8 Dyes, Stains and Antibiotics

The origins of modern drugs are profoundly mixed up with our interest in brightly coloured cloths and fabrics, and with the dyes that produced them. These dyes, in turn, derived from an accidental by-product of a new invention: gaslight.

In the 1790s, the inventor William Murdoch, working to help industrialise the Cornish tin mines, found that if coal was heated within an enclosed space, it gave off a flammable gas, one that 'burnt with great brilliancy'. By 1794 his house in Redruth was being lit by this 'gaslight'. By 1807 so was Pall Mall in London. Westminster Bridge followed in 1813, then the city of Baltimore in 1816, then Paris in 1820. Over the next few years Murdoch's gaslight began shining out from the world's most developed towns.

England, rich in coal, was not alone in finding that this wonderful new process had an unwanted consequence: a sticky, smelly waste residue called coal tar.

The German chemist Friedrich Runge, in 1834, was working with benzene. Benzene was one of the constituents of coal tar, by that stage a rapidly multiplying and unwanted commodity. Runge treated benzene with chloride of lime and produced a colour so blue that he named the substance cyanol. Other chemists made similar discoveries, giving them different names. In 1855 the German chemist August Wilhelm von Hofmann, looking at all these compounds, realised that they were the same thing. He called them aniline.

Possibly the implications of their colour should have occurred to him, as it also should have to Friedrich Runge. Hofmann admired the colour of his coal-tar-derived aniline, then moved on to studying more important aspects of it than its prettiness.

Brought to England in 1845 at the behest of Prince Albert, Hofmann was the founding director of London's new Royal College of Chemistry. His chemical 'first love' was aniline. The nature of it fascinated him, partly because of the way in which related chemicals had shared properties. This raised the tantalising possibility that their molecular structures – if such things could be worked out – would show similarities that corresponded to and explained their external and chemical ones. Benzene, derived from coal tar by one of Hofmann's English students, was at the core of the family of molecules that included aniline, and which Hofmann called 'aromatic'. He gave them the name because they smelt sweet.

Techniques for determining the structure of molecules were primitive, but chemists were effective at figuring out the elements that composed them. Benzene, for example, was C6H6, although the way those carbons and hydrogens fitted together was a mystery.* Hofmann, like many before him, wanted to synthesise quinine. In an 1849 report to the Royal College of Chemistry, he suggested that:

> . . . it is obvious that napthalidine, differing only by the elements of two equivalents of water, might pass into [quinine] simply by an assumption of water. We cannot, of course, expect to induce the water to enter merely by placing it in context, but a happy experiment may attain this end . . .

Four years after this, a fifteen-year-old boy named William Perkin came to study under Hofmann at the Royal College. For a youngster

* One famously solved by Kekulé dreaming of a snake swallowing its own tail, then waking to the realisation that benzene was a ring.

fascinated by the possibilities of chemistry, there was no mentor better. Not only was Hofmann brilliant, full of love for colleagues and chemicals alike, but his love and his brilliancy were catching. 'Who would not work, and even slave, for Hofmann?' recalled another student. 'There was an indescribable charm in working with Hofmann, in watching his delight at a new result or his pathetic momentary depression when failure attended the attempt to attain a result which theory indicated. "Another dream is gone," he would mutter plaintively, with a deep sigh.'

The natural world that Hofmann lived in was full of wonders. Perkin remembered him wandering around the laboratory happily, admiring the new compounds that were everywhere being derived for the first time, and joining in his students' explorations. 'Taking a little of the substance in a watch glass, he treated it with caustic alkali, and at once obtained a beautiful scarlet salt. Looking up at us in his characteristic and enthusiastic way, he at once exclaimed, Gentlemen, new bodies are floating in the air!'

It is difficult, living in a world that holds 'artificial' as a pejorative, to imagine how fresh and wonderful these colours seemed, the extent to which 'artificiality' meant a fertile combination of human talents and Nature's richness. Chemistry had the potential to offer compounds of power, and the attractions for the chemists were aesthetic as well as intellectual. Perkin and a friend, Arthur Church, were both keen painters. Colours attracted them and aroused their curiosity. While Hofmann viewed colour as something interesting for its chemical implications, Perkin and Church saw it as important in itself. In 1856 they submitted to the Royal Society a report 'On Some New Colouring Matters'. Distillations gave them oranges and crimsons and dark yellows, 'with a lustre somewhat similar to that of murexide'.

Murexide was a curious substance. The original source was a cone-shaped marine snail of the genus *Murex*, which could be

crushed to release tiny quantities of a precious purple dye. (An old myth told of Hercules walking his dog on the shore of the Mediterranean and the dog chewing snails and staining its mouth.) The Romans treasured it, not least because of its rarity – over 10,000 snails were needed to colour a single toga. The German chemist Carl Wilhelm Scheele described making murexide artificially in 1776 from the uric acid of human kidney and bladder stones. Then the doctor William Prout, much interested in the medical problems such stones caused, found that an even richer source for uric acid was the excrement of boa constrictors. (Reptiles, like birds, excrete their protein waste in a more concentrated form than mammals.) By chemical transformation Prout arrived at ammonium purpurate, and he called it murexide for the purple colour that so resembled that of the Phoenician sea snails. By analogy, he suggested that murexide might be useful as a dye – but that was as far as the idea went.

'I was ambitious enough to want to work on this subject,' said Perkin, about Hofmann's dream of making quinine from the coal tar residue napthalidine. He spent his spare hours during the spring and summer of 1856 in a room on the top floor of his father's East End house. Wreathed in the stink of ammonia, surrounded by his experiments in painting and photography, on a desk stained by all his various efforts, he changed the world.

Attempting to produce quinine, which was known to be colourless, Perkins ended up with something red. Wanting to understand where he had gone wrong, he tried a similar experiment using aniline as his starting material. This time he got something that was impressively black, then, after rinsing out the flask he had made it in, he noticed that the alcohol wash left a colour of startling hue and radiance. Perkin had produced mauve.

He found that his colour stained silk, and that the newly mauve fabric kept its appearance despite being washed and hung up in the

sun. At the end of August he filed a patent, and started to explore the potential for his discovery as a commercial dye. It exploded.

Queen Victoria wore mauve to her eldest daughter's wedding in 1858, then again in 1862 when she swept into the Great London Exhibition. In between, Charles Dickens's *All the Year Round* said Perkin's mauve made Tyrian purple look 'tame, dull and earthy'. It was part of a craze for the colour that made Perkin's fortune, stimulating the development of a synthetic dye industry that aimed to take advantage of the rainbow of colours hidden within coal tar. Perkin himself went on to develop Britannia Violet, Perkin's Green, and a method for commercially producing a brilliant red. Others rushed in with yellows and violets, blues and browns and blacks and every shade around them. By 1863 there was even a range of different magentas, named for their inventor: the Hofmann Violets, a belated contribution by the man who had initially failed to see the commercial importance of colourings. This was the new and wholly unexpected world of chemistry; imperial and potent and pregnant with synthetic power.

The impact of the artificial dyes was vast, not only in terms of fashion and economics but also in stimulating the development of organic chemistry. What had been an academic field became of immense industrial importance. This was scientific progress that people could *see*, a visible and vivid reminder of the power of invention, and the promise it held to add colour to people's lives.

Worries about toxicity developed early, partly in response to the real danger posed by arsenic in the manufacture of some dyes. These concerns were almost immediately exaggerated into a widespread belief that all aniline dyes, and possibly all the products of chemists and factories everywhere, were inherently poisonous. Brewers in the north of England discovered that beer made from Thames water had a pleasantly bitter taste by virtue of a molecule it picked up from the river. Picric acid, the molecule in question, began to be added by

northern brewers to produce the same effects. Even as chemists were able to establish molecular similarities – to show that picric acid added to Burton beer was the same as that which the waters of the Thames added in London – people were wondering if a molecule's power lay not in its structure but in its provenance, whether beer with naturally acquired picric acid was safer than when someone had tipped the ingredient in from a flask.

Most of the key steps in the developing dye industry were British. Faraday found benzene, Mansfield showed how to make it on a large scale from coal tar, Perkin and other of his compatriots came up with many of the early colours. Despite it all, in the wake of Prince Albert's death in 1861 Hofmann found that the British were not really interested in science at all, except as an amateur hobby. He returned to Germany. The British chemists that he left behind were struck by their country's lack of interest in encouraging competitive commercialisation of new chemical products. Britain began losing its early lead to German competition.

During the second half of the nineteenth century, Germany led in both industrial chemistry and medical research. Bismarck Brown rapidly joined Britannia Violet in the new pantheon of colours. The industry that Perkin founded in London was adopted far more vigorously on the banks of the Rhine than the Thames. Conducive patent laws, government encouragement and more thoughtful entrepreneurs all helped. Vital, also, was the contempt that scientifically minded Britons tended to feel for commercialising their work. 'England is not the land of science,' said a German delegate to the 1837 Liverpool conference of the British Association for the Advancement of Science. It worshipped the gentleman amateur to a damaging degree. 'There is only widespread dilettantism, [English] chemists are ashamed to be known by that name because it has been assumed by the apothecaries, who are despised.'

By 1879 there were seventeen dye-works in Germany, and only six

in England. When the First World War broke out, Germany was supplying three quarters of the world's dyes. England, despite having given birth to the industry, was importing from Germany 80 per cent of what it needed.

The immediate importance of dyes to medical research was through their power to expose the processes of both health and disease. The history of their use in this way was already old. In 1566, madder, the ancient vegetable dye, was noted to stain the bodies of the sheep that fed on it. An animal's bones turned red. Just over a hundred years later Raymond Vieussens, a French anatomist, was injecting saffron brandy into the necks of animals, pushing it into their carotid arteries to see which bits of their brains changed colour.

At the same time, in England, Robert Hooke was using his 'sharpen'd Pen-knife' to cut thin slices of cork. He went on to examine them with the use of a new tool, the microscope. Leeuwenhoek's demonstration of 'animalcules' with the microscope should have alerted the world to something important. Here was the opportunity to understand crucial new things about the way in which life worked. Had it sparked the interest it deserved, it could have led to the acceptance of germ theory centuries ahead of Pasteur. A few years after Robert Hooke sliced cork, Leeuwenhoek was doing the same. Contact with the Royal Society of which Hooke was a part gave Leeuwenhoek an audience for his research. A letter from Leeuwenhoek to the Royal Society in 1674 had some of his preparations attached to it – thin pieces of cork, of quill, of elder and 'the optic nerve of a cow'.

In a later letter, from 1714, Leeuwenhoek told the Royal Society about his efforts to combine such slices with coloured stains. Like Vieussens, Leeuwenhoek used saffron. He wanted to compare the muscle of a fat cow with that of a thin one, and wrote that:

> Since the fibres, cut into the thinnest possible layers, were so transparent that they could hardly be recognised, I have macerated a little crocus in brandy. To make the flesh particles more visible to the artist, I have merely moistened them with this wine, whereupon they were bright with a yellow colour.

Not many people read Leeuwenhoek's letter, then or after. A Harvard anatomist, Frederick Lewis, coming upon it during the Second World War, was so excited by the discovery that he repeated the experiment, simmering some saffron up in Boston tap water before applying it to a thinly sliced piece of steak and finding that 'the fibers indeed glow with a golden yellow color'.

People had been adding dyes to the soil and water of plants for a long time before – turning lilies red with powdered cinnabar, or using saffron to make roses yellow – but it was not until the early 1700s that Nicolas Sarrabat, a Jesuit priest and natural philosopher in Lyons, made use of the technique to try to determine how plants worked. He used the Mexican pokeweed berry, finding that its colour penetrated the smallest branches of the roots. When the plant under investigation was washed, the stain remained, visibly outlining the portions of the roots where the absence of its epidermis, the plant's skin, allowed the transfer of nutrients and water.

Despite these hints, people were slow to catch on that a dye's ability to stain selective parts of an organism provided a window onto life's inner workings. Intrigued by madder, in 1736 the British surgeon John Belchier sat down to eat a pig that had been fed on the stuff. The bones and the teeth were red. 'Neither the fleshy nor cartilaginous parts', he recorded, 'suffered the least alteration in colour or in taste.'

Attempts over the following twenty years to selectively dye plant structures led to mixed success. Charles Bonnet, a Swiss lawyer with an active interest in science, used madder and rose and black ink to

stain the roots of peas and beans. His efforts, he judged, were 'only weak attempts', but the method was 'a rich mine'. He put camphor in brandy and infused it into a living pear – the leaves took on the camphor's scent, but the fruit seemed not to. After reading Bonnet's 1754 work *Recherches sur l'usage des feuilles dans les plantes*, a medical student named Georg Christian Reichel showed that he could use red stains to prove that the spiral ducts of a plant distributed sap rather than air.

From here on interest accelerated. An English doctor, John Hill, used both cochineal and lead to substantiate his 1770 work *The Construction of Timber*. The vessels by which trees distributed the fluids essential for their lives could, by means of staining, be 'beautifully seen'. Hill developed a machine for cutting sections of his stained wood, a great improvement on Hooke's sharpened penknife, as well as ways of stiffening and blanching the slices that needed it.

Wilhelm Friedrich von Gleichen, who converted his unpromising beginnings to a career of courtly and military success, spent the second part of his life on science. Moved by the work of John Hill and by Leeuwenhoek's animalcules, in 1777 he showed that indigo and cochineal could illuminate the world of these microscopic creatures:

> The bones of animals coloured by the feeding of madder roots led me to this idea. So I coloured some water with carmine, and mixed it with an infusion of wheat in which a swarm of the largest ear-drop organisms and small oval animalcules had been living some months.

The animalcules might be small, but von Gleichen felt that their take-up of the dye was proof that, in some manner at least, they ate and drank like larger creatures. It was a discovery advanced in 1830 when

Christian Gottfried Ehrenberg figured out that only certain dyes were suitable for living creatures: 'These experiments', he noted, 'require organic dyestuffs.' The lead and other substances that dyers often used were too frequently fatal to the animals he wished to study.

Plant experiments dominated, but as the nineteenth century wore on, interest in using stains on larger animals grew. In 1851 the Marquis Alfonso Corti used a carmine dye to illuminate the structure of the inner ear. 'Under the microscope, I found that all its tissue was coloured red, being darker where it was thicker. The holes were clearly seen as small oval windows. I could easily be sure that there was really no tissue in the holes, and I could make out their borders with perfect distinctness.' He was describing the minute holes down which nerves travelled, holes being revealed for the first time by the stain. Carmine, pointed out the marquis, showed up the nuclei of cells. It was an observation of great potential, and situated as it was 'in a great paper in an important German journal' it should have won attention. Instead, in a mid-nineteenth-century world where anatomy, physiology and chemistry were increasingly dominated by the successful Germans, 'it attracted no notice: [since] it was written in French'.

Looking at the names of those who were working in microscopy, you get an impression of the reasons for much of the German success. The work there was being done by professionals, greatly supported by academies and universities. England relied on hobbyists. Lord Osborne demonstrated the staining of wheat cell nuclei to London's Microscopical Society in 1857, while pointing out that as a 'mere amateur' he 'made no attempt to resolve any question in chemistry'. The same year, Hermann Welcker, the gifted German doctor and anatomist, showed the value of stains in illuminating the nuclei of cells in frogs. Leading authors in England and elsewhere heard about Welcker's findings even as Osborne's failed to reach them.

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Adolf von Baeyer was fascinated by dyes from a young age. He dallied with physics and mathematics as a student at the University of Berlin, then went back to chemistry. From 1856 he was working for Bunsen in Heidelberg, and from the next year for Kekulé, of the benzene-ring dream, in Heidelberg and then in Ghent. From 1866, responding to the urging of Hofmann, the University of Berlin appointed von Baeyer as a senior lecturer, giving him no money but plentiful lab space. He worked on dyes, developing several new classes of chemical and industrial importance. Success, including a Nobel Prize in 1905, firmed rather than dissolved his belief in the essential humility required of a man in possession of theories rather than evidence. Those who designed experiments simply to confirm their prejudices were in danger, he felt, of designing bad ones, of misinterpreting their results, or even of fatally convincing themselves that their theories were too good to need such testing. 'I have never set up an experiment to see whether I was right,' said von Baeyer, 'but to see how the materials behave.'

Methylene blue is an aniline dye. In its powdered form it is a dark deep green; diluted in fluid it looks something like a clear and hopeful sky. It was discovered in 1876 by Heinrich Caro (involved in the development of Bismarck Brown) in collaboration with Baeyer. Caro was laboratory director at Badische Anilin & Soda-Fabrik – BASF. Set up in 1865 in response to the great opportunities for industrial chemistry in Germany, BASF's patent on methylene blue was Germany's first on a coal tar dye, and it became crucially important, through the work of Robert Koch and Paul Ehrlich, in the development of modern medicine.

Pasteur performed marvels in France in the 1860s, persuading the world of the truth of germ theory. A host of incomprehensible diseases were suddenly made clear by the idea of infection, the notion that invisible micro-organisms could invade the body and

turn health into illness. Here was suddenly a key to understanding, preventing and potentially treating a host of diseases in previously unthought-of ways.

Despite Pasteur's start, it was once more in Germany that the new techniques really shone. Robert Koch found the organism that caused anthrax in 1877, tuberculosis in 1882 and cholera in 1883. He even developed rules for other microbe hunters: 'Koch's postulates' – intellectual devices for reliably tying together diseases with their causative micro-organisms. Together with a climate of support for seriously conducted science, Germany forged ahead.

One of those supported and inspired by Koch was Paul Ehrlich. He was born in 1854, in Strehlen, Upper Silesia – then part of Prussia, now Poland – and his childhood passions were tied up with this new discipline of microbiology. As a schoolboy he tinkered with microscopes and was introduced to tissue-staining by his cousin, Karl Weigert, whose Breslau laboratory he later worked in. Weigert showed him how aniline dyes could colour cells and tissues, revealing their structure and relationships. Ehrlich was enthralled, 'awakened', as he later remembered, 'to the love and understanding of dyes that have accompanied me throughout my career'. He pursued the subject for his doctorate. Classmates remembered him as the man with the multi-coloured fingers. Over the next five years he used his dyes to explore blood cells, then bacteria. Frustrated after a time with injecting dyes into dead creatures, Ehrlich further developed 'vital staining', showing how methylene blue and other dyes could be injected into, as well as ingested by, living creatures. With encouragement, Ehrlich found, Nature not only showed her secrets, but did so in the most glorious of colours:

> If a small quantity of methylene blue is injected into a frog, and a small piece of the tongue is excised and examined, one sees

> the finest twigs of the nerves beautifully stained, a magnificent dark blue, against a colourless background.

It was Koch who showed how to use methylene blue to stain the tubercle bacillus. With the right dyes the cause of tuberculosis, this ancient and terrible disease, was not only discovered; it was displayed to the world in hues of beautiful pink and blue. Ehrlich was present at the meeting when Koch announced his discovery, and sat close enough to notice what years of work had done to Koch's hands. Their skin was dark and wrinkled, damaged by the stains and disinfectants that the tasks required. Ehrlich listened to Koch's announcement in wonder. 'I hold that evening', he said later, 'to be the most important experience of my scientific life.'

That was in 1882. Largely unwelcome in Berlin's Charité Hospital, where neither his ideas nor his Judaism were popular, Ehrlich became ill. Nevertheless he refined Koch's technique, and, in 1887, used the latest techniques to prove to himself that the spittle he was coughing up contained the tubercle bacillus. The discovery of the bug had not yet led to treatments. Ehrlich went to Egypt, hoping the climate would help his lungs heal. Two years later he returned, feeling somewhat better, this time to work as an assistant at Koch's new Institute for Infectious Diseases.

In Koch's laboratory, Ehrlich was at the heart of the world of medical research – a world that was still small. August von Wasserman, who found his own success researching syphilis, remembered the excitement of the concentration of talents:

> If a comparison of any sort is appropriate among such great men, I have to say that Paul Ehrlich was the champagne among the wines. While Koch appeared as the eternally serious-minded academic who thoughtfully weighed and stressed every word, disdaining all theory, observing only what was factual,

> and describing it in studied terseness, Ehrlich was literally bubbling over with brilliant ideas and views . . .

Ehrlich's laboratory, lined with the palette of his aniline dyes, was a startling sight. 'The visitor was confronted with a symphony of colours,' said Wasserman:

> without exaggeration, thousands upon thousands of glass bottles stood around, all filled with the brightest aniline dyes. Ehrlich . . . was involved in a highly stimulating exchange of ideas with the coal-tar industry. Thus, the industry sent him a sample of each new dye as soon as it appeared, and it was from that time onwards that his lifelong friendships and profound admiration for the creative geniuses and great names in the German dye industry derived.

For a time, Ehrlich left his beloved dyes behind, concentrating instead on the way in which animals seemed able to fight off infection. Serum is the name given to the fluid that blood moves in, the clear liquid turned red by the cells it contains. By exposing animals to infections, then bleeding them, Ehrlich found that their serum developed healing properties. Something in it contained the ingredients of immunity.

Serum therapy set Ehrlich wondering again about his coloured stains. It was clear that something in serum worked as an anti-toxin, specifically capable of fighting off infections like tetanus and diphtheria. These 'antibodies' must work, Ehrlich reasoned, something in the manner of 'magic bullets', ones with the power to find a particular target and destroy only that. He described, sketchily but for the first time, the way living cells could produce antibodies. A letter from 1901, arguing that Ehrlich should be awarded the inaugural Nobel Prize for medicine, noted that his 'explanation is

vastly different and much more innovative than anything that has been thought or written on the origin of antibodies so far'. It was, despite that, only one amongst a large number of deeply original pieces of work, including Ehrlich's 'earlier haematological work, the *discovery of the mast cells*, the histo-chemical staining of living nerve fibres with methylene blue, [and] his *vital staining*'. In the event the award of medicine's first Nobel was blocked by a chemist who incorrectly disagreed with some of Ehrlich's ideas, and who disliked the 'markedly Jewish atmosphere' he created.

When Ehrlich injected living rats with methylene blue, he found that the dye was taken up particularly by nerve cells. The stain had some selectivity for that bit of the body, a property that reminded Ehrlich of the manner in which antibodies seemed to pick out their targets. He set out to find chemicals that would work in the same way, mimicking the body's own ability to fight off infection, binding themselves only to the infecting organisms and killing only them.

It is easy to get an impression of Ehrlich as a man consumed with microbes and chemicals, just as it is straightforward to imagine that these successful new doctors were less concerned with individual suffering than their less effective predecessors of generations before. Neither impression is true. Ehrlich was neither cold nor consumed with any imaginary omnipotence. He knew that his tuberculosis might come back at any point, and that there was little anyone could do about it. And he worked in the wards as well as the laboratories.

> Whoever has seen Paul Ehrlich at a sickbed in one of the spreading wards of a large hospital, must have noticed that this extraordinary man embodied the humanist as physician. I was touched by the tenderness with which he took care of his child patients, how he joked with them and tried to soothe their discomfort by caresses, and yet, at the same time, I noticed his

> unease to be in the middle of an impersonal machinery whose wheels were turning in his name and by his authority.

Aniline dyes let Ehrlich understand more about the constituents of blood than anyone before him. An array of different cell types, previously unknown, appeared as he stained blood with these coal tar derivatives. They appeared in blushes of pinks and blues and greens, cells and structures gleaming into being. This was the work that led him to find the mast cells his admirer recommended he be rewarded for. They were white cells that existed plentifully in everyone's blood; without dyes, no one had understood them to be different from the other white cells around them.

In St Petersburg, in 1891, Yuri Romanovsky took blood from patients suffering from malaria and stained it. In patients treated with quinine, the malarial parasites were clearly damaged, the first definite indication that the drug acted by attacking the invader rather than supporting the host's defences. The same year Ehrlich, knowing that methylene blue stained the malarial *plasmodium*, gave capsules of the dye to two patients suffering from malaria in Berlin. Both recovered. Unable to deliberately infect animals with malaria, and busy with a project on diphtheria, he never followed the finding up.

From 1896 Ehrlich won his independence. His Institute of Serum Research and Examination was opened in Berlin. Three years later, in 1899, it moved to Frankfurt and was renamed the Royal Prussian Institute for Experimental Therapy. Ehrlich's collaboration with the dye companies continued. They sent him samples of the new colours they produced, and he tried to turn them to new uses.