categories in our H.A.T. conte

Funniest
Naughtiest
Craziest
Most feminine
Most masculine
Smallest
Most-apropos-for-a-histologist
Most creative

Our expert judges will pick six finalists with the
creative entries, and you'll determine the winner
your applause. A hat dance will follow the contest.
refreshments will be found next to the party hats.
you there.

Craziest

Most creative

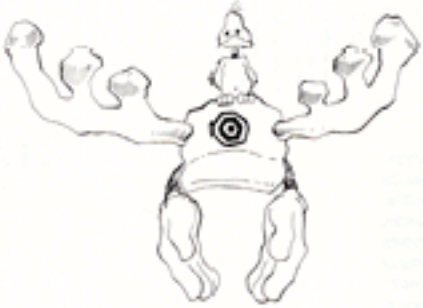
Most feminine

Most-apropos-for-a-histologist

Most masculine

Smallest

Naughtiest



Funniest

Tissue Identification Slide Training Sets Available

The Center for Histotechnology Training has available a number of normal* slide sets for sale. The sets were produced for histology technicians and are intended to provide a beginner's set of slides for studying identification of different tissue structures. The sets consist of 21 slides with the following tissues: skeletal and cardiac muscle, bone, skin, esophagus, stomach, small and large intestine, trachea, lung, kidney, liver, spleen, pancreas, cerebrum, cerebellum, spinal cord, adrenal, thyroid, testis, and prostate. There will be no handout provided since I feel that tissue identification should be studied by the user in conjunction with one or several of the many books in histology. A list of these books will be provided with the slide sets.

*The word *normal* suggests only that every effort was made to provide tissue with little or no pathology and/or other abnormal inclusions.

If you are interested in one or more of these sets, please send $55.00 per set to: Center for Histotechnology Training, P.O. Box 736, Olney, MD, 20832, attention Lee G. Luna.

To receive your own copy of *Histo-Logic*® or to have someone added to the mailing list, submit home address to: Miles Inc., Diagnostics Division, P.O. Box 70, Elkhart, IN 46515.

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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