Terri Staples Named New Scientific Editor of Histo-Logic

Brent Riley
Managing Editor

Staples succeeds Leonard Noble, who was named scientific editor of Histo-Logic in July of 1992 following the death of Lee Luna. Noble died of a heart attack in Chicago on February 3 of this year.

"I do not compare myself to Lee Luna or Leonard Noble," Staples said. "I hope one day to be the caliber of professional that they were. And I hope that I can do them justice in taking over this responsibility."

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Terri C. Staples, HT(ASCP) BS, HTL wasn't expecting the call. But she had little hesitation when phoned to ask if she would be interested in becoming the new scientific editor of Histo-Logic. In fact, she was delighted.

"I have a hard time saying no to anyone," Staples said, "but this decision was especially easy because the opportunity just seemed right for me."

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No reader should utilize or undertake procedures in Histo-Logic articles unless the reader, by reason of education, training, and experience, has a complete understanding of the chemical and physical properties of all materials to be utilized and the function of each material by which any procedure is accomplished. The procedures discussed in these articles represent the opinions and experiences of the individual authors. Miles Inc. assumes no responsibility or liability in connection with the use of any procedure discussed herein.

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Staples, who has been a histotechnologist for more than 18 years, is supervisor of the histopathology laboratory at Montclair Baptist Medical Center in Birmingham, Alabama. She supervises five full-time and three part-time histotechnologists and helps with the medical center's accredited school of histotechnology. On some evenings, she attends school herself, working on a graduate degree in Health Information Management from the University of Alabama, Birmingham.

Staples' career centers around education and communication. In addition to her responsibilities in the medical center's lab, she is in her third term as Region 3 Director for the National Society for Histotechnology. She is also on three NSH committees, including the Education Committee, the Quality Control Committee, and the Continuing Education Subcommittee.

Staples regularly gives workshops at local, state, regional, and national meetings. She will conduct two workshops at the 1993 NSH Symposium/Convention in Philadelphia. Her workshops are titled, "A Practical Approach to the HT/HTL Registry" and "Routine Special Stains in the Histology Laboratory." And she will be one of four panelists at the closing panel discussion.

Even with her heavy regional and national agenda, Staples finds time to devote to the Alabama Society for Histotechnology. She is the immediate past president and also editor of Histo-Info, her state society's newsletter.

Staples has written or co-authored a number of articles that have been published in various professional journals or newsletters, including Stain Technology, The Journal of Histotechnology, and Histo-Logic. Her first paper, "The Effect of Temperature on Argyrophil Impregnation — Development of a High Temperature Rapid Argyrophil Procedure," was published in Stain Technology. The paper resulted from her efforts to find better ways to stain the pancreatic and adrenal tissues that were used in endocrinology research by William Grizzle, MD at the University of Alabama, Birmingham. At about the same time, she had another article published in Laboratory Medicine. It, too, was about argyrophil impregnation.

Staples won the Golden Forceps award at the 1989 NSH Symposium/Convention for an article titled, "Methods for Staining Campylobacter Pyloris," which was published in September 1989. The Golden Forceps Award is sponsored annually by the Diagnostics Division of Miles Inc., and recognizes the writer of the best scientific article in Histo-Logic.

The article was originally written for her state society's newsletter. When Lee G. Luna saw it, he asked Staples to expand the article and submit it to Histo-Logic. It was Luna's encouragement that made her fully realize the importance of communication within the histotechnology profession.

"I think where people make the biggest mistake is thinking that everyone already knows the same thing they know," Staples explained. "I found that many of the things I know and have experienced that I didn't think were important were very helpful to someone else. But it took encouragement to convince me that other people needed to know these things.

"Now I'm very interested in sharing information with other histotechnologists, and I think Histo-Logic is a great place to do it. Information that may seem trivial in one lab may make a significant difference in another lab. To me, that's what Histo-Logic is all about, and I'm hoping to be able to solicit articles about the daily experiences of a typical histotechnologist — the tricks of the trade.

It might seem that histotechnology consumes every hour of every day for Staples. But she still finds time with her family, which includes her first grandson, who is now 9 months old. Occasionally, she even sings with a rock and roll band.

One important qualification Staples brings to Histo-Logic is her computer literacy. She is fascinated by the contribution computers can make in the laboratory, and she will not hesitate to take full advantage of computer technology in preparing articles for Histo-Logic.

Staples doesn't anticipate any drastic changes to Histo-Logic. "I feel that Histo-Logic is an excellent publication," she said. "I want to maintain its professional status and continue to send it around the world. I want to continue to demonstrate to readers just how important our profession is."

Staples would also like to see more information directed to the supervisor. She recognizes that histotechnology supervisors have worked their way through the ranks. They have to learn supervisory techniques on their own. "I'd like to include more articles in Histo-Logic that help these supervisors better understand their roles and management responsibilities," she said.

Staples sees a bright future for Histo-Logic. "I think we can continue to accomplish great things with Histo-Logic simply by encouraging people to write and submit articles," she said.
articles," she explained. "I plan to provide that encouragement by talking with people and, if necessary, helping them write. A lot of people think they don’t know how to write, or are discouraged because they can’t type or use a word processor. But that isn’t really that important. The important thing is to get the idea down on paper, and we can take it from there."

Perhaps the one thing that will help Staples the most to succeed as editor of Histo-Logic is her enthusiasm. She has a tremendous drive to reach her goals and the goals of the publication. She also has the positive attitude and persuasiveness to convince other histotechnologists to get more involved in Histo-Logic.

With that kind of attitude, it isn’t surprising that Staples welcomes this unexpected opportunity.

---

**Alcian Blue-Basic Fuchsin Stain for Acid Tumor Mucin**

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**Introduction**  
A simple thirteen-minute procedure is described for demonstration of adenocarcinoma tumor mucin. This stain may have numerous other uses outside the clinical lab. Use of dyestuffs certified by the Biological Stain Commission is also recommended.

**Materials and Methods**

1% Alcian Blue 8GX  
10% Ammonium-Alcohol  
Concentrated NH₄-OH  
Reagent alcohol SDA-3  
90.0 mL  
10.0 mL  
Acetone and deionized or distilled water are also needed.

**Microtomy**  
Cut paraffin sections at 6 μm. Use a positive control.

**Procedure**

1. Deparaffinize and hydrate to water as usual.
2. Stain in 1% alcian blue for 10 minutes.
3. Rinse off excess stain in running tap water.
4. Place in ammonium alcohol for 1 minute.
5. Rinse in running tap water for 1 minute.
6. Rinse in deionized water.
7. Stain in 0.1% pararosaniline hydrochloride for 1 minute.
8. Rinse off excess stain in deionized water.
9. Rinse six times in two changes of acetone.
10. Clear in three changes of solvent and coverslip with appropriate mounting medium.

**Results**

Acid tumor mucin ......................... bright blue  
Nuclei ................................. red

**Note**  
This is an excellent stain for poorly differentiated adenocarcinomas of the lung and bowel. There are instances, however, where it will be necessary to do an alcian blue/PAS technique to stain both neutral and acidic mucin.

Alcian blue is a very large molecule and I feel that it is prone to premature oxidation in solution. Stock solutions in my hands have broken down in as little as one month. It would, therefore, make sense to prepare a small amount weekly to insure optimal reacting stain.

Use of capillary action staining technique is recommended to conserve reagents for this procedure.

**References**

Verhoeff's Elastic Stain — Picric Acid Differentiation

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In recent years, the quality of picric acid as a component of certain histological stains appears to be less than satisfactory. The bright yellow staining of RBCs and muscle with the classical Van Gieson as a counterstain for Verhoeff's Elastic staining method frequently shows up at best as an anaemic yellow-green or brownish color.

In an effort to overcome this, it has been found that the use of picric acid as a differentiating agent, instead of the conventional ferric chloride, is effective in lessening the green-brown overtones, thereby achieving a more distinctive contrast when reinforced by the Van Gieson counterstain.

Solutions

5% Alcoholic Hematoxylin
Hematoxylin: 5.0 g
Absolute alcohol: 100.0 mL

10% Ferric Chloride
Ferric chloride: 10.0 g
Distilled water: 100.0 mL

Iodine Solution
Iodine: 4.0 g
Potassium iodide: 8.0 g
Distilled water: 200.0 mL

Verhoeff's Elastic Stain
5% Alcoholic hematoxylin: 20.0 mL
10% Ferric chloride: 8.0 mL
Iodine solution: 8.0 mL

Van Gieson Stain
Saturated aqueous picric acid: 100.0 mL
1% Acid fuchsin: 10.0 mL
Concentrated HCl: 0.25 mL

Saturated Aqueous Picric Acid Solution
95% Alcohol
5% Sodium Thiosulfate
1% Potassium Permanganate
1% Oxalic Acid

Method
1. Hydrate sections. (It is not necessary to remove mercury pigment as that is dissolved by the iodine in subsequent staining.)
2. Stain in Verhoeff's Elastic stain for 15 to 20 minutes.
3. Wash slides in running tap water for 1 minute.
4. Place slides in 5% sodium thiosulfate or 95% alcohol for 3 to 5 minutes.
5. Wash slides in running tap water for 3 minutes.
6. Differentiate in saturated aqueous picric acid for 1 minute.
7. Rinse quickly in fresh change of saturated aqueous picric acid.
8. Place slides, without rinsing, into the Van Gieson stain for 3 to 5 minutes.
9. Quickly rinse in water to get rid of excess acid fuchsin.
10. Rinse in 95% alcohol to remove any residual iodine.
11. Dehydrate in two changes of absolute alcohol after which a few drops of saturated alcoholic picric acid are added. (Prolonged exposure to these alcohols causes the picric acid stain to wash out.
12. Pass through xylene to clear.
13. Mount slides with appropriate mounting medium.

Results
Elastic fibers: black
Nuclei: black
Collagen: red
Muscle and RBCs: yellow

Pretreatment with 1% potassium permanganate for 5 minutes followed by 1% oxalic acid improves sharpness and intensity of staining.

Note
The use of picric acid instead of ferric chloride produces significantly better differentiation, hence giving a more picturesque appearance. The substitution is simple, effective, and inexpensive way of improving this stain.

References
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Recovering Antigen-Antibody Reactions from Long-Term Stored Tissue Sections Mounted with a New Mounting Medium

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A new mounting medium for preserving alcohol-soluble chromogen reactions in immunostained tissue sections has been developed in our laboratories. One of the several advantages of this medium is the ability to keep the intensity of the chromogen reaction for a significantly longer period of time.\(^1\) The purpose of this study is to see whether this medium can also preserve the tissue structure and contents so that other antigen-antibody reactions may be applied at a later time.

Materials and Methods
Ten 3-μm sections from rat submandibular glands that had been previously immunostained and mounted with our medium and stored at room temperature or 4°C for 18 months were used.

1. Anti-SMG-C and anti-B1 antibodies\(^2\)
2. ABC Immunostains Kit — Biomeda Corporation
3. Amino-ethyl carbazole (AEC) — Biomeda Corporation
4. Mounting medium\(^1\)

The slides containing the tissue sections were immersed in 60 to 65°C water to remove the cover-glass and immunostained as previously described.\(^3\)

Results
Ten out of ten sections show very strong positive antigen-antibody reactions without any obvious background. Figures 1-2 and 3-4 show two sets of adjacent sections that were immunostained and stored at 4°C for 18 months. Note that the morphology and location of the positive cells of the present preparation are the same as the sections that had been previously stained and stored for 18 months.

Discussion
In addition to the advantages demonstrated before,\(^1\) our mounting medium is able to preserve the tissue structure and contents for a significantly longer period of time. This allows investigators to easily retest their old preparations for other purposes.

References

Fig 1. — Tissue 1, stained with anti-C and reacted with AEC on July 13, 1990.

Fig 2. — Adjacent section of tissue 1, stained with anti-B1 and reacted with a black chromogen on July 13, 1990, and re-stained with anti-C and reacted with AEC on January 26, 1992.
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In its 20-year history, the National Society for Histotechnology Symposium/Convention has grown from 9 to 94 workshops, from 24 to more than 65 exhibitors and from 300 to over 1000 attendees. This year, the big event will be held in Philadelphia, at the new Convention Center, from October 2 to 8. To obtain more information, or to register for the meeting, contact:

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The Neuropathology of Schizophrenia

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Abstract
Brains of schizophrenics show evidence for specific pathology by imaging studies with CAT and PET scanners, planimetric studies of postmortem tissues and cytoarchitectonic studies. The brains of schizophrenics exhibit signs of atrophy. Planimetric studies localize the atrophy in temporal lobe structures. Cytoarchitectonic studies point to cell loss and disarray in the hippocampus, parahippocampal gyrus, and insular cortex. The atrophy and/or aplasia may be developmental in origin. Many authors posit the structural change was present before the onset of symptoms and remains constant. Structural changes in the brains of schizophrenics are well documented, however the etiology of this disease remains to be elucidated.

Introduction
Plum (1972) referred to schizophrenia as the "graveyard of neuropathologists." Schizophrenia is a severe, destructive mental illness in which the patient's experiences include hearing strange voices, bizarre delusions, and the paralysis of will. The diagnostic features of schizophrenia are listed in Table 1. Kraepelin's concept of dementia praecox (from his treatise on schizophrenia) describes an organic and progressive disease. The current concept of this illness still implicates an underlying organic pathology. Numerous neuropathological investigations of schizophrenia have sought its physical basis. Nissl and Alzheimer made early advances in the field of organic dementias, which gave impetus to numerous studies on the brain in schizophrenia. These yielded little reproducible data, since little was known at the time about artifacts created during tissue processing, or the changes occurring in the agonal state. Thus, many of the early findings were due to age, artifacts, or intercurrent disease. By 1934, Wertham and Wertham, upon reinvestigation of earlier findings, reported no evidence for organic neuropathology in schizophrenia. Claims of pathologic changes have been renewed. More than 250 articles on the neuropathology of schizophrenia had been published by 1952, yet none could agree on the specificity of morphological changes and the whole area again fell into disrepute.

Atrophy
In the 1930s, a number of studies reported cortical atrophy and abnormalities of the meninges in psychotics undergoing neurosurgery. The atrophy was supported by pneumoencephalographic studies. Lemke, in 1935, compared 1000 schizophrenics and 100 age-matched neurologic and psychiatric controls and found significant atrophic changes in 85% of schizophrenics as opposed to 12% of controls. Increased ventricular size was related to the degree of atrophic changes and severity of clinical course. Subsequent negative studies on the specific neuropathology of schizophrenia caused these findings to be ignored until much later.

Evidence for atrophy continued to build. Studies of brain weight in schizophrenics versus controls yielded small but significant differences. By the early seventies, neuropathologists had yet to demonstrate the specific neuropathology of schizophrenia. By the mid seventies, serial studies of CAT scan changes, confirmed earlier pneumoencephalographic data suggesting cerebral atrophy. Schizophrenic planimetric studies lent further support to evidence of atrophic changes in the brains of schizophrenics. The atrophy was less severe in degree than that found in brains of patients with Alzheimer's Disease, however the atrophy was particularly prominent in medial temporal lobe structures (hippocampus and associated cortex). The relationship between the temporal lobe (limbic system) and schizophrenia had long been suspected.

A prospective study showed schizophrenic patients with larger ventricles to have more frequent hospitalizations and a poorer outcome, including an increased tendency to develop the movement disorders associated
with neuroleptic treatment. Atrophy also correlates with peripheral neuro-chemical results. Patients with ventricular enlargement had lower CSF levels of 5HIAA, HVA, dopamine, and cyclic adenosine monophosphate (AMP), and higher whole blood serotonin levels.

CAT studies also confirmed that the atrophy was present at the first episode and, therefore, was not merely a feature of the end stage of the disease, nor an artifact.

The changes in the temporal lobe have been suggested as being lateralized to the left. This finding was consistent with the left-side lateralization of the temporal lobe epilepsy model of schizophrenia-like psychosis.

**Microscopic Studies**
No pathognomonic brain lesion has as yet been demonstrated for schizophrenia.

**Temporal Lobe**
Cytoarchitectonic studies indicated that cell loss and disarray were present in the hippocampus, parahippocampal gyrus, and ventral insular cortex in schizophrenics. Kovelman and Scheibel's studies suggested

### Table 1
**Schizophrenia: The Disease**

Presence of characteristic psychotic symptoms in the active phase: Either a, b, or c for at least 1 week (unless successfully treated):

- a. Any two of the following:
  - delusions
  - incoherence
  - hallucinations
  - catatonic behavior
  - flat or inappropriate affect

- b. Bizarre delusions, such as thought broadcasting or being controlled by a dead person.

- c. Prominent hallucinations of a voice with content having no apparent relation to elation or to depression, or a voice keeping up a running commentary on the person's behavior or thoughts, or two voices conversing with each other.

- d. During the course of the disturbance, the patient's functions in areas such as work, social relations, and self-care are markedly reduced.
a defect in neuronal migration, with cytoarchitectural disruption maximal in the middle and anterior portions of the hippocampus and at the interface with the hippocampal formation, and hippocampal segments CA1, CA3, and CA4 were decreased in the left hemisphere. Pyramidal cell loss was more noticeable in paranoid than catatonic patients. Pyramidal cell numbers were unaltered elsewhere. Hippocampal cell numbers were unaltered elsewhere and were not accompanied by gliosis. Jakob and Beckman found cytoarchitectonic changes in the ventral insula and rostral entorhinal cortex in the parahippocampal gyrus. Findings were also pronounced on the left. No correlations between the degree of abnormality and durations of hospitalization were present.

Other Cortical Regions
A morphometric study of the motor, anterior cingulate, and prefrontal cortex in schizophrenia showed a trend to fewer neurons in some cortical layers. As in the temporal lobe studies, the lower neuronal counts were also associated with lower glial cell counts. Thus the neural-glial ratio was the same in normal and schizophrenic cortices. This argued against a neuronal degeneration theory for the origin of schizophrenia. Previous reports of cell loss in the striatum, thalamus, and substantia innominata have not been confirmed by recent morphometric studies. Similarly, reports of lesions in the nucleus accumbens and the substantia perforata have not been substantiated.

In summary, neuroradiological and neuropathological work completed since 1975 has been broadly consistent. CAT scan studies demonstrated the presence of ventricular enlargement in the brains of schizophrenics. The degree of atrophy is positively correlated with clinical deterioration and worsening of prognosis as well as with a number of peripheral neurochemical indices. Planimetric studies on postmortem material also demonstrate atrophy and localize the atrophy largely to temporal lobe structures.

Cytoarchitectonic studies demonstrate cell loss and disarray in the hippocampus, parahippocampal gyrus, and ventral insular cortex of schizophrenics. The significance of these changes is yet to be established.

Following nerve cell injury, glial cells proliferate to form glial scars. Therefore, gliosis marks the site of some patholgy of the brain. Studies have found gliosis in the diencephalon, in the cranial nerve nuclei, reticular formation, periaqueductual septal and hypothalamic grey matter. These findings were consistent.

Immunoreactivity attributable to glial fibrillary acidic protein (GFAP) has been quantified in two series of reports. No increase was found, however GFAP, a structural protein of astrocytes, was increased in areas of gliosis. Similarly, a study of monoamine oxidase (MAO-B), also increased in gliosis, also proved negative in schizophrenic brains, although there have been scattered reports of increased MAO-B in the temporal lobes of schizophrenics.

However, gliosis only accompanies neuronal loss in the developed brain and is not seen in neonates and fetuses. The concept of a developmental lesion in schizophrenia arose. At present, the model by Weinberger is of a developmental lesion that becomes manifest only as the patient faces the stresses of maturity.

Conclusion
The presence of structural changes in the brains of schizophrenics has been firmly established. Recent work suggests that the medial temporal lobe is preferentially affected. Atrophies and aplasias may be developmental in origin. These findings may herald new interest among pathologists in the study of postmortem material from schizophrenic patients and portend a better understanding of the neuroanatomical basis of schizophrenia.

Fig. 2 — Top View of limbic system
1. Parahippocampal gyrus
2. Dorsal gyrus
3. Hippocampus
4. Stria terminalis
5. Olfactory bulb
6. Medial longitudinal stria
7. Indusium griseum covering superior surface of body of corpus callosum (continued on page 32)
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Chromogen overstaining of immunohistochemical preparations may be induced by different factors, such as excess concentration of antibodies or too much incubation time in the chromogen, which can make the observation of the preparations difficult or impossible. This situation has occurred in our lab and may be encountered in other labs, so we have developed a simple method to rescue these overstained preparations within 5 to 10 minutes.

**Materials and Methods**
1. Acetone — Fisher Scientific
2. AEC — Biomeda Corporation
3. Slides overstained with AEC chromogen

The overstained slides were put in 100% acetone until the AEC was completely removed. They were washed in water for 5 minutes, rinsed in distilled water, and incubated with AEC that was diluted to half strength of the manufacturer's recommendation, or they were incubated with AEC at full strength for a shorter period of time. This procedure could be repeated several times until satisfactory results were obtained.

**Results**
All of the slides that had the AEC removed were able to be restained with another AEC incubation without repeating the incubation with the primary and secondary antibodies.

**Discussion**
Although alcohol can remove the AEC chromogen, we have found that in order to re-stain the preparation it is necessary to re-incubate with the primary and secondary antibodies. Our method allows us to remove the AEC and re-stain the preparation without re-incubation of the primary and secondary antibodies.

**References**

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**A Simple Method to Rescue Immunohistochemical Preparations Overstained with the AEC Chromogen**

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Introducing a New Miles Member
Sue Stafford

Brent Riley
Managing Editor

We are pleased to announce that Sue Stafford has joined the Marketing Department at Miles Inc. In her new position as a US Marketing Manager, Sue will be responsible for providing histology/cytology products and services that better address customer needs. Sue will also be directly involved in the production of Histo-Logic, and readers should expect to see her contributions in future issues.

Sue brings to Miles extensive experience in both the technical discipline of histotechnology, as well as marketing and business management. Sue’s career as a histology professional began in 1972 with enrollment in a Histology Training Program, achieving HT (ASCP) certification. In Sue’s 14-years as a histotechnologist, she supervised histology departments in Illinois and California, completed her HTL (ASCP) certification, and held numerous positions in state and national organizations.

Sue jumped from the bench to the business side of histotechnology in 1987, when she accepted a position as Product Specialist with Histoline, Inc. She continued her business career, joining Instrumentation Laboratory in 1988, where she supported the Histology product line as Technical Manager, Product Manager, and Sales Specialist.

Please join us in welcoming Sue Stafford to Miles and to Histo-Logic.
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What Is Really Killing Health-care Workers?

The public is scared to death of AIDS. Health-care workers, however, have another disease to worry about. The Centers for Disease Control estimate that to date, 400 health-care workers have died from contracting AIDS in the work environment. But each and every year, 9,500 health-care workers become infected with hepatitis B, and 200 die from this disease.

"Depending on the area that you serve," Steven Nuenberger, MD, said, "your risk could be very high or low." But according to Dr. Nuenberger, the point is there are many cases of highly transmissible hepatitis B.

Unlike the AIDS virus, hepatitis B does not automatically kill its victims. That's the good news. The bad news is that the virus can cause years of illness and shortened lives—if it doesn't kill its victims first. Patients who contract the hepatitis B virus have a 2 percent chance of dying. Between 7 and 18 percent of those who contract the virus get chronic hepatitis.

"Even though there are modes of treatment now that may be effective," said Dr. Nuenberger, "it's still a devastating illness that leads to liver cancer in a large percentage of people, and sclerosis in an even larger percent."

Chronic hepatitis can affect more than the worker who contracts it, he noted. Children who contract the disease have a great risk of getting active hepatitis. "If you take that virus home to your daughter or son," he said, "you're signing a death sentence for that kid." A child with the disease won't die right away, he said, but he or she may face death as early as age 50.

The bad news about hepatitis B is balanced by the good news: Hepatitis B is preventable through vaccination. Dr. Nuenberger said that in the state of Illinois, the hepatitis B vaccination costs just over $100. And for those considering the vaccination, there's even better news: vaccines, such as Recombivax HB and others, are extremely safe.

"If you can justify a measles vaccination," Dr. Nuenberger said, "if you can justify a polio vaccination, if you can justify any vaccination you've ever had, you've got no reason to avoid getting the hepatitis B vaccination."
Histopathologic Techniques and Color Atlas of Special Stains and Tissue Artifacts

by Lee G. Luna, D. Lt. (Hon), HT (ASCP)

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