100 YEARS OF BIOMEDICAL SCIENCE

IBMS

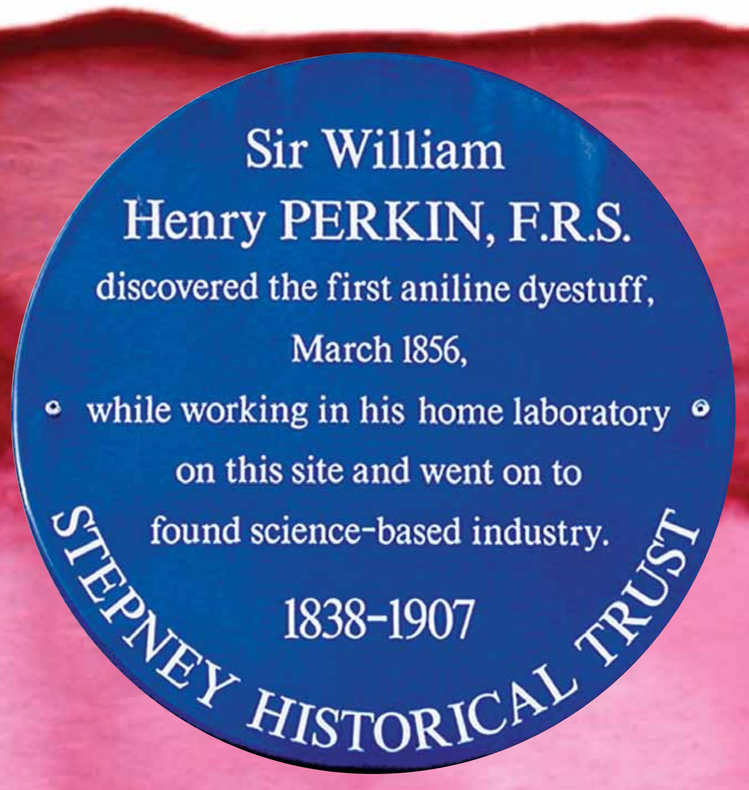
1912 - 2012

Dyeing for a Diagnosis

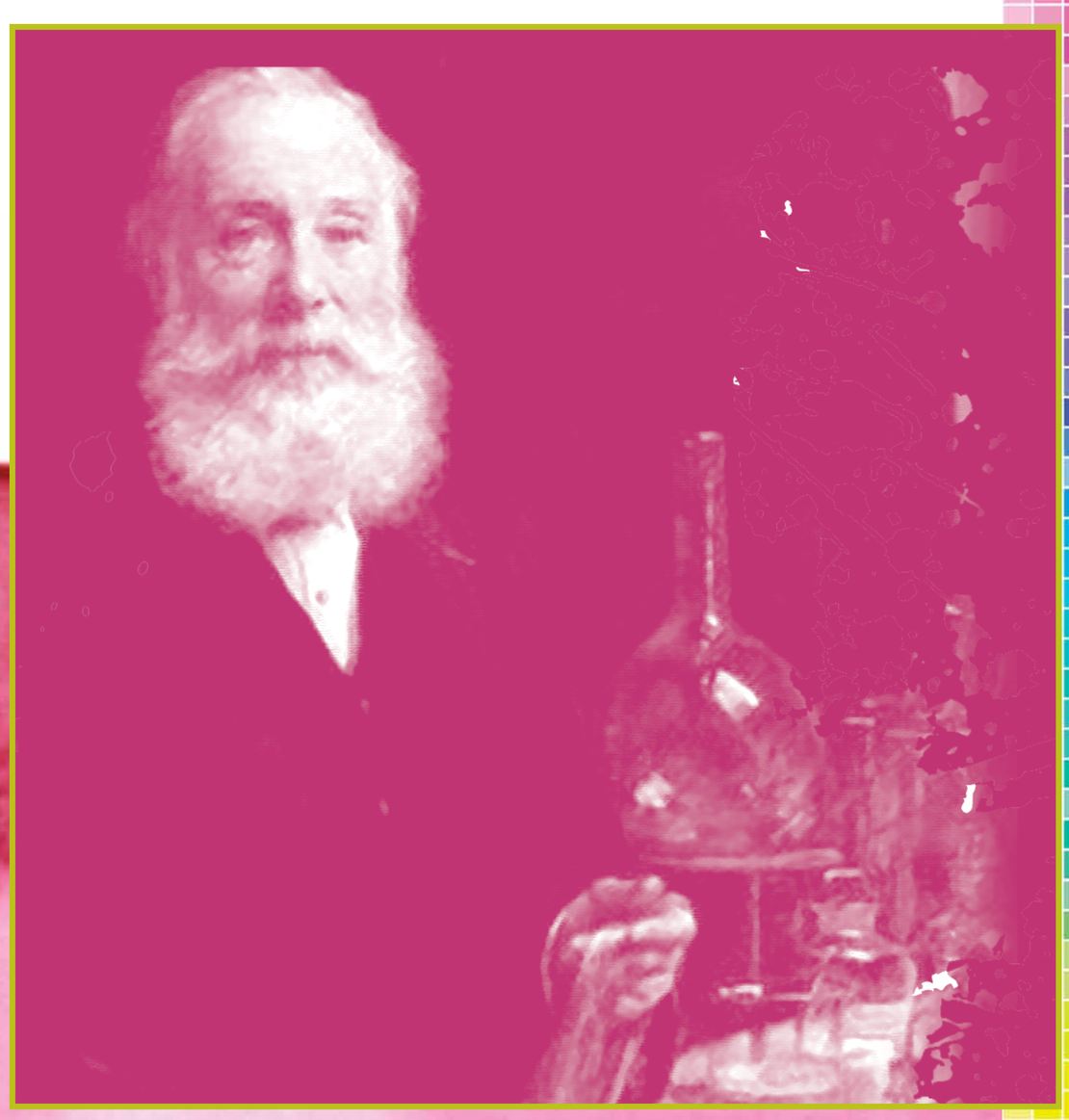
Where would pathology be without Sir William Henry Perkin?

(1838-1907)

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



The Blue Plaque where No 3 King David Lane, Shadwell, once stood – the birthplace, home, and laboratory of William Perkin.



How?

In Perkins' own words: (Whilst 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 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 Farenheite, 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 spirist ... which dissolves out the new colouring matter".

328, 238, 434, and 27.6%.

At an event in the Midland Hotel in Manchester in 1956 the attendees gathered to celebrate "100 Years of Mauve".

One exhibit gave the CIE system co-ordinates for the colour that changed the world. These define the redness, greenness, blueness, and lightness of the colour.

So – if you want to see what "Mauve" really looks like, here is your answer.

..and Perkin's 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 Perkins work led to groundbreaking advances in pain relief for those with cancer.
- Led to the discovery of saccharin by Hugo Schweitzer

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

- 1860s Beneke (Marburg) – used mauve.
- Joseph Janvier Woodward – Fuchsine and Aniline Blue
- Paul Ehrlich – Methyl Green, to stain nuclei green and cytoplasm red (reactive dying)
- Carl Weigert (Ehrlic's cousin) – Methyl Violet to stain bacteria in tissue
- Robert Koch – Methylene Blue to stain tubercul bacilli.

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 diphtheria
- Scarlet 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 Paraquinoid ring, an Orthoquinoid ring, two azo-coupled 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 commonest of these and formed the basis of the original dye industry.

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

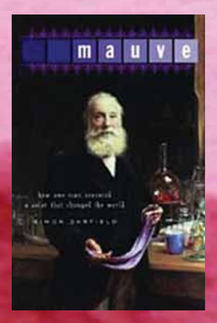
- Basic and Acid Fuchsin
- Crystal Violet
- Aniline Blue
- Eosin
- Thionin
- Methylene Blue
- Neutral Rerd
- Carminic Acid
- Bismark Brown

...and just for interest. The names of colours.

In 1956 Deane B Judd of the National Bureau of Standards in Washington.

Of the 7500 colour names in use at the time:

- 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 peoples
- 214 were derived from substances
- 200 had personal names
- 183 were derived from botanical names



A "Must Read" for anyone interested in dye technology, social history, fashion, or industry.
MAUVE – How one man invented a colour that changed the world.
Simon Garfield 2000.
Faber and Faber. ISBN 0-571-20197-0

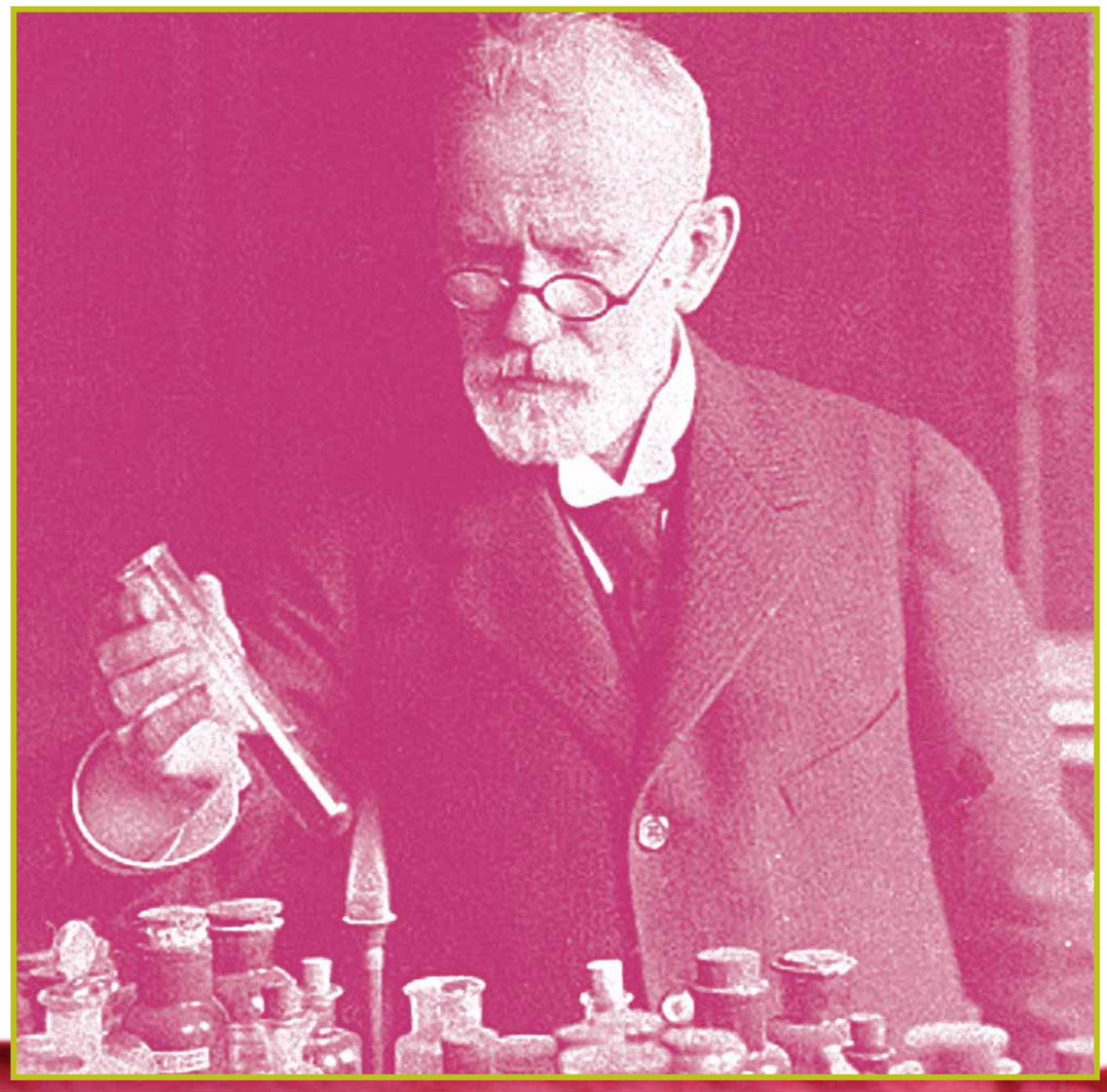
Dyeing for a Diagnosis

Produced by the Institute of Biomedical Science History Committee

100 YEARS OF BIOMEDICAL SCIENCE

IBMS

1912 - 2012



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 30% 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

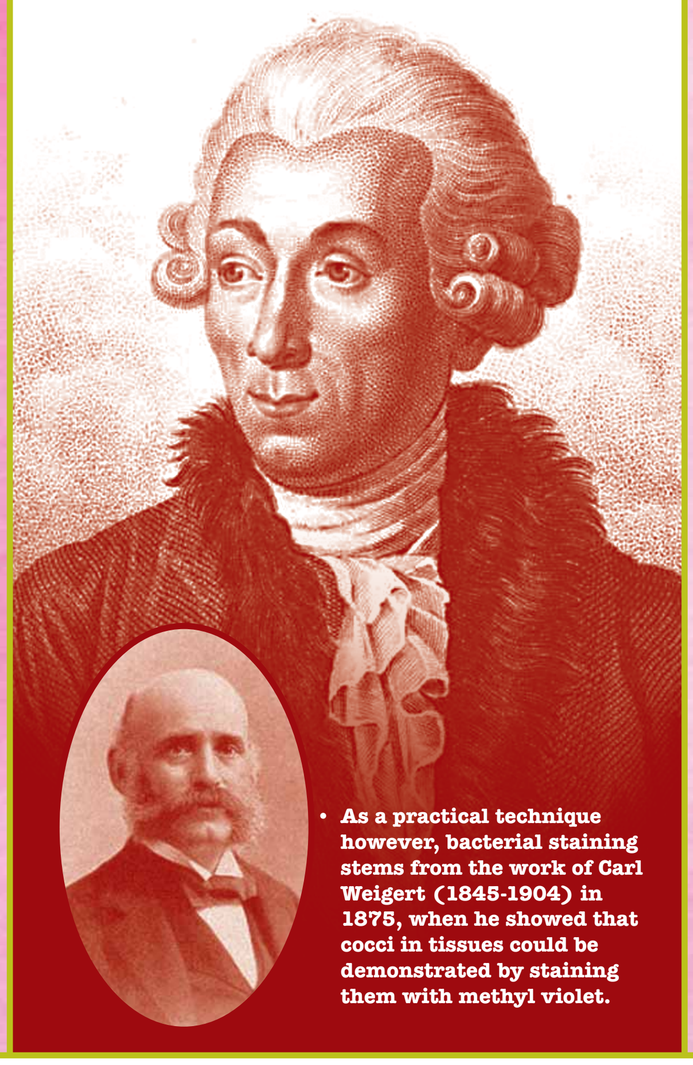
Born – Manchester 1820

- 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 Tyrian Purple (Royal Purple) – from the shellfish Nucella lapillus – 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).



- **As a practical technique however, bacterial staining stems from the work of Carl Weigert (1845-1904) in 1875, when he showed that cocci in tissues could be demonstrated by staining them with methyl violet.**

Ira van Gieson

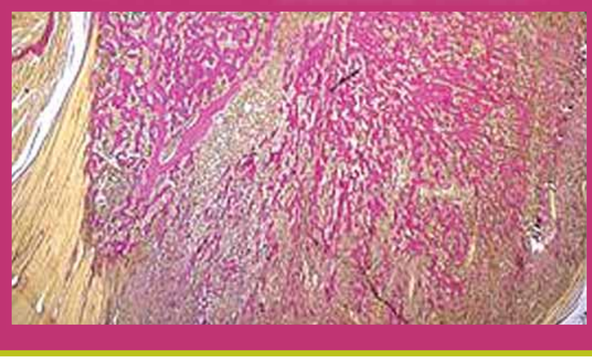
1866 - 1913

- American neurologist, psychiatrist, bacteriologist and neuropathologist.
- Introduced his Picric Acid / 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 mixture prepared as follows: A few drops of a saturated aqueous solution of Grübler's acid fuchsin is added to one hundred c.c. of a saturated aqueous solution of picric acid, until the mixture has a dark-garnet color. 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 color. The axis cylinders are stained red and the myelin is stained yellow. This stain is used considerably in the laboratory in place of carmine.



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.
- The term eosinophilic is literally "a love for eosin" and is used to describe any structure that has an affinity for the dye eosin – e.g. eosinophils, erythrocytes, intra- and extra-cellular proteins of all kinds.
- How many of you knew of the derivation of the terms from the Greek Goddess of the Dawn?

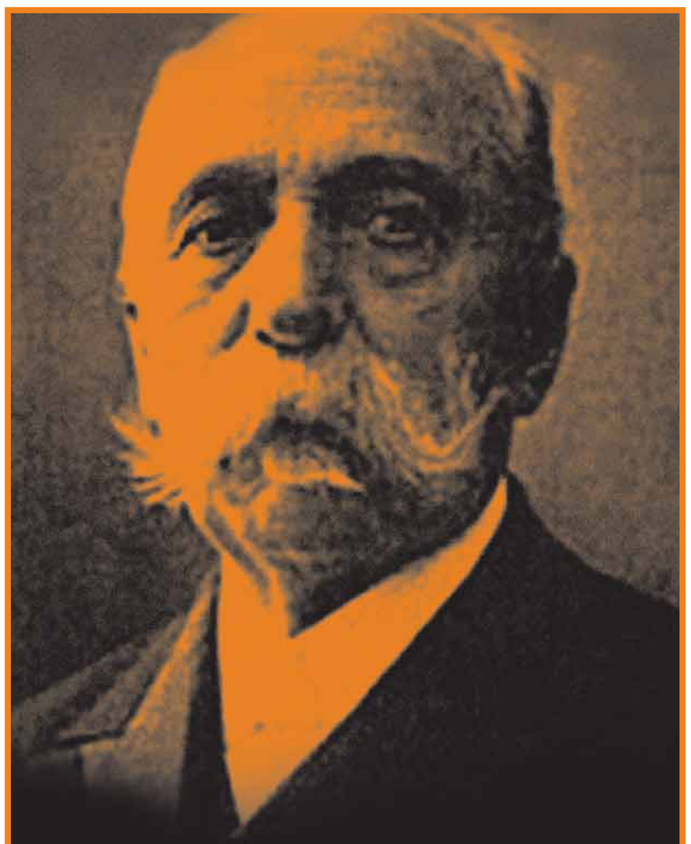
Dyeing for a Diagnosis

Produced by the Institute of Biomedical Science History Committee

100 YEARS OF BIOMEDICAL SCIENCE

IBMS

1912 - 2012

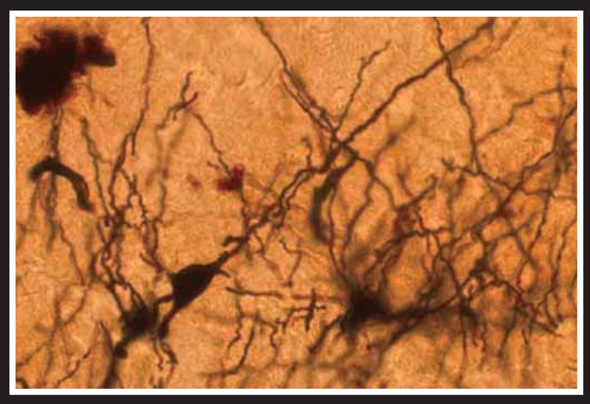


Camilo Golgi

1843-1926

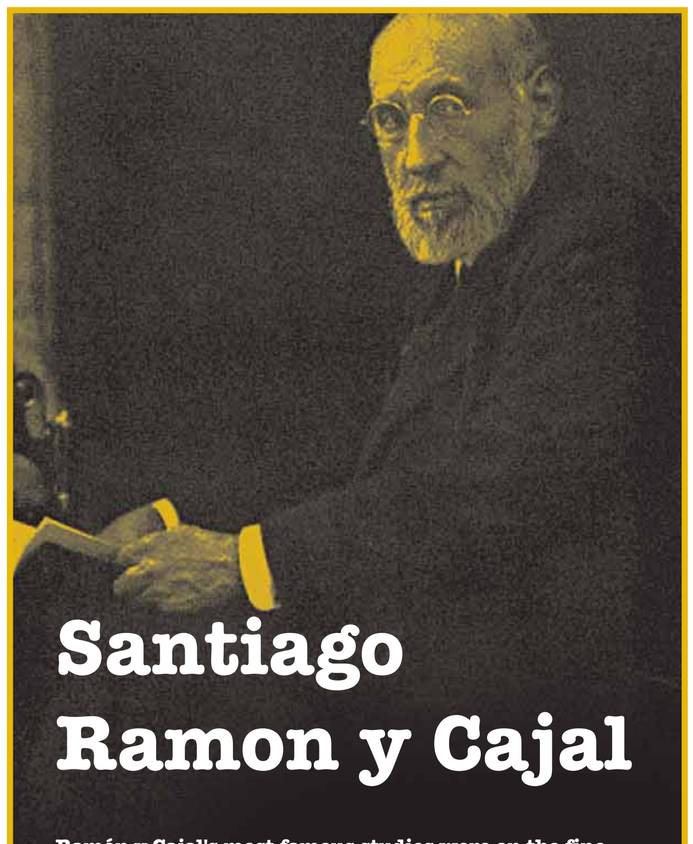
Golgi's method for was initially named the black reaction (la reazione nera) by Golgi, but it became better known as the Golgi stain or later, Golgi method.

- **Cells are filled by microcrystalisation of silver chromate.**



“The method of staining, once having taken root in the animal histologist, grew and grew, till to be an histologist became practically synonymous with being a dyer with this difference, that the professional dyer knew what he was about, while the histologist with few exceptions did not know, nor does he to the present day”.

Gustav Mann, Physiological Histology, 1902



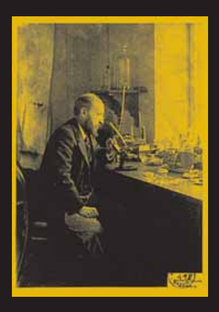
Santiago Ramon y Cajal

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 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-fictional "Vacation Stories" under the pen name "Dr. Bacteria."

The asteroid 117413 Ramonycajal is named in his honour.

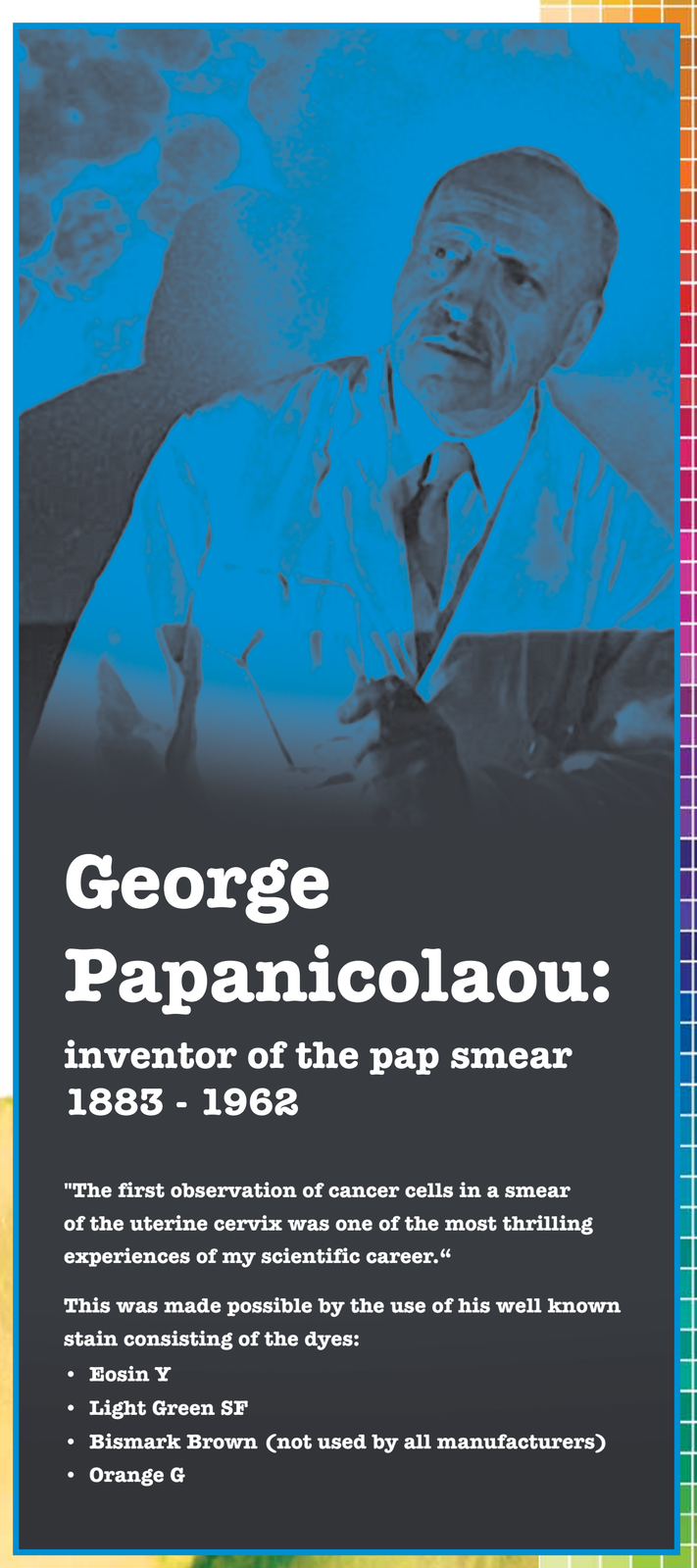


Drawing of Purkinje cells (A) and granule cells (B) from pigeon cerebellum by Santiago Ramón y Cajal, 1899. Instituto Santiago Ramón y Cajal, Madrid, Spain.

Natural dyes

Until the advent of synthetic dyes in the second half of the 19th century, all dyes were derived directly from natural sources – roots, leaves, flowers, berries, stems, roots, insects, shellfish, and minerals.Some of these were:

- Weld – yellow
- Woad – blue
- Madder – red
- Kermes – red
- Shellfish – purple (see Royal Purple)
- Logwood – haematoxylin
- Cochineal - Scarlet



George Papanicolaou:

inventor of the pap smear
1883 - 1962

"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 his well known stain consisting of the dyes:

- **Eosin Y**
- **Light Green SF**
- **Bismark Brown (not used by all manufacturers)**
- **Orange G**

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, scarlet, grey, maroon, crimson)
- 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 Haematoxylum campechianum
- Orcein (Archil, Orchil, Lacmus, Litmus, Citrus Red 2, Neutral Red 28)
- Orcinol is extracted from the Aricil lichen Rocella tinctoria and is converted to orcein by the action of ammonia and air.
- Carmine - from the Cochineal insect
- Czocor'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.

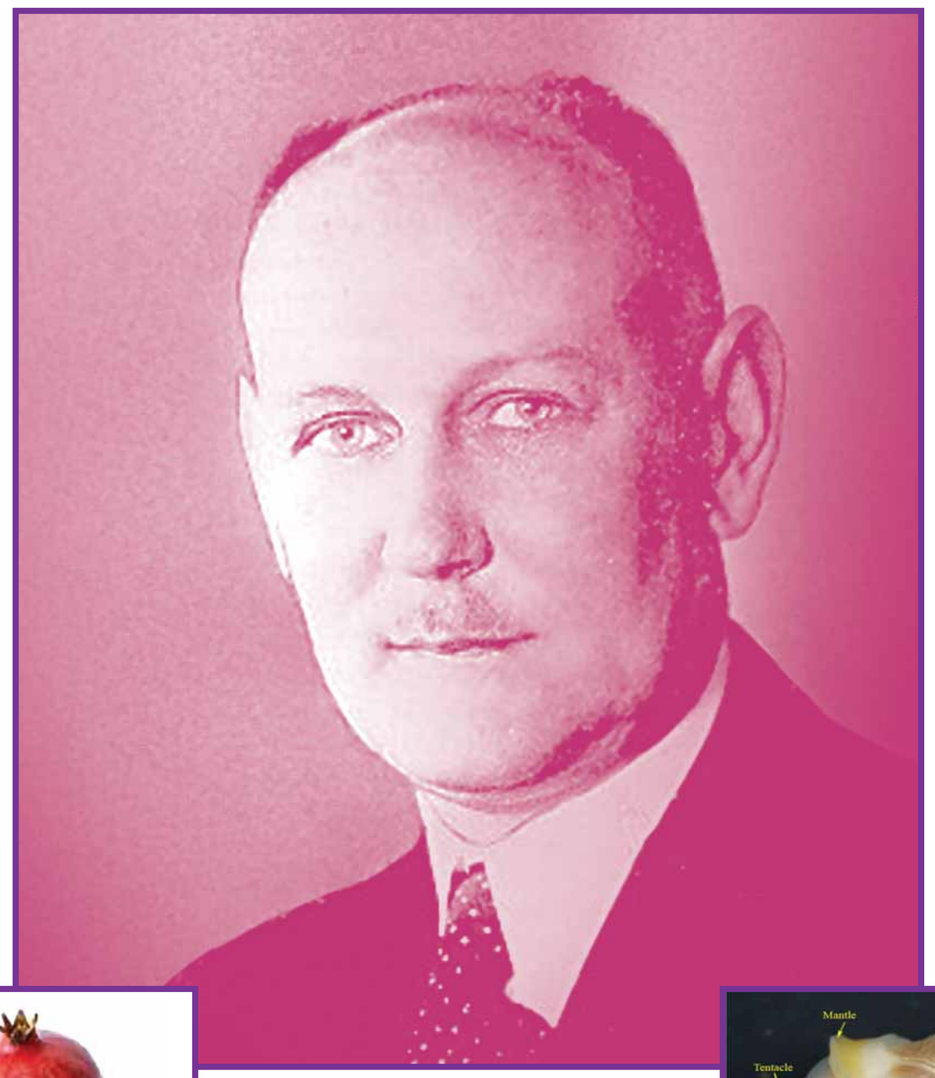
Dyeing for a Diagnosis

Produced by the Institute of Biomedical Science History Committee

100 YEARS OF BIOMEDICAL SCIENCE

IBMS

1912 - 2012



Prontosil – the miracle drug

Paul Ehrlich was the first to postulate that, since part of an aniline dye was responsible for attachment to a micro organism 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 1 the experimental pathologist Gerhard Domagk (see photo) recognised the antibacterial properties of sulfonomide-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.

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 (following oral, rectal, and intravenous treatment) her temperature had returned to normal.

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 such as chemicals like benzene, toluene, aniline, phenol, and naphthalene.**
- **It was thought that since these compounds came from the destruction of organic compounds maybe 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 great German chemist, Wilhelm Hofmann, as director.**
- **In 1856 Hofmann's assistant, William Perkin, set out to synthesize quinine from coal tar. He reacted aniline with potassium dichromate, a strong oxidizing agent. the resulting black goo 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 didn't 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 realization 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 colors. There seemed to be few colors which could not be fashioned by art and ingenuity from coal tar.**
- **In France, synthetic dye manufacturers charged so much for their products that demand there 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) – 1861.**
- **Farbwerke Hoechst – 1862.**
- **Freidrich Bayer -1863.**
- **Kalle & Co – 1864.**
- **'Aktien-Gesellschaft für Anilin-Fabrikation', AGFA. - 1873**

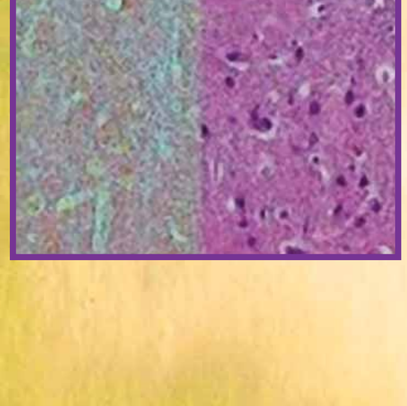
Pomegranate

Gharravi, Golalipour, Ghorbani, and Khazaei have found (2006) that a dye extracted from Pomegranate skin stains CNS cells differentially.

- Astrocytes – yellow
- Astrocytes (Hippocampus) – deep yellow
- Neurones (Hippocampus) – pale red
- Purkinje cells (Cerebellum) – pale red
- Granular cells (Cerebellum) – yellow

How to do it –

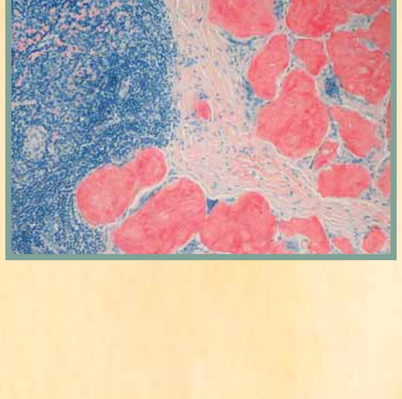
- Pomegranate skin is allowed to dry in light and added to boiling water.
- The concentrated juice is allowed to cool and is filtered.
- Sections are stained for 12-24 hours in the dark
- Sections are washed in running water for 20 minutes
- Counterstain with Eosin for up to 2 mins.
- Remove excess Eosin with 95% and absolute alcohol (2 mins each)
- DCM

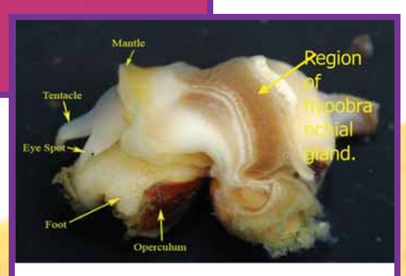


Why "Congo" Red?

In 1883 a young chemist, Paul Bottiger, was attempting to develop a dye that would work as a pH indicator; 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).

- In 1884 Bottiger patented this dye and then sold the patent to AGFA.
- In 1885 Chancellor Otto von Bismark presided over 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.





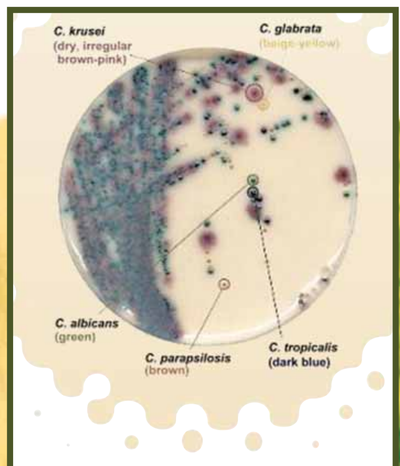
Imperial Purple

aka Royal Purple, Tyrian Purple, and Imperial Purple.

This dye is an oxidation product of a mucus secretion from the hypobranchial gland - located on the dorsal surface of the mantle cavity and running parallel to the gill - of Murex brandaris (the Spiny dye-murex). The animal 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. Because of 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 dye unnecessary – but not until 1856.



Enzyme-dependant colour production and consequent differentiation of clinically significant Candida spp on a chromogenic medium for the detection of hexosaminidase and alkaline phosphatase activity.

Chromogenic media in bacteriology

Chromogenic media contain a chromogenic substrate – a compound or substance that contains a colour-forming group.

Such chromogenic substances produce variable 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
- Nitroaniline

Ref: "Culture" Vol 28, No 2, September 2007 – Oxoid

Questions

- **Why was Alizarin helpful to Alexander the Great?**
- **How did Prussian Blue get its name?**
- **Why did women with long finger nails once venture out on before sunrise with lanterns?**
- **What was supposedly discovered when Hurcules' dog bit into a marine gastropod?**

Dyeing for a Diagnosis

Produced by the Institute of Biomedical Science History Committee