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Marine Pathology and Aquaculture, Its Evolution, Disease Mechanisms, and Impact on Histologic Technique: A Challenge for the '90s

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Introduction

As the science of marine and aquaculture pathology dramatically expands throughout the world, the need to develop standard procedures for the laboratory analysis of specimens, especially those techniques that involve tissue preparation, is essential, particularly in light of new Federal regulatory guidelines for inspections soon to be implemented that will essentially make laboratory diagnostics mandatory. Expanding laboratory facilities for examination of marine specimens will no doubt increase the demand and opportunities for experienced Histotechnologists. The importance of the exciting challenge for the development of marine and aquaculture technology is apparent—the increasing world demand for an inexpensive and easily obtainable source of dietary protein is extremely important for future food production throughout the world. As the disciplines of marine and aquaculture pathology evolve, there will be an increasing need for the development of techniques for diagnostic testing and analysis for the control, prevention, and treatment of disease. An exciting challenge faces marine histologists. They must be prepared to utilize all facets of histologic technique. They must be prepared to adapt and modify techniques when development of procedures is necessary. They must be prepared for dealing with numerous unknowns and ready to establish new "standards" for an exciting new frontier—diagnostic marine histology.

There are a number of marine animals that are commercially significant in the Americas and throughout the world today. These animals include shrimp, crabs, lobsters, oysters, clams, salmon, bass, turtles, catfish, redfish, mullets, and crawfish, to name just a few. The commercial applications of marine marketing and aquaculture clearly represent a significant and rapidly growing industry with a substantial economic potential. For instance, worldwide culturing of marine shrimp has reached an all-time high production level. In 1986, 310,000 metric tons of shrimp were harvested with an estimated harvest of over 500,000 expected in 1990. Striped bass represent a harvest of over 2,268 metric tons a year, and this yield is steadily increasing.[1,4] Other mentioned

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species are equally impressive in their commercial impact. Commercial operations are rapidly establishing reproducing populations in freshwater and saltwater environments throughout the United States. Perhaps less apparent is the steadily increasing demand for more exotic delicacies, such as soft-shell crawfish.

A problem that confronts marine and aquaculture specialists is the loss of animals due to disease either by slow continuous attrition or by a sudden catastrophic incident.[12] The practice of short-term holding of specimens for market, such as lobsters held in ponds or crabs in shedding tanks, also presents problems. The practice of disease control research in aquaculture and commercially marketed marine animals is not new to marine technology. For several decades, progress has been made, but major advances in the understanding and control of disease in marine aquaculture are more recent, largely within the last 20 years.[12] However, there are enormous gaps in our knowledge and understanding of marine and aquaculture disease processes and their pathology. A number of organizations, including the International Council for the Exploration of the Sea (ICES) and the World Aquaculture Society, provide information regarding important diseases of fish and shellfish and are an important resource for investigators. Unfortunately, the bulk of important information still remains widely dispersed throughout the scientific world, making dissemination of information difficult. Currently, investigators in marine science and aquaculture development, seeking specific information, have limited scientific resources at their disposal. This academic deficit, however, is greatly and rapidly improving as interest in the industry increases.

There is much to be learned about aquaculture technology concerning inexpensive defined diets, maintenance of water quality, larval survival, and disease control. As greater numbers of investigators look for diseases and their solutions, there will emerge a greater number of observations of disease and its control and treatment. The most important need of marine and aquaculture specialists today is the development of accurate, rapid diagnostic procedures for the identification of marine and aquaculture diseases.

Regarding the role of disease diagnosis and prevention and its relationship to advancement and evolution of the technology, marine studies and aquaculture differ only slightly from the husbandry of other animal species. There are, however, significant physiological differences between these animals, whether invertebrate shrimp or vertebrate fishes and the vast majority of other cultured animals. These differences are substantial enough to have restricted advances in disease diagnosis while techniques and methodologies were developed for use in marine and aquaculture investigation.[1]

Opportunity for New Technologies

Opportunities for the Histologist in the marine specialities are exciting. Although there are many fine resources currently being developed, there remains a tremendous amount of work to be done regarding the development and standardization of histologic techniques applicable to the numerous marine specimens examined. Technologists, particularly those accustomed to mammalian histology, must familiarize themselves with the marine characteristics and presentation of disease, many of which are very different from those associated with mammalian disease. Bacteria, viruses, and parasites associated with aquatic disease may differ in their presentation of disease from their mammalian counterparts, and an understanding of physiology and histology, as well as water chemistry, is required. However, the general principles of fundamental, established pathology still apply, and lesion-interpretation is essentially the same in aquatic animals as in the "higher" vertebrates.[3] For instance, infectious disease problems in the crustacean culture are mostly microbial in origin—viruses, bacteria, fungi, and protozoans. All are significant. Although minor compared to systemic infections, bacteria contribute to shell-destroying disease. The recognition and diagnosis of virus disease are becoming extremely significant as reports of virus disease in marine and aquaculture facilities are steadily increasing. Fungi are common. In fact, shrimp demonstrate nearly 30 described diseases and disease symptoms. We can be certain that there remain a number of poorly understood and defined or as yet unrecognized but important diseases to be identified.[1]

Effective disease control depends on diagnosis, disease prevention, and treatment of aquaculture specimens. Correct diagnosis, including understanding the life cycle and ecology of the pathogen, is obviously a critical step in any control program.[12] Although the usual emphasis is directed toward the pathogens, it should be understood that poor water quality and inadequate nutrition are often the basic determinants of disease outbreaks. Techniques developed and expertise expressed in the Histology Laboratory will play a vital role in resolving these diagnostic problems.

Histologic Technique

The development of histologic technique for marine pathology begins with a thorough understanding of one physiological fact. Unlike warm-blooded mammals, which tend to cool down after death, thereby slowing autolysis, marine and aquaculture specimens out of water often warm up. Tissues must therefore be placed into fixative as quickly as possible before autolysis begins and hinders or makes interpretation of sectioned material impossible.[3] Rapid tissue autolysis is dramatically demonstrated in the gills when even a 5-minute delay in fixation may produce lammellar epithelial swelling and lifting from the basement membrane. The use of scrapes, smears, squashes, and whole mounts from live and anesthetized or freshly dead specimens are also valuable diagnostic tools. Collections from the skin and gills are especially important where protozoan or other parasitic disease are suspected. Blood samples and microbial cultures may provide additional information. While these samples may provide diagnostic information, they are generally not as useful as in mammalian specimens. Blood samples may also be collected for serology and clinical chemistry. Specimens for ultrastructural examination should be collected at this time. Obviously, the Technologist must be prepared to work quickly and efficiently when collecting marine specimens. The Technologist who has been primarily involved with mammalian histologic techniques may find it necessary to review techniques and methods that may be unfamiliar, such as those primarily used in microbiology, hematology, parasitology, and toxicology disciplines.

The gross examination and dissection of tissue (depending on the species and investigation) prior to fixation is an extremely important procedure. Small fish under 4 cm may be collected whole, with the removal of the operculum and incised abdominal cavity (to facilitate the penetration of the fixative) being the only dissection. However, other specimens, such as shrimp, may require injection fixation with little or no dissection prior to fixation. In some specimens, such as larger fish, a combination of smears, scrapes, squashes, microbial, and blood collections may be incorporated with the specimen necropsy and tissue collection. Necropsy procedures vary from species to species. However, the most important aspect of necropsy is consistency so that accurate comparisons can be made from examination results.

Several physiological factors can significantly affect the techniques applied in marine pathology. There are dramatic differences between the general pathology of mammals and that of many marine species. For instance, compared to mammals, fish demonstrate a number of significant physiological differences. Marine specimens do not appear to demonstrate an especially pronounced acute inflammatory response as seen in mammalian specimens; i.e., heat, redness, swelling, or loss of organ function. This difference in the manifestation of the inflammatory response occurs, despite the fact that many aquatic species possess the cells and most of the organ systems required to elicit such a response familiar in mammals. The formation of obvious pus is not a usual feature of marine animal inflammation, although aggregates of neutrophils are sometimes seen.

Mycobacterium marine may cause an extensive granuloma formation response, and it can be so extensive that it virtually obliterates the involved organ. A prominent feature of chronic inflammatory responses, in some species, is the presence of melanin or other pigment-containing melanomacrophages. In fish, although seen in normal specimens to some degree, melanomacrophages are frequently demonstrated in the spleen, kidney, and, to a lesser extent, the liver. They are also noted within the encapsulating response to many foreign bodies or parasites. The Technologist must be able to recognize these conditions microscopically and, where appropriate, prepare differential special stains for their demonstration and diagnosis. Immunohistochemistry will no doubt play a very important role in the diagnostic procedures as expertise in this area steadily increases, and the Technologist must be prepared to apply these procedures.

When considering the speed of an inflammatory response, it is important to note the effect of the water temperature and, therefore, the body temperature of the animal. A drop of 10°C will reduce the metabolic rate of a marine animal roughly by half, slowing the response to disease. Yet with rising water temperatures, these animals can succumb rapidly to diseases, especially bacterial diseases that thrive in warmer temperatures. With these factors in mind, the Technologist must recognize seasonal variations in wild catches, as well as environmental controls in cultured specimens.

Hyperplastic responses are well developed in the marine animal as is neoplasia. Classification of these lesions in marine specimens is based largely on established mammalian criteria, but with the exception of the hepatic carcinoma, few are observed to metastasize. Finally, it is important to note that repair and regeneration of epithelial lesions are extremely efficient in marine animals. This is especially well demonstrated in fish. Even very severe lesions may heal with little gross evidence of tissue scarring.

Fixation

Fixation presents significant technical problems to Technologists in marine pathology because of rapid tissue autolysis. Particularly delicate organs, such as the liver and the hepatopancreas, may represent the primary target of many disease processes and, therefore, are vital in diagnostic evaluations. Improperly fixed specimens can lead to misinterpretation of sectioned material. Because of the great variation in marine specimens, the Technologist must be able to apply knowledge of a number of suitable fixatives to the specimens.

A fixative is selected for its ability to stop metabolic processes quickly, preserve cytologic and histologic tissue elements (while maintaining actual form), harden or give consistency to soft materials, and to mordant the tissue for differential staining.[2] Fixatives frequently used in marine specimens include neutral buffered formalin; seawater formalin; and Bouin's, Helly's, Lillie's, and Davidson's fixatives. Many of the marine specimens, such as the crustaceans, demonstrate an external skeleton containing chitin that may be variably hardened by calcium salts. Fixatives, such as Davidson's, Lillie's, and Bouin's, have proven to be effective for these specimens because of their ability to penetrate tissues rapidly, and minimize postmortem degeneration of the tissues and inherent decalcifying properties (acetic and formic acid) on the chitinous exoskeleton or bones of the animal.

Effective collection and fixation of marine and aquaculture specimens is dependent on a number of important factors. Specimens should be fixed immediately after removal from water, if possible. Sick but alive specimens usually yield the most valuable diagnostic information. Freshly dead and cooled (not frozen) specimens are a second choice. (Do not collect specimens that are dead unless it can be positively determined that they have died within the last few minutes or unless an obvious abnormal state exists. The condition of the specimen should be clearly stated in the specimen history.) Stress caused by handling live specimens should be kept to a minimum. Because of the relatively impervious nature of these specimens (chitinous exoskeleton, shell, or scales), simple immersion in a fixative does not allow for adequate fixative penetration. Some specimens, such as shrimp, may require injection of the fixative into vital areas prior to immersion in fixative. (While death by injection is recommended in many species, anesthesia and euthanasia of marine specimens are frequently necessary in the collection of specimens.[3]) Other specimens, such as fish, require slitting the specimen open prior to immersion in the fixative.[1] The rate of fixative to tissue (for immersion of tissue) should be at least 10 parts of fixative to 1 part tissue volume.[5] The use of vacuum infiltration techniques during fixation may prove to be a useful adjunct to the problem of marine specimen fixation.[9] Procedures creating vapors or fumes should always be performed in a well-ventilated or hooded area. Such fixatives are frequently used in marine pathology. Appropriate use of latex or surgical gloves is strongly recommended. For those Technologists not familiar with these fixatives, it may be necessary to review the literature for specific instructions for their composition, handling, storage, and disposal.[5, 7, 11] It should be particularly noted that because these fixatives frequently contain decalcifying agents or acids, these tissues must be thoroughly rinsed to remove all traces of fixative before processing.

Following fixation, specimens are trimmed, and specific tissues are selected for processing and histologic examination. The Technologist must have a broad knowledge of specimen anatomy and appropriate tissue selection. Some specimens may be very small and provide only a limited amount of tissue. Exact orientation of the specimen must be achieved or the entire organism, tissue, or lesion may be destroyed or lost. For instance, the ventral nerve cord of the penaeid shrimp is examined for evidence of certain virus disease. Incorrect preparation can easily result in the loss of this nerve for microscopic evaluation. Also, extremely small larval or postlarval specimens may require microscopic examination. These specimens may be so small that a dissecting microscope is frequently used for collection and orientation of the specimens. The Technologist may find excellent resource material in recently published works on specific tissue collection, such as shrimp, fish, and crab.[1, 3, 6] However, material is yet to be presented dealing with a wide variety of other species. The Technologist is frequently challenged with the development of species-specific procedures and must become knowledgeable in a variety of tissue specimen preparations.

Tissue Processing

Another challenge to the Technologist is that of tissue processing. A variety of processing programs may be used, including hand processing. The program chosen depends on the species, type, and size of the tissue. The use of vacuum infiltration is particularly valuable in marine histology because it releases tiny air bubbles that may be trapped beneath a carapace or exoskeletal shell.

Specimens vary considerably from one species to another or one growth stage to another. Numerous "unknowns" that do not necessarily conform to "standardized" histologic technique must be determined as different marine species are submitted for evaluation.

Embedding of marine specimens is similar to that of other histologic evaluations in that orientation of the tissue is of utmost importance. Again, the Marine Technologist must demonstrate a broad knowledge of the many species presented. Tiny larval or postlarval tissue may require the use of magnifying glasses and the use of ultrafine forceps or delicate brushes as instruments. The ingenuity of the Technologist in determining "what works" remains essentially standard throughout established histologic technique as new techniques are developed.

Standardized, established histologic technique is usually followed when staining marine tissues. Of course, there may be significant differences in staining requirements from one specimen to another. A hematoxylin and eosin stain is routinely used for the initial evaluation of specimens. Eosin-phloxine staining is also used. Because of the frequent use of fixatives that contain decalcifying agents, staining times may be extended due to the acid treatment. Special stains generally follow the same guidelines as those used in histologic disciplines other than marine pathology. Fungal and bacterial stains prominently head the list. (The development of the use of Pap stain for the demonstration of virus disease in smears has shown great promise.[8])

Perhaps the biggest challenge facing the Technologist involved in marine pathology and aquaculture is the potential for the examination of the histologic slides. Technologists traditionally examine preparations for technical quality, such as specimen adequacy, fixation, sectioning, and staining qualities. However, in many institutions, the Technologist not only is responsible for the technical presentation of the specimen but may be charged with the initial diagnostic evaluation of specimens. Specimens are examined for evidence of fungal and bacterial infections, parasite infestation, viral disease, and inflammatory response or neoplasia. As Technologists examine each specimen, they can determine the successes or inadequacies of the preparation. This process of in-depth evaluation and discovery significantly enhances the development of improved techniques.

As the disciplines of marine pathology and aquaculture develop, there will be an increasing need for diagnostic testing for the control, prevention, and treatment of disease. There will be an increased need for the well-trained Technologist. For the young Technologist entering the field of marine pathology, there is an exciting future. For the more experienced Technologist entering the field of marine pathology for the first time, this exciting new environment will be met with the need to think "marine," think "aquaculture." Both Technologists will find an environment filled with new and exciting technical challenges.

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Three Simple Methods to Reduce the Background of the Counterstain in Immunohistochemical Preparations

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Abstract

In the immunohistochemical preparation, inadequate or excess counterstain can sometimes produce distorted or unclear results. We have used the following methods to eliminate or avoid this problem.

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1. Acid water can be used to remove excess counterstain with no discoloration or loss of intensity of the aminoethylcarbazole (AEC) chromogen.
2. Harris' hematoxylin can be used to give excellent nuclear detail without much background in the cytoplasm and also with no loss of intensity to the AEC chromogen.
3. Prestaining with hematoxylin before the primary antibody can also present the opportunity to control the intensity of the nuclear stain.

Introduction

In immunohistochemical staining, the counterstain helps to demonstrate the morphology of the cellular structures. However, the hematoxylin counterstain can sometimes overlap the enzymatic reaction, which can make the interpretation of results more difficult, especially if the enzymatic reaction is weak. Also, it can be difficult to obtain sharp, clear photographs of the reaction. We have devised three methods that allow us to control the intensity of the counterstain so that the chromogen can be clearly demonstrated.

Method 1. Acid water: Counterstain the slides with hematoxylin. If the counterstain is too strong, dip in 0.5% acid water (0.5 mL of concentrated HCL to 99.5 mL of distilled water), 5 to 10 quick dips. Wash slides in water for a few minutes, blue with phosphate buffered saline (PBS), and observe with microscope. The same procedure can be repeated until desired intensity is obtained.

Method 2. Harris' hematoxylin: After enzymatic chromogen reaction (AEC), wash slides in water. Stain in Harris' hematoxylin for one minute, and rinse in water. In most situations, this will give a very sharp nuclear stain. If the background in the cytoplasm is too strong, it can be removed by using the procedure described above.

Method 3. Prestaining: Hematoxylin is applied to the slide for one minute prior to incubation with primary antibody. If the counterstain is too strong, remove excess with acid water, as described above, until desired intensity is obtained. Wash the slides with water for five minutes, rinse in distilled water, and then follow the immunostaining procedure provided by the manufacturer.

Results

Acid water can completely remove any excess hematoxylin and not affect the AEC reaction (Figs. 1-4). Harris' hematoxylin can produce excellent nuclear staining without excess stain in the cytoplasm (Figs. 5-6). Prestaining with hematoxylin prior to applying the primary antibody will allow you to control the desired intensity (Fig. 7).

Note the multinucleated giant cell in the center of Figure 1 prior to counterstain. A weak positive reaction is found in this cell. Figure 2 shows this reaction was overlapped by the counterstain. Figures 3 and 4 show that the acid water can remove the excess counterstain without decreasing the intensity of the enzymatic reaction.

Discussion

The conventional method of counterstaining is performed after the chromogen reaction. Sometimes this can create a problem because it can be difficult to control the intensity of the counterstain. As illustrated below (Fig. 1), a weak enzymatic reaction, such as is seen in the multinucleated giant cell in the center of the herpes labeled slides, may be difficult to detect if the counterstain is too intense. Our methods show a few ways to control this problem. Prestaining allows us to determine the desired intensity of the counterstain for a variety of tissues. Harris' hematoxylin, which some sources say should not be used with alcohol-soluble chromogens,[1] gives us sharp nuclear detail. We used the chromogen supplied by Biomeda and did not experience any change in the chromogen reaction when counterstaining with Harris' hematoxylin, although several sources suggest using an aqueous-based hematoxylin.[2] Acid water allows us to control the intensity of the counterstain, which may vary due to fixatives or the thickness of the sections.

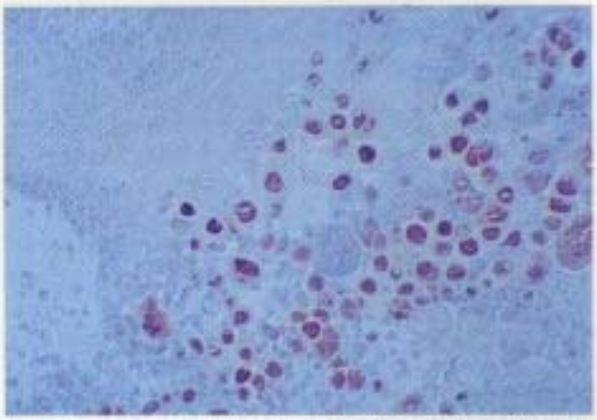

Figure 1: A case of Herpes II with no counterstain. Notice the clarity of the virus demonstrated. (×20)

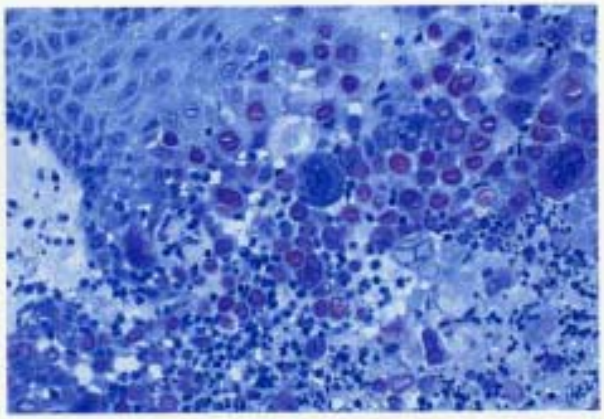

Figure 2: Duplicate section from Figure 1 but over counterstained with Harris' hematoxylin. Note the poor differentiation of the virus. (×20)

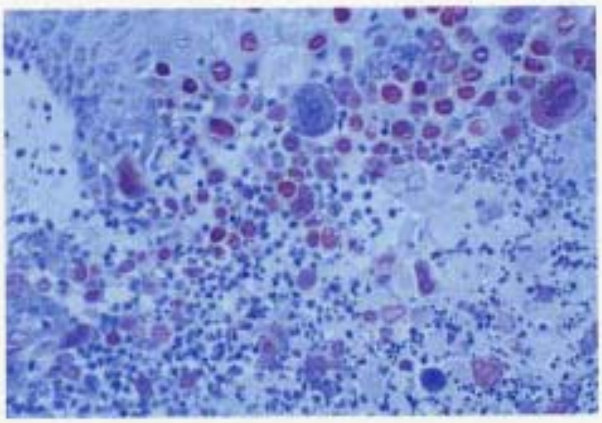

Figure 3: Duplicate section decolorized in 0.5% acid water for 5 dips. (×20)

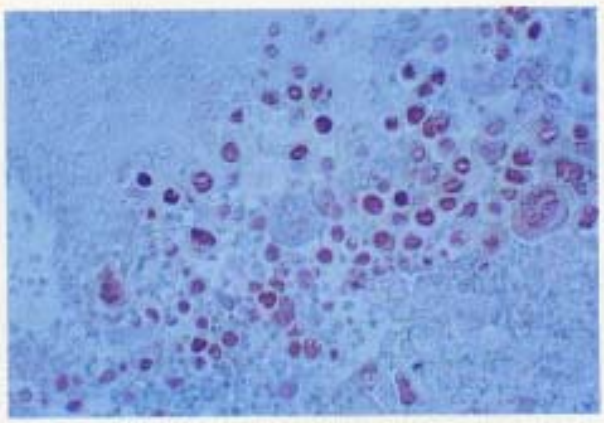

Figure 4: Duplicate section decolorized in 0.5% acid water for 10 dips. Notice the similarity to Figure 1. (×20)

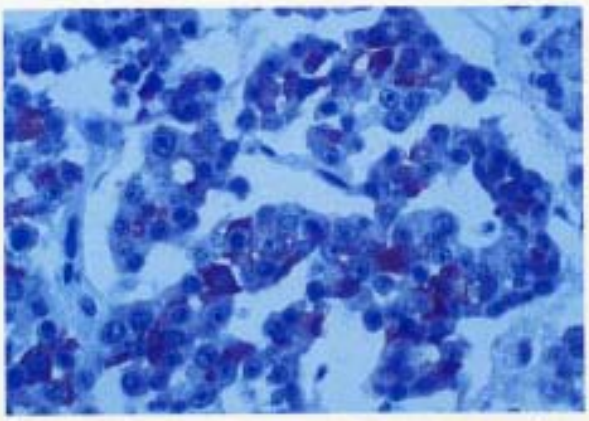

Figure 5: A section of pancreas immunostained for insulin. In this case, Harris' hematoxylin was used as the counterstain. (×40)

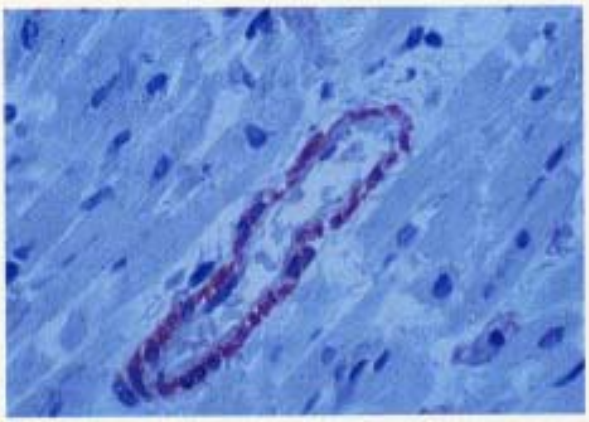

Figure 6: A section of heart muscle immunostained for smooth muscle action. Harris' hematoxylin was used as the counterstain. (×40)

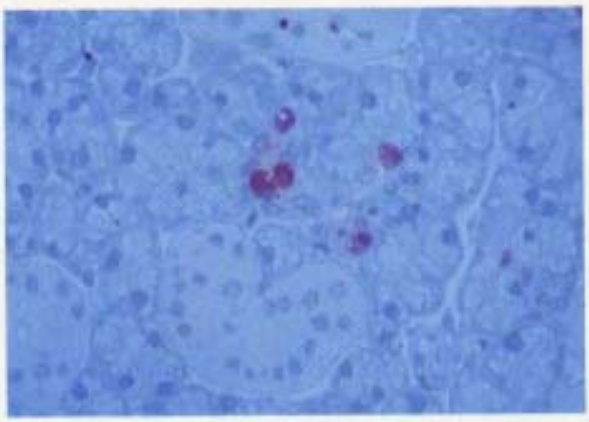

Figure 7: A section of salivary gland prestained with hematoxylin. (×40)

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A New Oxidant For Harris' Hematoxylin

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Some Technologists have expressed concern about the use of mercuric oxide (red) as the oxidant in the preparation of Harris' hematoxylin. The toxicity of mercuric oxide has encouraged the use of alternate reagents, such as sodium iodate, as the oxidant in the preparation of Harris' hematoxylin. Unfortunately, hematoxylin solutions prepared with some of these alternate oxidants do not appear to provide crisp nuclear staining.

Described herein is the use of commercial JAVEX (Clorox [5.6% sodium hypochlorite]) as the oxidant in the preparation of modified Harris' hematoxylin. The resultant solution gives nuclear staining comparable to hematoxylin using mercuric oxide (red) as the oxidant. Apart from common use as a laboratory disinfectant, this reagent also has the advantage of effecting bacterio-stasis of the solution.

Staining Solutions

Hematoxylin powder	2.5 gm
Potassium or ammonium alum	50.0 gm
Distilled water	500.0 mL
Sodium hypochlorite (JAVEX)	2.0 mL
Glacial acetic acid (concentrated)	2.5 mL

Method
Dissolve the alum (ammonium or potassium) in distilled water in a conical flask, preferably with the aid of a magnetic stirrer. Add the hematoxylin and mix. Cover the flask and leave mixture in a 60-65°C oven overnight. Remove from oven and add 2 mL of JAVEX (commercial preparation of 5.25% sodium hypochlorite). Cool. Solution should be ready in about 1 week. Add glacial acetic acid just before use. Filter before use.

Comments
It has been found that allowing the solution to further "ripen" for a few more weeks improved the intensity of the stain to a remarkable degree, and the solution deteriorated less rapidly than conventional Harris' hematoxylin.

The use of commercial JAVEX in this preparation makes economic sense because, in most institutions, it is already used as the standard disinfectant. Addition of acetic acid is optional.

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Acknowledgments
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Figure 1: This section demonstrates the staining details of the modified hematoxylin.

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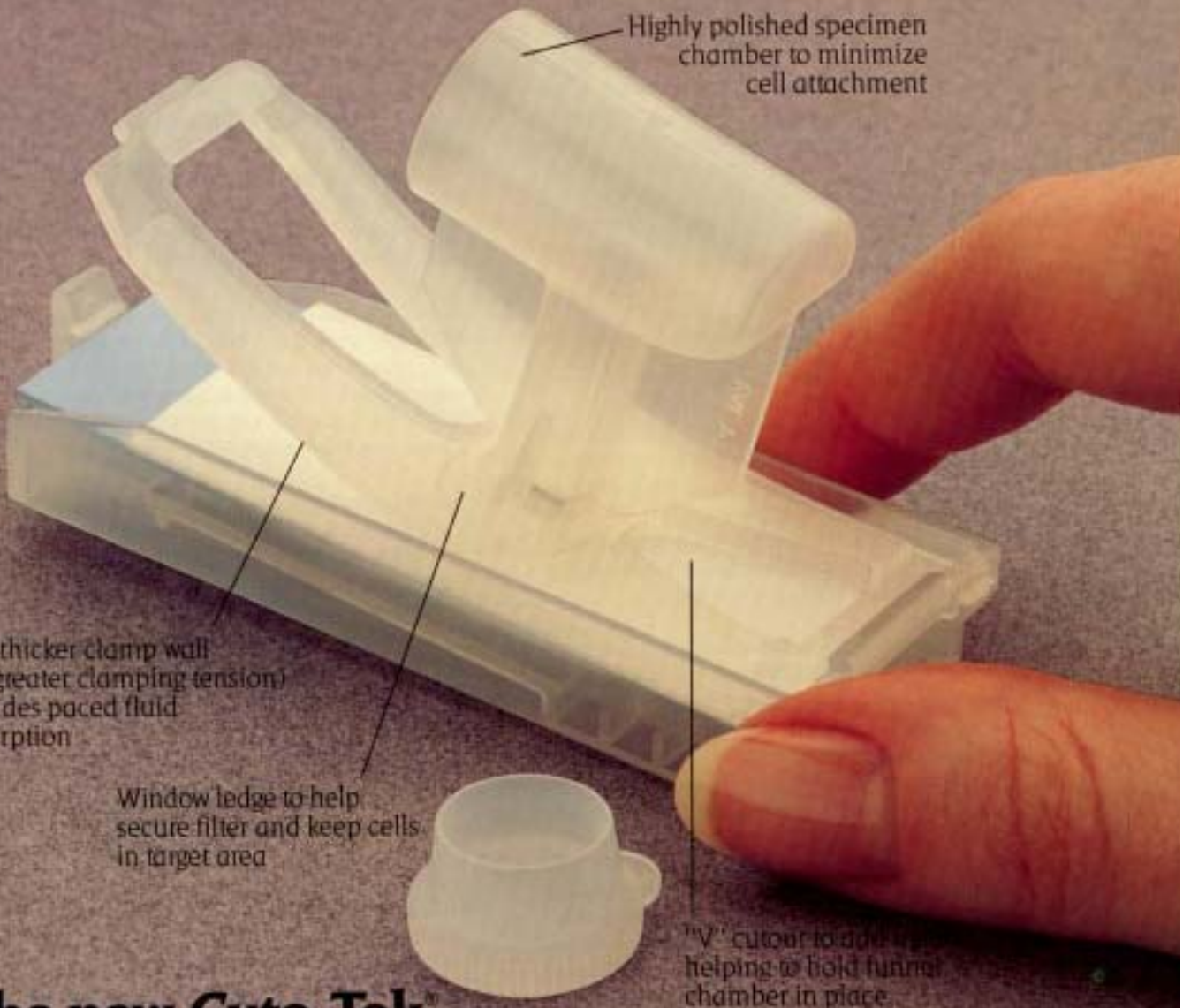

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Histotechnologist of the Decade: Lee G. Luna

Brent Riley
Managing Editor

On September 13, at the 1990 NSH Symposia in San Antonio, Texas, Lee G. Luna, HT (ASCP), D. Lit. (Hon) became the first official recipient of the NSH Histotechnologist of the Decade Award. (The 1980 award was sponsored by the Maryland Society of Histotechnology.) The award recognizes outstanding contributors to the field of histotechnology.

From humble beginnings, Luna became the first Chairman of the Board of NSH, NSH Convention Chairman, and chairman of a histopathology department. He was also the first Histotechnologist to lecture to pathology organizations, serve on the editorial board of a professional journal, write chapters in pathology books, and be invited to teach in another continent.

Luna was born in Cherow, Colorado, and grew up working in onion and beet fields. His mother was Mexican, and he never knew his father. But he survived poverty and an apparent lack of opportunity through sheer determination.

In 1947, Luna left home to join the army, concealing his actual age of 15. "I think they were ready to grab anything back then," he laughed. After 3 months of basic training in Fort Lewis, Washington, Luna was assigned to Madigan General Hospital in Tacoma, Washington. There, he spent 3 months on KP duty. Although it was his first exposure to a hospital environment, it wasn't exactly the opportunity that would open the door to his illustrious career.

Luna was scheduled to be shipped to the Panama Canal Zone, but 2 weeks before he was about to leave, his orders changed. Instead, he was sent to the Armed Forces Institute of Pathology (AFIP) in Washington, D.C. Maybe it was a fluke. Maybe it was just fate. But it was the beginning of a distinguished career in histotechnology.

For the first 6 months, Luna worked as a utility man in the institute's lab. He ran errands. He cleaned cover glasses. He cleaned slides. And he cleaned floors. He did whatever needed to be done, and whatever no one else wanted to do.

"In those days, we used to get cover slips that were very dirty," he recalled. "So every day I'd have to stop and clean many slides and cover slips by submerging them in acid alcohol and wiping them clean."

But even at these apparently menial tasks, Luna was making his mark. Everything he did, he did exceptionally well. "I'm the type of individual," he admits, "who will clean right up to the corner rather than slop around and leave a half-moon around the corner. Everything I did, they seemed to be pleased with."

Soon he was given the responsibility of sharpening knives, a job that was done by hand back then. He sharpened them so well that they began to teach him to do H and Es, then to cut. By then, he was well on his way.

Luna served a little more than 6 years in the army, including a 14-month tour of duty in Korea during the Korean War. While there, he became one of the youngest soldiers to achieve the rank of Sergeant First Class. In Korea, Luna supervised the army's first medical field laboratory, a 79-member unit that provided pathology lab services in the field. The unit had two primary responsibilities: performing autopsies and doing research in an effort to discover the source of Hemorrhagic fever, a disease that produced potentially fatal hemorrhaging in its victims. "We used to go near the front lines to catch animals and see if we could identify the entity that was responsible for Hemorrhagic fever," Luna recalled.

In 1953, Luna returned to Washington, D.C., and continued his work in the AFIP laboratory as a civilian. It was then that his unique contributions to the histotechnology profession began.

"I think my greatest accomplishment was going from a poor, uneducated farm hand to a recognized professional,"

Luna said. "I've lived under the strains of segregation. I literally slept on dirt floors as a kid." Now, Luna is probably the best-known Histotechnologist in the world. He is so well known that he can walk into any histology laboratory and be recognized, at least by name.

Although as a young man Luna had no aspirations to become a Histotechnologist, he seemed to be a natural fit. "I picked up an immediate interest because when I was assigned to the institute, even before I got settled in Washington, D.C., I started going to night school and taking laboratory science courses—basic zoology, bacteriology, physiology." He isn't sure exactly what motivated him to take those courses. Most likely, it was just his desire to perform his job to the best of his ability. He continued to take night classes for 15 years, earning more than enough hours but never taking the time to apply for a degree. He does, however, hold an honorary doctorate of literature from Florida Beacon College.

Luna credits Elson Helwig, his boss and Chairman of the Department of Pathology at the AFIP, with having the biggest impact on his life. He also acknowledges the role his family has played in his success.

The AFIP laboratory was divided into four separate labs: eye, bone, neural, and general. Luna worked in the general laboratory for about 2 years, then he was assigned to the bone laboratory. After 3 years, he was made supervisor of the bone laboratory. Two years later, the AFIP was reorganized into 10 specialty laboratories. Luna became supervisor of the newly created veterinary lab.

In 1961, Luna was promoted again, this time to what is perhaps the highest position ever created for a Histotechnologist. At the age of 30, he was made Chairman of the Department of Histopathology for the AFIP and was responsible for all 10 labs. Luna is believed to be the only individual who has ever been officially designated "chairman" of a Department of Histopathology, a title that implies a higher status than "director" or "supervisor."

In his new position, Luna took personal responsibility for fulfilling the mission of the AFIP. The institute's charter from the Department of Defense states that the mission is to provide consultation, education, and research. Although those tasks were not spelled out in Luna's job description, he dedicated himself to fulfilling those three missions.

In all, Luna devoted 40 years to the AFIP. He retired in 1986 with between 50 and 60 military and civilian employees working under his direction in 11 different laboratories.

Luna's contributions to histotechnology have gone far beyond the AFIP. He was instrumental in forming the National Society for Histotechnology and conducted the first national symposium for the AFIP, which later evolved into the annual symposium/convention of the NSH.

The first AFIP symposium was held in 1965. It was the first national histology meeting ever held. At the time, Luna's director was skeptical. He was afraid that the institute would look bad if nobody registered for the meeting. "After 2 years of my badgering, he finally agreed," Luna recalled. In fact, 150 people registered for the 1-day meeting, and it was a tremendous success.

The symposium continued to grow, and in 1974, Luna turned it over to the NSH. He knew that a ready-made convention would help make the newly formed society a success. "I almost got fired over it," Luna remembered. "When I went to my director and told him what I wanted to do, he said, 'If it goes, you go with it.' I had to get legal assistance to be sure I was not derelict of my duties or in a conflict of interest."

Today, Luna is Vice President of American HistoLabs, Inc., a national histology laboratory he helped form in 1973. The lab was initially formed to perform the histology portion of toxicity studies. The company has since evolved into a multidisciplined laboratory. Luna is still actively involved, serving as Director of Histo-evaluation, Director of the Center for Histotechnology Training, Director of the lab's quality assurance program, editor of *Histo-Logic*, and a consultant.

Luna conceived the original idea of *Histo-Logic* and has served as editor since the publication first appeared in 1971. He also served as editor of the *Journal of Histotechnology* for 2 years.

Awards are certainly not new to Luna. He has received more than 50 in his career. One of the most meaningful is the Meritorious Civilian Service Award from the Department of the Army. He is one of only two nonphysicians who have ever received the award. Luna has also received awards from the American Society of Clinical Pathology, the Veterans Administration, the American Society of Medical Technologists, as well as the NSH, which includes the first J.B. McCormick Award and the first President's Award. He received the Superior Performance Award for 10 straight years while at the AFIP.

Luna has written 2 books and is currently working on a third. He has also written chapters in 3 pathology

textbooks and has published 114 technical papers. His current book will be titled *Histological Procedures and Special Stains: A Practical Guide*. It will contain more than 500 pages, including 50 pages of four-color photographs. The book covers many theories in the area of histologic technique. Much of the subject matter is previously unpublished. "It will include many schematics to explain difficult concepts in simple ways," Luna said.

In the early 1970s, Luna was the first Histotechnologist invited to speak outside North America. His speaking engagements have taken him to Japan, Venezuela, Brazil, Costa Rica, New Zealand, and Australia. While in Venezuela and Brazil, Luna helped establish their national histotechnology societies.

For 27 years, Luna supervised the production of microscopic slides for the annual Anatomic Pathology Slide Seminar of the ASCP, and for a period of 16 years was the Director of the semiannual histotechnology courses of the ASCP. He served 8 years on the HT and HTL committees of the Board of Registry, in addition to grading sessions and business meetings.

Luna lectured and conducted workshops for 13 years at the annual meeting of the American Society of Medical Technologists and was recipient of seven ASMT awards. Other contributions include seven yearly seminars for the International Academy of Pathology and several lectures for the American Academy of Oral Pathology and the American Academy of Dermatology. He is presently a member and honorary member of 13 national and state societies.

"Part of my motivation was the fact that for every channel I pursued, there appeared to be a dozen that opened up," Luna said. "So I always had a lot of things I wanted to look into. I don't ever remember being bored because of the tremendous interest I've had in getting answers to the things I had questions about.

"When I first arrived at the institute, I became very interested in learning anatomy," he continued. "From there, I developed an interest in other sciences. The more I learned about those things, the more I wanted to learn. The same has been true of the practical end of histotechnology. There were so many things I didn't know. I was very inquisitive as to why certain things happened. Whenever a question came to mind, I would develop a research approach to determine why it was happening. This research approach led to the discovery of many artifacts.

"Even today, I find it very exciting," Luna said. "Sometimes I wish my career was just beginning because of all the new technology and all of the potential. I see so many exciting new things, like morphometrics and DNA probes, etc. Histotechnology is an emerging science. Now would be the time to really get going. My 40 years of experience in histotechnology has established a foundation which lends itself to the emerging new technologies of today."

In fact, Luna is still doing plenty. Even though he was diagnosed with cancer in 1988, and has since had two major operations, a stroke, and phlebitis in both legs, he still works full time, lectures, conducts workshops, provides consultations, and spends time with his family. Luna and his wife, Iris, have three grown children and two grandchildren. One of his sons works with him at American HistoLabs, Inc., and assists him in conducting some workshops.

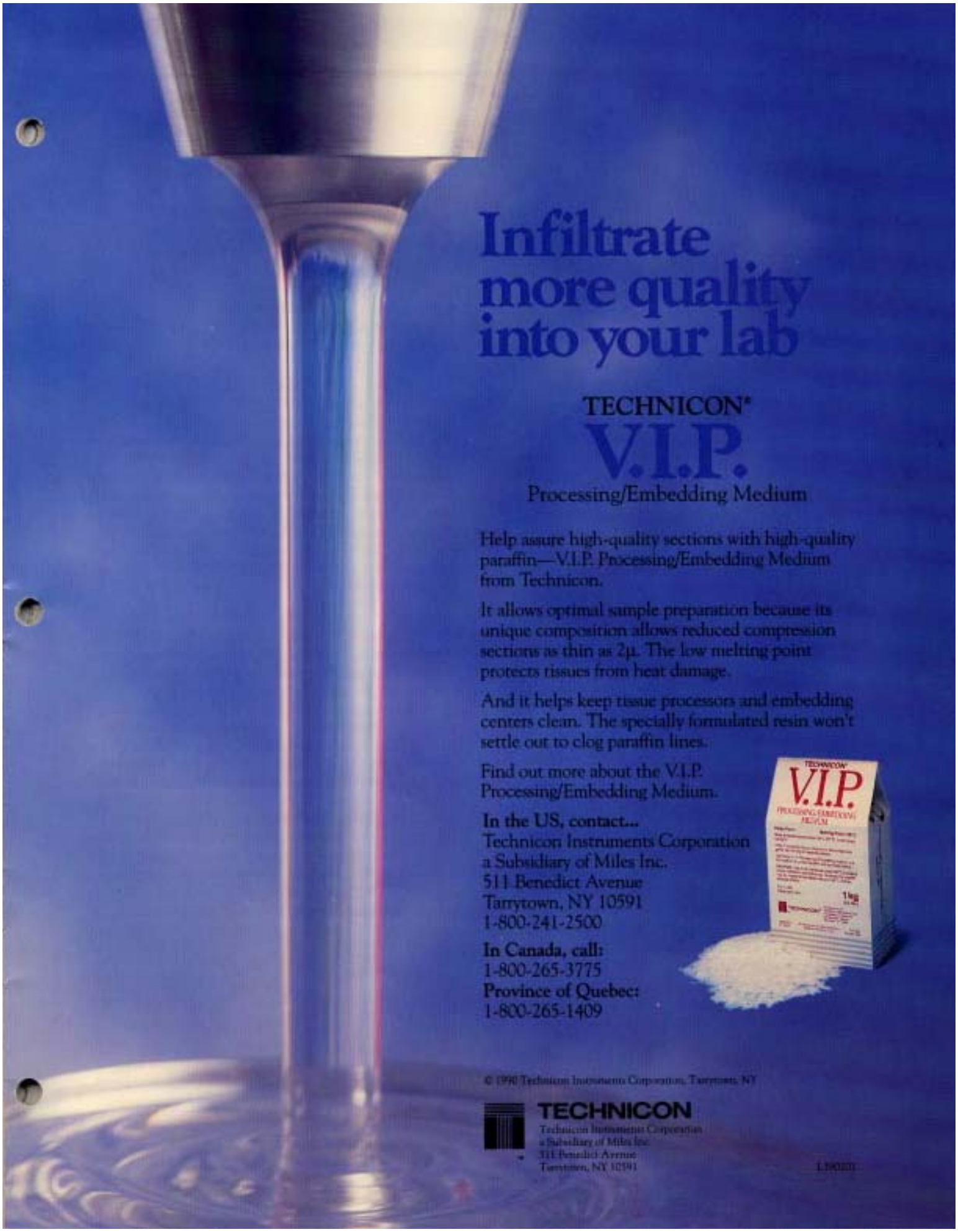
"There are three things that I am very proud of," Luna said. "My family, my Mexican heritage, and that I am an American. I'm one of those people who almost cries when I hear the national anthem. I came from very humble beginnings and lived through many difficult times. But I have no regrets."

With all of his accomplishments—all of his firsts—Luna has always made histotechnology and other Histotechnologists his foremost priority. His first thought when told that this article would be written was to be sure that the purpose of the article would be to help others, not to praise himself. He wanted it to be motivational.

But simply telling Lee Luna's story is helpful to all those who read it. His career—indeed, his entire life—is an inspiration. One can't help but come away with something positive.

No matter what profession he would have chosen, Luna would have excelled. Luna often wonders what he would be doing now if he had stayed in the onion fields instead of joining the army. One thing is certain: he would be the best onion topper that ever lived.

Through an overwhelming desire to bring recognition to the histotechnology profession, and a lifetime of dedication to this accomplishment, we salute Lee G. Luna as the Histotechnologist of the Decade. Focusing on the future, Luna anticipates the 1990s as another "Decade of Progress."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Biotechnic & Histochemistry
Editorial Statement of Content Call for Manuscripts

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

Biotechnic & Histochemistry, formerly *Stain Technology*, originally a journal for reporting investigations concerning biological stains and their practical applications, has, over the years, expanded its scope to include investigative and technical reports concerned with the demonstration of biological entities at morphological, chemical, or physical levels. Thus, *Biotechnic & Histochemistry* presently embraces all aspects of histochemistry and microtechnics. Papers currently are published on topics as diverse as in situ hybridization, cytochemical probes, autoradiography, light and electron microscopy, tissue culture, image analysis, cytogenetics, and automation or computerization of investigative procedures.

Biotechnic & Histochemistry is truly interdisciplinary. It spans the spectrum of the biological sciences, from botany to cell biology to medicine.

Published papers are categorized by length as either regular papers (more than 1½ pages) or notes on technic (usually 1½ pages or less). Review articles concerning topics of current interest are also welcome.

All papers are peer-reviewed by experts in the appropriate field. A list of reviewers appears annually in the November issue.

The editorial staff of *Biotechnic & Histochemistry* is always eager to expand its coverage and invites contributions from all fields of biological research. Instructions to authors appear at the front of every issue of this journal. Send manuscripts to:

Dr. G.S. Nettleton, Editor
Anatomical Sciences and Neurobiology
University of Louisville
Health Sciences Center
Louisville, KY 40292
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The Biological Stain Commission—What It Is and How It Functions

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

The objectives of the Biological Stain Commission are: 1) to ensure uninterrupted supply of dyes used in biological and medical applications, 2) to promote cooperation and dialogue among manufacturers, vendors, and users of dyes for histochemical applications, 3) to ensure the quality of dyes through independent testing, according to appropriately rigorous chemical and performance criteria, 4) to educate users of biological stains about sources of reliable dyes and how they might best be used, and 5) to publish information concerning new or improved uses for biological dyes and related histochemical technics.

These objectives are met by: 1) analysis in the Commission's laboratory of dye content and composition of samples supplied voluntarily by dye manufacturers or vendors; 2) testing performance of dye samples in rigorous standardized procedures known to be discerning tests of the staining quality of the dye; 3) issuing certification labels to be attached to the containers used by companies marketing accepted dyes to assure consumers that these dyes have met the performance criteria of the Biological Stain Commission; 4) conducting and supporting research on biological dyes and histochemical technics requiring them; 5) publishing books concerning biological dyes and histochemical technics, and *Biotechnic & Histochemistry*, a bimonthly journal of microtechnic and histochemistry; and 6) maintaining, through correspondence and annual meetings, active dialogue among scientists, manufacturers, and vendors concerned with biological stains.

The Biological Stain Commission is considering the feasibility of certifying immunocytochemical reagents and has supported investigation by an independent ad hoc committee of the technical, logistical, and financial issues involved in such an enterprise.

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Trustees of the Biological Stain Commission. *Back row, L-R,* LaVelle, Mowry, Horobin, Luna, Wittekind. *Front row, L-R,* Penney, Kasten, Nettleton, Schenk. Not pictured is Longley.

Meeting Dates

May 16-18, 1991
Georgia Society of Histotechnology Annual Symposium
Holiday Inn at Buckhead, Atlanta, Ga

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Control Tissue Network Up and Going

Brent Riley
Managing Editor

The NSH Control Tissue Network is in place and ready to go, according to Rhonda Rogers, Chairman of the NSH Quality Control Committee.

"Labs sometimes have difficulty obtaining certain positive tissue specimens to use as controls," Rogers explained. "Our goal is to let members know that there are other members out there who need certain control tissues and to encourage labs to save control tissues for sharing. The idea of our network is to exchange names between members who have certain tissues and members who need them.

"We didn't want to exchange the tissue ourselves," she explained. "We didn't want tissue coming into the NSH. The idea is simply to put people in touch with people."

The NSH Board of Directors determined that the Control Network would be worthwhile and asked Rogers to spearhead the project. Much of the work involved planning and devising the necessary forms.

Now Rogers must publicize the program. The Network was first announced in the November issue of *NSH in Action*. Copies of the forms were also inserted in the issue, and a number of them have been returned already.

Rogers hopes to be able to respond to most requests within 1 or 2 weeks. "At first, I expect to get more requests for controls than people who have surplus," she said, "but our idea is to get people to start saving their surplus." If a particular type of tissue is not available through the network, requests are kept on file until the tissue does become available.

"The network should be very helpful to small labs that don't have as great a variety of cases as larger labs," Rogers continued. "It provides them with an opportunity to replace control blocks before they're down to their last slide." Although control blocks are available commercially, Rogers knows of no other control block network.

Rogers is attempting to computerize the Network, and hopes to eventually turn the program over to the NSH office so it can be on their computer.

The Network is available only to NSH members. "We want people to know that membership in the NSH provides benefits they otherwise might not have access to," Rogers said.

The Quality Control Committee is currently working on two other significant projects. One is a study of reagent shelf life that will result in published standards. The other project involves establishing guidelines for writing histology laboratory procedure manuals to comply with NCCLS requirements.

Rogers, who is Supervisor of the Histology Lab at University Hospital in Augusta, Georgia, has been Chairman of the NSH Quality Control Committee for three years. She has served on the committee for many years. She has been a Histotechnologist for 23 years and is a charter member of the NSH.

Management Corner
Effective Time Management

Brent Riley
Managing Editor

If there is one drawback to being a Histotechnologist, it's the fact that the work must be done on a timely basis. When the workload is particularly heavy, someone has to work extra hours to get it done. While this scenario is unavoidable at times, there are other times when an increased level of efficiency can make the difference between overtime and regular hours.

There's a popular theory known as the 80-20 rule. It says that you accomplish 80% of what you achieve in 20% of your time. If you fit into this theory—and most of us do—then you're getting only 8 hours of quality working time into a normal 40-hour week.

Believe it or not, the way to improve your productivity is not to work harder but to work smarter. One way to do this is to spend more time preventing problems. That way, you'll spend less time resolving them. Make sure all of your equipment is maintained properly and on a timely basis. Make sure supplies are ordered well in advance. If you run out of a reagent, think how much time is wasted waiting for a new supply.

You can also save time by anticipating problems and planning a course of action to follow when certain problems do arise. Be prepared to move on to another task if the project you're working on is temporarily delayed.

It is also important that you attempt to minimize the number of interruptions. This, of course, is difficult in a Histology Laboratory, but you can at least minimize the impact. If the cause of the interruption can be set aside, get back on task right away.

It is easy to become overly concerned with trivial matters. Don't let it happen. Some parts of your job may be more fun than others, but they might also be less productive or less results-oriented. Set daily goals for yourself and stick with them. Set priorities. Get the important goals accomplished first.

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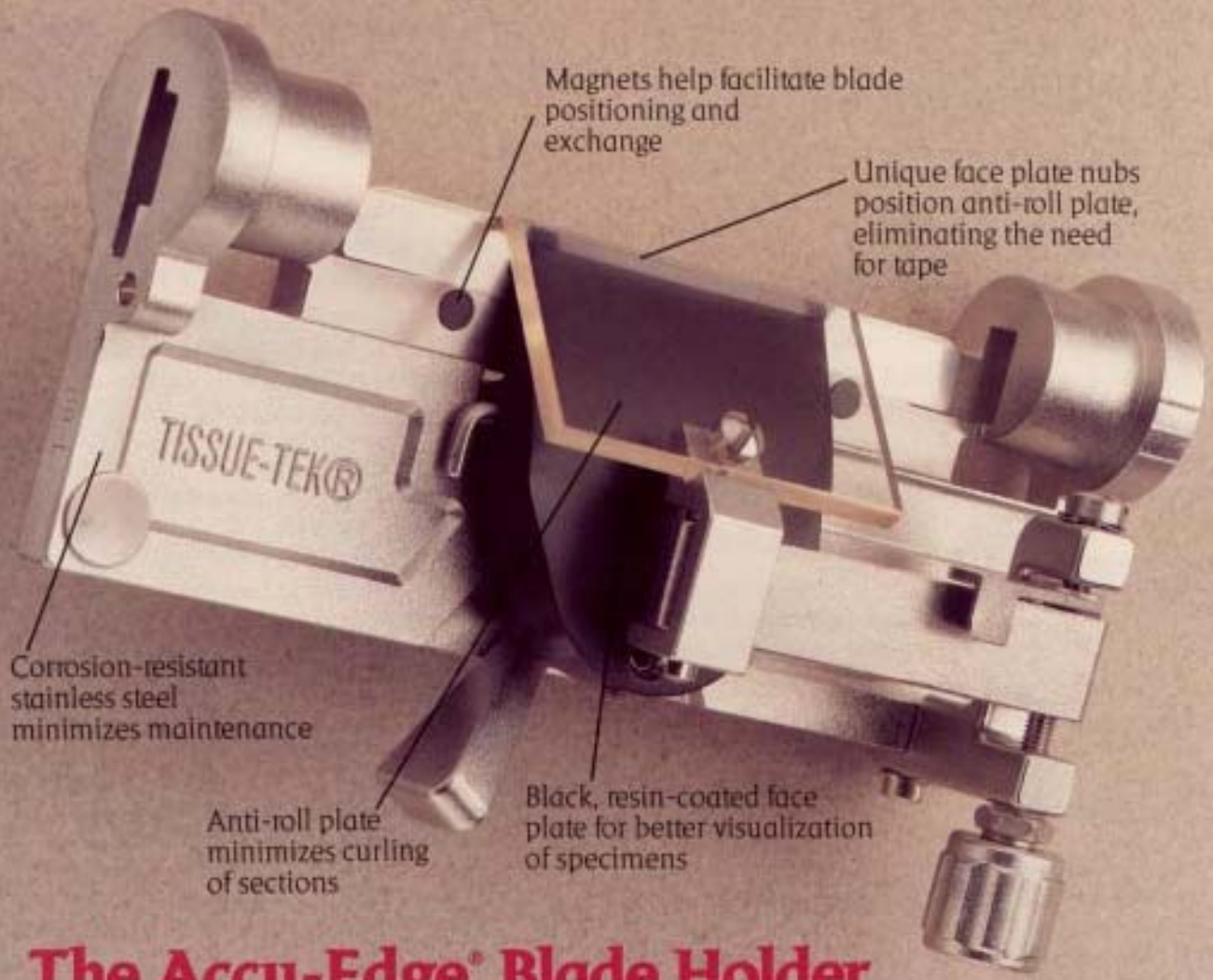

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If you're a Supervisor, don't be afraid to delegate to another person. Chances are, your people are more capable than you think, especially when they are given specific duties. And most people are eager to take on new responsibilities.

If you really want to improve your productivity, do an audit of your time for a week or two. Write down everything you do, the time it takes to do it, and the time of day. Then study it to determine if your priorities are in order. Are you spending too much time on unimportant tasks?

You can also use your time audit to determine what time of day you are most productive. If you are most productive first thing in the morning, schedule your most difficult or important projects then. As the day progresses and your energy level drops, you can handle the less strenuous or less important jobs.

Effective time management is not as difficult as it might seem. And it isn't unusual for someone to find an extra hour or two in each day. If you can use that time to accomplish more on the job, or even to relax more at home, you've made a very worthwhile effort.

Freida Carson
She's Only Just Begun

Brent Riley
Managing Editor

When Freida Carson, HT, PhD, retired, she didn't retire from histotechnology. She just wanted more free time, and more time to devote to the promotion and improvement of histotechnology, a profession she dearly loves.

Carson recently retired after 35 years in the Histopathology Lab at Baylor University Medical Center in Dallas. She had a very distinguished career there. And although she will no longer be working full time, she's not ready to give it all up just yet.

Actually, Carson never intended to become a Histotechnologist in the first place. In fact, she literally became one overnight.

Carson graduated from Texas Woman's University in Denton, Texas, with a degree in chemistry. "I had planned to be a home economics major when I enrolled in college," she said. "When I got into my first chemistry course, I liked it so much that I decided to major in chemistry instead." Chemistry courses were required for a home economics degree.

After graduating, she went to work as a Chemist for a potash company in Carlsbad, New Mexico. After 9 years, she became homesick and moved back to Dallas, her home town. She didn't have a job and wasn't having much luck finding one in chemistry.

Finally, she went to Baylor University Medical Center hoping to find a job in the Chemistry Laboratory. The only opening, however, was in the Histopathology Laboratory.

"I had no experience," Carson recalled. "I didn't know one end of a microscope from the other. But because I had a degree and a background in chemistry and because they were losing their supervisor in 6 months, they hired me as Supervisor of the Histopathology Lab. At that time, there were six people in the lab, and none of them were certified. Everybody had been trained on the job. So I trained very quickly, took my certifying exam the next year, and passed. Then I started training people myself. So I kind of got into it by the back door.

"I always liked analytical chemistry," Carson continued. "I liked the staining aspect and working on fixatives because they related to my chemistry background."

The physical skills required of a Histotechnologist came naturally to Carson. "I've always been involved in crafts and art," she said, "so I really enjoyed and picked up rather quickly the manual skills. I have good hand-eye coordination and manual dexterity.

"The theory is what I really had to work on," she explained. "While I was learning the theory, I was lecturing to med tech students, and I was just staying one jump ahead of them. That was really a big challenge."

When Carson retired, she was Director of the Histopathology Laboratory, supervising 15 employees. For the past 5 years, her responsibilities have centered around specialized areas of histopathology. "I've been doing primarily practical research and development, and direct supervision of immunohistochemistry, enzyme histochemistry, and other specialized areas," she said.

After passing her certification exam, Carson continued to attend night school. "By then, I was so interested in histology," she recalled, "that I applied for graduate school and got my master's degree in anatomy from Baylor University. My thesis was on fixatives and the effect of the buffer vehicle, and the effect on the ultrastructure of blood cells.

"I really didn't plan to go any further with my education, but it was so easy because I could go right out the back door of the hospital and go to class at Baylor's dental college. I took one course a semester and spent every Saturday down there." Soon, Carson had earned a doctorate degree in anatomy.

"I got into some very unusual research when I was doing my dissertation," she said. It involved studying changes in the growth plate of ricketic chickens. "Because it was a dental school, they were very interested in hard tissue," she continued, "but sick chickens are not much fun to work with."

Carson has always been active in the NSH, as well as the Texas State Society. She is a charter member of the NSH and has been Chairman of the Judicial Committee, as well as Speaker of the House of Delegates. She has been the NSH representative to the Board of Registry for the past 7 years. There are 2 years remaining in her current 3-year term. However, Carson has been involved with the Committee for the Board of Registry since the early 1970s. As such, she is not eligible to take the HTL exam.

Educating Histotechnologists has always been a priority with Carson. She used to run an accredited program through Baylor University. "Most of my teaching has been to residents in the pathology program or to students of histotechnology," she explained. She was also actively involved in the Thomas Edison program, outlining courses and helping to write instructional material and exams. She has also conducted workshops at the NSH Symposium/Convention and this year will codirect two workshops at the meeting.

"Had I been younger when I started all this," she said, "I probably would have tried to teach in a medical school because I really do enjoy teaching. That probably was the most rewarding aspect of my job."

Carson recently completed a book that was published by the ASCP Press. The book is titled, *Histotechnology: A Self-Instructional Text*. "It's a collection of my 35 years of teaching material plus some additions," she said. "I really tried to target on-the-job trainees. A lot of them don't have much help in studying or figuring out what to do in their lab. Hopefully, this will give them a good starting place.

"I think histotechnology is becoming more complicated," Carson said. "To understand what you're doing and to have a thorough knowledge of the problem-solving that's involved in some of these techniques, it takes more education. And I think the Technologists are becoming better educated.

"I think the field has improved as to the ability to produce the quality of work that we should be producing," she continued. "The NSH and state Societies have helped a great deal toward this end."

It is with that in mind that Carson intends to stay involved. "I want to stay active professionally for awhile," she said. "I'm not ready to just totally let all that go." Along with her involvement in the NSH, Carson will serve as a consultant for Baylor University in their efforts to computerize the anatomical portion of the lab.

After 35 years, Freida Carson just isn't ready to quit.

Dave Cavanaugh Sings!

Dave Cavanaugh
Iowa State University
Ames, Iowa 50011-1250

The following verses were written for the entertainment portion of the Iowa Histotechnology Society meeting in 1990. We thought some of you might enjoy them.

It Had To Be Blue

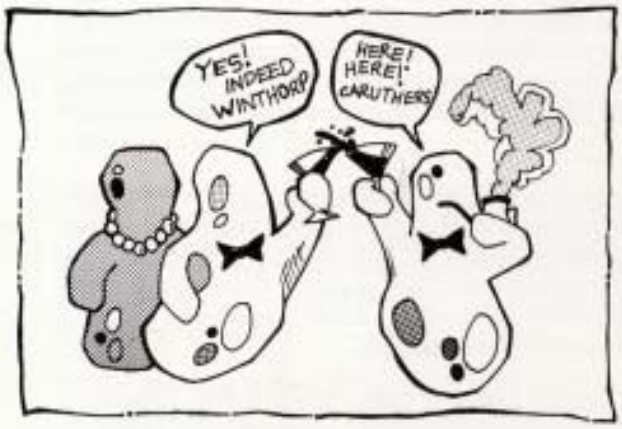
Sung to the tune of "It Had To Be You"

It had to be blue, the collagen too.
I wandered around and finally found,
the dye that would do.
Could make the stain true,
could make the slide blue,
and even be pure to find the cure,
thinking of you.
Some others I tried
could make Dr. _____ cry.
Might never stain in when sections are thin,
so they wouldn't do.
For nothing else gave me a thrill.
With all my errors, you fit the bill.
I'll use the dye, Aniline Blue.
It had to be you.

Sweet For-Ma-Lin

Sung to the tune of "Sweet Georgia Brown"

No fix, made has got a shade
on Sweet For-ma-lin.
Can't stand heat but oh so neat,
has Sweet For-ma-lin.
We all sneeze and want a breeze
for Sweet For-ma-lin.
I'll tell you just why,
You know I don't lie, not much!
It's been said she knocks 'em dead
when she's in the lab.
Since she came, why it's a shame
how she brings 'em down.
Smellers — she can't get are
Smellers — she ain't met.
Aldehydes claimed her, chemists named her,
Sweet For-ma-lin.



Tissue Culture

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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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