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The long history of hematoxylin

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Abstract

Hematoxylin is a naturally occurring chemical used as the basis of a dye in laboratories throughout the world to stain nuclei in microscope slide preparations. This chemical is extracted from the logwood tree *Hematoxylon campechianum* and was discovered by Spanish explorers to the Yucatan in 1502. A vigorous trade soon developed related to growing and preparing hematoxylin for use in dyeing fabrics in Europe. In the mid 1800s, amateur microscopists first used hematoxylin to stain cellular components. Later scientists developed a wide range of techniques to demonstrate different cellular components. Hematoxylin remains the most popular nuclear stain in histology. This paper briefly describes the history of hematoxylin production and use in histology.

Key words: *Haematoxylon campechianum*, hematoxylin, hematein, logwood extract

It is interesting that in these days of rapidly advancing laboratory technology, the most commonly used stain in biology is based on hematoxylin, a naturally occurring compound derived from the logwood tree, *Haematoxylon campechianum.* This dye, arguably first used for histology about 1830 (von Waldeyer 1863), has a long history; it had long been used by the native population in the Caribbean area where the tree grows naturally. Hematoxylin as found in the tree is colorless. The different staining solutions are based on its oxidized form, hematein. By convention, histologists call all of these solutions ''Hematoxylin.''

Hematoxylin is used most widely in the field of surgical pathology where each working day in laboratories around the world, millions of microscope slides stained with hematoxylin and eosin (H & E) are prepared and viewed by pathologists as part of the diagnosis process. In the United States, such laboratories use commercially prepared ready to use hematoxylin solutions, many based on the Harris formula (Harris 1900). Such solutions stain chromatin in formalin fixed, paraffin embedded sections of tissue in 2–5 min. Following counterstaining with eosin, pathologists make important diagnoses based on cellular morphology and staining reactions.

In this paper, I briefly review the history, use and production of hematoxylin, and its use in histology laboratories today.

Geographical range and synonyms

The logwood tree, *Haematoxylon campechianum* is a member of the legume (pea) family Fabaceae and is native to Central America where it grows wild and often is used as a hedge or barrier. The tree grows to about 40 feet high, has a short crooked trunk, spines on the branches, and light yellow flowers (Fig. 1). The dye is extracted from the heartwood, which is dark and dense. The trees were originally located on the shores of the Gulf of Campeche, but have been transplanted throughout the West Indies, Brazil, India, Ghana and Madagascar. Hematoxylin has had several synonyms through history including Campeachy wood, block wood and logwood. In fact, hematoxylin is most widely known in industry today as ''logwood extract.'' Logwood trees can grow in southern parts of the US, but will not survive freezing. The bark and gum had uses in earlier days as a subastringent

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Fig. 1. A naturalist's drawing of the foliage of *Haematoxylon campechianum* published in 1812 (Titford 1812).

and enema, and as a treatment for dysentery (Titford 1812)*.

History

Logwood was discovered in 1502 by Spanish explorers in what is now the Mexican State of Campeche. The Maya Indians of that area used logwood to stain cotton and for medicinal purposes (Kahr et al. 1998). Following importation to Spain, it was quickly utilized by other European countries, and by the mid 16th Century, a brisk trade had developed. In the early days before aniline dyes, fabrics and clothing most often were dyed using colors obtained from plants and lichens. Some of these colors were not ''fast'' and faded easily in sunlight or with repeated washing. Large sums of money could be made supplying the textile industry with dependable dye compounds. Early Spanish and Portuguese explorers also harvested another dye wood from the coasts of Central and South America. This was Brazil wood from trees of the *caesalpina* species from which the red dye brazilein is extracted. The red heartwood of these trees reminded Portuguese sailors of red glowing embers or coals (brasa in Portuguese) and thus Brazil was named.

With the warring European nations competing for land in the New World, frequent sea battles and tales of piracy were common. England's Sir Walter Raleigh, at one time a privateer, would lie in wait off the Azores to capture Spanish ships carrying goods from Spain's empire in the New World including dye woods that the privateers would sell in England (Kahr et al. 1998, Winton 1975). The Spanish desired to retain their monopoly and considered logwood contraband if it was carried on a ship not registered in Spain. Against the wishes of the Spanish, British pirates and others, using native labor, started logging operations in the Caribbean. Plantations were started in Jamaica in the 16th Century and hematoxylin production soon became a flourishing business (Kahr et al. 1998).

Early fabric dyeing methods involved placing bags of logwood chips in the vat with the clothes to be dyed (Bronson and Bronson 1817). Hematoxylin was used to dye the uniforms of American soldiers during the Civil War and World Wars I and II, and is still used to dye some leathers, silk and surgical suture (Stevens 1948, OSHA 2004). Silks are of interest, because they are sold by weight and the tin mordant used in the dyeing adds weight to the silk.

Logwood extraction

The dye hematoxylin (empirical formula $C_{16}H_{14}0_6$; C. I. 75290) is colorless and is found in the heartwood of the logwood tree. Chemically, hematoxylin is a flavonoid, a widely distributed group of plant chemicals, some members of which are involved in plant coloration. When exposed to air, hematoxylin is oxidized to reddish brown hematein (Fig. 2). Hematein should not be confused with the hematology pigment hematin. Further confusion can

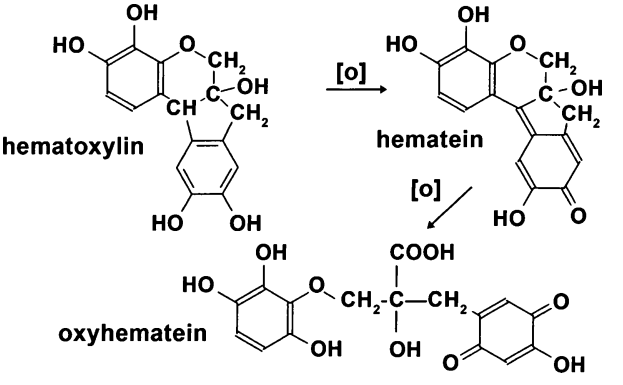

Fig. 2. Oxidation of hematoxylin (Horobin 2002).

* William Jowett Titford (1784–1823) a plantation owner and amateur naturalist in Jamaica was a distant relative of the author.

arise owing to the British spellings of haematoxylin and haematein.

Hematoxylin is abundant in the upper roots, heartwood of the trunk and larger limbs of the logwood tree. After cutting, the sap wood is removed and the trunk is cut into 6 foot lengths. In a processing plant, the cut logs are chipped into $1/2 \times 1/2 \times 1/6$ inch pieces. During extraction, hot water is passed over the chips at atmospheric pressure, first over the most extracted chips, then onto the least extracted and ''virgin'' chips. The resulting liquor containing about 4% hematoxylin is directed into evaporators. The specific gravity is about 1.1, or 20 degrees twadell in textile terminology. Water is evaporated until the hematoxylin concentration is 54%, or 51 degrees twadell. This solution is termed ''logwood extract'' and is drawn from the evaporator. To produce a powder, the liquor is placed on trays and dried under vacuum. The fresh wood contains about 8–10% hematoxylin. This is called the ''French method'' (Kirk-Othmer 1979, Dalkey 1986).

The ''American'' method uses steam distillation with steam pressure of 15–30 pounds per square inch (Kirk-Othmer 1979, Dalkey 1986). The trees from Campeche give the best yields. At the present time, hematoxylin is almost exclusively used as an extract (''logwood extract'') prepared near the site of the logwood plantation.

Most batches of ''hematoxylin powder'' contain approximately 12% naturally oxidized hematein, although in the 1970s, Lillie reported that the amount could vary up to 100% (Lillie and Fullmer 1976). For textile dyeing, 75% hematein is required. This is achieved in the dye industry by chemical oxidation. If histology laboratories use hematoxylin powder that has been prepared from logwood chips that contained much sapwood or if much of the hematoxylin was oxidized to hematein, variable staining can result.

A number of factories produced logwood extract in the US during the late 19th and early 20th centuries. These included the New York Dyewood Mills, American Dyewood Company, Smith Hartshorne and Company, Sharpless Dyewood and Extract Company, Harway Dyewood Company and the New York and Boston Dyewood Company (Fig. 3). The Compagnie Haitienne operated in Haiti (Stevens 1948, Dalkey 1986).

Worldwide production of hematoxylin was listed as 70,000 tons in 1943. At that time there were four plants around the world extracting hematoxylin. A factory in La Harve, France, worked with chips from Haiti, while the British Dyewood Company operated a plant in Glasgow, Scotland. The J.F. Young Company operated a plant in Baltimore, and the West Indies Chemical works operated in Spanish Town, Jamaica. Processing plants closed in the 1970s for several reasons. One was the lack of logwood chips, because hematoxylin tree plantations were replanted with sugar cane (Dalkey 1986).

Levels of current production, purification methods, and sites are notoriously difficult to obtain, but it appears that most of the supply in the western hemisphere is produced by the company, Mexicana De Extractos S.A. located in Campeche, Mexico since the 1970s. The company has over 200 employees and produces approximately 1200 tons of logwood each year (Mexicana de Extractos 1999). Mexico does not allow the exportation of logwood chips for industrial use, so in effect a monopoly has been created.

Use as a textile dye

Early fabric dyers found the colors of hematoxylin to be ''fugitive'' or short lasting. The discovery of heavy metal salts as mordants to bond the hematoxylin chemically to the fabrics opened up a whole new field of dying. The rise of the aniline dye industry in the 1830s, however, lessened the need for hematoxylin.

The early dyers discovered that the use of different heavy metal salts as mordants resulted in different hues in the stained fabric. Iron salts gave gray to black colors, copper salts gave green-blue to black colors, chromium salts gave blue to black colors, and aluminum salts gave violet to gray colors. This information was not lost on early histologists who developed a range of different mordants to use with hematoxylin.

Use in histology

Hematoxylin stains poorly by itself and for this reason some authors claim it is not a dye. In the histology laboratory, however, when oxidized to its hematein form and combined with a mordant, usually a metal salt, hematoxylin stains tissue sections a deep blue to black color depending on the staining method. By itself, hematoxylin is also amphoteric in its hematein form; it is red at acid pH and blue at alkaline pH. Differentiation following hematoxylin staining removes nonspecific staining. Counterstaining the cytoplasm, if required, adds more tinctorial qualities.

In the routine H & E technique used in surgical pathology laboratories, 4 μm sections of paraffin embedded tissues are dewaxed in xylene, hydrated

Fig. 3. An 1892 period drawing of the New York and Boston Dyewood factory in Boston. Schooners, shown moored, were used to bring the logwood from Central America (Stevens 1949).

to water, and stained in hematoxylin. Sodium iodate is a popular oxidant and aluminum potassium sulfate a common mordant. Nonspecific staining is removed by differentiation with acid alcohol. Treatment with a weak base, such as dilute ammonia, creates a stable blue color. Counterstaining the cytoplasm with eosin usually follows. Slides are dehydrated and coverslipped using a mountant that when dry has the same refractive index as glass. The result when viewed microscopically is of a monolayer of cells apparently embedded in glass. Hematoxylin also is used almost exclusively in cytology laboratories to stain nuclei in ''Pap smears'' named after Dr. Papanicolaou. Cytotechnologists and cytopathologists study the structure of the nuclei and cytoplasm for signs of malignancy (Papanicolaou 1941). In this method, nuclei in smears of cells on glass slides are stained initially with hematoxylin followed by counterstaining with orange G, then a mixture of light green FCF, Bismarck brown and eosin Y.

Although hematoxylin is used most widely for routine H & E staining, it is with other staining methods that its versatility is best demonstrated. It can be used for staining a wide variety of tissue structures using a variety of oxidants, mordants and differentiating agents, sometimes followed by counterstains.

A wide variety of oxidants may be used including sodium iodate, hydrogen peroxide, potassium permanganate, iodine, and mercuric oxide. Depending on the chemical oxidant used, the hematoxylin can become over-oxidized to oxyhematein and fail to stain correctly (Fig. 2). It generally is true that the quicker the oxidation reaction, the shorter the shelf life of the solution. In more recent years, histologists have devised solutions where the hematoxylin and the oxidant are more balanced or the oxidant is slightly deficient resulting in longer shelf life for the solution (Gill et al. 1974). Oxidation also can be accomplished over a long period naturally by exposure to ambient air (atmospheric oxidation) as in Ehrlich's hematoxylin method (Ehrlich 1886) and Mallory's phosphotungstic acid hematoxylin (Mallory 1897).

A metal salt mordant is used in nuclear hematoxylin methods. The ''Alum'' methods, e.g., Harris' (Harris 1900), Ehrlich's (Ehrlich 1886), and Mayer's (Mayer 1903), use ammonium or potassium aluminum sulfates (these are interchangeable). Ehrlich's method uses potassium alum, and if naturally oxidized, retains its staining power for years. Mayer's method uses sodium iodate and

potassium alum, while Harris' hematoxylin method uses in its original formula mercuric oxide and ammonium alum, although now with environmental concerns, sodium iodate is used instead of mercuric oxide. In Heidenhain's (1892) method, the tissues are treated first with the mordant, ferric ammonium sulfate, then stained.

After mordanting and staining, the tissue sections are usually over-stained and require differentiation to remove nonspecific staining. When an acid is used for differentiation, nuclear staining is accomplished as in most routine H & E methods.

Some methods use differentiation with the mordant, which gives a variety of results depending on the method, tissue and mordant. Verhoeff's elastic stain (Verhoeff 1908) uses hematoxylin, iron chloride as the mordant, and iodine as the oxidant. This results in blue-black staining of elastic fibers and nuclei. In Heidenhain's method (Heidenhain 1892), the mordant iron alum is used first on the tissues, which then are over-stained with the hematoxylin, then iron alum is used again as a differentiator to remove excess stain. This results in both nuclear and cytoplasmic staining.

In the Weil method for myelin in brain tissue (Weil 1928), sections are stained in hematoxylin, mordanted in iron alum, differentiated initially in iron alum, then differentiated further in borax ferrocyanide resulting in dark gray to black staining of myelin. Mayer's mucihematein method (Mayer 1896) is a progressive method using sodium iodate to oxidize and aluminum chloride as the mordant for staining mucin.

Mallory's phosphotungstic acid hematoxylin method (Mallory 1897) uses naturally or chemically oxidized hematoxylin and includes phosphotungstic acid as the mordant. The method is progressive and excess stain is removed with alcohol. The result is a polychrome effect with cytoplasm stained shades of blue and connective tissue stained yellow to brick red.

The Weigert's iron hematoxylin method (Weigert 1904) is progressive and stains nuclei blue-black. The dye lake formed is fairly resistant to destaining by counterstains and is widely used in the Masson trichrome method (Masson 1929) and with the van Gieson method (van Gieson 1889).

Other chemicals are included in hematoxylin stains for a variety of reasons. Glycerol evens the coloration in nuclear stains and prevents overoxidation. Acetic acid enhances nuclear staining and reduces cytoplasmic staining. Some methods omit a specific oxidant, and oxidation is carried out by another chemical in the procedure, or there is enough naturally occurring hematein present to produce staining (Lillie and Fullmer 1976).

Arguably, suitable replacements for hematoxylin as the nuclear stain in the routine H & E regimen are available, but pathologists and others trained using H & E stained slides are disinclined to change and point out that they make life and death decisions based on the subtleties of H & E stained slides. Chrome alum gallocyanin is one stain that is easily prepared and could be modified to be a suitable replacement for hematoxylin in the routine H & E (Einarson 1932).

Hematoxylin remains the most popular stain in histology. Despite the advent of molecular methods in histology and the replacement of microanatomical studies with cytological studies and immunohistochemistry, alum hematoxylins are used almost exclusively for nuclear counterstaining in the newer methods. Verhoeff's is the most popular elastic stain, and Weigert's iron hematoxylin method often is used as the nuclear stain in multicolored staining methods. Should the specialty of histopathology move toward automated screening, new nuclear staining methods that do not involve hematoxylin may be adopted that better facilitate the screening.

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