Origins

Haematoxylin—from the wood

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In the early days of microscopy very many plants with distinctive colours were examined to see if they possessed the necessary constituents to add colour to microscopic specimens. Indeed, Mann[1] stated that to be a microscopist became synonymous with becoming a dyer. However, the advent of coal tar (aniline) dyes, usually accredited to Perkin although apparently not published by him,[2] led to the gradual demise of natural dyes for staining tissue sections, although carmine remained a favoured substance well into the synthetic age. An obvious exception to this is haematoxylin which, over 100 years later, remains the cornerstone for staining tissue sections. Indeed this compound is, to a very large extent, the foundation upon which modern histopathological diagnosis is built.

Haematoxylin is derived from the heart wood of the logwood tree (fig 1). Baker[3] states that "the dyeing property of the heart-wood was known to the natives of Campeche (Mexico) before the arrival of the Europeans. The Spaniards brought [it] to Europe shortly after the discovery of America. It [was] brought to England early in the reign of Queen Elizabeth [I] . . . . . .The tree was introduced into Jamaica in 1715, where it is still cultivated . . . . . Extracts of [the] logwood were first used in microtechnique in the eighteen-forties."

Bracegirdle[4] considers it likely that Reichel[5] introduced the use of logwood for microscopic staining, using a simple unmordanted solution to colour plant tissues. Quekett,[6] in a single paragraph devoted to mounting sections in balsam, was the next to mention haematoxylin, long before the usually accepted date for its introduction as a stain. Waldemeyer,[7] wrongly in Bracegirdle's opinion,[4] is usually credited with its general introduction. Böhmer[8] was the first to use logwood in combination with a mordant, his inspiration coming from textile dyeing of the time.

By the end of the 19th century haematoxylin, or logwood, had been described as "without equal" as a tissue stain by Cole,[9] although for others carmine was considered to be the "staining agent *par excellence*" (Bolles Lee[10]). However, chromic and osmic acids were the commonest fixatives of the time and following their use haematoxylin gave better results. Hence haematoxylin was destined to secure the central role in biological staining. Only the preparation of the stain—"by no means simple"(Bolles Lee[10])—was a drawback.

Delafield, whose formula is probably the oldest one currently used, never formally published his method. Ehrlich[11] published his acid haematoxylin formula in 1886. Mayer[12] is given credit for discussing ripening of the dye and, with Nietzki,[13] for showing that the active ingredient was haematein in 1891 (Bracegirdle).[4]

With the unexpected donation of a piece of logwood from the *Haematoxylon campechianum* tree (fig 1), an opportunity arose to make haematoxylin from first principles. The tree is usually felled at about 10 years, the bark and sap wood chipped off, and the heart wood exported in three foot long pieces. The logs are reduced to chips to extract the dye haematoxylin, according to Baker.[3] In this paper I describe my efforts to repeat this process, and illustrate the results.

Cook[14] published one of the first protocols for preparing and using aqueous haematoxylin stains in 1879, using an alum/copper sulphate "extraction" procedure to remove the haematoxylin from the logwood. Note that in this procedure, despite the work of Mayer[12] and Nietzki[13] and Cole's observation[9] that such extracts contain both haematoxylin and haematein ("differing from one another by two equivalents of hydrogen"), haematein—which was insoluble in the sulphate solution—was considered to be of no use to the histologist. Cook's procedure for extracting haematoxylin was:

"Grind the alum, logwood extract, and sulphate of copper in a mortar, and when powdered, add sufficient water to form a thin paste,

Figure 1 Cross section of trunk of Haematoxylon campecheanum from the Cayman Islands. Longest dimension approximately 15 cm. The sample is darkening with age.

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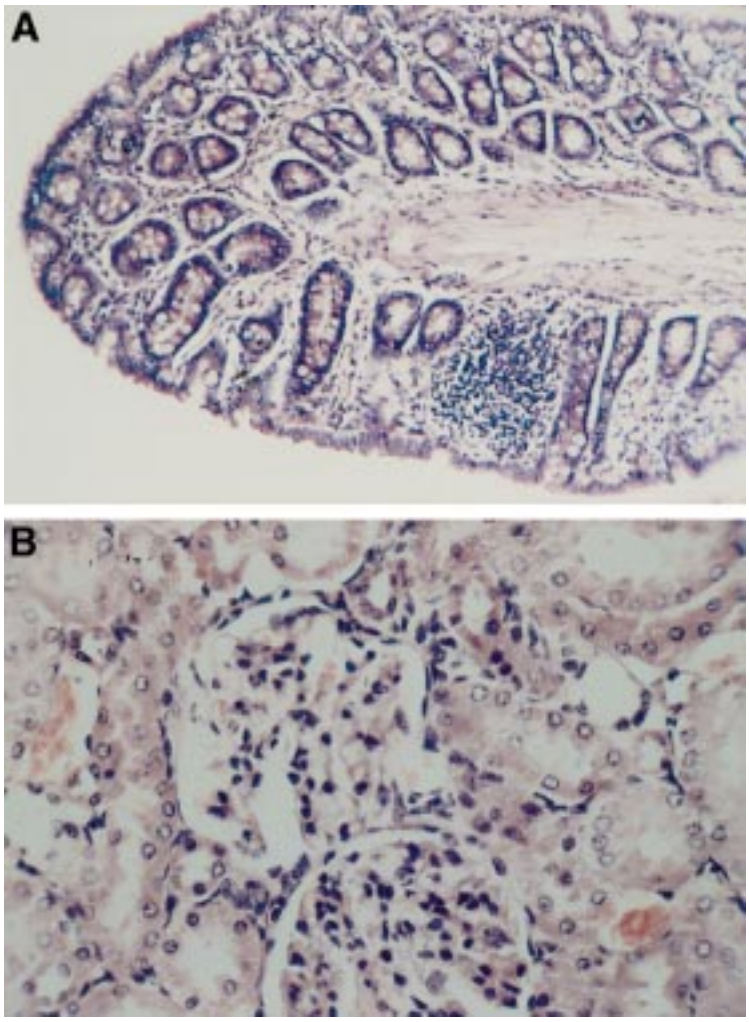

Figure 2 Sections stained with haematoxylin prepared from the illustrated wood and counterstained with aqueous eosin.

leave for one or two days with occasional stirring, and then filter. The haematein contained in the logwood extract will be retained by the filter with the dirt, and the solution consists of haematoxylin, alum and sulphate of copper, to which a crystal of thymol may be added to preserve it from mould.

Logwood extract 6 parts
Alum 6 parts
Sulphate of copper 1 part
Water 40 parts
all ingredients to be free of iron."

It was attempted to follow this procedure exactly. However, published reports of the time gave no explanation of how the logwood was to be prepared. Later methods refer to "finely ground" logwood, a simple description belying the difficulty of grinding this extremely hard wood. A first attempt to grind small sawn pieces in a mortar and pestle proved useless, as did subsequent attempts using a ball mill, when the wood chips proved efficient wedges to restrain the movement of the enclosed ball bearings. Finely ground wood was finally obtained by using the waste from fine drill holes made into the wood. From that stage on, the procedure was without problems.

The extracted haematoxylin efficiently stained nuclei and other basophilic structures, when used for 12 minutes on deparaffinised tissue sections, with the result as illustrated (fig 2). Staining also conformed to the criterion of Mayer[12] that it should be "of a violet colour with a decided touch of blue and should not be at all reddish."

Interestingly, in the light of Cole's comments on haematein,[9] simple alcoholic extraction of the reportedly "useless" dirt residue following filtration resulted in a stronger solution, judged by simple paper chromatography and staining. Entirely satisfactory staining was achieved when a 1% aqueous eosin counterstain was applied (fig 2, A and B). Cell nuclei of a 4 μm section of lymph node were crisp and differentiated the cell chromatin well, using either the aqueous filtrate or the alcohol extracted residue of haematein.

One can only marvel at the initiative of those who originally discovered the dye and extracted it from such hard wood. The only clue to the presence of a dye in the wood used in this experiment came with the colouration of the hands and shirt collar of the investigator. Even that was readily removed, supporting the Elizabethan prohibition, which lasted nearly a century, that the dye not be sold in England owing to its lack of permanence.[3]

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