Abstract

Many of the time-honored methods for histopathological analysis of tissue sections have been superseded by new histological techniques, immunohistochemistry, or other diagnostic tests. Some of the dyes that were used for generations are no longer available, and chemicals used in some older methods are now considered hazardous. Additionally, the histotechnologists and pathologists familiar with these techniques are now retiring and leaving the field, resulting in a loss of experience. To provide a sense of the rich legacy of histopathological analysis, this article reviews the development of special stains in histopathology, describes staining techniques, and discusses the wide range of applications. Numerous staining techniques mentioned in this article were originally published many years ago, some in obscure journals in foreign countries, and are not readily available to practicing histotechnologists. To encourage further study, references in English are given, many to popular histotechnology texts found in most histopathology laboratories or biomedical libraries. (The J Histotechnol 32(1):9–19, 2009)

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Introduction

The use of histological techniques to aid in the diagnosis of diseased tissues is a relatively new application. In the late nineteenth century, hospital-based surgeons served as their own pathologists, examining tissues removed from patients during operations and determining the type of tumor, in addition to whether it was malignant or benign. The pathologists of that period meanwhile, were more interested in performing autopsies, clinical pathology, and microbiology, and they considered surgical pathology as being out of their field.

Starting in the late nineteenth century, however, the larger medical centers hired full-time physicians to handle surgical pathology. The Bellevue Hospital in New York hired William Henry Welch in 1878. Welch later worked at Johns Hopkins in Baltimore (1). The Mayo Clinic hired their first surgical pathologist in 1904 (2,3), and the College of Physician’s and Surgeon’s, also in New York, hired their first surgical pathologist in 1905 (4) (Figure 1). These early pathologists developed the intraoperative frozen section technique and adapted special stains techniques for use in histopathology. Paraffin infiltration was developed at approximately the same time (5).

Staining Techniques in History

The techniques used by early surgeons and pathologists were derived from techniques developed by seventeenth-century amateur scientists such as Leeuwenhoek. These forerunners of modern histologists used naturally occurring substances such as madder, saffron, and indigo to color tissues, which they then studied under rudimentary microscopes (6, p. 67). These researchers used microanatomy, the relationship of one group of cells to another, to elucidate the normal structure of plants and animals. Later techniques were devised to study cellular details. Early microscopists used a variety of laboratory chemicals to preserve the tissues in a life-like state and to prepare them for later staining.

Joseph Von Gerlach is thought by some to have founded microscopical staining when, in 1858, he successfully stained cerebellum with ammoniacal carmine. These early researchers used readily available laboratory chemicals such as potassium dichromate, mercuric chloride, and alcohol to harden the tissues so thin slices could be prepared for microscopical examination. Some of the fixation and staining methods devised were ingenious. Soon, multicolored staining methods were in use. Special stains still play an important role in surgical pathology and some at this time are irreplaceable, such as the trichrome methods for renal and liver biopsies and silver nitrate methods for organisms. The mid-1800s also brought about the development of improved microscopes with corrected spherical and chromatic aberration, most successfully in Germany by Ernst Abbe working at the Zeiss Factory. The final step that accelerated the development of histopathology staining was the creation of theiline dye industry in 1856, which created an avalanche of new dyes, some with applications in histopathology.

As knowledge about the normal structure of human tissue became available, physicians and others used that...
knowledge and the newly developing histology techniques to study diseased tissues. Much was already known about the effect of disease on tissues. Malignant and nonmalignant tumors and metastases were already understood and bacteria had been accepted as a cause of disease in the first quarter of the nineteenth century (3).

**Important Dyes Used in Histopathology Staining**

Some of the dyes, especially the naturally occurring ones used in histopathology, are extremely versatile, and researchers have devised numerous ways to use them in histology to stain tissues. Some examples are listed in the sections to follow. Many of the staining techniques use methods first used in the textile industry where dyes have their greatest use.

**Carmine**

Carmine is a commonly used dye in histology and is quite versatile. Early botanists such as John Hill, who published his work in 1770, also used carmine in their studies (6, p. 67; 7, p. 44). Carmine is most commonly used in an ammoniacal solution to study microscopic tissue structures. It is still used today by microscopists in different fields. The colored compound is obtained by grinding the dried bodies of the female *Coccus cacti* insect found in Mexico. From the insect is obtained cochineal, which contains carminic acid. Further treatment with alum and calcium salts results in carmine. (C. I. 75470) (8). The dye was previously available from a related insect in southwest Asia, but it was with the Spanish conquest of Mexico that carmine became popular. Rudolph Virchow (1821–1902), the “Father of Pathology,” used carmine in his microscopy studies, but as was common in the practice of his day, gave scant technical information in his publications (9). In another use, boiling carmine with acetic acid creates aceto-carmine, which is still used for staining plant chromosomes (10, p. 397). In 1896 Mayer (11, p. 168) devised the mucicarmine stain by the addition of an aluminum mordant. The method was modified by Southgate in 1927 (11, p. 169). These stains were popular before alcian blue became available. The mucicarmine stain is used to identify mucin production in adenocarcinomas, in signet ring carcinomas, and in some mucin-containing fungi such as *Cryptococcus neoformans*. Carmine combined with a potassium salt will stain glycogen, as in the Best’s carmine method published in 1906 (11, p. 167). In the Orth lithium carmine method modified by Mallory in 1938, the dye is used in conjunction with lithium carbonate; following staining, the sections are treated with acid alcohol to fix the dye in the nuclei (12, p. 111). Grenacher’s borax carmine method and that of Orth are useful for counterstaining fat stains in frozen sections (12, p. 290). Carmine stains of frozen sections are generally resistant to bleeding.

**Hematoxylin and Hematein**

Hematoxylin is another naturally occurring substance with useful properties and a long history in histopathology. It was reportedly first used by Wilhelm von Waldeyer in 1863. Hematoxylin is obtained from the logwood tree, *Haematoxyylon campechianum*, found mainly in Central America. Hematoxylin itself is a weak dye, and the different staining solutions are based on its oxidized form, hematein. Hematein, when combined with a mordant, an oxidizer, and sometimes a differentiating agent, can be used to identify a wide variety of cellular components (13). Solutions prepared from hematein are usually called “hematoxylin.”

Because of this versatility, there are many hematoxylin methods. Used daily in histopathology laboratories today are alum-based hematoxylin nuclear stains such as those of
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Ehrlich (1886) and Mayer (1903) (11, p. 142). Harris’s formula (1900) (11, p. 142) results in a shorter staining time, making it suitable for automatic stainers. Weigert’s iron hematoxylin introduced in 1904 (11, p. 146) has wide applications today as a nuclear stain in trichrome methods. This progressive nuclear stain is resistant to subsequently applied acidic solutions, making it suitable for procedures with multiple steps.

The Verhoeff elastic method published in 1908 (11, p. 196) uses ferric chloride as the oxidant and mordant, and then ferric chloride again to differentiate the resulting blue–black nuclei and elastic fibers. Elastic stains are helpful in delineating blood vessel wall structures in diseases such as temporal arteritis and to visualize atrophy in emphysema in the lung. Mayer’s muchhematein method was first used in 1896 and is used much as the mucicarmine stain is (12, p. 213). The original Weil method for myelin published in 1928 uses hematoxylin, with iron alum as the mordant. Differentiation follows, first in iron alum, and then in a borax–potassium ferricyanide solution (11, p. 261; 14). The phosphotungstic acid hematoxylin method (PTAH) (1897) (11, p. 193) gives a polychrome effect with cytoplasm stained shades of blue and connective tissue stained yellow to brick red. This stain still has occasional applications in histopathology. There are very many other hematoxylin methods devoted to both nuclear and cytoplasmic staining.

Silver Nitrate

Silver nitrate has had a long use in histopathology and is still used in several different methods. Silver nitrate and other metals used in histotechnology are not dyes but are grouped along with dyes because they also are used to visualize tissue structure. The early microscopists used silver nitrate to study tissue structure, either by rubbing solid silver nitrate into tissue and then studying the tissue microscopically, or by immersing tissues in a silver solution and then reducing the silver and studying the tissue structure in that fashion. The presence of calcium carbonate and calcium phosphate is demonstrated by the von Kossa reaction (first published in 1901) and its modifications (11, p. 227). The silver in this technique combines with calcium phosphate and calcium carbonate to create a yellow to brown coloration at the site of the phosphate or carbonate. Use of a bright light during the reaction results in a black deposit. Other compounds may stain nonspecifically and confirmatory tests may be required.

In argentaffin reactions such as the Masson–Fontana originating in 1914 (11, p. 277), silver nitrate in an ammoniacal solution is reduced by numerous substances including argentaffin cells which are found mainly in the epithelial lining of the intestine and lungs, melanin, chromaffin and some lipofuscin. With so many compounds staining with this ammoniacal silver nitrate solution, confirmatory tests are required. The presence of many of these substances is also demonstrated incidentally by other silver methods.

A similar group of cells is involved in the argyrophil group of silver reactions. These methods are based on a silver reaction similar to the Bielschowsky nerve method published in 1904 (11, p. 254). Argyrophil substances in the tissue combine with the silver nitrate but are unable to reduce it. Reduction is accomplished by the use of an external reducing compound such as hydroquinone or formalin. Argentaffin compounds are also demonstrated. In a little twist, in some methods, tissues are pretreated to render certain tissue components argyrophilic.

Additional methods have been devised that “tailor” these argyrophil reactions for certain substances, including reticulum (Gomori 1937) (10, p. 345), fungi (Grocott-Gomori 1955) (11, p. 245), basement membranes (Gomori 1946) (11, p. 187), organisms (Steiner and Steiner 1944) (11, p. 241), and nerves (Holmes 1947) (10, p. 360). Reticulin is commonly called “reticulin.” There are many methods. Reticulin fibers support tissue and the pattern of reticulin is altered or missing in some disease processes as in cirrhosis of the liver and fibrosis of the lung. The Gomori reticulin method is popular in the United States, whereas the Gordon and Sweets 1936 method (10, p. 347) is popular in the United Kingdom. New uses for silver stains are still being found. The Grocott-Gomori method used to demonstrate Pneumocystis jiroveci (formerly called P. carinii) increased in popularity during the 1980s during the onset of HIV/AIDS, and some laboratories use older silver stain methods, for example, the 1927 Dieterle method (11, p. 241) to demonstrate the presence of Helicobacter pylori, an organism involved in gastritis, which can develop into gastric and duodenal ulcers.

Other Important Staining Procedures Used in Histopathology

The start of the aniline dye industry in 1856 made available a multitude of new dyes, many of which researchers tested for use in histology. Waldeyer (6, p. 70) was reportedly an early user of the dyes mauve and Paris blue in his studies, whereas later Ehrlich introduced at least 12 dyes to histology (6, pp. 70–71). In the histopathology laboratory of today, the special stains used will depend on factors such as the workload of the laboratory, type of work, where the pathologists were trained, and what type of special stains they are familiar with, as well as the skill of the histotechnologists.

The well-known pathologist Dr. Juan Rosai (15) has published a list of groups of special stains that are useful for the work performed by his laboratory. They are 1) periodic acid Schiff, 2) organisms stains, 3) argentaffin and argyrophilic stains, 4) amyloid stains, 5) reticulin stains, 6) trichrome stains, 7) phosphotungstic acid hematoxylin, 8) stains for melanin, calcium and iron, 9) stains for neutral lipids, 10) mucin stains, 11) Giemsa stains, 12) elastic stains, 13) myelin stains, and 14) formaldehyde-induced fluorescence (which is beyond the scope of this article). These methods and others are listed below.

Hematoxylin and Eosin

Just about every specimen processed through a histopathology laboratory receives the hematoxylin and eosin staining treatment (i.e., H&E). This was reportedly first devised by Wissowzky in 1876 (6, p. 71). There are numerous H&E methods, but most follow the same general procedure of staining the nuclei in an alum mordanted hematoxylin, followed by differentiation in dilute acid alcohol and bluing in running tap water or slightly alkaline water. The cytoplasmic components are then stained in eosin (either alcoholic or aqueous). Pathologists examine the pattern of cells, ratio of nuclei to cytoplasm, and the density and pattern of the chromatin. The pathologist likewise examines the structure, pattern and color of the cells’ cytoplasm. As many as five shades
of pink can be achieved when using eosin depending on the staining method, fixation, tissue, etc.

An amazing variety of histopathological conditions can be studied and diagnosed just on the basis of the H&E staining method alone. The method is cheap, quick to perform, and amenable to automation. Still, there are some drawbacks. The H&E method does not answer all questions and, as a result, additional “special stains” must be used to reveal specialized features or substances. Furthermore, the exact appearance of an H&E-stained slide often depends on the tastes of the pathologists viewing the slide; the pathologists may prefer the slide to be “light” or “dark” (usually depending on their training). In the early 1980s, Richard Allen Medical Industries (Kalamazoo, MI) made available the Specimen Management System™. This program was an effort to standardize and improve the preparation of microscope slides in the histochemistry laboratory and to thereafter provide consistent, high-quality results. The company supplied the fixative, processing chemicals, wax, stains, etc., including their proprietary products, and schedules for to be followed for the preparation of microscope slides. Schedules for H&E staining were adjusted to suit the tastes of the pathologists reading the slides. The program did not attain wide acceptance. Most likely the company found that, unlike the rest of the clinical laboratory, there was much variation and personal taste involved in the preparation of H&E stains. Additionally, the program may have been before its time.

Romanowsky Stains—Giemsa Stains

In 1891 Dimitri Romanowsky, and perhaps at the same time Malachowski (1891), devised the popular stain for parasites in blood smears that is still widely used for this and other purposes today. The Romanowsky stain replaced Ehrlich’s triacid stain (16, p. 320) as a popular multicolored stain. Ehrlich had earlier created the field of hematopathology when he demonstrated that certain white blood cell structures had different affinities for the same dyes based on their acid or alkaline composition (17,18).

The Romanowsky method uses eosin, methylene blue, and the oxidation products of methylene blue to stain blood smears, resulting in a variety of colors from a pale pink, through orange, to red-purple and an almost black color. The boiling or aging of the methylene blue (polychroming) generates products of demethylation (the azures) that provide a purple rather than blue coloration of leukocyte nuclei when present in a solution that also contains eosin (19, p. 308). Nuclei of protozoa acquire a color closer to red. Some modern blood stain solutions and powders add dyes to achieve these colors.

Improvements to the original method occurred very soon with Unna (1891), Jenner (1899), Lieshman (1901), Wright (1902), and Giemsa (1902) all devising changes in the formula to improve staining. Giemsa is probably the most popular. There are many other variations to these methods. Fortunately for histology, some of these stains are adaptable to formalin-fixed, paraffin-embedded sections and are used for cell maturation studies in bone marrow biopsies, organism detection, and other specialized uses (17).

Acid-Fast Bacilli

The demonstration of the organism Mycobacterium tuberculosis was of extreme interest to early pathologists. The poor living conditions of the general population (high infection rates, lack of suitable medications, and poor nutrition) made tuberculosis a major disease in early times. Early researchers found the organism was difficult to stain at first due to its lipid coat.

Robert Koch in 1882 was the first to develop a workable method for the demonstration of this organism. In his method, the smears were treated with alcoholic methylene blue mixed with potassium hydroxide. After extended staining, the smears were again stained in concentrated Bismarck brown which differentiated out the methylene blue. When viewed microscopically, the organism was colored blue, with cells and cell nuclei staining brown (other organisms also stained brown). The method could be shortened to one hour by heating the staining solution in a water bath.

This method of Koch laid the foundation for all of the subsequent methods, for it involved a chemical to aid in stain penetration, heat, and decolorization. Within months Paul Ehrlich improved upon the method by staining the organisms with a solution of either aniline basic fuchsine or aniline methylene violet, and by decolorizing with a mineral acid, hence the “acid fast” tag. Ehrlich was followed by Franz Ziehl, who recommended the use of carboxic acid (phenol) to aid in dye penetration, and by Rindfleisch (1882), who recommended heating the slide, and then by Neelsen (1883), who joined Ehrlich’s basic fuchsine stain and Ziehl’s phenol (20). The Ziehl Neelsen stain commonly used today is applicable to paraffin sections and is widely used. The method will also stain other “acid-fast” organisms such as Mycobacterium avium—intracellulare (MAI) complex, some lipid-containing pigments, and a few other organisms such as Nocardia species, as well as nonpathogenic acid-fast organisms.

Some acid-fast organisms contain reduced amounts of lipid in their coats, and routine paraffin processing further decreases this important feature required for the Ziehl Neelsen special stain. To cope with the resulting poor staining, a variety of methods have been developed that involve dewaxing the paraffin sections with different oils that purportedly retain or even restore some of the acid-fastness of the organism, while at the same time removing the paraffin. Faraco (1938) recommended olive oil or mineral lubricating oil. Fite and others in 1947 recommended 30% vegetable oil such as cottonseed or peanut oil for dewaxing slides during staining of Mycobacterium leprae, the acid-fast organism of Hanson’s disease, and later in 1952 Wade recommended rectified turpentine mixed with liquid petrolatum (21, p. 335).

Most histopathology laboratories have created their own modification of these techniques, often based on the Wade–Fite methods of 1957. Patients receiving chemotherapy and those with HIV/AIDS may have impaired immunological systems and can develop acid-fast organisms as opportunistic diseases. This has increased interest in acid-fast methods.

Fite also published in 1940 a fuchsin–formaldehyde method in which the sections stained in carbol fuchsin are treated with formaldehyde before decolorization in hydrochloric acid, which permitted counterstaining later in hema-toxylin and Van Gieson (11, p. 238). In the histopathology laboratory of today, while the Ziehl Neelsen method is widely used, a fluorescent method using the stains auramine-rhodamine is favored because sparse, acid-fast bacilli can easily be seen fluorescing reddish yellow on a green-black background (11, p. 239).
Mallory’s Phosphotungstic Acid Hematoxylin

First published by Mallory in 1897 (11, p. 193), this method again demonstrates another application of hematoxylin. In this progressive method, chemically or naturally ripened hematoxylin stains polychromatically using phosphotungstic acid as the mordant. Cytoplasm is stained shades of blue and collagen tissue yellow to brick red. It is especially suitable for the demonstration of muscle striations, intercalated discs, nervous tissue, and fibrin. However, because these items can be identified easily with other methods, it is less used today.

The use of phosphotungstic acid by Mallory is an excellent example of how early researchers found uses for obscure chemicals in histological techniques.

Gram Stain

Microscopists had been able to stain micro-organisms beginning around 1875 using dyes such as Bismarck brown, fuchsin, and carmine. In 1881 Paul Ehrlich introduced methylene blue. The great difficulty at that time was for microscopists to be able to separate the appearance of organisms from the accompanying cells and cellular debris.

In 1884, while studying pneumonia, Hans Christian Gram published a method that involved staining the smears in a solution Ehrlich had devised of aniline oil and gentian violet, followed by treatment in Lugol’s iodine, decolorization in alcohol, and counterstaining in Bismarck brown (22). Organisms stained an intense blue with a brown background. Gram was saddened that not all organisms stained! He had noted that some organisms did not take up the stain (these were probably the Gram negative organism Klebsiella pneumoniae). Only later did other researchers recommend the method for the differentiation of bacteria.

This method and its variations is still widely used today and is applicable to paraffin sections. Modern methods often use Hucker’s solution of crystal violet with ammonium oxalate (published in 1927) (11, p. 234), which acts as an accentuator to enhance differentiation, and a stronger iodine solution for the post mordant or “trapping.” Acetone is often used as the decolorizer, and neutral red or safranine is used as the counterstain. Gram-negative organisms are stained by the counterstain. The many modifications of the Gram stain method used in histology laboratories today include the Gram-Twort method, introduced in 1924 (10, p. 320); the Brown and Brenn method, first used in 1931 (23); and the Brown and Hoppes method (1973) (11, p. 235).

To greater or lesser degrees, the Gram method also stains other substances in tissue sections such as fibrin, fungi, sweat gland ducts and internal elastic lamina. The Gram–Weigert method published in 1887 (10, p. 319) built on this ability and stains Gram-positive organisms and other structures in paraffin sections. In the method, the nuclei are stained in an alum hematoxylin, followed by a phloxine counterstain. Gram-positive organisms and structures are then stained in aniline crystal violet, treated with Lugol’s iodine and differentiated in aniline xylene. Gram-positive organisms and structures are stained blue-black, nuclei blue, and cytoplasm shades of red.

Other Organism Stains

Many of the organisms that caused large-scale diseases and plagues in history have decreased with the arrival of modern healthcare, antibiotics, and improved public health. Syphilis was a common disease studied by the medical community in history and the spirochete was demonstrated by several different silver stains. The Warthin–Starry method introduced in 1920 (21, p. 347) and the Warthin–Faulkner method (10, p. 325) used silver impregnation of the section at a specific pH followed by development in hydroquinone. The Dieterle method (1927) (11, p. 241) and the Steiner and Steiner method (1944) (11, p. 241) use treatment with uranyl nitrate, followed with silver nitrate and hydroquinone developer in a gum mastic solution. Bulk silver methods such as the Levaditi published in 1905 (10, p. 325) were also used, and a Hage Fontana method reported in 1925 (21, p. 345) was available for smears. Some spirochete methods will also stain other organisms, such as Legionella pneumophila and the Gram-negative bacillus of cat scratch disease.

Today in histopathology laboratories, the Gridley (1953) (11, p. 246) and Grocott-Gomori methods (1955) (11, p. 245) are used for fungi, and the alcian blue–periodic acid Schiff method (11, p. 172) for Cryptococcus neoformans. The presence of inclusion bodies such as in cytomegalovirus (CMV) and rabies is demonstrated by Macchiavello’s stain (developed for Rickettsia), dated 1937 (12, p. 413), or Lendrum’s phloxine tartrazine introduced in 1947 (11, p. 248). There are many other methods for microorganisms, and new methods are still being devised. The Leung method (24) published in 1996, for example, is recommended for Helicobacter pylori. A modified Giemsa method can also be used for this organism.

Important Substances and Structures Demonstrated by Histopathology Staining Methods

Carbohydrates

Carbohydrates are a large group of substances found both in normal tissues and under pathological conditions. There have been several attempts at their classification based either on their structure, site in the body, or their staining reactions. Kiernan (25, p. 286) has classified them broadly as polysaccharides (for example, glycogen), proteoglycans, and glycoproteins. Histopathologists look for the absence or increase in these substances, together with the staining reactions and distribution, to make a diagnosis. The presence or absence of a carbohydrate may also indicate a genetic disorder.

McManus developed the periodic acid Schiff method (PAS) for carbohydrates in 1946, and it has become one of the most useful histological stains (11, p. 166). In this method, glycol groups of carbohydrates are oxidized by periodic acid to form aldehydes. These aldehydes are then stained red by Schiff’s reagent in an application of the Schiff reaction for aldehydes used in chemistry. Hematoxylin is often used as a counterstain.

A well-known use of the PAS method is to demonstrate the presence of glycogen in tissue sections. Glycogen is normally found in the liver, endocervix, and fresh muscle. Glycogen is present pathologically in some adenocarcinomas, mesotheliomas and seminomas (26, p. 183), in clear cell carcinomas, and as a PAS-positive granular material (in Whipple’s disease, for example); it may also be pathologically absent. As with other special stains, control methods must be used with the PAS technique. The most well known is the use of diastase (now replaced by alpha amylase) to remove glycogen. Mentioned earlier was the Best’s carmine method for glycogen (11, p. 167), in which an alcoholic solution of carmine combined with potassium carbonate in
an alkaline environment stains glycogen bright red. Of more complexity, basement membranes of renal glomeruli contain epithelial cell carbohydrates whose PAS demonstration provides an excellent view of the glomerular basement membrane structure. Collagen, umbilical cord (Wharton’s jelly), and thyroid colloid are also PAS-positive. Abnormal sites of carbohydrates include carcinoma of breast, amyloid, and myxoid liposarcoma (26, p. 183). Combined with hematoxylin, control methods, and other stains such as alcian blue, PAS remains an important special stain in histopathology. Some examples of diagnosis involving PAS are shown in Table 1 (26).

**Mucins**

Mucins have the ability to absorb ferric iron from colloidal iron solutions. This feature is put to use in the Hales colloidal iron method (introduced in 1946) and its modifications (11, p. 171). After treatment with colloidal iron, an iron stain is performed. Acid mucins and mucosubstances are stained blue. Some pathologists maintain the colloidal iron method is more sensitive than the alcian blue. Mucins also have an affinity for basic dyes and stain with polychrome methylene blue, thionine, toluidine blue, azure II and safranine. The high-iron diamine method introduced in 1965 (26, pp. 202–203) identifies sulfated mucins with a brown–black color.

There are many control methods available for use with carbohydrate stains as well as the alpha amylase method including saponification, acetylation, methylation, and sialidase and hyaluronidase digestion. In the complicated borohydride–PAS method (1975) for mucin in primary tumors of the lower gastro-intestinal tract, sections are oxidized in periodic acid, blocked with sodium borohydride, and saponified in potassium hydroxide; the resulting sites are demonstrated by Schiff’s reagent. Some metastases from adenocarcinomas of the lower GI tract that contain mucin are identified by this method (11, p. 170).

The alcian blue method was devised by Steedman in 1950 as a stain for acid mucins and it is used combined with many other staining techniques (11, p. 172). Used alone or with a counterstain at pH 2.5, it will stain most acid mucins and mucosubstances. At pH 1.0 it will stain sulfated mucosubstances (1964) and, when used at different electrolyte concentrations using magnesium chloride at a higher pH, different mucosubstances can be demonstrated (11, p. 173), although the method is disputed. Alcian blue can be combined with the PAS method, and methods for high-iron diamine published in 1965 (26, p. 203) and aldehyde fuchsin (1960) (26, p. 201).

**Pigments**

The demonstration of pigments in tissue sections covers a large and interesting section of histopathology methods.

Many pigments can be found in the human body under normal or pathological conditions. They include pigments from the fixation process, naturally occurring pigments, pigments created by a disease process, and pigments introduced into the body from outside, perhaps during work-related activities or from the living environment.

The use of certain fixatives will create pigments in the tissue. The use of nonbuffered formalin can create formalin pigment in tissues, especially in hemorrhagic areas. Mercury and potassium-dichromate-based fixatives can deposit pigments in tissues. Exogenous pigments are taken into the body, usually through the lungs, and are found microscopically in the tissues. Carbon in the lungs of smokers and city dwellers is a good example. Some exogenous pigments are associated with certain trades or life styles. Coal dust in the lungs of mine workers (“black lung”) and tattoo pigment are examples. Asbestos fibers found in the lungs of shipyard workers and pipe fitters are classified along with pigments. These pigments when present in large amounts can be readily visualized on a routine H&E section. Some are optically active; that is, they have the ability to rotate the path of light when using polarizing lenses. The final group, endogenous pigments, are of the most interest to pathologists. These are pigments created by the body’s metabolism. Examples include iron (hemosiderin), melanin, chromaffin substance, lipochrome, lipofuscin, hemoglobin, and Dubin–Johnson pigment.

Perl’s Prussian blue method for hemosiderin remains the most important staining method for a pigment in histopathology and has been virtually unchanged since its publication in 1867 (11, p. 218). Hemosiderin (hereafter called “iron”) is a naturally occurring pigment in the body, resulting from the recycling of red blood cells. Iron stains are commonly used to evaluate normal iron levels in bone marrow biopsies and aspirates. In the technique, the masked ferric iron in the tissue is unbound by exposure to a strong mineral acid, and the iron then combines with potassium ferrocyanide to create the Prussian blue color, ferric ferrocyanide. Iron is partially depleted by exposure to acid during decalcification.

Melanin is a naturally occurring pigment found in the skin and the substantia nigra in the midbrain. Its presence in pathological situations may indicate a melanin-producing neoplasm such as a nevus, or a melanoma, an aggressive, fast-growing tumor. Methods to demonstrate the presence of melanin include Masson’s 1914 modification of Fontana’s 1912 method (11, p. 277), with and without melanin removal with an acidified potassium permanganate–oxalic acid sequence, and the Schmorl ferric-ferricyanide method (11, p. 223). Also grouped with these pigments are the argentaffin and argentophil cells of the intestinal and respiratory tract epithelium. These cells can form carcinoid tumors. The argentaffin cells are demonstrated by the Schmorl and Masson Fontana methods, and also by the Diazoo method introduced in 1931 (11, p. 278). The argentophil cells are demonstrated by specialized silver methods. The pH of the solutions used is critical and a variety of methods have been devised to demonstrate them. These include the Hellerstrom and Hellman method (1960) (27, p. 286), the Grimelius method (1968) (27, p. 285), and the Churukian and Schenk method (1979) (27, p. 285). These methods will also demonstrate the pancreatic alpha cells.

Table 1. Some examples of carbohydrates of diagnostic significance (26)

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<thead>
<tr>
<th>Carbohydrate</th>
<th>Diagnostic Significance</th>
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<tbody>
<tr>
<td>Glycogen</td>
<td>Involved in cancers of bladder, liver, kidney, ovary, pancreas and lung, some mesothelioma, and juvenile rhabdomyosarcoma.</td>
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<tr>
<td>Hyaluronic acid</td>
<td>Involved in myxoid liposarcoma</td>
</tr>
<tr>
<td>Neutral mucins</td>
<td>Carcinoma of stomach (diffuse type)</td>
</tr>
<tr>
<td>Sialomucin</td>
<td>Carcinoma of rectum and colon</td>
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</table>
The demonstration of other endogenous pigments, while important, is less common. Malaria pigment resembles the fixation pigment of formalin and has similar staining characteristics but can be distinguished by its intracellular location. Chromaffin cells, found naturally occurring in the adrenal medulla, are also found in pheochromocytomas; they are demonstrated by the Schmorl and ammoniaca1 silver methods, and are green after Giemsa staining. The lipofuscinics are an ill-defined group of pigments associated with cellular aging and are variously positive with the PAS, Masson Fontana, Schmorl, long Ziehl Neelsen and Sudan Black methods, and also with Nile blue sulphate. Bile is demonstrated by the Fouchet method introduced in 1917 (11, p. 219) and Steins iodine method dated 1935 (11, p. 219), in which the bile is oxidized to green biliverdin. The interest in bile demonstration is to differentiate bile from lipofuscin in liver biopsies. There are many other pigments of lesser importance including lipochrome, hemofuscin, and pseudomelanin. Fixation affects many of these staining reactions. Gout is demonstrated by methenamine silver without oxidation. Dithio-oxamide (rubenamic acid) (1958) (11, p. 229) was originally recommended for the demonstration of copper accumulation in the liver such as occurs with Wilson’s disease (hepatolenticular degeneration), but it has now been replaced by the p-dimethylaminobenzylidene rhodanine method (1969) (28, p. 341).

There are also several methods to demonstrate hemoglobin, which is blue with the Mallory PTAH, dark blue with the leuco patent blue method (1938) (21, p. 251) and dark brown with the 1941 benzidine method (11, p. 216).

**Lipids (Fats)**

A wide range of stains for lipids (commonly called “fat stains” in the histology community) are possible on histological sections. Fat is normally found surrounding some organs of the body and in the skin as packing, but is indicative of a pathological process when found inside an organ, such as in cases of fatty liver, or as a lipoma or liposarcoma. Muscle biopsies for neuromuscular disorders routinely include a fat stain to indicate the metabolic state of the muscle. Most fats are dissolved out of tissues during normal paraffin processing and cryostat sections are normally required for their demonstration.

In 1896, Daddi introduced Sudan III as a fat stain. Previously, fats in tissues were demonstrated with the use of osmium tetroxide. Osmium coats the lipid, is reduced by it, and turns black at the site of the lipid. After Sudan III, several other fats stains were introduced such as Sudan IV in 1901, oil red O in 1926, and Sudan black in 1935 (29, p. 220). Most fat stains work on a preferential solubility principle. The fat stain is initially dissolved in a diluted organic solvent. When the section containing the fat is introduced, the dye is more soluble in the fat and passes from the solution into the dye. Counterstaining usually follows. Technique is important in demonstrating fat stains. The fat stain must dissolve in the solvent, not dissolve fat in the section; the stain must pass from the solvent into the fat. Many solvents have been tried. Although 70% alcohol will work, it is thought to dissolve out most of the fat. Lillie and Ashburn’s method (published in 1943) uses a supersaturated solution in isopropyl alcohol, whereas Chiffelle and Putt’s method of 1951 uses propylene glycol (11, p. 205).

One way to classify lipids is as simple lipids, compound lipids and derived lipids. Compound lipids would include phosphoglycerides, glycolipids and sulfatides. Sudan black stains a wide range of lipids and can stain phospholipids in paraffin sections, but the results are disappointing. Less commonly used fat stains include Bakers acid hematein (11, p. 208), introduced in 1946, for phospholipids, and the Luxol fast blue methods published in 1953 for phospholipids and neuronal storage disorders (28, p. 316). (Some researchers question whether Luxol fast blue stains the myelin, or attaches to the myelin basic protein.) In 1947 Cain published a Nile blue sulphate method (11, p. 207) in which two components in the solution stain neutral lipids red and free fatty acids blue. Other, more complicated, lipid methods such as the OTAN (osmium tetroxide alpha naphthylamine) for cholesterol esters (1959) (28, p. 317) and gold hydroxamate methods (1963) for phosphoglycerides (29, p. 228) stain specific lipid groups and are used to study storage disorders. Some methods such as the Feulgen and Voit’s 1924 plasmal reaction for plasmalogens are often mentioned in histotechnology textbooks, but are rarely used in pathology (21, p. 303).

**Elastic Fibers**

The 1908 Verhoeff elastic method mentioned previously is easily the most popular elastic stain used today in histopathology laboratories. Using hematoxylin, the method stains both elastic fibers and nuclei and can be quickly performed in the laboratory. This method is often combined with the Van Gieson (1889) (11, p. 196) connective tissue stain, which gives blue–black elastic fibers, yellow cytoplasm, and red collagen. In early times the orcein elastic stain (dated 1890) was popular (30, p. 68). The basis for this stain is a colorless compound originally obtained from certain lichens that, following chemical treatment, develops staining properties. It is now prepared synthetically. The stain is dissolved in an acidified alcohol solution and slides are stained overnight. Orcein is reputed to be the stain of choice to stain delicate elastic fibers in skin and was favored by dermatopathologists and also reportedly stained the hepatitis surface antigen (31). Gomori’s aldehyde fuchsin introduced in 1950 (11, p. 197) is a progressive method using basic fuchsin combined with paraldehyde to stain not only elastic fibers but also mast cell granules, mucin, the beta cells of the pancreas and the pituitary, zymogen granules, salivary glands, and chief cells of gastric glands. Of interest to histologists is the Weigert’s elastic stain, reported in 1898, and its modifications. A variety of dyes can be used depending on the color of elastic desired. In the presence of resorcinol, Victoria blue TS stains elastic fibers blue; saffron O will stain elastic fibers red; basic fuchsin and crystal violet combined give a black color; and malachite green gives green elastic fibers. Sections are initially given the Mallory bleach (acidified potassium permanganate) followed by oxalic acid. The method is progressive. Excess stain is removed with alcohol (21, p. 177).

**Amyloid Fibrils**

Amyloidosis is a group of diseases resulting in the deposition of insoluble protein in the interstitial spaces of blood vessels and various organs. There are at least five types, and staining reactions can be variable depending on composition of the amyloid. A popular amyloid stain is Benhold’s Congo red (1922). In an alkaline environment and a saturated salt solution, Congo red stains the amyloid fibrils a light red
color. Following Congo red staining, amyloid will polarize, giving what is popularly described as an “apple green” color. In this phenomenon, amyloid in the tissue, following staining, is able to turn the plane of the light when the section is viewed microscopically between polarizing lenses (the section must be thicker than normal for this to occur, perhaps 8–10 μ). The carbohydrate component of amyloid can be demonstrated by the PAS method and by iodine; it is metachromatic, and is positive with the thioflavine T (1959) fluorescence method (11, p. 177–178). Secondary amyloid is usually created in response to chronic inflammatory processes including pulmonary infection, tuberculosis, and rheumatoid arthritis, as well as some neoplasms. Pretreatment with potassium permanganate will reduce secondary amyloid staining (32).

Endocrine Glands

Considering the importance of the endocrine glands in the functioning of the human body, it is surprising that there are not more histological staining methods devoted to their demonstration. The cells in the medulla of the adrenal glands contain catecholamines which have an affinity for chromium salts (the “chromaffin reaction”). These tissues will develop a brown color following a dichromate-based fixation, and then will stain greenish-yellow if subsequently stained with a Romanowsky type (1891) method (18). Chromaffin-positive cells are also Schmorl-positive (11, p. 223).

The pancreas has both endocrine and exocrine functions. The basal portion of the pancreatic acini cells contain zymogen granules that stain with the PAS, and stain darkly with hematoxylin in a normal H&E. A variety of different trichrome stains can be used to demonstrate the endocrine cells of the pancreas. These cells, located in the islets of Langerhans, can be classified by special stains as alpha (synthesizing glucagon), beta (synthesizing insulin) and delta (synthesizing somatostatin and a polypeptide), but cannot be distinguished using the H&E method. Tumors can arise from these cells and special stain reactions indicate which cells are involved. With Gomori’s chrome alum hematoxylin phloxine stain introduced in 1952 (11, p. 269), beta cells are blue and alpha cells red, whereas the aldehyde fuchsin method (1950) (11, p. 270) stains beta cells deep purple. Many other methods are available.

The anterior lobe of the pituitary contains three cells types when viewed after a trichrome type stain and each has an endocrine function. However, the staining reactions are not specific for the hormone produced, just the cell type (immuno-histochemical methods disclose more cell types). These cells can give rise to tumors. The alpha cells are acidophilic and are stained red by most trichrome methods. The orange fuchsin green method (1961) is suitable for pituitary staining (27, p. 279). The beta cells contain a glycoprotein and are PAS-positive. The third cell type, the chromophobe, usually stains poorly. The “Tripas” method of Pearse introduced in 1949 uses a PAS, hematoxylin, orange G in phosphotungstic acid sequence to stain the beta cells magenta, the acidophil cells orange, and nuclei blue black (11, p. 271).

RNA and DNA

During training, histotechnologists will have been taught about the Feulgen nuclear reaction for nucleic acids (1924) (11, p. 150). In this method, nucleic acids are hydrolyzed by hydrochloric acid to create aldehydes, which are then demonstrated by Schiff’s reagent. However, this method has limited applications in histopathology. Usually histopathologists are interested in the size and distribution of nuclei and in the pattern of chromatin material as seen in a routine H&E slide. The H&E method (depending on pH and formula) will also demonstrate RNA in Nissl cells and in pancreatic acini cells. The chrome alum gallocyanine method of Einarson, dated 1932, was designed specifically to stain nucleic acids (21, p. 280). Basic dyes such as toluidine blue and thionine, as well as safranine and neutral red are general stains for nucleic acids.

An intriguing stain developed long ago to stain nucleic acids was the methyl green–pyronine method devised by Pappenheim (1899) and improved by Unna (1902), and many others. The dyes methyl green and pyronine Y, a red dye, would normally stain both nucleic acids. Pappenheim discovered that at a certain pH, both stains act, but in a different manner, with the methyl green predominately staining the nuclei (DNA), and the pyronine Y predominately staining the cytoplasm (RNA) (11, p. 151). This technique was invaluable to pathologists interested in studying the cell population of tumors. Fixation in Carnoy gave best results. Impure methyl green was treated with chloroform prior to use. Methyl green is no longer available and has been replaced by ethyl green (33, p. 196).

Myelin

Myelin is the material that forms the extended surface membrane of Schwann cells in the peripheral nervous system and of oligodendrocytes in the central nervous system. It forms a multilayer sheath around the axons of neurons and provides electrical insulation. However, the axons can become diseased and undergo demyelination. In the past, hematoxylin was used to demonstrate myelin in tissue sections. The Weigert Pal (1886) (11, p. 262), Kultschitsky (1890) (21, p. 264), and Loyez (1910) (34, p. 114) methods were commonly used. The PTAH technique (1897) will also demonstrate myelin.

Modern methods for staining myelin include the Luxol fast blue (1953) (11, p. 264) and eriochrome cyanine R (developed in 1965 and called by its synonym, solochrome cyanine R, in old texts) (35, p. 355). As previously mentioned, it is unknown if the Luxol fast blue stains the myelin, or the protein attached to the myelin. If a neuron is damaged, the surrounding myelin sheath deteriorates and degeneration products can be demonstrated by the Marchi method published in 1886. In the original method, tissues are placed in a solution of potassium dichromate and osmium tetroxide. The degenerating myelin is stained by the osmium, but the normal myelin remains unstained. The potassium dichromate prevents reduction of the osmium by the normal myelin, but the breakdown products, cholesterol esters and unsaturated fatty acids, are stained black by the osmium. Sections are then prepared (11, p. 262). A more recent modification is the Swank and Davenport method published in 1935 (35, p. 357; 36).

In 1956 a Luxol fast blue/oil red O method was published for use with frozen sections to demonstrate degenerating myelin (11, p. 265). Sudan black will also demonstrate myelin in paraffin sections, but faintly.

Nerve Cells, Axons, and Neurofibrils

There are a variety of different nerve cells present in the central nervous system, each with different structures and
functions. These neurons can undergo changes due to age, disease or injury. A variety of methods are available to demonstrate these neurons, the neurofibrils within them, and their processes. Histopathologists examine the processes, neuronal reaction to damage, nerve cell atrophy, and degeneration (37, p. 1342–1346).

The classic stain for the demonstration of neurofibrils in nerve cells is the Bielschowsky silver method (1904) (11, p. 254). This method stains the neurofibrils (bundles of neurofilaments in the axons) black and also stains some cell bodies, larger dendrites and pathological structures such as neurofibrillary tangles and plaques. In the original method, frozen sections are immersed in silver nitrate solution, and then in ammoniacal silver nitrate. Later treatment with formalin and toning in gold chloride results in brown to black neurofibrils, axons and dendrites. There have been many modifications, some designed for paraffin sections. A suitable modern silver method for nerves published in 2001 omits the formalin and gold chloride steps and gives a dark brown to black nerve and neurofibril elements, and golden brown plaques on a light brown to yellow background (38). Other popular methods include that of Marsland, Glees and Erikson’s (1954) (35, p. 347) and Palmgren (1948) (35, p. 348). The methods of Davenport (1930) (39) and Holmes (1947) (11, p. 256) use an initial high concentration of silver nitrate, followed by impregnation and reduction, while the method of Bodian (1936) (11, p. 256) uses a silver proteinate, Protargol, which slowly releases silver into the solution. Copper wire or shot present during impregnation is thought to compete for the silver and reduce staining of connective tissue.

In recent years there has been an increased interest in neurological degenerative diseases such as Alzheimer’s and Pick’s diseases and a corresponding interest in histopathological methods to demonstrate them. As with other special stains, there is not one method that will demonstrate all the pathological features in which a researcher may be interested. Researchers choose techniques that demonstrate best the structures they are studying and then with repetition and experience become experts with that particular method. The Gallyas silver stain published in 1971 (40) is said to demonstrate more pathological lesions than any other method, but each neuropathologist has his or her favorite method. Histopathologists also look for other signs of disease, infectious agents, and deposits such as lipofuscin that may be part of disease process or aging.

Neuroglia

The glial cells are the supporting cells of the central nervous system. There are generally assumed to be four different types of neuroglial cells—astrocytes (fibrillary and protoplasmic), oligodendroglia and microglia, and to these some add ependymal cells, the cells lining the ventricles of the brain. As in other tissues, histopathologists look for microscopical changes in these cells that indicate damage or disease. Tumors of glial cells include astrocytomas, oligodendrogliomas, and ependymomas. An important pathological change in astrocytes is gliosis: hypertrophy or hyperplasia due to disease or injury.

Light microscopy stains for glia include Holtzer’s method (11, p. 260), dated 1921, which utilizes a complicated formula that includes crystal violet, phosphomolybdic acid, potassium bromide, chloroform and aniline, and Anderson’s Victoria blue method introduced in 1929 (21, p. 273). A separate group of gold and silver stains is available to demonstrate glial cells. These were devised by a group of researchers in Spain in the early 1900s. Cajal’s gold chloride sublimation method (1913) (11, p. 259) uses an initial fixation in formal ammonium bromide (FAB), followed by impregnation in a gold chloride-mercuric chloride solution. Astrocytes are seen as purplish-black. This method was developed by Santiago Ramón y Cajal of Spain (1852–1934), who made many discoveries in histology and cancer research but is best known for his “neuron doctrine,” which holds that each nerve cell is independent of any other nerve cell. A group of like-minded histologists who made other discoveries formed around Cajal (41). Among these was Pío del Río-Hortega, who first described microglia and oligodendroglia (42). Hortega’s silver carbonate method for astrocytes (1918) uses the same FAB fixation, followed by an ammoniacal silver nitrate–lithium carbonate solution. After several steps, the astrocytes are seen as black (42).

Trichrome Stains

Staining methods that will simultaneously color various tissue components in a histological section have long appealed to histopathologists, and there are a multitude of histological methods that give multiple colors. It is understandable that early microscopists who used carmine and other monochromatic stains would have appreciated some differentiation by color of the tissues they were studying. Initially, just two colors were used, as in the picro–nigrosine method introduced in 1883 (43, p. 162) and the methylene blue–eosin method dated 1894 (43, p. 162). The first reported triple stain was that of Gibbs in 1880 (6, p. 72). However, a better-known triple stain was Ehrlich’s “triacid stain” published in 1888 (7, p. 113), which used methylene blue, acid fuchsin, and orange G, a stain with which he discovered granulation in neutrophils. A “trichrome” in today’s histopathology laboratory usually means a section with the nuclei stained with hematoxylin, and the connective tissue and cell cytoplasm stained different colors, rather than just a section with three colors. Most popular in present times is the Masson’s trichrome introduced in 1929 (11, p. 190), that stains nuclei blue–black, cytoplasm red, and connective tissue green or blue depending on the particular stain chosen. Pretreatment of the section with Bouin’s fluid, or picric acid solution alone, is common before trichrome staining.

Many trichrome methods will demonstrate fibrin, which is useful to histopathologists. Fibrin in tissue sections is a product of clotting, and its site and structure give much information. Fibrin stains on lung, for example, will demonstrate lobar pneumonia. Areas of the lung become hemorrhaged and develop fibrin. Fibrin is pink on a routine H&E, is sometimes PAS-positive, and stains with the PTAH and Gram-Weigert (10, p. 319) methods. A similar material, fibrinoid, which contains fibrin as well as other materials, is also found under similar conditions. Several trichrome methods have been devised with the intention of improving the demonstration of fibrin, among other things. Other connective tissue stains include the picro Mallory (Lendrum) dated 1949 (44) in which nuclei are stained blue black, erythrocytes yellow, connective tissue blue, muscle light red, and fibrin deep red. In the United Kingdom, Lendrum’s martius scarlet, blue (i.e., MSB) introduced in 1962 (44) is popular.
and gives blue nuclei, yellow erythrocytes, blue collagen, red muscle, and fibrin (early fibrin may stain yellow and old fibrin blue).

The trichrome stains are a good example of how some complicated staining methods have evolved. Starting in the 1940s, Lendrum and others (44) demonstrated how tissues of different fine structure and consistency are stained by dyes, with small molecules staining tissues with the finest structure, and larger molecule dyes staining more porous tissues. Dyes are replaced by other dyes during the staining, and physical changes in the dye solutions further change the staining characteristics. In trichrome methods, phosphotungstic acid behaves like a colorless dye, replacing other dyes. There are many other popular trichrome methods, with laboratories choosing their methods depending on their particular situation. The Movat pentachrome stain (1955) (11, p. 198) is occasionally still used in some laboratories. Unfortunately, to get the best results with many of these methods, formalin-fixed tissues require treatment in heavy metal salts such as mercuric chloride and potassium dichromate, which precludes their utilization in modern laboratories.

Fluorescent Methods

Quite a few fluorescent methods for applications in histopathology have been developed, but most are seldom used. Improvements in fluorescence microscopes should have increased their ease of use. The earliest fluorescence microscopes used visible light at the blue end of the spectrum. Since the 1970s, epi-illuminated fluorescent microscopes with strongly emitting ultraviolet mercury vapor bulbs have been used, with a "cube" in the light path which permits visible light to pass up from the slide to the eyepieces. The correct filtration must be selected for the fluorochrome used.

An excellent fluorescence method is the auramine-rhodamine technique of Kuper and May introduced in 1960 (10, p. 501) for the demonstration of acid-fast bacilli in tissue sections. This method will detect the orange-stained bacillus on a green background and will often disclose solitary organisms overlooked by a routine Ziehl Neelsen staining. Also useful is the thioflavine T method of Vasser and Culling (1959) mentioned earlier for amyloid and Paneth cells. Methods were also devised for RNA and DNA (Bertalanffy 1960), 3:4 benzpyrene and Phosphine 3R methods for Lipids (Berg 1951, Popper 1941), Mucin (Hicks and Mathaei 1958), calcium (1960) and the acridine orange (1961) and calcofluor white (1984) methods for fungi (Chick 1961) (10, p. 258, 390, 497, 496; 45).

Metachromasia

An uncommon staining phenomenon called metachromasia relates to the ability of some tissue structures to aggregate dye particles and create a new color. This phenomena was first described by Cornil in 1875 (6, p. 70). The most common group of dyes exhibiting this effect are members of the thiazine group (thionine, methylene blue, and toluidine blue) and the tissues that can be stained metachromatically include mast cell granules, the matrix of cartilage, Wharton's jelly in umbilical cord, Nissl substance, some mucins, thyroid colloid, vitreous humor of eye, amyloid (with methyl violet dyes), and several other tissues. These blue dyes normally stain metachromatically a red or purple color. pH and dye purity play a role in metachromatic staining. Some methods involve pretreatment of the section with the Mallory bleach. The aqueous environment also plays an important role in metachromatic staining; dehydration and clearing will usually destroy or diminish the staining. The method of Hugheson (1949) (26, p. 199) involves differentiation of the staining with uranyl nitrate, followed by air drying of section and clearing in xylene. The methylene blue extinction test (1926) uses solutions of methylene blue at various pH to differentiate acid mucopolysaccharides (46, p. 341).

Techniques for Uncommon Tissue Abnormalities

A survey of the histological methods that have been devised during the years will reveal numerous techniques that demonstrate pathologies so unusual or uncommon that they have infrequent or no use in the routine histopathology laboratory, depending on the caseload. However, these same techniques may have a use in a specialized histopathology or research laboratory. For example, Dr. Frank Johnson at the Armed Forces Institute of Pathology developed a method to demonstrate calcium oxalate in tissue sections in hereditary hyperoxaluria, a genetic disorder (1962) (47). Attwood's method demonstrates keratin in amniotic fluid embolism (48) in the lung and is a variant of an earlier Lendrum method (44). The hematoxylin-basic fuchsin-picric acid method (1971) demonstrates early myocardial ischemia (11, p. 199).

There are many other similar techniques.

Conclusion

The past 30 years have witnessed great changes in histotechnology and histopathology. Already, although some old, popular, special stains remain in use, many others have been replaced by either newer staining methods or immunohistochemical techniques. Still other techniques have been abandoned because they require chemicals now considered toxic. Additionally, histotechnologists familiar with the older methods are retiring and leaving the field. With this shift in the workforce, the knowledge required to perform these techniques may be lost. Although the roles of tomorrow's histotechnologists and histopathologists will undoubtedly change as new methods and materials evolve (49), future generations can amplify their understanding of the discipline by looking to the past for its legacy of stains and staining techniques.

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References
