Dyeing for a Diagnosis

Where would pathology be without Sir William Henry Perkin? (1838-1907)

The man who accidentally discovered the colour mauve and led to the production of a rainbow of colours from coal tar aniline.

How?

In Perkins' own words: (Whilst attempting to synthesise quinine)

“...It took a cold solution of sulphate of aniline, or a cold solution of sulphate of toluidine, or a cold solution of sulphate of alysidine, or a mixture of any one of such solutions with any others or other of them, and so much of a cold solution of a soluble bisulphate 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 ten or twelve 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 degrees centigrade, or 212 degrees Fahrenheit, and digest it repeatedly with coal tar naphtha, until it is free of neutral sulphate. I then mix the solutions, and add just enough of a cold solution of a soluble bicarbonate as contains base enough to convert the 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 sulphate of aniline, or a cold solution of sulphate of toluol, or a cold solution of sulphate of alysidine, or a mixture of any one of such solutions with any others or other of them, 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 ten or twelve 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 degrees centigrade, or 212 degrees Fahrenheit, 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”.

328, 238, 434, and 27.6%.

...and Perkin’s work with coal tar.

• Enhanced 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.
• Led to the discovery of saccharin by Hugo Schweitzer.

Aniline dyes in therapeutic medicine.

• Joseph Lister - Methylene Blue as an antiseptic
• Methylene Blue - transformation of haemoglobin into methaemoglobin for treating cyanide poisoning
• Congo Red - to treat infectious rheumatism and syphilis
• Sulfacet Red - to treat chronic ulcers and burns by stimulating cell growth
• Acridine Yellow - antibacterial agent used in First World War
• Mercurochrome (fluorescein dye) - a disinfectant for small wounds
• Gentian Violet - antibacterial and antifungal therapy

And 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 parquinoid ring, an orthoquinoid ring, two naphthyl benzene rings, or a naphthyl group attached to a benzene ring. These dyes are not “fast” without the addition of an xanthene chromophore. The 6H group is one of the constant of these and forms the basis of the original dye industry. Amongst the dyes used in pathology that are derived from these structures are:
• Basic and Acid Fuchsin - Green, to stain nuclei and cytoplasm red
• Gentian Violet - antibacterial and antifungal therapy

How aniline dyes contributed to the study of cells and tissues.

• 1870s Bunsen (Marburg) - used mauve.
• Joseph Laccier (Wochward) - Fuchsin and Aniline Blue.
• Paul Ehrlich - Methyl Green, to stain nuclei green and cytoplasm red (reactive dying).
• Carl Weigert (Berlin's cousin) - Methyl Violet to stain bacteria in tissue.
• Robert Koch - Methylene Blue to stain tubercular bacilli.

300 were derived from substances
• 239 were derived from fruit
• 292 had personal names
• 243 from other foods
• 200 had pigment names
• 230 were derived from flowers


The 7500 colour names in use at the time:
• 528 were derived from minerals
• 427 had place names
• 340 were pure colour names

1886 Benzole (Marburg) - used mauve.
• Joseph Laccier (Wochward) - Fuchsin and Aniline Blue.
• Paul Ehrlich - Methyl Green, to stain nuclei green and cytoplasm red (reactive dying).
• Carl Weigert (Berlin's cousin) - Methyl Violet to stain bacteria in tissue.
• Robert Koch - Methylene Blue to stain tubercular bacilli.

A “Must Read” for anyone interested in dye technology, social history, fashion, or industry.

Paul Ehrlich
(1854-1915)
Organic chemist, Histologist, Immunologist, Haematologist, Pharmacologist.

“The Father of Immunology”

• Whilst a student – discovered and named the Mast Cell.
• Doctoral thesis was titled: “Contributions to the Theory and Practice of Histological Staining”.
• In 1887 confirmed Tuberculosis in himself (sputum) using his own stain – Carbol Methyl Violet in Aniline oil and differentiated with 50% Nitric acid Acid and counterstained with Vesuvin (Bismark Brown Y).
• 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.

Ehrlic’s Triacid stain
Gradually pour saturated aqueous Methylene Green into saturated aqueous Acid Fuchsin until granules precipitate. Redesolve with additional Acid Fuchsin. Add Orang G.

Henry Edward Schunk
Born – Manchester 1820
• Studied dyeing with the aim of taking over his fathers textile industry but, in his spare time:–
• (Whilst studying) isolated Lecanoric acid, Erythric acid, and Orsellinic acid from lichens – the precursors of Orcinol (the origin of Lichen Purple)
• Isolated Alizarin and Purpurin from Madder
• Extracted Indigo Blue from the Woad plant
• Identified Tyrann Purple (Royal Purple) – as an indigo derivative.
• Identified the similarities in structure between chlorophyll and haemoglobin.

Herman Hoffmann
Professor of Botany
– Geissen University
• The study of bacteria by means of dyes grew out of staining methods used in histology.
• An attempt to stain bacteria, using carmine and fuchsin in watery solutions, had been made in 1869 by Hermann Hoffmann, professor of botany (and a mycologist) at Giessen University (aka Justus Liebig University).

Ira van Gieson
1866 - 1913
• American neurologist, psychiatrist, bacteriologist and neuropathologist.
• Introduced his Fuchsin / Acid Fuchsin stain initially for use in neurohistology.
• Laboratory notes of technical methods for the nervous system:
  Acid Fuchsin And Picric-acid Mixture For Staining Sections Of The Peripheral Nerves And Central Nervous System.
  Sections which have been properly hardened in Müllers fluid and then in alcohol are stained rather deeply with haematoxylin – preferably Delafield’s solution – to color the nuclei. They are then washed in water and left for three to five minutes in acid fuchsin and picric-acid muriatic acid mixture project at which a drop or two are applied to the section. The mixture of acid fuchsin and picric acid a mixture of equal parts of acid fuchsin and dilute acid. The mixture has the characteristic rose-red color. The sections are then rapidly washed in water and in two volumes of alcohol and dried. The sections are then stained in Delafield’s solution of haematoxylin. The nuclei stain deep blue and the cytoplasm is stained yellow. The myelin sheath is stained yellow and the axons are stained red. The vacuoles and the cytoplasm are stained blue.

Eos (“dawn”) was, in Greek mythology, the Titan goddess of the dawn, who rose from her home at the edge of Oceanus, the Ocean that surrounds the world, to herald her brother Helios, the sun. As the dawn goddess, she opened the gates of heaven (with “rosy fingers”) so that Helios could ride his chariot across the sky every day.

Eosinophilia/Eosinophilic
(der. Eos, the goddess of the dawn)

• The term eosinophilia is used to indicate an increase in the number of eosinophilic granulocytes (eosinophils) occurring in the blood. Normal counts are about 5%, but can be raised to 10%-80% in disease.
• How many of you knew of the derivation of the terms from the Greek Goddess of the Dawn?

Dyeing for a Diagnosis
Produced by the Institute of Biomedical Science History Committee
Santiago Ramón y Cajal

Ramón y Cajal's most famous studies were on the fine structure of the central nervous system. Cajal used a histological staining technique developed by his contemporary Camilo 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-fictional “Vacation Stories” under the pen name “Dr. Bacteria.”

The asteroid 117413 Ramonycajal is named in his honour.

Some natural dyes

- Brazil Wood extract - (Brazilin [leuco form], and Brazilein [oxidised form] and Neutral Red 24)
- On dying with different mordants can give 8 different colours (brown, pink, black, violet, marlet, grey, marcon, orange)
- Brazil derived its name from the tree – not the other way around.
- Logwood extract – (Haematoxylin, and Natural Black 1)
- An easily extracted compound from the tree Haematoxylovum campechianum
- Green (Archil, Gerbil), Launus, Lethrin, Chrus Red 3, Neutral Red 28
- Drolin is extracted from the Arctis Ilchen Rooselik tinctoria and is converted to carmine by the action of ammonia and air.
- Carmine - from the Cochineal insect
- Czoter’s Cochineal Solution. 7 gm. of powdered cochineal (scale insect) and 7 gm. of roasted alum are kept suspended in 100 c.c. of water by stirring while the mixture is boiled down to half its volume.
- Ref: A text-book of histology including microscopic technic by a. A. Bohm, m. D., And M. Von davidoff, m. D. – 1904
- Morinda lucida (Brimstone tree) – origin Nigeria
- An anthraquinone extracted from the root of Morinda lucida has been used as a stain for histology, staining collagen fibres, muscles and red blood cells golden yellow-brown.
Prontosil – the miracle drug

Paul Ehrlich was the first to postulate that, since part of 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 World War I the experimental pathologist Gerhard Domagk (see photo) recognised the antibacterial properties of multime-containing an dyes. On Christmas Day 1932 G Parten (a research collaborator) applied for a German patent for the red dye Prontosil (“prontosil rubrum”) as a therapeutic drug.

December 4th 1935

Domagk’s daughter suffered a needle stick injury to her hand and wrist, leading to a severe streptococcal infection. This later led to inflammation of her whole arm. Domagk had the permission of his daughter’s treating surgeon to treat her with Prontosil. Within 4 days (bilirubin reduced, rectal, and intravenous treatment) her temperature had returned to normal.

A short history of aniline dyes and the industry they spawned

• In 1856 Michael Faraday insisted on monochromatic destructive distillation of coal tar.
• In 1856 Otto Unverdorben isolated aniline from floggan.
• In 1859 Prussian Blue isolated anilines and phthal from coal tar.
• Non-destructive distillation started producing chemicals from coal tar such as chemicals like benzene, toluene, aniline, phenol, and naphthalene.
• It was thought that since these compounds came from the decomposition of coal tar compounds maybe it would be possible to synthesise useful things by putting them back together again.
• As that time coal tar was 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 great German chemist, Wilhelm Hofmann, as director.
• In 1856 Wilhelm Hofmann, the great German chemist, William Perkin, set out to synthesize the red dye Alizarin from coal tar. He reached anilines with phenyl substitution, a strong oxidizing agent, the resulting thing was a quinine, but it made a beautiful purple solution in alcohol. Perkin called it “mauve” and dropped out of college at the age of 28 to work on this new synthetic dye.
• Scottish chemists didn’t think that mauve would catch on, but the French fashion houses liked it so much that Perkin was able to retire at the age of 36 almost a millionaire.
• The realization that dyes could be made from coal tar led to variations on the original symbiosis with natural dyes from aniline: aniline red, aniline violet, indigo blue, brown, and blue. Substituting phenyl for aniline produced derivatives like benzidine and derivatives like artificial colors.
• The synthesis of these color which could not be made from coal tar.
• In France, synthetic dyes mass manufactured and were much for their products that demanded small amounts of natural and imported dyes.
• German anilinewerke in 1872 made patent enforcement much more difficult and the case, but by that time the big players in German were:
• Badische Anilin und Soda Fabrik (BASF) - 1861.
• Farbwerke Hoechst – 1862.
• Barmische Anilin und Soda Fabrik (BASF) – 1861.
• Farbwurke and GM – 1862.
• Friedrich Bayer - 1863.
• Kalle & Co - 1865.
• ‘Aktion-Gesellschaft für Anilin-Fabrikation’, AGFA. – 1873

Dyeing for a Diagnosis
Produced by the Institute of Biomedical Science History Committee

Questions

- Why was Alizarin helpful to Alexander the Great?
- How did Prussian Blue get its name?
- Why did women wear long fingernails once venture out before sunrise with lanterns?
- What was supposed discovered when Hurcules’ dog bit into a marine gastropod?