Dyeing for a diagnosis

Where would pathology be without Sir William Henry Perkin? He was the man who accidentally discovered the colour mauve and his pioneering work led to the production of a rainbow of colours from coal tar aniline.

Sir William Henry Perkin (1838–1907) was a chemist, and his discovery of a mauve-coloured dyestuff was accidental. In Perkin's own words – while attempting to synthesise quinine – "I took a cold solution of sulphate of aniline, or a cold solution of sulphate of toluidine, or a cold solution of sulphate of xylidine, or a mixture of any one of such solutions with any others or other of them, and as much of a cold solution of a soluble bicarbonate as contains base enough to convert the sulphuric acid in any of the above-mentioned solutions to a neutral sulphate. I then mix the solutions and allow them to stand for 10 or 12 hours, when the mixture will consist of a black powder and a solution of neutral sulphate. I then throw the mixture upon a fine filter, and wash it with water until free of neutral sulphate. I then dry the substance thus obtained at a temperature of 100˚C, or 212˚F, and digest it repeatedly with coal tar naphtha, until it is free from a brown substance which is extracted by the naphtha. I then free the residue from the naphtha by evaporation, and digest it with methylated spirit… which dissolves out the new colouring matter."


Sir William Henry Perkin.

THE COLOUR PURPLE

At an event held in the Midland Hotel in Manchester in 1956, the attendees gathered to celebrate '100 Years of Mauve'. One exhibit gave the CIE system coordinates (328, 238, 434 and 27.6%) for the colour that changed the world. These figures define the redness, greenness, blueness and lightness of the colour. So, if you want to see what mauve really looks like, the answer is in the numbers.

WORKING WITH COAL TAR

In addition to Perkin's discovery of purple, his work with coal tar enabled Paul Ehrlich to pioneer immunology and chemotherapy, helped Robert Koch with his discovery of tuberculosis and cholera bacilli, led Dr Hugo Schweitzer to suggest that Perkin's work led to groundbreaking advances in pain relief for those with cancer, and led to the discovery of saccharin by Schweitzer.

ANALINE DYES IN PATHOLOGY

Synthetic dyes are derived from the modification of the benzene ring, either as benzene, quinone or aniline, by the addition of a chromophore. This can be a paraquinoid ring, an orthoquinoid ring, two azocoupled benzene rings, or a nitro-group attached to a benzene ring. These dyes are not 'fast' without the addition of an auxochrome. The NH_2 group is one of the most common of these and formed the basis of the original dye industry.

Among the dyes used in pathology that are derived from these structures are:

- Aniline blue
- Basic and acid fuchsin
- Bismarck brown
- Carminic acid
- Crystal violet
- Eosin
- Methylene blue
- Neutral red
- Thionin

Aniline dyes also contributed to the study of cells and tissues. For example:

- Beneke (Marburg), in the 1860s, used mauve
- Joseph Janvier Woodward used fuchsin and aniline blue
- Paul Ehrlich used methyl green to stain nuclei green and cytoplasm red (reactive dyeing)
- Carl Weigert (Ehrlich's cousin) used methyl violet to stain bacteria in tissue
- Robert Koch used methylene blue to stain tubercle bacilli.

ANILINE DYES IN THERAPEUTIC MEDICINE

- Joseph Lister used methylene blue as an antiseptic
- Methylene blue was used to transform haemoglobin into methaemoglobin for treating cyanide poisoning
- Congo red was used to treat infectious rheumatism and diphtheria
- Scarlet red was used to treat chronic ulcers and burns by stimulating cell growth
- Acridine yellow was used as an antibacterial agent in the First World War
- Mercurochrome (a fluorescein dye) was used as a disinfectant for small wounds
- Gentian violet was used in antibacterial and antifungal therapy.

THE NAME OF THE COLOUR

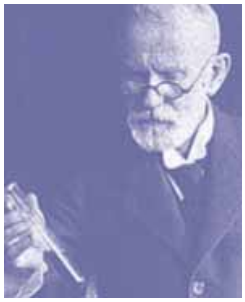
In 1956, Deane B Judd of the National Bureau of Standards in Washington provided a breakdown of the sources of the 7500 colour names in use at the time. Among these:

- 528 were derived from flowers
- 427 had place names
- 340 were pure colour names
- 290 were pigment names
- 254 were derived from fruits
- 239 from other foods
- 221 had names of people
- 214 were derived from substances
- 200 had personal names
- 183 were derived from botanical names

STAINING EPONYMS

Paul Ehrlich

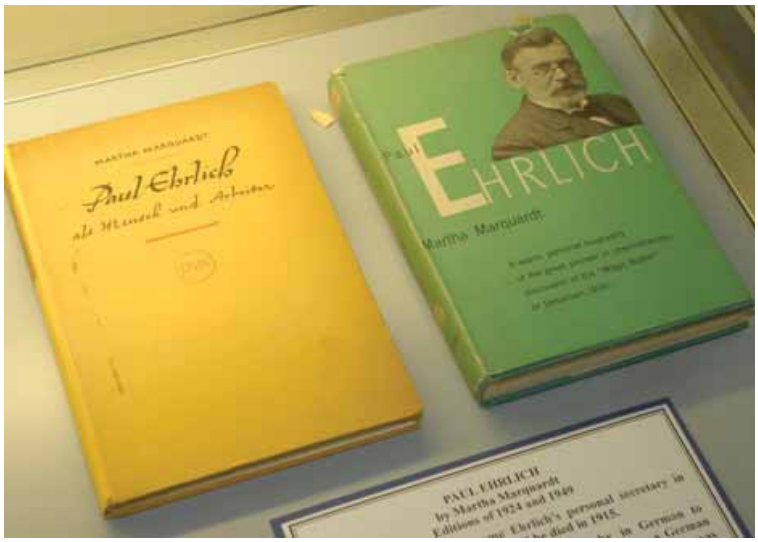
Paul Ehrlich (1854–1915) was called the 'Father of Immunology'; he was an organic chemist, histologist, immunologist, haematologist and pharmacologist. While a student, he discovered and named the mast cell. His doctoral thesis was entitled 'Contributions to the Theory and Practice of Histological Staining'. In 1887, he confirmed tuberculosis in


Paul Ehrlich.

himself (via a sputum sample) using his own stain – carbol methyl violet in aniline oil, differentiated with 30% nitric acid, and counterstained with Vesuvin (Bismarck brown Y).

Ehrlich identified three types of white blood cell by their affinities for alkaline, acidic and neutral dyes, as well as normoblasts, megaloblasts and leukaemic cells. In 1879, he developed a neutral stain that could simultaneously stain acidophil and basophil leucocytes. This stain also demonstrated the violet granules of neutrophil leucocytes.

Ehrlich's triacid stain was prepared by gradually pouring saturated aqueous methylene green into saturated aqueous acid fuchsin until granules precipitated. These were redesolved with additional acid fuchsin, and finally orange G was added.



Two rare books on Paul Erhlich formed part of the History Committee's display, Dyeing for a Diagnosis, at the IBMS Congress.

Ira van Gieson

Ira van Gieson (1866–1913) was an American neurologist, psychiatrist, bacteriologist and neuropathologist. He introduced his picric acid/acid fuchsin stain initially for use in neurohistology. In laboratory notes of technical methods for the nervous system, he wrote: "Sections which have been properly hardened in Müllers fluid and then in alcohol are stained rather deeply with haematoxylin – preferably Delafield's solution – to colour the nuclei. They are then washed in water, and left for three to five minutes in acid fuchsin and picric acid mixture prepared as follows: a few drops of a saturated aqueous solution of Grübler's acid fuchsin is added to one hundred c.c. [*sic*] of a saturated aqueous solution of picric acid, until the mixture has a dark garnet colour. The sections are then rapidly washed in water and in two volumes of alcohol, cleared in oil of origanum, and mounted in balsam. This stain selects the ganglion cells, neuroglia, blood vessels and sclerotic areas, distinctly giving them a garnet colour. The axis cylinders are stained red and the myelin is stained yellow. This stain is used considerably in the laboratory in place of carmine."

Santiago Ramón y Cajal

Ramón y Cajal's most famous studies were on the fine structure of the central nervous system. Cajal (1852–1934) used a histological staining technique developed by his contemporary Camillo Golgi. Golgi found that by treating brain tissue with a silver chromate solution, a relatively small number of neurons in the brain were darkly stained. This allowed Cajal to resolve in detail the structure of individual neurons and led him to conclude that nervous tissue was composed of individual, autonomous cells, instead of a continuous web. Cajal was instrumental in compiling work to support the 'neuron doctrine', the idea that neurons are the basic structural and functional units of the nervous system. He published over 100 scientific works and articles in French, Spanish and German. Among his most notable were Rules and advices on scientific investigation, Histology, Degeneration and regeneration of the nervous system, Manual of normal histology and micrographic technique, Elements of histology, Manual of general pathological anatomy, New ideas on the fine anatomy of the nerve centres, Textbook on the nervous system of man and the vertebrates, and The retina of vertebrates. In 1905, he published five science fiction 'Vacation Stories' under the pen name Dr Bacteria. The asteroid 117413 Ramonycajal is named in his honour.

Gerhard Domagk.

George Papanicolaou

George Papanicolaou (1883–1962) invented the stain that bears his name and which is inextricably linked with the screening process to detect premalignant change in the cervical epithelium. According to Papanicolaou, "The first observation of cancer cells in a smear of the uterine cervix was one of the most thrilling experiences of my scientific career." This was made possible by the use of the now well-known stain consisting of the dyes eosin Y, light green SF, Bismarck brown (not used by all manufacturers) and orange G.

PRONTOSIL – THE MIRACLE DRUG

Paul Ehrlich was the first to postulate that, as part of an aniline dye was responsible for attachment to a microorganism and part was responsible for the colour, then this molecular structure could be used to carry a toxic component to kill an infectious agent without harming the host (patient). During the First World War, the experimental pathologist Gerhard Domagk (1895–1964) recognised the antibacterial properties of sulphonamide-containing azo dyes. On Christmas Day 1932, I G Farben (a research collaborator) applied for a German patent for the red dye Prontosil (prontosil rubrum) as a therapeutic drug. In December 1935, Domagk's daughter suffered a needlestick injury to her hand and wrist, which led to a severe streptococcal infection involving inflammation of her entire arm. Domagk gained the permission of his daughter's surgeon to treat her with Prontosil. Within four days (following oral, rectal and intravenous treatment) her temperature had returned to normal.

CHROMOGENIC BACTERIOLOGY

Chromogenic media contain a chromogenic substrate – a compound or substance that contains a colour-forming group. Such chromogenic substances produce various colours depending on the enzyme activity of different bacteria species. Colour-forming groups (chromophores) that can be attached to the substrate include derivatives of indoxyl nitrophenol and nitroaniline

WHY 'CONGO' RED?

In 1883, a young chemist, Paul Bottiger, was attempting to develop a dye that would work as a pH indicator; but instead he came up with a brilliant red dye that would stain textiles without the use of mordants (all previous aniline dyes needed this extra step).

Bottiger patented this dye in 1884 and then sold the patent to AGFA. In 1885, German Chancellor Otto von Bismarck presided over

Murex brandaris.

the Berlin West Africa Conference that discussed free trade issues with the Congo River Basin area – a well-publicised conference at a time of colonialisation – and a diplomatic flashpoint. Three weeks after this conference, AGFA filed a patent for Congo red – using the highly publicised conference as a marketing aid for the introduction of the dye to the market place.

IMPERIAL PURPLE

Imperial purple – also known as Royal purple and Tyrian purple – is an oxidation product of a mucous secretion from the hypobranchial gland of *Murex brandaris* (the spiny dye-murex). This sea snail uses the secretion to warn off predators – where it turns purple in sea water. It also uses it as an antimicrobial agent on its eggs. The method of collection was to insert a hair into the gland and drag out the secretion. It can only be imagined how labour-intensive and time-consuming this was. Owing to its scarcity, the Roman Imperial families banned its use for dyeing cloth to everyone but themselves. The purple dye produced by William Perkin made the collection and production of this natural dye unnecessary – but not until 1856!

This article is based on material presented by the IBMS History Committee at the recent Biomedical Science Congress.

A short history of aniline dyes and the industry they spawned

- In 1825, Michael Faraday isolated benzene from the destructive distillation of whale oil
- In 1826, Otto Unverdorben isolated aniline from indigo
- In 1834, Friedlieb Runge isolated aniline and phenol from coal tar
- Non-destructive distillation started producing chemicals from coal tar – chemicals such as benzene, toluene, aniline, phenol and naphthalene
- It was thought that as these compounds came from the destruction of organic compounds, perhaps it would be possible to make good and useful things by putting them back together again
- At that time, and as a result of colonialism, malaria was a problem for the British Empire, and the best treatment for malaria was quinine, extracted from the bark of a South American tree
- To promote chemical innovation, the British started up the Royal College of Chemistry in 1845, with the German chemist Wilhelm Hofmann as director
- In 1856, Hofmann's assistant, William Perkin, set out to synthesise quinine from coal tar. He reacted aniline with potassium dichromate, a strong oxidising agent. The resulting black material was definitely not quinine, but it made a beautiful purple solution in alcohol. Perkin called it 'mauveine' and dropped out of college at the age of 18 to develop his new synthetic dye
- British dyers did not think that mauve would catch on, but the Paris fashion houses liked it so much that Perkin was able to retire at the age of 36, almost a millionaire
- The realisation that dyes could be made from coal tar led to variations on the original synthesis, producing dozens of dyes from aniline: aniline reds, aniline violets, aniline greens, yellows, browns and blues. Substituting phenol or naphthalene for aniline produced two more distinct families of artificial colours. There seemed to be few colours that could not be fashioned by art and ingenuity from coal tar
- In France, synthetic dye manufacturers charged so much for their products that demand shifted to natural and imported dyes
- German unification in 1871 made patent enforcement easier than had previously been the case, but by that time the big players in German dyestuffs were established: Badische Anilin und Soda Fabrik (BASF) in 1861; Farbwerke Hoechst in 1862; Freidrich Bayer in 1863; Kalle & Co in 1864; and Aktien-Gesellschaft für Anilin-Fabrikation (AGFA) in 1873