PHENOTYPE

Issue 25 | Michaelmas Term 2016

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Celebrating 75 years of penicillin in Oxford

Exclusive insights into the new exhibition, *Back from the Dead*: Oxford's Pencillin at the Museum of the History of Science p9

Special features on antibiotic research across Oxford: p6-21

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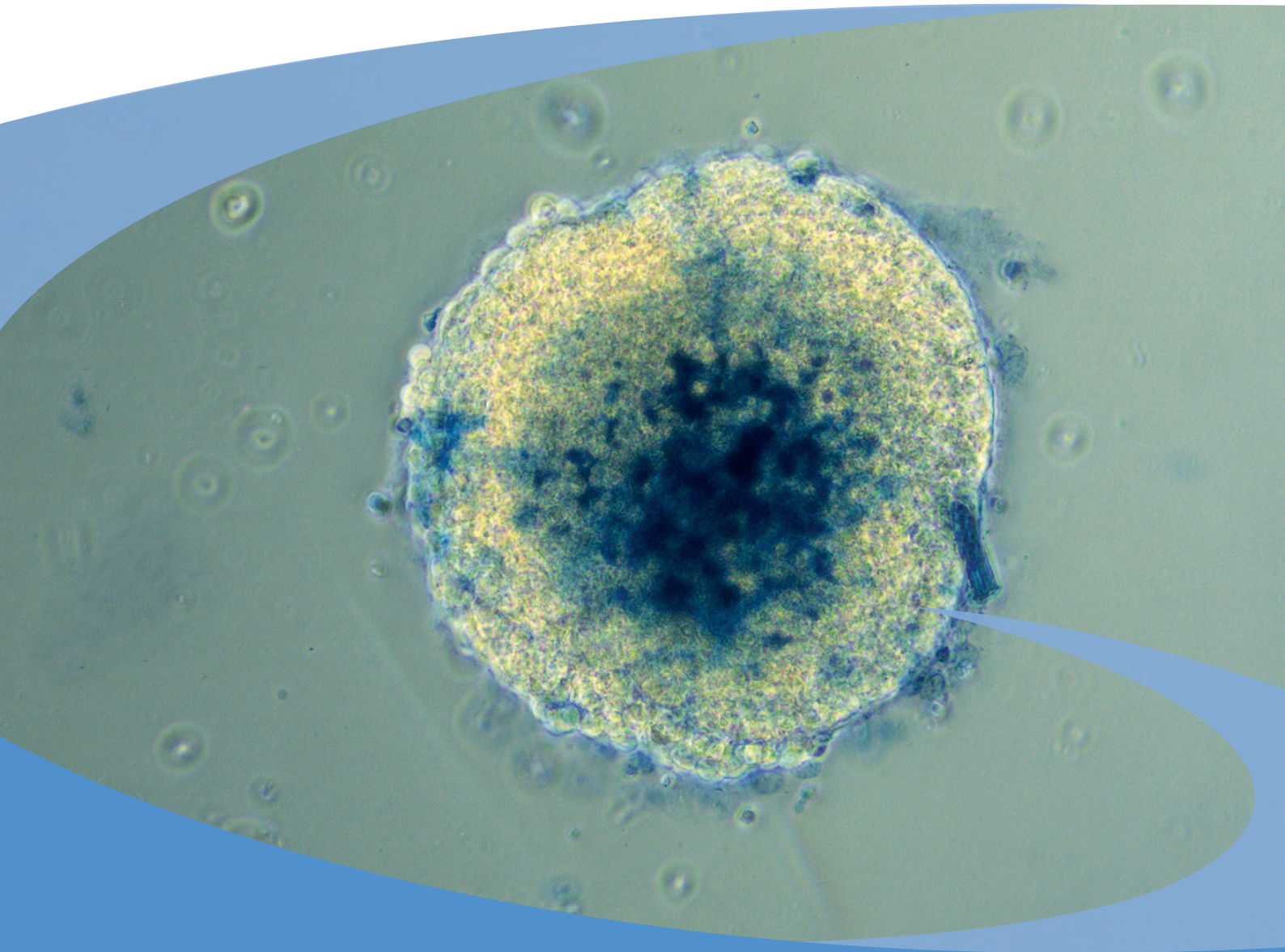

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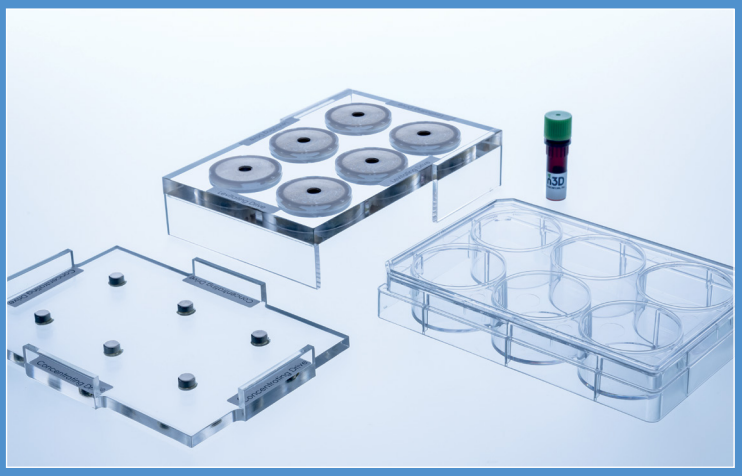

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EDITORIAL

A very warm welcome to the 25th issue of Phenotype!

As well as our 'Silver Anniversary,' this issue is celebrating 75 years since the first successful use of penicillin in a human. The story of penicillin is linked inextricably with Oxford, in particular with the Dunn School of Pathology, where Howard Florey, Ernst Chain and Norman Heatley defied the odds to produce penicillin and revolutionise the treatment of infection against the backdrop of World War II. A new exhibition at the University's Museum of the History of Science, entitled Back from the Dead: Oxford's Penicillin, *details their incredible story, and we are very fortunate to have an exclusive insight into the exhibition on pages 9-11.*

A contemporary twist on this story has inspired several of our authors this issue, who focus on the global health threat that is antibiotic resistance. On pages 6-8, Prof Craig MacLean explains his work on determining the drivers behind the evolution of antibiotic resistance in bacteria, and how that can help us slow down resistance in the clinic. Dr Burcu Anil Kirmizitas gives us hope for an imminent antibiotic revolution on pages 16 & 17, with her article on the discovery of Teixobactin, while on pages 20 & 21, Martine Abboud profiles β-lactamase-mediated resistance and the novel inhibitors helping to fight this. To round this all off, turn to the centrefold, where our incredible research infographic by Dr Anne C Wolfes will inspire you to get your thinking caps on and solve the antiobiotic crisis!

Continuing on the theme of global health crises, Sanskrithi Sravanam gives us the lowdown on current research into the ZIKV (Zika) virus and microcephaly on pages 22-23, highlighting the questions that remain to be answered.Yet another significant health burden, in the form of Alzheimer's disease, comes under the spotlight on pages 24 & 25, thanks to Helle Bogetofte Barnkob's fascinating article on 'Flushing the brain.' On a different note, Dr J Rubén Goméz Castellanos discusses an E.U. Horizon 2020 Initiative centred around Biocatalysis, emphasising the key contribution of European scientists to the burgeoning biotechnology sector.

Our Science and Society section turns away from health this issue, and instead features a thought-provoking piece from Rachel Wheatley on the nitrogen crisis, and the envronmental and ecological consequences this will have on our growing population. Turn to page 26 for more. We also step away from the lab in our Careers Insight, in which Dr Eleanor Healey takes us through her move to JA Kemp, where she is training to become a patent attorney. Find out what she loves about her new role on page 30!

Finally, I have to draw your attention to our Regular items, which are of an exceptional quality this term. On page 5, Dr Rachel Mulvaney has delved into the recent literature from the University and rounds up two excellent studies with significant impact to both obesity and diabetes. Dr Suvi Honkanen, James Eaton and Dr Cristina Marculescu have each reviewed a recent title from our partners at Wiley Blackwell. Their reviews cover books on such diverse topics as pathogen detection, wine production and autoimmunity, so if you're looking to add to your bookshelf, look no further than pages 28 & 29.

Our amazingly beautiful cover image this term comes from Dr Sonia Muliyil and Dr Clémence Levet, of the Freeman Lab, Dunn School of Pathology. We profile the scientists behind the art on page 31. Finally, Fish challenges you to his 10th cryptic crossword for Phenotype, this term on the subject of antibiotics- test your grey matter on our back page!

All that remains for me to say is that this is my last issue at the helm of Phenotype. It has been a fantastic privilege to work with a very talented team of contributors and editors, and I have no doubt that there are more excellent issue to come. I hand over to our current Co-Editor-in-Chief, Heather Booth, who I know is excited to receive your ideas, contributions and editorial assistance. Contact her at heather.booth@st-annes.ox.ac.uk to get involved!

Enjoy this issue and your Michaelmas Term!

Becky Hancock
Editor-in-Chief

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Phenotype is also available to read online via our website: **www.phenotype.org.uk**

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RESEARCH HIGHLIGHTS

by Dr Rachel Mulvaney

Merkestein, M *et al.* (2015) Nature Communications 6:6792.
DOI: 10.1038/ncomms7792

FTO influences adipogenesis by regulating mitotic clonal expansion

Common variants in the first intron of the fat mass and obesity related gene (*FTO*) were the first polymorphisms shown to be associated with obesity. Whether their observed effect on obesity arises due to effects on *FTO* itself or altered expression of adjacent genes is still under debate. *In vivo* mouse models have previously shown that an *Fto* knock-out (*Fto*-KO) or impaired function of *Fto* reduces fat mass and body weight, whilst overexpression of Fto increases fat mass and body weight. However, the underlying mechanisms are still poorly understood.

Here, the Ashcroft and Cox groups have compared adipogenesis (generation of new adipocytes) in mouse embryonic fibroblasts (MEFs) and primary adipocytes from *FTO*-KO and *FTO*-overexpression (*Fto*-4) mice. Using histological and qPCR approaches it was found that *Fto*-KO had reduced adipogenic capacity, which was linked with a reduction of mRNA levels of genes that play critical roles in adipogenesis. Conversely, *FTO*-4 had increased adipogenic capacity. Mice overexpressing *FTO* and fed a high fat diet for two months also showed an increase in the number of adipocytes when compared to WT mice.

Mitotic clonal expansion (MCE) is pre-requisite for adipocyte differentiation and occurs within 48 hours of adipogenic stimulation. To assess when *FTO* exerts its effect on adipogenesis, Fto was knocked down using siRNA 48 hours after adipogenic induction in *Fto*-4 MEFs. No difference in adipogenic gene expression was observed, suggesting that *FTO* exerts its effect before MCE. It was also shown that *FTO* overexpression enhanced the expression of the short isoform of runt related transcription factor - RUNX1T1-S, which stimulates adipogenesis. Merkestein *et al* suggest that it is possible that *FTO* mediates its effect on adipogenesis through the increased expression of the pro-adipogenic RUNX1T1-S transcription factor.

This study contributes to unraveling the mechanism of body mass regulation, which could help in rational drug design and future therapeutics to help tackle the obesity epidemic.

Ramracheya, R.D. *et al.*, (2016) Cell Reports 15, 944-950
DOI: http://dx.doi.org/10.1016/j.celrep.2016.03.091

PYY-dependent restoration of impaired insulin and glucagon secretion in type 2 diabetes following Roux-En-Y gastric bypass surgery

With the current epidemics of obesity and type 2 diabetes (T2D), growing numbers of patients are undergoing Roux-En-Y gastric bypass (RYGB) surgery in a bid to lose weight. It has been observed that within days of surgery up to 90% of patients have undergone a full and durable remission of T2D without losing any weight. The underlying mechanisms of this effect remain unknown.

T2D is characterised by the dysfunction of glucose-stimulated insulin secretion (GSIS) and inappropriate regulation of glucagon production from the pancreatic islet cells (ß- and α- cells respectively). In this study, Ramracheya *et al* have used Goto-Kakizaki (GK) rats, which develop T2D early in life, to assess the effects of RYGB on islet function, morphology and investigate the underlying mechanisms responsible forT2D remission. It was found that RYGB restored islet morphology and increased the number of insulin positive ß-cells relative to sham-operated GK rats.

Previous studies have observed an increase in peptide tyrosine tyrosine (PYY) concentrations following RYGB. PYY is a hormone that is postprandially secreted from the L-cells of the gastrointestinal tract and acts to reduce appetite. Levels of PYY are drastically reduced in obese patients and those suffering from T2D. In this study it was found that PYY plasma concentrations were markedly increased within 10-14 days post-RYGB and remained elevated for up to 8 months. Islets from diabetic GK rats cultured with either PYY or serum from RYGB rats for 48-60 hours also showed improved GSIS and normalised glucagon secretion. This effect was reversed by neutralisation of PYY using a PYY-specific antibody. These findings indicate that the mechanism underlying T2D remission may be mediated by PYY and suggest that drugs promoting PYY release or action may restore pancreatic islet function in T2D.

Evolutionary drivers of antibiotic resistance in pathogenic bacteria

by Professor Craig MacLean

Antibiotics have made an important contribution to improving human health and increasing our lifespan, but the recent spread of resistance in pathogenic bacteria has undermined the utility of most antibiotics that are currently in use. Whilst difficult to fully assess the medical and economic costs of resistance, an influential recent report predicts that antibiotic resistant infections will result in approximately 10 million deaths per year by 2050, unless we find new ways to combat bacterial pathogens (1). This crisis forces us to overcome two challenges. The first is to develop new antibiotics that will allow us to treat infections caused by drug-resistant pathogens. The second is to change the way that we use antibiotics so that we slow down the spread of antibiotic resistance. Research in my lab is aimed at addressing this second challenge by elucidating the evolutionary drivers of resistance.

The spread of antibiotic resistance in pathogenic bacteria is a simple and elegant example of adaptation by natural selection. Pathogenic bacteria acquire resistance by spontaneous mutation or horizontal gene transfer, and exposure to antibiotics selects for resistant clones because they have higher fitness in the presence of antibiotics. This is Darwinian natural selection in its purest and simplest form. How, then, can we utilise the principles of evolution to help us fight the spread of resistance?

Pathogenic bacteria inhabit a heterogeneous world characterised by brief periods of intense exposure to antibiotics, for example during infection, and extended periods of exposure to low doses of antibiotic, for example in environmental reservoirs. Evolving antibiotic resistance by spontaneous mutation or by acquiring mobile genetic elements tends to be accompanied by reduced fitness in the absence of antibiotics. This 'fitness cost' is thought to limit the ability of resistant strains to effectively persist in bacterial populations. Two of the main objectives in my lab have been to quantify the cost of resistance and to determine the mechanisms underpinning this cost. This is important because understanding the origins of these costs could help us fight against resistance by using antibiotic treatment strategies that maximise the cost of resistance.

The approach that we take to measure the fitness of resistant bacteria is to directly compete antibiotic sensitive and resistant strains against each other under controlled lab conditions. It is possible to measure fitness by determining changes in the abundance of strains during co-culture, typically using cells that express fluorescent proteins. Most of our work in this area has focused on resistance to rifampicin in the pathogenic bacterium *Pseudomonas aeruginosa* (2). One reason that we were attracted to this system is that the molecular basis of rifampicin action and resistance are well understood. Rifampicin inhibits bacterial gene expression by binding to a highly conserved domain on the β-subunit of RNA polymerase (RNAP), blocking transcription initiation. High-level resistance to rifampicin arises from mutations in at least 50 sites in *rpoB*, the gene encoding the β-subunit of RNAP, that prevent rifampicin-RNAP binding by altering the structure of this domain. To assess the cost of rifampicin resistance, we have competed resistant mutants against a wild-type strain in the absence of rifampicin. These experiments show that rifampicin resistance almost always reduces competitive fitness (Figure 1A). This cost arises because resistance mutations reduce the transcriptional efficiency of RNA polymerase, providing a simple link between antibiotic resistance, enzyme activity and bacterial

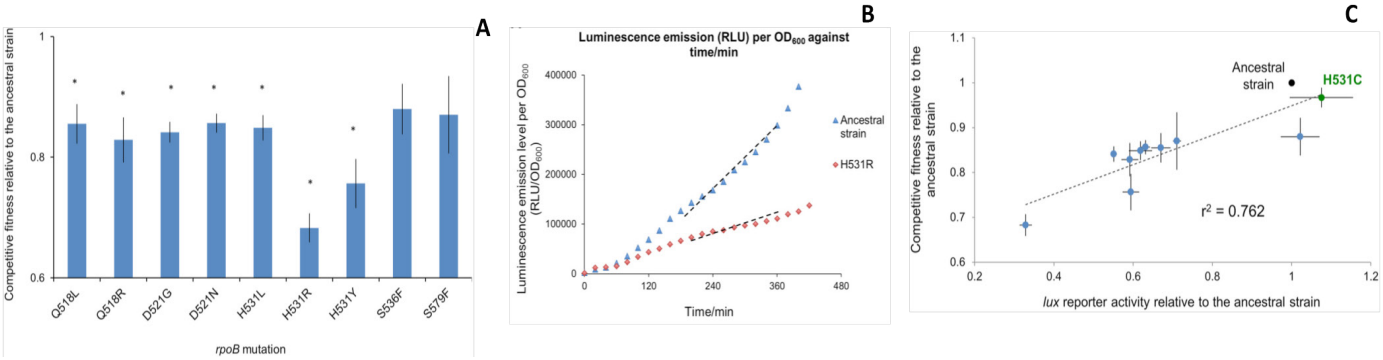

Figure 1: Fitness costs of resistance. A. Fitness of rifampicin resistant mutants of *P. aeruginosa*, as judged by competition experiments against an antibiotic sensitive tester strain. **B.** Efficiency of transcription of a luminescence reporter construct in a rifampicin sensitive strain (ancestral) and a resistant mutant (H531R). The dotted line shows the inferred transcription rate. **C.** Graph showing the link between transcriptional efficiency and fitness. Figures reproduced from (2) under a Creative Commons Attribution–Share Alike 3.0 Unported (CC BY-SA 3.0) iicense, © 2014, Qi *et al.*

fitness (Figure 1B,C).

Given that resistance carries a cost, one strategy for managing resistance is simply to stop using an antibiotic once resistance begins to appear. Unfortunately, restricting antibiotic use has had mixed success in clinical trials, and in some cases resistance to antibiotics has persisted for years after antibiotic use was discontinued. How can we explain this?

Imagine a bacterial population that is made up of a single clone carrying a costly resistance mutation. In this case, because of the high prevalence of resistance, the use of the antibiotic would be discontinued. We might expect that reversion mutations that lead to the loss of antibiotic resistance would eventually appear in the population, and these mutations should spread because they restore fitness. This simple scenario illustrates how evolution could potentially lead to the loss of resistance in a bacterial population. A number of studies have now tried to create this scenario, by propagating populations of antibiotic resistant strains in antibiotic-free culture medium for hundreds to thousands of generations (Figure 2A). Rifampicin resistant populations, for instance, usually recover fitness to wild-type levels within 300 generations of evolution in the antibiotic-free culture medium (Figure 2B) (3). These experiments provide ample evolutionary opportunity for the reversion of antibiotic resistance, and yet this outcome is rarely observed. Instead, resistance tends to stably persist in the absence of antibiotic use.

One of the most interesting findings from these reversion studies has been that compensatory mutations can help maintain resistance by ameliorating its costs. Compensatory mutations often occur in the same gene as resistance mutations, and they recover fitness by eliminating the biophysical defects associated with resistance mutations. For example, mutations in *rpoB* coding for the region outside of the rifampicin-binding pocket recover the fitness cost of rifampicin resistance by restoring transcriptional efficiency back to wild-type levels (Figure 2C) (2). One interesting feature of compensatory mutations is that they interact epistatically with resistance mutations, so that it becomes costly to lose resistance in strains carrying compensatory mutations. Populations that have evolved compensatory adaptations are, therefore, incredibly unlikely to ever evolve back to being antibiotic sensitive. The available evidence suggests that compensatory mutations are much more common than reversion mutations, and it is likely that this asymmetry explains why reversion to antibiotic sensitivity is such a rare evolutionary outcome. From a resistance management perspective, this represents a grim scenario where there is no reason to expect that restricting antibiotic use will reduce resistance.

Most studies on the evolution of antibiotic resistance have focused on looking at fitness costs and compensatory evolution in the absence of antibiotic use. However, the effects of antibiotic exposure can be very dramatic on antibiotic-naïve pathogenic bacteria. In order to fill the information gap that we have about these effects, my lab turned to a very simple model system involving *P. aeruginosa* and a small, multi-copy plasmid, pNUK73, that carries a plasmid replication gene and an aminoglycoside (a type of antibiotic used against Gram-negative bacteria such as *P. aeruginosa*) antibiotic resistance gene (Figure 3A) (4). In spite of its small size, pNUK73 carriage imposes a massive fitness cost,

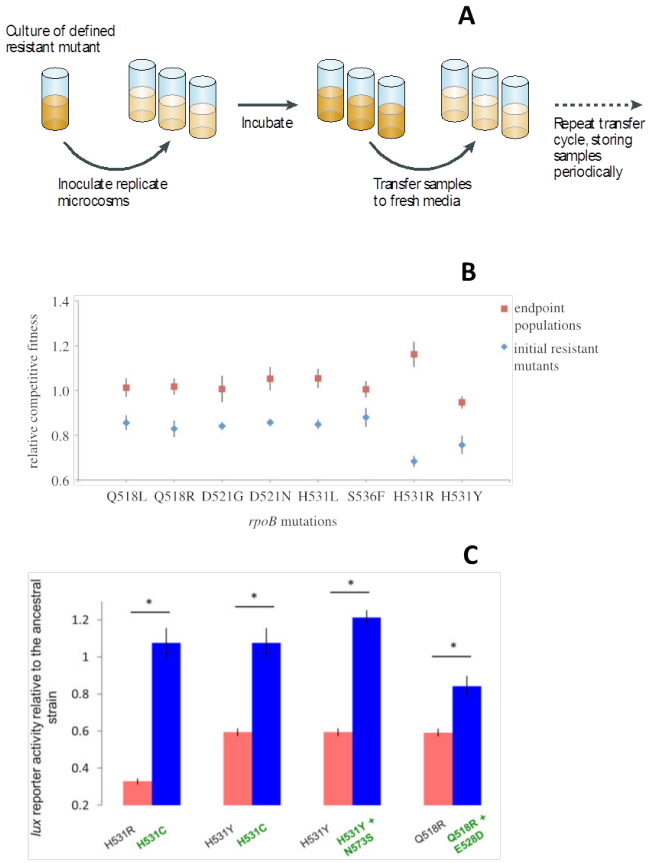

Figure 2: Compensatory adaptation. A. Design of a simple experiment to study the evolution of bacterial populations in the lab. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Genetics* (5) copyright 2010. **B.** Fitness of rifampicin resistant populations of *P.aeruginosa* before (blue) and after (red) 300 generations of evolution in the lab. Fitness was measured using competition experiments against a wild-type strain. Figure reproduced from (3) under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, © 2016, Qi *et al.* **C.** Transcriptional efficiency of rifampicin resistant strains (red) and strains carrying second site compensatory mutations in rpoB (blue). Figure reproduced from (2) under a Creative Commons Attribution–Share Alike 3.0 Unported (CC BY-SA 3.0) license, © 2014, Qi *et al.*

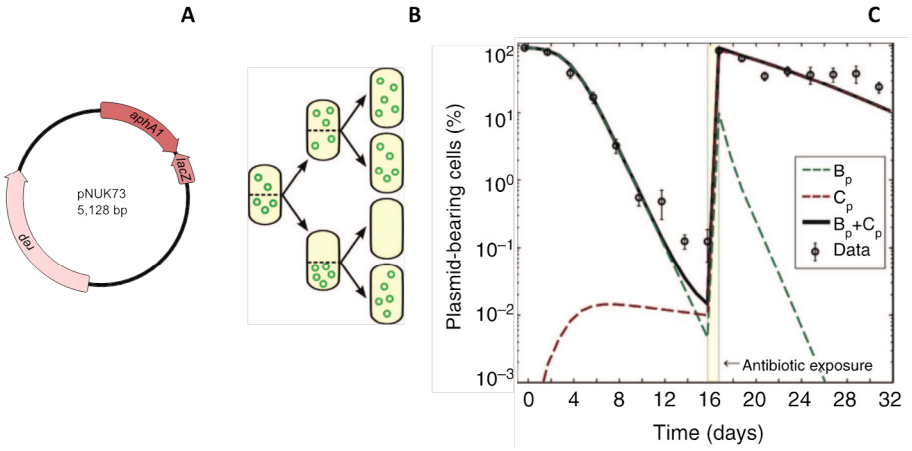

Figure 3. Antibiotic use stabilizes resistance. A. Map of the resistance plasmid pNUK73. **B.** Scheme showing the potential for loss of plasmid carriage during cell division. **C.** The dynamics of pNUK73 carriage during experimental evolution. Plasmid prevalence rapidly declines during the first 14 days of the experiment. Following antibiotic treatment at day 16 the plasmid declines slowly. Dotted lines show the predicted frequency of pNUK carrying cells with (Cp) and without (Bp) compensatory mutations. Panels B & C reproduced from (4) under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, © 2014, San Millan *et al.*

on the order of 20%. Copies of pNUK73 are randomly distributed to daughter cells during cell division (Figure 3B), meaning that approximately 1/100,000 cells inherit no copies of this plasmid. The key advantage a system like this offers is that the large fitness costs of resistance and rapid rate of segregational loss ensure that the dynamics of this system are very rapid, making it ideal for studying dynamic responses of bacterial populations to antibiotic exposure (Figure 3C).

In our experiments we observed some interesting phenomena. Firstly, exposing cultures of bacteria to a large dose of neomycin (an aminoglycoside) rapidly increased plasmid prevalence up to 100%, because only plasmid-bearing cells could survive the antibiotic. Following exposure to antibiotics though, owing to the cost of plasmid carriage, the frequency of plasmid-free cells rapidly increased in *Pseudomonas* populations. Interestingly, however, the rate at which the plasmid was lost from populations began to slow down later on. This occurred because compensatory mutations that eliminate the cost of plasmid carriage spread through the plasmid-bearing population, increasing the stability of the resistance plasmid. So in this system, compensatory mutations in chromosomal genes down-regulate the expression of the plasmid replication protein, eliminating the cost of plasmid carriage (5).

To examine the consequences of antibiotic use, we then exposed populations to a second treatment of neomycin. As expected, plasmid prevalence rapidly increased to 100% upon treatment. After antibiotic use was discontinued, the frequency of the plasmid decreased very slowly because bacteria carrying compensatory mutations dominated the plasmid bearing population at the time of antibiotic exposure. Compensatory adaptation and antibiotic use therefore interact to stabilise resistance plasmids in bacterial populations. Interestingly, there is an evolutionary feedback between these processes. Antibiotic use increases the population size of resistant lineages, and this increases the rate at which resistant lineages evolve compensatory adaptations. Compensatory adaptation, in turn, makes resistance more stable after antibiotic use. The applied lesson from this is simple: antibiotic resistance becomes increasingly stable every time an antibiotic is used.

In his Nobel Prize acceptance speech, Sir Alexander Fleming warned that the overuse of antibiotics could result in a loss of their efficacy. More than 1 million tons of antibiotics have now been used, and this has resulted in the incredibly rapid evolution of resistance. It is clear that new antibiotics are now needed to treat infections caused by resistant organisms, but it is also imperative that we begin to use the principles of evolution to help slow down the spread of resistance in pathogen populations. This is a daunting challenge that will require researchers to move well beyond the simple *in vitro* experiments that I have described in this article. The next stage of research in this field will be to use a combination of lab experiments and bacterial genomics to understand the evolutionary drivers of resistance in clinical settings.

Craig MacLean is Associate Professor and Royal Society University Research Fellow, Department of Zoology, and Hugh Price Fellow in Evolutionary Biology, Jesus College, University of Oxford

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Back from the Dead: Oxford's Penicillin

The rise of the superbug: a pressing contemporary issue? Or a problem dating back 80 years? Will modern medicine save us from antibiotic resistance, or will we have to save ourselves?

by Marie-Louise Kerr and Dr Stephen Johnston

Back from the Dead, a new exhibition at the Museum of the History of Science on Broad Street, explores these questions alongside the history of penicillin in Oxford. Combined with an exciting programme of public events and learning opportunities, *Back from the Dead* aims to juxtapose past and present in antibiotics, bring new perspectives to familiar stories and recover voices that have previously been lost.

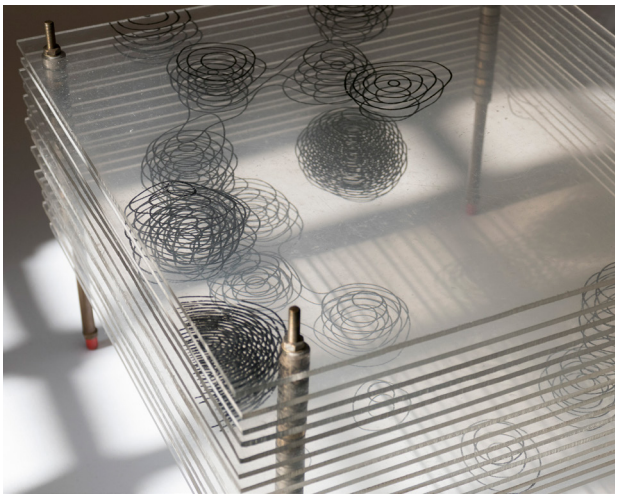
Today, penicillin is typically associated with Sir Alexander Fleming and his disorganised workbench. In September 1928, Fleming discovered a mould, surrounded by a sterile ring, growing on a culture plate of *Staphylococcus* bacteria. In an era when there was no medicine against *Staphylococcus,* Fleming understood the importance of his observation, and identified the antibacterial mould as *Penicillium notatum*. However, he was unable to either isolate or purify the substance, or to effectively test it on animals or humans.

Thirteen years later, Howard Florey, Professor of Pathology at the Dunn School in Oxford, supervised the first clinical trials and batch production of penicillin. In the midst of World War II, penicillin's military and civilian potential as a treatment against infections was seized upon, and by the end of the war large-scale production was underway in the United States. The achievement of Florey and his international team makes this episode one of the most celebrated in the history of Oxford science, and 2016 marks the 75th anniversary of penicillin's first successful human use.

Back from the Dead recreates the context for the development and testing of penicillin at the Dunn School. Drawing on the Museum's own collections, the exhibition highlights the hand-to-mouth character of research in the early days of World War II. Bed pans, biscuit tins, and sheep dip cans were all initially used as convenient vessels in which to culture the penicillium mould. This conjures a world of make-do and mend, where the insights of Ernst Chain, a Jewish German refugee biochemist, were balanced by the ingenuity of Norman Heatley, who conceived the temperamental apparatus by which penicillin was purified.

Only a few fragments of Heatley's apparatus survive today, and the exhibition includes resources from other collections to paint a broader picture of microbiological research during the 1940s. Loans from the Science Museum, the Wellcome Collection and the Imperial War Museum reveal the high expectations created by existing 'magic bullet' drugs, the laboratory work at the Dunn

Above. Original penicillin culture and specimen, Howard Florey and colleagues, Oxford, c.1940–1
The dried-up contents of the cotton-sealed flask are one of the original cultures that Florey's team used to extract penicillin.
When administered, not all the penicillin was metabolised and some could be recovered from the urine of patients and reused, as witnessed by the tiny glass vial dated 25 June 1941
Inventory Numbers 23034 & 14439. Museum of the History of Science, University of Oxford.



Right: Model of the structure of penicillin, Dorothy Crowfoot Hodgkin, Oxford, c.1945
The model gives a three dimensional map of part of one of the crystal salts of penicillin. The contours are lines of electron density and show the positions of individual atoms in the structure. The diagram shows two schematic views of the structure.
Based on X-ray crystallography work by Dorothy Crowfoot Hodgkin and Barbara Low (Oxford) and C.W. Bunn and A. Turner-Jones (I.C.I. Alkali Division, Northwich).
Inventory Number 17631. Museum of the History of Science, University of Oxford.

School, and the experience of patients when penicillin was administered not as a convenient pill, but as a painful injection – one patient likened it to the sensation of having boiling water injected into their veins (1).

The first steps in the new era of antibiotics were faltering. After an initial response to treatment, one of the first patients suffered a relapse and died within a month, largely because not enough penicillin could be produced. To supplement the very short supply, the team even recycled the penicillin excreted in patients' urine. They would collect the urine of patients from the Radcliffe Infirmary and then cycle it back to the Dunn School laboratory so that the penicillin could be extracted for re-use; they affectionately named this team "P-Patrol"(2).

The urgent need to step up production led Florey and Heatley to leave for a secret mission across the Atlantic. They reached the United States and persuaded local researchers and pharmaceutical companies to take up the penicillin challenge. A new method of deep tank fermentation was designed, which resulted in a dramatic increase in penicillin production.

While this development was underway in North America, researchers in other countries hoped that a better understanding of the penicillin molecular structure would enable the drug to be chemically synthesised, rather than extracted from a mould. The crucial work was again carried out in Oxford, by Dorothy Crowfoot Hodgkin and a small team of X-ray crystallographers. Still the only British woman to receive a Nobel Prize for science, Crowfoot Hodgkin's work is revealed in the *Back from the Dead e*xhibition, both through the painstaking labour of molecular model-making, and the vivid insights of her personal correspondence. Loans from her papers in the Bodleian Library reveal details of her research on penicillin and its remarkable power to bring people seemingly 'back from the dead'.

Limitations to penicillin's use were nevertheless understood from the start. Alexander Fleming spoke of the risk of antibiotic resistance in his 1945 Nobel Lecture. This warning has increased significance today. The twentieth-century 'golden age' of antibiotics, which saw a proliferation in newly-created drugs, advancements in surgery and organ transplantation, and a drop in deaths from infection from 43% to 7% in Europe, is now widely claimed to be over. The UK's Chief Medical Officer has stated that the current era of modern medicine will collapse by 2050 if Fleming's historical caution is not heeded.

Back from the Dead explores some of the current responses to the challenge of antibiotic resistance. There are striking continuities with the work of the 1940s. X-ray crystallography – now operating at a scale, intensity and speed not dreamt of 75 years ago – is still a vital tool to study not just drugs, but also their targets and mechanisms. With advice from colleagues in the Dunn School and the University's Chemistry Research

Laboratory, the exhibition opens up the realm of contemporary research for the public.

Florey and his team would be surprised by the many forms of research that their pioneering work has inspired. Through collaboration with the multi-disciplinary team of The Oxford Martin School Programme on Collective Responsibility for Infectious Disease, the exhibition includes an interactive station that will question visitors about ethical medical dilemmas to further psychological research. No longer conceived as passive consumers, patients are now active partners in the successful delivery of modern health programmes, and the exhibition offers visitors an opportunity to contribute to current research on the public understanding of the use of antibiotics.

To testify the link between science and art, the exhibition includes the work of bio-artist Anna Dumitriu, who creates artwork through the direct use of bacteria. When the exhibition closes, her intervention will expand to take over the space, so that even the bacteria will come back from the dead...

Back from the Dead is led by the Museum's Director Dr Silke Ackermann and delivered with the help of its collections, education, design, public engagement, web and technical staff, along with specialists in digital animation. The exhibition has been made possible by the generous support of the EPA Cephalosporin Fund. It will open to the public on 3 November 2016 and will run until 21 May 2017. A new permanent display will follow in the Museum's Basement Gallery, after the closure of the temporary display.

If you are interested in contributing a personal story to the exhibition's online presence, or sharing related research you are working on, please contact the exhibition's curators Dr Sophie Waring (Modern Collections Curator, sophie.waring@mhs.ox.ac.uk) or Marie-Louise Kerr (Special Exhibition Curator, marie-louise.kerr@mhs.ox.ac.uk).

Above: Penicillin Specimens. Inventory Number 16920, 26401 & 14439. Museum of the History of Science, University of Oxford.

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Marie-Louise Kerr is Specicial Exhibition Curator and Dr Stephen Johnston is Assistant Keeper at the Museum of the History of Science, University of Oxford. Thanks also to Robyn Haggard, Public Engagement Officer at the Museum.

Leadership in enabling industrial technologies in the European Union: Robust oxidative biocatalysts in the Horizon 2020 Initiative.

by
Dr J. Rubén Gómez Castellanos

For the European Union (EU), biotechnology is one of six Key Enabling Technologies (KETs), defined as multi-disciplinary, knowledge and capital-intensive technologies. Their importance resides in the fact that they cut across many diverse sectors, providing the basis for a significant competitive advantage for the European industry sector by stimulating growth and creating new jobs. A strong scientific, technological, and innovation base in biotechnology will support European industries to secure leadership in this key enabling technology. This position will be further strengthened by integrating the safety assessment and management aspects of the overall risks in the deployment of biotechnology (1).

This project is funded by the European Union

ROBOX

For this reason, biotechnology is included in the Industrial Leadership Programme of Horizon 2020. This programme financially implements the European Council's Innovation Union, a Europe 2020 flagship initiative aimed at securing Europe's global competitiveness. Horizon 2020 (2) is the largest ever EU Research and Innovation programme, with nearly €80 billion of funding available over 7 years (2014 to 2020), in addition to private investment. These public-private synergies promise more breakthroughs and discoveries by taking great ideas from the lab to the market.

One of the most pressing matters for the industrial biotechnology sector is the substitution of conventional chemical reactions, which are often energy intensive, inefficient and require the use of harmful stoichiometric reagents, for biocatalysts such as enzymes produced by microorganisms. Biocatalysts offer an attractive and sustainable alternative to conventional chemistry but need to be tailored to the chemical conditions that prevail in large-scale chemical production. Specifically, oxygen moieties are a key functional group in many chemicals and materials; the efficient introduction of these into raw materials is key to the production of bulk and fine chemicals. Innovative biocatalytic oxidation routes using molecular oxygen from air under benign conditions (such as ambient temperature, pH and pressure) can greatly improve the sustainability and economics of industrial processes. Particularly in the pharmaceutical sector, enzyme-catalysed steps often represent the highest added value. Limited enzyme production and catalytic efficiency cause the high price of the end product, around €100 - €1,000/kg. (4)

In order to increase the industrial application of enzymatic bio-oxidation processes in lower-price chemical markets, a consortium was established to carry out the project ROBOX (expanding the industrial use of ROBust OXidative biocatalysts for the conversion and production of alcohols). ROBOX (www.h2020robox.eu) will demonstrate the technological and economic viability of bio-transformation for four types of robust oxidative enzymes:

- P450 monooxygenases (P450s): monooxygenases that obtain their electrons from the nicotinamide cofactors nicotinamide adenine dinucleotide hydrate (NADH) or nicotinamide adenine dinucleotide phosphate hydrate (NADPH). These are versatile biocatalysts that catalyse the regio- and stereospecific oxidation of non-activated hydrocarbons under mild reaction conditions, which is one of the most challenging tasks in chemical catalysis.
- Baeyer-Villiger monooxygenases (BVMOs): flavoenzymes that catalyse a wide variety of oxidative reactions such as regio-, chemo-, and enantioselective Baeyer-Villiger oxidations and sulfoxidations. BVMOs catalyse the transformation of linear and cyclic ketones into their corresponding esters and lactones

by inserting an oxygen atom into a carbon-carbon bond.

- Alcohol dehydrogenases (ADHs): these catalyse the oxidation of alcohols using NAD+ or NADP+ as the electron acceptor; the reaction is reversible and substrates include a variety of primary or secondary alcohols, ketones, aldehydes and hemi-acetals, with excellent regio- and chemo-selectivity compared to chemical oxidation.
- Alcohol oxidases (AOXs): these enable highly regio- and chemo-selective oxidation using soft chemistry (*i.e.* without the use of oxidative reagents such as chromium-based catalysts), and convert alcohols to aldehydes or ketones, and lactols or sugar derivatives to lactones.

For the industrial biotechnology sector to consider enzymes robust, they must fulfil the following performance characteristics:

- sufficient activity (substrate turnover per unit time);
- excellent chemo-, regio- or enantioselectivity (capacity to target specific chemical bonds in a molecule, form bonds in one specific direction, or form specific types of enantiomers);
- solubility under the required pH and temperature conditions;
- tolerance to high concentrations of substrate and product; correct substrate specificity; and minimal degradation; and,
- the enzyme should be efficiently produced and applied in both small and large settings.

ROBOX has outlined five Work Packages to streamline the work of the consortium: 1) **enzyme engineering and identification**, to enhance enzyme stability, activity and efficiency; 2) **enzyme production**, to establish competitive fermentative production systems that feature robust strains, high yields and efficient extraction methods; 3) **biocatalytic process design and validation**, to develop efficient biocatalytic processes and technologies that optimise the performance of the enzyme during the reaction; 4) **demonstration**, to bridge the gap between lab and market introduction by means of pilot scale demonstrations of biocatalytic processes and 5) **process benchmarking and evaluation**, to perform a full process evaluation of the demonstrated biocatalytic processes and the technologies of the ROBOX platform and to pave the way for further replication of the technology in a range of new markets.

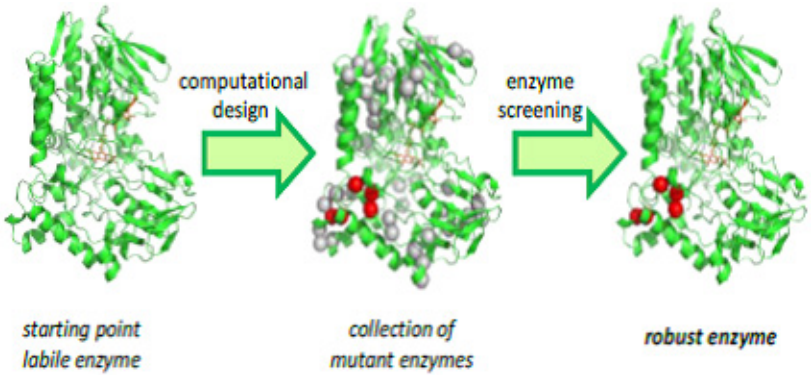

Figure 1. Computational approach to creating stable enzymes, showing mutations (grey and red spheres) that contribute to the stability of the enzyme. The grey mutations indicate changes that did not yield an improvement, while the red mutations indicate improvement; the main cofactor flavinadeninedinucleotide can be seen in the centre in red.

As a key partner, the University of Pavia is collaborating closely with the University of Groningen in targeting the robustness of BVMOs. In order to improve the performance of an enzyme, detailed knowledge of the enzyme's 3D structure and reactive site is essential. Furthermore, the 3D structure determines the stability of an enzyme. Most enzymes are relatively unstable under industrial conditions, which is not unexpected as most of them have not evolved to operate at high temperatures or in the presence of organic solvents. However, thermostability is an important property of industrial enzymes as it serves as a proxy for robustness. This also applies under extreme pH conditions, high substrate/product concentrations, or in the presence of organic solvents. Despite the initial computational effort, improvements to thermostability still require the creation and screening of large mutant libraries, which primarily help to increase the number of improved variants per screening round (3). In most proteins, stabilising mutations cluster in a limited number of critical regions, suggesting that early unfolding events start at these regions. Figure 1. Computational approach to creating stable enzymes, showing mutations (grey and red spheres) that

contribute to the stability of the enzyme. The grey mutations indicate changes that did not yield an improvement, while the red mutations indicate improvement; the main cofactor flavinadeninedinucleotide can be seen in the centre in red. shows how directed evolution towards a more robust enzyme can take place.

Currently, we are focusing our efforts on improving the thermostability of BVMOs. The engineering approach described above is complemented by *in silico* screening for robust variants using Framework for Rapid Enzyme Stabilization by Computational libraries (FRESCO). In parallel, sequence databases can identify enzymes that are predicted to perform the desired reaction that originate from organisms that tolerate relatively high temperatures. This will yield robust enzymes with the potential for immediate application. Of particular interest are various monooxygenase and oxidase variants that feature specifically modified reactivity properties with oxygen. Applying these techniques, the Universities of Groningen and Pavia have enabled the conversion of monooxygenases into oxidases and of oxidases into dehydrogenases (5).

Through the introduction of robust bio-oxidation for alcohols, ROBOX is expected to bring substantial reductions in cost (up to -50%), energy use (-60%), chemicals (-16%) and greenhouse gas emissions (-50%) (4). Since the overall contribution of biotechnology to economic growth is expected to increase from €60 billion annually up to €300 billion/year in 2020 (4), ROBOX will help Europe maintain its global leadership in the industrial biotechnology sector.

J. Rubén Gómez Castellanos, DPhil is a Research Fellow in the Department of Biology and Biotechnology at the University of Pavia. *The research for this work has received funding from the European Union project ROBOX (grant agreement no. 635734) under the EU's Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1.*

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Revolutionising the world of antibiotics

by
Dr Burcu
Anil
Kirmizitas

Revolutionary findings in science occur when highly skilled and motivated scientists combine hard work with teamwork. A revolution in the fight against bacterial infections occurred within a time frame of 17 years in the mid-20th century. The main characters involved were Alexander Fleming, Howard Florey, Ernst Chain, and Dorothy Hodgkin. Fleming discovered penicillin in 1928, and Florey and Chain managed to isolate it from the microorganism *Penicillium notatum* in 1940. The effectiveness of penicillin and the prevalence of infections in wounded soldiers during the Second World War created an urgent need for synthetic penicillin, since it was impossible to produce sufficient quantities by isolating it from *P. notatum*. In order to do this, scientists needed to know the composition and structure of penicillin, however by 1943 only the composition was known.

In 1943 Hodgkin was highly regarded in the field of X-ray crystallography, having already solved the crystal structures of insulin and cholesterol. She was running her own laboratory at the Dunn School of Pathology in Oxford and started working on the structure of penicillin after receiving it in crystalline form from the US pharmaceutical company Squibb (1). Nowadays, when conducting X-ray crystallography experiments, computer programs are used to model complex X-ray diffraction patterns. Back in the 1940s this was not possible. It used to take scientists like Hodgkin to crack X-ray diffraction patterns manually, and she solved the structure of penicillin by combining her creativity, in-depth knowledge of chemistry, and hard work. The structure was completely solved in 1945 (Fig. 1) and was chemically synthesised for the first time in 1957. Since then, many different successors to penicillin have been derived, and many other antibiotics have been discovered. The fight against bacteria was won, and we were never to go back to the difficult days of bacterial infections. Or so we thought.

It has been apparent for some time that the fight is not over. Resistance to antibiotics is becoming an increasingly serious problem. In 1928 Fleming himself predicted that exposing microbes to non-lethal quantities of antibiotic could prompt them to develop mutations that ensure their survival. A bacterium with a mutation that makes it resistant to one type of antibiotic has the ability to pass this mutation on to its progeny and even its neighbours, resulting in many more of the mutant bacteria. Bacteria can also become resistant to multiple types of antibiotics by accumulating several different mutations, creating a "superbug". For example, there are documented cases of infections that cannot even be eradicated with colistin, an antibiotic that is only used as a last resort due to its serious side effects (2).

Needless to say, there is a huge effort to try and overcome this problem; governments, doctors, and scientists are hard on the case. Despite the bleak outlook, recent studies suggest that we may be on the brink of another revolution. In their 2015 *Nature* paper, Losee L. Ling and colleagues reported a remarkable study on a new antibiotic called teixobactin. This compound is produced by the novel microorganism *Eleftheria terrae*, the discovery of which is also described in the study (3). Teixobactin is extremely effective against Gram-positive bacteria, which lack an outer cell wall. Ling and colleagues

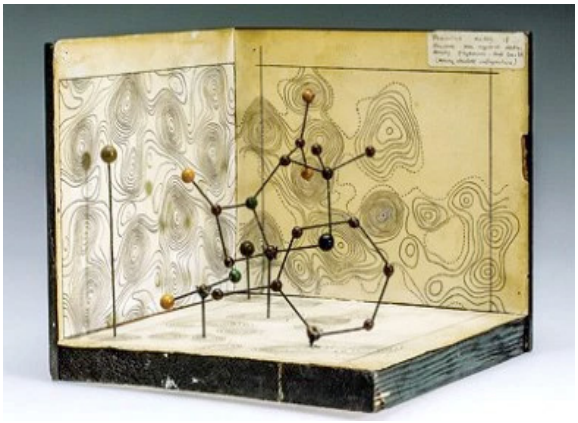

Figure 1: The X-ray crystal structure of penicillin shown in a model built by Dorothy Hodgkin, on display at the Science Museum in London.

reported that it was efficacious against all tested antibiotic-resistant strains.

The path that led to the discovery of *Eleftheria terrae* was paved by two authors of the study, Kim Lewis and Slava Epstein. It all started with a phenomenon called 'The Great Plate Count Anomaly' (4). The standard method of cultivating microorganisms in the laboratory is by growing them in plates that contain agar enriched with growth medium. 'The Great Plate Count Anomaly' refers to the fact that only 1% of bacteria can be cultivated using this method. Slava Epstein and Kim Lewis hypothesised that the nutrients in the agar growth plates were not appropriate for most bacteria and decided to find a way to cultivate soil bacteria in their native environment. They developed a multi-channel device called the iChip, which was designed to preserve the ability to clonally isolate specific bacterial strains while also enabling their growth in native conditions by immersion of the whole chip containing single bacteria in soil (5). Intriguingly, they noticed growth in some iChips that looked quite curious; bulbous and "sticky". They isolated the bacteria that grew in these iChips and confirmed that they belonged to a bacterial strain that nobody had cultivated before. They named this new strain *Eleftheria terrae* (3). They then tested extracts from *E. terrae* for antimicrobial activity. The extracts displayed effective antimicrobial properties, and the subsequently purified antibiotic was named teixobactin. Teixobactin was superior to other antibiotics commonly used in the treatment of bacterial infections, for example caused by the bacterium *Staphylococcus aureus* (Fig. 2). The extraordinary advantage of teixobactin is that it is effective against all known Gram-positive bacteria. Lewis and Epstein are now co-founders of a company called NovoBiotic, which runs the preclinical development of teixobactin as a new antibiotic (4). While this lengthy process ensues, they continue to study the resistance profile of teixobactin-sensitive bacteria. The team is also searching for new antibiotics and new microorganisms. Meanwhile, the iChip technology is being used by other scientists around the world, who are beginning to report new findings of their own.

The continuing search for effective antibiotics also includes those that target Gram-negative bacteria. Gram-negative bacteria have so far proven the harder case to crack when it comes

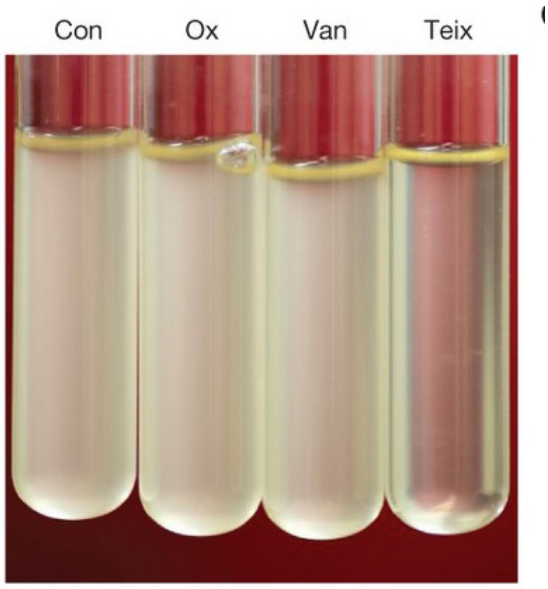

Figure 2. Teixobactin (Teix) lyses late phase growth S. aureus more effectively than oxacillin (Ox) and vancomycin (Van), the antibiotics normally used to treat this infection (Con: control sample) Figure reprinted by permission from Macmillan Publishers Ltd: *Nature* (3), copyright 2015.

to antibiotic resistance. These bacteria have an extra layer surrounding their cell membrane that is difficult for small molecules, including drugs, to penetrate. Hopefully, innovative studies like those conducted using the iChip will also discover new antibiotics against Gram-negative bacteria. Such a discovery may well become as famous as the story of penicillin.

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ANTIB

DEFINITIONS & FACTS

antibiotics are used to treat bacterial infections, but are sometimes mixed up with antimicrobials

In England, **1 in 3 people** take one or more courses of antibiotics / year

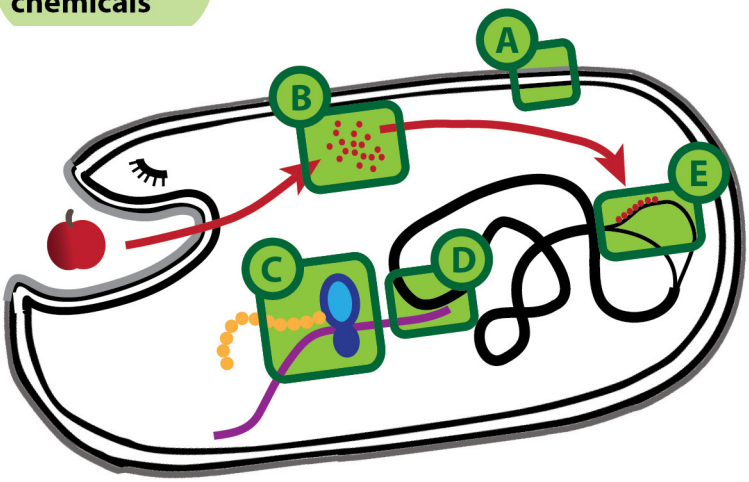
antimicrobials tackle:
- bacteria (anti-bacterials)
- viruses (anti-virals)
- fungi (anti-fungals)
- worms (anti-parasitics)

1 in 3 patients in English hospitals are prescribed antibiotics

Drug-resistant bacteria cause
25,000 deaths / year
1.5 billion € loss / year
(and this is in the EU alone!)

antimicrobials are also used for: **animals** (farm, wildlife, pets), **plants** (vegetation, farming), **aquaculture**, industrial and household **chemicals**

MECHANISM OF ACTION

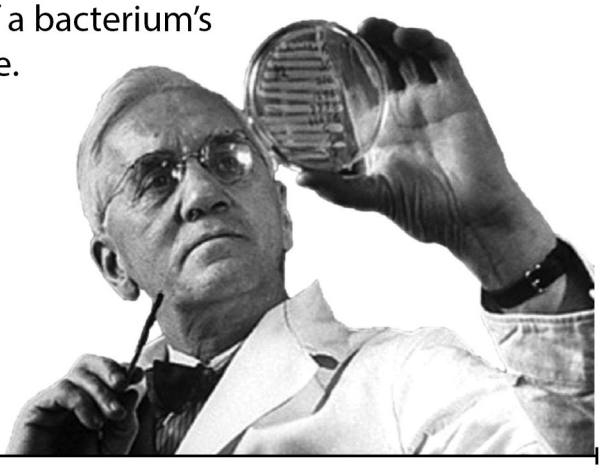


Antibiotics target bacterial:

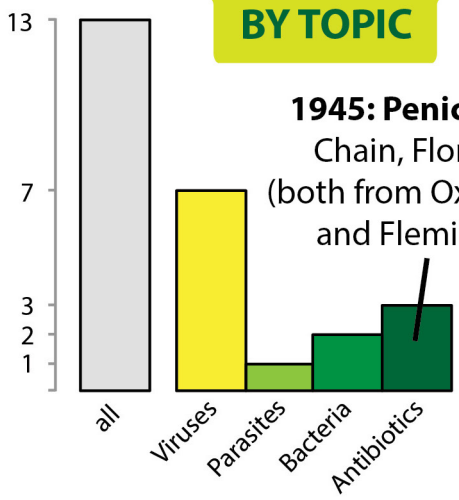
- **A** cell walls & membranes
- **B** folic acid metabolism
- **C** protein synthesis
- **D** RNA synthesis
- **E** DNA synthesis

Human cells have neither cell walls nor folic acid metabolism, so antibiotics targeting these only tackle bacteria - but this may include bacteria that you actually rely on, e.g. gut bacteria that support human digestion.

Same-class antibiotics often target the same areas of a bacterium's life cycle.



NOBEL PRIZES BY TOPIC



1945: Penicillin Chain, Florey (both from Oxford), and Fleming

Pre-antibiotic era

1640, England: John Parkington suggests mould as a treatment (as in ancient Greece, Serbia, and India)

1897, France: Ernest Duchesne cures typhoid in guinea pigs using mould

1899: Rudolph Emmerich & Oscar Löw test the first antibiotic, pyocyanase, but find that it is toxic to humans

1910: Paul Ehrlich & Sahachiro Hata discover Salvarsan

1928: Alexander Fleming finds that penicillin killed bacteria in an old, fungus-covered Petri dish

1932: Gerhard Domagk discovers prontosil

by Dr Anne C Wolfes

IOTICS

WHEN TO USE ANTIBIOTICS

Antibiotics treat infections of the:

- **lung** (in the case of bacterial pneumonia and pertussis)
- **eye**
- **urinary tract** and **genitalia**
- **ear** (otitis), **nose** (sinusitis), and **throat** (pharyngitis)
- **skin**
- **digestive system**, e.g. serious gut infections can cause diarrhoea

WHAT CAN YOU DO?

Stop the spread of antibiotic resistance by:

- washing your hands
- using antibiotics only when needed (i.e. not for the common flu); 1 in 3 antibiotics prescriptions is unnecessary!
- finishing your prescribed course of antibiotics: bacteria become resistant more easily if only little antibiotic is around

ANTIBIOTICS IN THE NEWS

2005 EU bans antibiotics for use in boosting animal growth

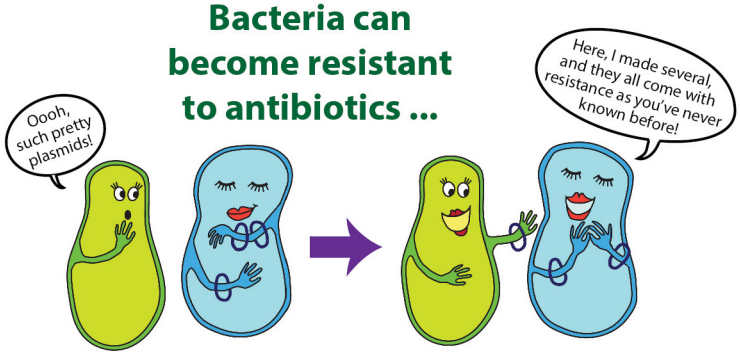
2007 - 2013 drug-resistant forms of Bubonic plague appear, from which Kyrgyzstani herder dies

2015 bacteria resistant to colistin (a "last resort") appear in the UK

2016 "superbug" resistant to all antibiotics appears in the U.S.

GENE TRANSFER IN BACTERIA

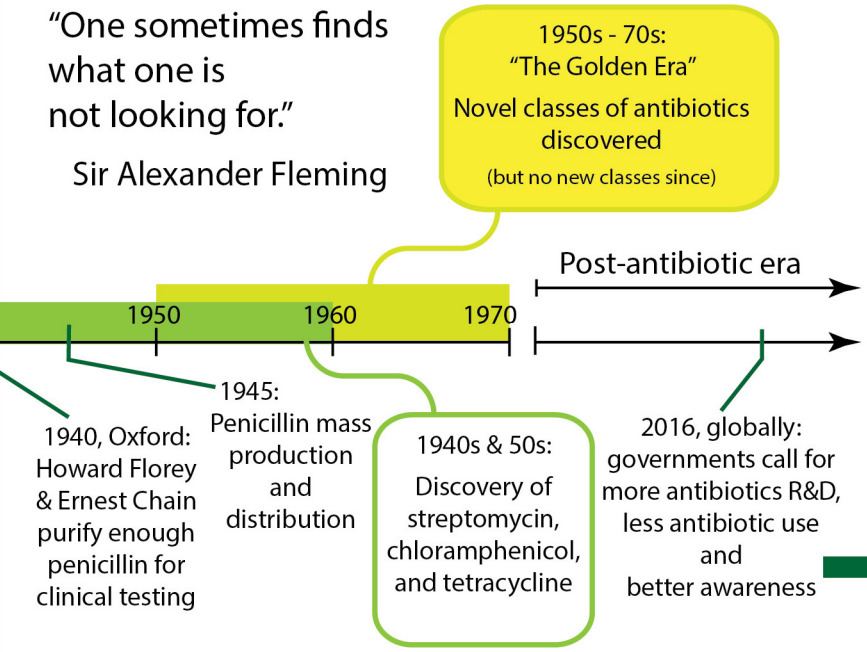
Bacteria can become resistant to antibiotics ...



... via spontaneous mutation or gene transfer, i.e. from plasmids provided by other bacteria. Plasmids (small circular pieces of DNA) can come from different strains or even entirely different types of bacteria!

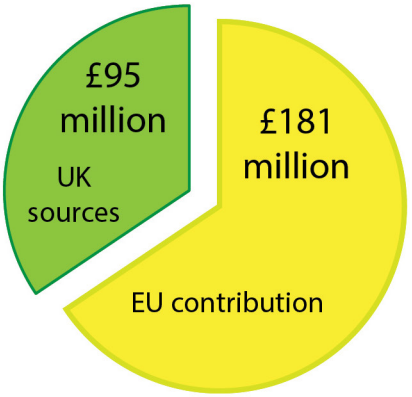
"One sometimes finds what one is not looking for."

Sir Alexander Fleming



UK-based research funding on antibiotics:

£276 million in total



... but this represents **less than 1%** of the UK's gross research funding in 2008-13

Good news:
More governmental support

This could be the time to start a career in antibiotics research!

β-Lactamase-mediated antibiotic resistance and the importance of avibactam

by Martine Abboud

Antibiotic resistance is a major and growing global health threat. The World Health Organisation (WHO) report on antimicrobial resistance suggested that a post-antibiotic era in which common infections and minor injuries can kill is a very real possibility for the 21st century. Hospital-acquired infections (HAIs) account for a growing rate of mortality and represent an increasing financial burden. It is estimated that within the UK 8.2% of hospitalised patients contract a HAI (1) costing the UK National Health Service over £1 billion per annum (2). β-Lactams, such as penicillins, cephalosporins, and carbapenems, remain the most important class of antibiotics, representing 60% (3) of all antibiotics used. Today, however, the use of these life-saving β-lactam antibiotics is threatened by antimicrobial resistance which constitutes an immediate and growing threat to public health.

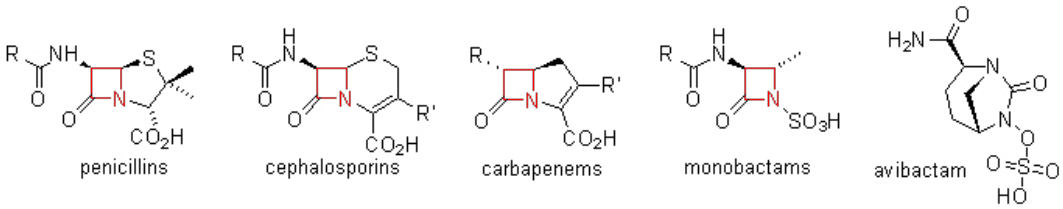


Figure 1. Major classes of clinically used β-lactams and avibactam. The β-lactam core is highlighted in red.

Peptidoglycan cross-linking

β-Lactam antibiotics mostly target peptidoglycan cross-linking in bacterial cell walls. Peptidoglycan is a highly cross-linked polymer composed of glycan strands with peptide cross-links. Because peptidoglycan is unique to bacteria and its presence is essential for maintaining the structural integrity of the cell, it constitutes an excellent target for selective toxicity. The glycan strands of peptidoglycans consist of alternating units of two amino sugars, *N*-acetyl glucosamine (GlcNAc) and *N*-acetyl muramic acid (MurNAc), which are linked by β-(1,4)-glycosidic bonds. A peptide stem that forms a cross-link with the peptide stem of an adjacent glycan unit is attached to each MurNAc unit. The peptide side chains are linked together by extensive cross-linking reactions, mediated by penicillin-binding proteins (PBPs). PBPs react *via* an active site serine residue with the D-Ala-D-Ala terminus of a pentapeptide to break the D-Ala-D-Ala linkage and form a Ser-D-Ala ester bond, leading to an acyl-enzyme intermediate (Figure 2). The carbonyl carbon of the sub-terminal D-Ala is then attacked by the side chain of an amine donor to cleave the ester linkage of the acyl-enzyme and form a cross-linking peptide amide bond. Hence, the free amino group is linked to the carboxyl group of the subterminal D-alanine by carboxypeptidation, releasing the terminal D-alanine (4).

β-lactam mode of action

β-lactam antibiotics are characterised by a four-membered β-lactam ring that resembles the D-Ala-D-Ala strained conformational structure. BLAs are able to bind to the active site of the transpeptidase enzymes where they form an acyl-enzyme with the active site serine. This acyl-enzyme is sterically blocked for attack by the pentapeptide nucleophilic residue and hence, the covalently bound β-lactam is a long-lived inhibitor of PBPs blocking peptidoglycan cross-linking. β-lactam antibiotics are special by nature and their side chains have been functionalised to achieve better activities.

Production of β-lactamases

Bacteria have, however, evolved efficient mechanisms of AMR, including PBP mutations to lower efficient binding of BLAs, the production and modifications of porins and efflux pumps to export β-lactam antibiotics (5). The most common mechanism of resistance in Gram-negative bacteria is the production of β-lactamases that catalyse the hydrolysis of the amide bond in the β-lactam ring to generate ineffective products. The incidence of HAIs by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* or *Acinetobacter baumannii* is increasing. According to the Ambler classification (7), β-lactamases can be subdivided to four classes: class A (penicillinases), class B (carbapenemases), class C (cephalosporinases) and class D (oxacillinases) based on structural and mechanistic characteristics (6). Classes A, C and D are serine-β-lactamases (SBLs) which use an active-site serine residue to catalyse the hydrolysis of the β-lactam ring. Class B are metallo-β-lactamases (MBLs) which use zinc ions in their active site to hydrolyse the β-lactam ring (8).

The special nature of avibactam

Avibactam is the first non-β-lactam based β-lactamase inhibitor to reach the market (in combination with ceftazidime, it is branded as

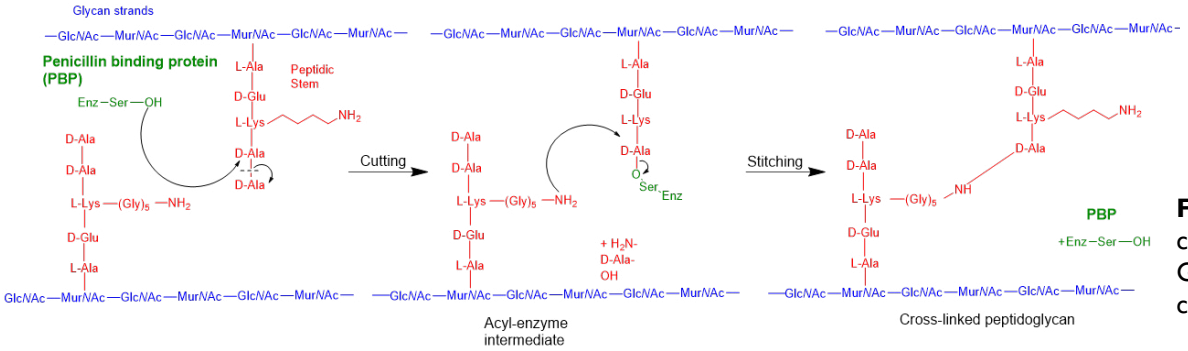


Figure 2. Peptidoglycan cross-linking in the Gram-positive bacterial cell wall.

Avycaz). Avibactam is a potent broad-spectrum inhibitor of class A, class C, and some class D SBLs. Like β-lactam antibiotics, avibactam has a core ring which is subject to degradation by SBLs *via* covalent interaction with their catalytically active site serine residue. In contrast to β-lactam antibiotics which react irreversibly with SBLs and become inactive after hydrolysis, avibactam has been shown to react reversibly with SBLs *via* recyclisation of its core ring. Accordingly, avibactam is more efficient than BLAs in terms of the number of molecules required to inhibit each β-lactamase; it requires only 1-5 avibactam molecules per β-lactamase compared with 10s-100s molecules of BLAs per β-lactamase due to hydrolysis competing with inhibition. Avibactam also forms a long-lived complex with its SBL target (t1/2>7 days). As the first clinically useful non-β-lactam β-lactamase inhibitor, avibactam represents a major breakthrough in the β-lactamase/PBP antibacterial field. The advent of avibactam should inspire further efforts to identify non-β-lactam penicillin-binding protein/serine β-lactamase inhibitors (9).

The challenge

However, avibactam does not bind and/or is poorly effective against MBLs (10). Some reports estimate that within 5-10 years up to 1 billion people could be infected with New Delhi MBL-1-carrying organisms (11). Whilst inhibitors of Class A β-lactamases, e.g. clavulanic acid (partnered with amoxicillin) have proved to be very successful in extending the lifetime of penicillins, MBLs are not inhibited by any of the currently available SBL inhibitors (12). MBLs have a broad-spectrum activity and can catalyse the hydrolysis of almost all β-lactams except monobactams (13). They are not inhibited by SBL inhibitors and/or a mechanism-based inhibitor such as clavulanic acid, sulbactam or tazobactam. Thus, the challenge nowadays is to develop MBL inhibitors that have broad-spectrum activity, do not interfere with human enzymes which possess the same MBL αβ/βα fold and are not subject to readily bacterial resistance by simple mutations. The biophysical and biochemical properties of MBLs and their mode of action need to be better understood. Furthermore, it is important to raise awareness of antimicrobial and important hygiene measures and encourage the public to follow prescriptions accurately and avoid excessive use of antibiotics. Fighting antimicrobial resistance is a global challenge and requires unification of efforts.

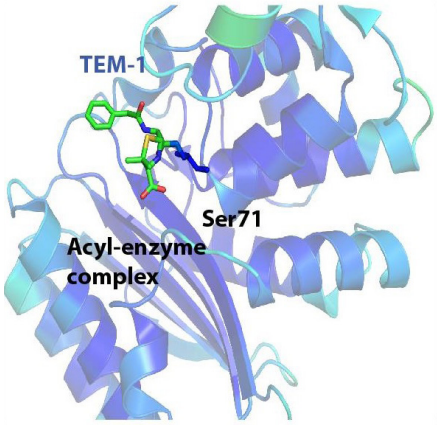


Figure 3. View from a crystal structure of a serine-β-lactamase in complex with hydrolysed penicillin G.

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Profile: ZIKV and microcephaly

by Sanskrithi Sravanam

With active transmission reported in over 63 countries, Zika virus (ZIKV) has caused increasing concern over public health. Relatively unheard of for over 60 years by anyone other than a few specialised researchers, the virus is now causing massive outbreaks in Asia and the Americas. Although there is an increasing body of knowledge about the virus and its effects on those who become infected, there are many questions yet to be answered.

The arbovirus, which belongs to the genus *Flavivirus*, was first discovered in 1947 in the Zika forest of Uganda in samples taken from captive sentinel monkeys. In 1948, the virus was recovered from *Aedes africanus* mosquitoes, also caught in the Zika forest, and the first formal description of the virus was published in 1952. For more than 60 years, there were no large outbreaks of the virus, with only sporadic human infections, totalling around 14 cases (1). In recent times, however, ZIKV is making headlines around the world due to its link with birth defects in children born to infected mothers. The virus was declared a Public Health Emergency of International Concern by the World Health Organisation (WHO) in February 2016 (2).

The transmission cycle of ZIKV is similar to dengue virus (DENV). ZIKV in East Africa is maintained in a sylvatic cycle in wild animals. It causes cyclical infections amongst non-human primates and is transmitted via a wide variety of mosquito species, notably *Aedes* mosquitoes. *Aedes* mosquitoes have been domesticated over the years by encroachment of the urban landscape into their habitat. Multiple species of *Aedes* have so far been shown to be capable of transmitting ZIKV (3), we can therefore infer that any country with endemic DENV, where *Aedes* mosquitoes are present, is at risk of ZIKV transmission. However, unlike DENV, ZIKV is an emerging virus and populations will be immunologically naïve, with virtually no protection.

The first ZIKV epidemic in humans was recorded in 2007, when it caused an outbreak on Yap Island, part of the Federal States of Micronesia. Since then ZIKV infections have been reported all over the world, including Asia; French Polynesia and other Pacific islands; and South, Central and Northern America. A study published in *The Lancet* by Cauchemez and colleagues analysed data from the ZIKV outbreak in French Polynesia to explore the link between ZIKV and microcephaly, a congenital condition that results in smallness of the head due to incomplete brain development (4). The study established the greatest risk of microcephaly to be maternal ZIKV infection during the first trimester. Furthermore, the team compared the risk of birth defects and the prevalence of ZIKV with other viruses, including cytomegalovirus (CMV), rubella, and parvovirus B19 (Table 1). The data clearly showed that, although the risk of birth defects associated with ZIKV infection of a pregnant female may be very low, there is an incredibly high prevalence of the virus in the population, which increases the number of cases that are likely to arise due to ZIKV presence in an area.

Virus	Risk of birth defects	Incidence of virus in population
ZIKV	1%	66% (FP), 73% (Yap)
CMV	13%	1-4%
RUBELLA	38-100%	<10 cases per year (Fr)
PARVOVIRUS B19	10%	0.6-1.2%

Table 1. Comparison of the risk of birth defects and viral incidence of various viruses (FP: French Polynesia, Fr: France). Data from Cauchemez, *et al.* (4).

Clinically, almost 80% of those infected with ZIKV will be asymptomatic. In the remainder, infection usually presents with low-grade fever (<38.5°C), transient arthritis or arthralgia, maculopapular rash and conjunctivitis, myalgia, lethargy and headaches. The incubation period is between 3-12 days and the symptoms are short-lived, lasting up to a week. Most infections are not recognised or are misdiagnosed as DENV or chikungunya virus (CHIKV) in the absence of laboratory testing. Currently, ZIKV can be confirmed in the laboratory via detection of viral RNA from clinical specimens, usually blood. However, the period in which the virus is present in the blood is short, lasting only 3-5 days after onset of symptoms. Serological assays can detect ZIKV antibodies 5-6 days after the onset of symptoms, but false positive results can occur due to cross-reactivity with related *Flaviviruses* such as DENV or yellow fever (3).

Investigations to date have confirmed the presence of viral RNA in brain tissue, placenta, and amniotic fluid of some infants with microcephaly and from post-mortem samples of foetuses from mothers

infected with ZIKV during pregnancy. However, it is still not known whether ZIKV is the causative agent for any of the reported foetal complications, which include microcephaly, foetal loss and Guillain-Barré syndrome, a rare but serious neurological disorder.

Several studies have investigated the potential pathogenic mechanisms of ZIKV. Experiments in mice have shown that the highest level of ZIKV RNA in infected pregnant females was found in the placenta (6), which might facilitate transmission of the virus to the foetus. Other studies demonstrate that ZIKV is a highly neurotropic virus, meaning that it can infect nerve cells. Infected foetal mouse brains exhibit characteristic irregularities in their development including decreased size, similar to microcephaly as seen in human counterparts (7, 8). A recent study has identified the AXL receptor, a *Flavivirus* entry receptor, as enriched in certain types of brain cells, such as radial glial cells, astrocytes, and endothelial cells, suggesting that these may be especially prone to ZIKV infection in the developing brain (9).

ity of the virus to affect foetal development is of great concern. Children with microcephaly are at risk of long-term developmental problems. Although there is currently no available treatment, early diagnosis and intervention may improve the child's quality of life. As ZIKV research increasingly focuses on the effects of the virus on neurodevelopment, new mouse models, and lessons learnt from other Flaviviruses, will enable a much deeper understanding of the mechanisms that facilitate viral pathogenesis.

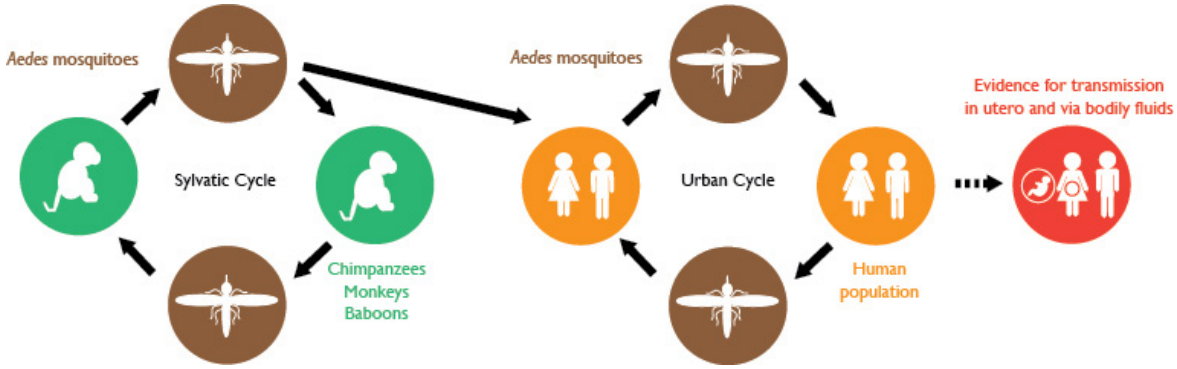

Figure 1. The virus is maintained in a sylvatic cycle in non-human primates in tropical rainforests. It can be transmitted to humans via mosquitoes, which are attracted to pools of standing water in warm urban environments.

Although many of the big questions about ZIKV are being answered, many more remain to be tackled, such as:

- Can the virus be transmitted through bodily fluids?
- Are all ZIKV strains equally able to cause infection and induce neurological birth defects?
- Can the maternal immune system prevent infection of the placenta?
- As there is increased risk of microcephaly in the first trimester as compared to later stages, does the immune system differ during trimesters?
- Does ZIKV infection cause neuronal damage directly, or does placental damage indirectly contribute to pathogenesis?

These questions are among many others that are continually raised through research and clinical findings.

ZIKV is spreading, and it is spreading fast. The ability of mosquitoes to transfer the virus and the capac-

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Flushing the brain

by Helle Bogetofte Barnkob

The 'glymphatic system' is a phrase coined by the Danish neuroscientist Maiken Nedergaard, who was one of the first to propose this paradigm-shifting hypothesis of how the brain gets rid of its waste. The word glymphatic is a contraction of the words glia and lymphatic. The hypothesis puts a spotlight on the importance of the brain's glial cells and addresses the long-unanswered question of why sleep is vital for us.

Although nerve cells are responsible for the brain's main function, synaptic and electrical signalling, glial cells actually outnumber the nerve cells by a ratio of around 3 to 1. Their many roles include maintaining homeostasis, and protecting and supporting the nerve cells. One subtype of glial cells, the astrocytes (or astroglia), seem to be particularly important for sustaining the appropriate chemical environment for the nerve cells to signal. One way they perform this function is by extending numerous processes to surround the blood vessels in the brain, thereby supporting the blood-brain barrier formed by the endothelial cells. New research indicates that astrocytes are part of the glymphatic system and are crucial for waste disposal in the brain (1).

Brain waste

In addition to astrocytes, the other component of glymphatics is the lymphatic system. As well as being an important part of our immune system, the lymphatic system is vital for transport of excess fluid, extracellular proteins and metabolic waste products away from tissues and into the blood circulation. If this function is disrupted, the osmotic pressure and homeostatic regulation of the tissue is compromised, resulting in oedema.

For decades it was believed that the central nervous system (CNS) was different from the rest of the body, in that it completely lacked a lymphatic system. Lymphatic vessels in the CNS had simply never been documented. Early theories of how the CNS cleared waste without a lymphatic system suggested that cerebrospinal fluid (CSF), although not in direct contact with the brain and spinal cord, was responsible. How exactly this worked was, however, not clear. The distance between the CSF compartment and the deeper brain tissues is far too great for larger compounds to be removed by simple diffusion. This problem puzzled researchers for many years and various theories on the subject were proposed and rejected.

Fluorescent flow

In 2012, Professor Maiken Nedergaard and her research group published their first paper on the glymphatic system (2). They used a simple but very convincing strategy to prove their theory. By injecting fluorescent tracers into the CSF of mice, they showed that the CSF enters the brain tissue through spaces that surround cortical arteries. Furthermore, fluorescent tracers injected directly into specific brain areas were removed together with the extracellular fluid, also called interstitial fluid, through the larger veins leaving the brain tissue (2).

The group hypothesised that astrocytes were partly responsible for the flow of fluid into and out of the tissue and that this happened through water channels composed of the protein Aquaporin-4 (AQP-4). The theory underlying this idea was that AQP-4 channels are found at a very high concentration on the surface of the astrocyte processes that surround the blood vessels of the brain. To further investigate this hypothesis, Nedergaard's team studied transgenic mice lacking AQP-4, and identified that they showed a large decrease in both entry of CSF into the brain tissue and clearance of interstitial solutes from it (2). Three main conclusions about the glymphatic system were drawn from these initial experiments (Figure 1):

1. The CSF enters the brain tissue through an entry route surrounding arteries in the brain.

2. The extracellular fluid leaves the brain tissue through an exit route around the major veins in the brain.

3. Flow of interstitial fluid in the brain tissue, between these entry and exit routes, is facilitated by AQP-4 channels in astrocytes, which drives the clearance of interstitial fluid, protein and waste products from the brain (3).

Sleep and the glymphatic system

The need for sleep is conserved across almost all animal species. When animals or humans are significantly sleep deprived, this can impair cognitive abilities and ultimately lead to seizures

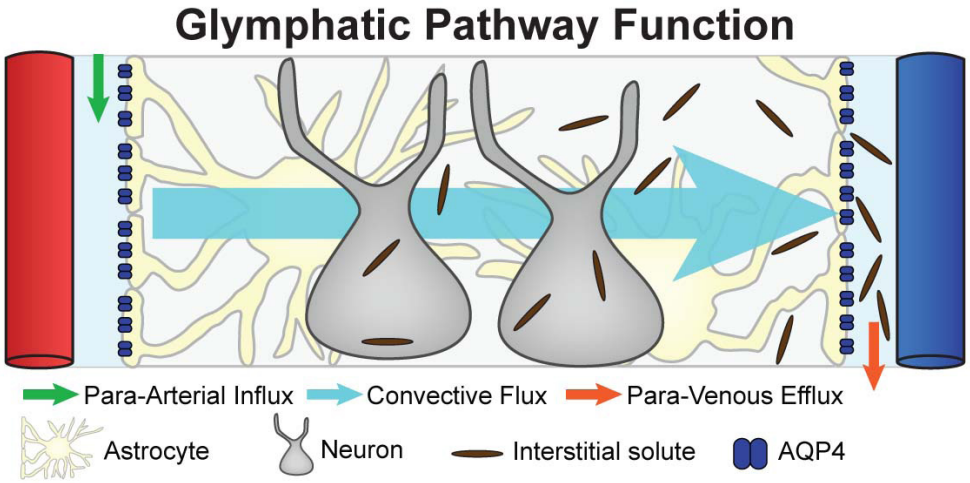

Figure 1. Schematic showing how the glymphatic system transports waste products out of the brain tissue and is facilitated by Aquaporin-4 channels on astrocytes.

and even death. However, we do not fully understand why sleep is so vital or what influence it has on our brains. The effect of sleep on the glymphatic system could perhaps give an indication of the answer. After their initial discoveries, Nedergaard's research group went on to show that glymphatic flow is dramatically increased in the brains of mice when they are asleep (4). This increase in glymphatic activity was correlated with an increase in the extracellular space in the brain tissue during sleep. Although the mechanisms that underlie expansion of the extracellular space were not examined further, one hypothesis is that the glial cells, by changing shape and size, are responsible. The conclusion, and indeed title of the resulting paper, was that "sleep drives metabolite clearance from the adult brain" (4)

Cleaning out Alzheimer's

The group has also established a role for the glymphatic system in the development of neurodegenerative diseases such as Alzheimer's. They injected fluorescently-labelled Amyloid-β, a protein that accumulates in the brains of Alzheimer's patients, into the brains of mice. The proteins were found to follow the same exit pathways around the veins as the fluorescent tracers used in the group's previous studies. Furthermore, in mice lacking AQP-4 channels, the removal of Amyloid-β was strongly inhibited, highlighting the importance of the glymphatic system for the clearance of these potentially toxic proteins (2). The removal of Amyloid-β was, as expected, a lot more effective in sleeping mice (4). Given that ageing is the most important risk factor for neurodegenerative diseases like Alzheimer's and Parkinson's disease, it is also interesting that glymphatic flow has been shown to decrease with age in mice.

The secret lymph

In 2015, another ground-breaking discovery was made in the field. Two different research groups independently discovered and published that lymphatic vessels can be found around arteries in the meninges, the membranes that cover the brain and the spinal cord (5,6). Due to difficulty in preserving the meninges during dissections, these lymph vessels had not previously been noticed. Both research groups suggested that these lymphatic vessels act as an entrance and exit point for lymphatic fluid and immune cells moving between the meningeal space and the glymphatic system, thereby tying together their work with previous findings (6). The combined discovery of the glymphatic system and a classical lymph pathway in the brain has not only changed our fundamental understanding of the anatomy of the brain, but could also help advance our understanding of why we sleep and how sleep impacts on neuroinflammatory and neurodegenerative diseases.

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A problem for the 21st Century: tackling the Nitrogen Crisis

by Rachel Wheatley

Nitrogen is a building block of life: it is a constituent of nucleic acids, amino acids and ATP. It is abundantly available in the atmosphere (~78% of air), but in an unreactive dinitrogen form that is inaccessible to plants. Therefore, nitrogen deficiency is a major factor limiting plant growth and crop yields. Food production was consequently restricted to using naturally occurring sources of reactive nitrogen, such as manure, until the development of the Haber-Bosch process during the early 1900s. The Haber-Bosch process allows us to fix reactive nitrogen and then apply it to crops as nitrogen fertiliser, and this has allowed food production to roughly keep pace with population growth over the last 50 years.

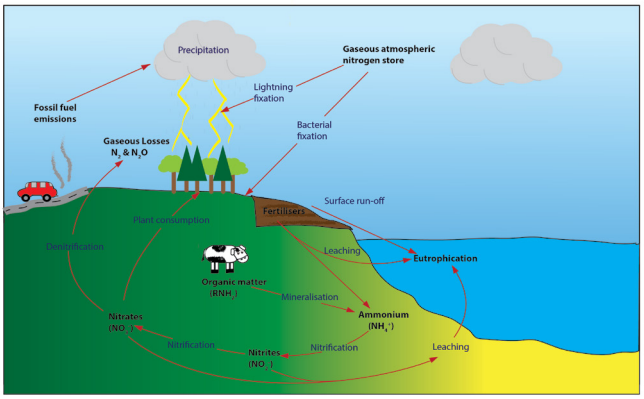

Figure 1. The nitrogen cycle

While adding nitrogen fertiliser enhances plant growth, there is a 'critical load' to the nitrogen that plants can absorb, after which nitrogen will leach into the water system and atmosphere. Over its long lifetime in the environment (~120 years) nitrogen can change forms and detrimentally affect a myriad of different systems, before eventually denitrification releases it back into the atmosphere (1). Overloading nitrogen in the environment drives biodiversity loss, contributes to global warming and causes human health problems. Nitrous oxides are a major contributor to smog and cause air pollution that decreases life expectancy in the areas affected by it (1). Nitrogen entering water systems promotes algal blooms, which harm water quality and cause low-oxygen zones and death of aquatic organisms. In addition, heavily nitrogen-polluted water is unsafe to drink, as the elevated toxins and bacterial growth within it are damaging to human health.

There has been an eightfold increase in nitrogen fertiliser application on agricultural land in the developed world in the last 50 years, and only roughly half of this applied nitrogen is actually absorbed by crop plants (1, 2). What's more, nitrogen fertiliser production and fossil fuel combustion have doubled the amount of reactive nitrogen entering the nitrogen cycle each year (3). The resulting major global shifts in the nitrogen cycle mean we now face a number of environmental and ecological consequences, together with issues affecting human health. Paradoxically, in other regions of the world with insufficient capital and infrastructure for the distribution of fertilisers, a lack of reactive nitrogen is one of the major restrictions on food production. Nitrogen deficiency in the soil causes substantial yield losses, and this further increases the inequality in our global food systems.

In short, the challenge of the nitrogen crisis that we face is to balance our use of reactive nitrogen so that we can sustain food production and population growth, while minimising the damage to the environment and ecosystems. This challenge will no doubt require the integration of numerous scientific, social and political innovations.

One exciting scientific possibility is to take advantage of the naturally occurring symbioses between plants of the legume family and nitrogen-fixing bacteria (*Rhizobia*). The *Rhizobium*-legume symbiosis efficiently supplies the legume hosts with their nitrogen demands, without producing excess soluble nitrogen that runs off into the soils and pollutes the environment. Farmers recognised the benefits of growing legume crops in rotation with others over 8,000 years ago. The legume crops provide nitrogen-rich soils and also produce nutritional protein-rich seeds.

A major focus in the field is to optimise the pre-existing symbiotic nitrogen fixation, by identifying the most effective nitrogen-fixing bacterial strains, for instance, and by developing the best inoculation strategies for the legume crops. Another major focus is on engineering this symbiosis into cereal crops, targeting globally important crop plants such as maize, wheat and barley. This would alleviate the need for a large proportion of the chemical nitrogen fertilisers that are applied, while still allowing crop yields to continue to increase (4). A number of engineering solutions have been proposed, such as expressing the nitrogen-fixing enzyme nitrogenase in mitochondria, modifying bacteria that are naturally present within cereal roots to fix nitrogen, and producing synthetic symbioses (4).

Numerous social and political innovations have also been proposed, including changes to agricultural practises and our diet. In terms of agricultural practice, this could include improving the distribution of the reactive nitrogen fertiliser we already produce, and avoiding applying fertiliser near water systems or on heavily sloping land. With regards to dietary changes, the suggestion is to reduce meat product consumption in favour of plant-based foods. Halving meat and dairy product consumption in the European Union alone would reduce nitrogen emissions by 40% (5).

In summary, it is clear that proper nitrogen management will be key to ensuring that food production matches future population growth, while minimising damage to the environment and to human health. There is an urgent need to balance the input of reactive nitrogen into our biosphere with crop plant nitrogen requirements, while achieving a more even distribution of reactive nitrogen on a global scale to combat the inequalities in food production in nitrogen-deprived areas.

Rachel Wheatley is a second year DPhil student supervised by Professor Philip Poole in the Department of Plant Sciences

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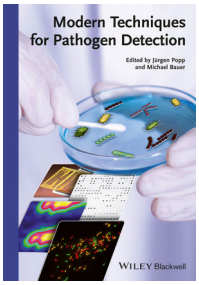
BOOK REVIEWS



Modern Techniques for Pathogen Detection

Ed. Michael Bauer & Jürgen Popp

ISBN: 978-3-527-33516-9 Wiley Blackwell (2015)

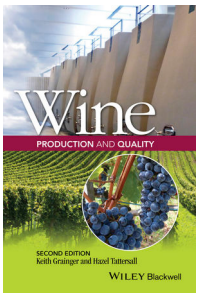
352 pages: Hardback, £90/ eBook, £81.99

Reviewed by Dr Suvi Honkanen

Infectious diseases caused by microbial pathogens (bacteria, viruses, fungi and parasites) are a major cause of income loss, disability and death worldwide. While infectious diseases such as tuberculosis, malaria and AIDS remain particularly prevalent in developing countries, increased bacterial antibiotic resistance and recent major disease epidemics are likely to keep microbial pathogens in the headlines globally. Each microbial pathogen has its own distinct characteristics, which define the type of treatment likely to be most effective. Therefore, in order to provide each patient with optimal treatment, as well as to prevent further spread of the disease, it is crucial to identify the disease-causing microbe as quickly, accurately, and cost effectively as possible.

Modern Techniques for Pathogen Detection summarises the latest as well as older but still valid methods for identifying microbial pathogens. The book contains 330 pages and is split into seven chapters. Chapter one focuses on the management of life-threatening infections, which due to their acute nature require particularly fast intervention. Chapter two provides a very brief overview of the conventional methods used for detecting microbial pathogens, whereas chapters 3-7 give a more detailed description of both conventional and novel methods based on nucleic acid amplification, microarrays, mass spectrometry, infrared and Raman spectroscopy, and fluorescence in situ hybridisation (FISH), respectively. Each of these chapters first summarises the background of the technique, and then describes its variations, discussing their strengths and weaknesses in different contexts.

The book is intended to provide an overview of the methods available for pathogen detection without providing the level of technical detail necessary to carry out each method in practice. This makes the book easily readable for anyone with a basic understanding of molecular biology. *Modern Techniques for Pathogen Detection* is a useful resource for anyone interested in diagnostic methods. In particular, I would recommend this book to researchers focusing on human microbial pathogens, as it gives an idea of the methods currently available, and perhaps provides a starting point for exploring how the methods used for detecting infections caused by their pathogen of interest could be improved.



Wine Production and Quality: 2nd Edition

Keith Grainger & Hazel Tattersall

ISBN: 978-1-118-93455-5 Wiley Blackwell (2016)

328 pages: Hardback, £65 / eBook, £58.99

Reviewed by James Eaton

Wine, to many, is a completely subjective topic. Usually described with words like "deep" or "complex", it can seem totally inaccessible to those without an extensive and expensive cellar. However, with their book *Wine Production and Quality,* Keith Grainger and Hazel Tattersall show how the often impenetrable world of wine can be enjoyed by anyone who can "see, smell and taste the difference between oranges, lemons and grapefruit".

The first half of the book takes the subjectivity out of wine and quite plainly describes how and why, climate, soil, environment and several other factors can affect the quality of wine. This gives a whole new meaning to the term "terroir" and provides countless facts and stories that will undoubtedly impress even the most seasoned of wine connoisseurs.

The authors then describe in exquisite detail the numerous ways in which red and white wine can be made. Two chapters are dedicated to barrel maturation and preparing the wine for bottling, giving an informative look at two important yet often overlooked steps of the wine making process. Each chapter has plenty of examples relating every step to a real world situation, ensuring that the book does not become too abstract.

The second half of the book is devoted to wine quality: how it can be measured, enforced and what it actually is. It begins by explaining how to look at, smell and taste wine to assess quality, with each sensation given its own chapter. The scientific style of "why" and "how" that dominates the first section is not lost here; at one point there is a neuroanatomical description of how people perceive taste.

The authors then go on to explain what wine quality really is. While this seems hard to define at first, by the end the concept is clear: "Good-quality wines excite and stimulate with their palettes of flavours and tones, their structure and complexity. Fine wine can send a shiver down the spine, fascinate, excite, move and maybe even penetrate the soul of the taster".

An informative and interesting book for those wishing to learn more about wine, *Wine Production and Quality* will suit both seasoned oenophile and curious wine lover alike.

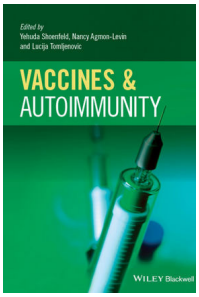
BOOK REVIEWS

Vaccines and Autoimmunity

Ed. Yehuda Shoenfeld, Nancy Agmon-Levin, Lucija Tomljenovic

ISBN: 978-1-118-66343-1, Wiley Blackwell (2015)
Hardback, 384 pages, £113/ eBook, £1091.99

Reviewed by Dr Cristina Marculescu



Vaccines and Autoimmunity is a collection of extensive reviews on autoimmunity as response to vaccines, written by world experts in this complex field of research.

While a few decades ago, the majority of people would have regarded vaccines as one of the greatest inventions in the history of humankind, recent years have witnessed a sudden change. The question whether there is a connection between vaccination and autoimmune disease is now surrounded by controversy and has deeply penetrated into the public media. Intense debates are going on at all levels of society regarding the causality between vaccines for diseases such as measles and hepatitis B virus, and multiple sclerosis. Tetanus toxoid, influenza vaccine, polio vaccine and others have been related to phenomena ranging from autoantibody production to rheumatoid arthritis. Conflicting data exists regarding the connection between autism and vaccination with measles vaccine.

It is now clear that certain genetic factors and pre-existing medical conditions such as autoimmune inflammatory rheumatic diseases influence the likelihood of side-effects following vaccination. *Vaccines and Autoimmunity* provides an excellent summary of the relevant evidence available at the moment and brings a strong argument towards personalised vaccination programmes.

At the same time, the authors shine light on aspects of vaccine formulations less appreciated and investigated. Questions ranging from the role and mechanism of action of various types of adjuvants (aluminium-based, oil-based, virosomes), the correlation between the adjuvant used and the type of vaccine, and ultimately the potential side-effects are all considered. In this context, a few chapters are dedicated to the Autoimmune Syndrome Induced by Adjuvants and offer an extensive summary of our current understanding.

Several of the most commonly employed vaccines such as those for measles, mumps and rubella, hepatitis B, influenza, human papilloma virus and yellow fever are discussed at length.

While undoubtedly *Vaccines and Autoimmunity* is indispensable for researchers working in the field of vaccine development, this book would also be highly valuable to medical doctors and anyone who would like to have a deeper understanding of what could be done in order to minimise the risks currently associated with vaccination.

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Career Insights: Patent Attorney

by Dr Eleanor Healey, J A Kemp

Ellie Healey is a first year trainee patent attorney at J A Kemp. She has an undergraduate degree in Natural Sciences from the University of Cambridge and a DPhil in Structural Biology from the University of Oxford.

What is a patent attorney?

Patent attorneys advise clients on protecting their inventions, draft patent applications and then steer the applications through examination by patent offices. Patent attorneys have science or engineering degrees and often PhDs, and they are trained in patent law.

Why did you choose a career as a patent attorney?

Although I enjoyed my PhD, I was looking to escape some of what I saw as the downsides of a career in academic research, such as an uncertain career path, short-term contracts and the pressure to publish. I first discovered the world of patents in the third year of my DPhil whilst googling "what jobs can I do with a PhD in biochemistry". I was looking for a job that would be related to science, intellectually challenging and diverse on a day-to-day basis.

What is the training process like?

The training process is not for the faint-hearted! It is long (about 5 years) and requires passing a number of exams. At J A Kemp we have on-the-job training under the guidance of a qualified mentor supplemented by tutorials, journal clubs and external courses. After we have been with the firm for a year, we get the opportunity to attend a one-term course in intellectual property at Queen Mary College in London.

What do you enjoy most about the job?

I get to learn about scientific innovations that have real world applications. The process of understanding a patent examiner's objection, working out how to get around it and then explaining this in writing to the client is satisfying.

Do you miss science?

The beauty of this job is that I am still connected to science. If the question was do I miss the lab work… my answer would be not really. If the practical side of science is what you really enjoy then this is not the job for you. However, if, like me, you enjoy problem solving and written communication and like the idea of working in varied and often unfamiliar scientific fields, then you should look into becoming a patent attorney.

What is the worst thing about the job so far?

You work on lots of different cases that are all at different stages in the application process, so things can get quite confusing, particularly when you are new to it all. There are a lot of deadlines, and juggling all the different tasks can be tricky. That said, at the end of each day I always feel as if I have made progress on a case, which is very different from how I felt about work in an academic lab where it often felt like months went by without making any progress.

Do I need a PhD to become a patent attorney?

No, but you do need a science or engineering degree. Some firms like recruiting people with PhDs, but it is not normally a requirement. I learned valuable skills during my PhD that I can now apply to my current job, such as clear scientific writing, critical analysis of documents and independent time and project management.

What is the work-life balance like?

Very good… so far! Sometimes a critical deadline is approaching and I have to stay to finish a draft, but I usually know in advance if that will happen and can plan for it.

Do you have any further advice for someone interested in becoming a patent attorney?

Most of the large patent firms have a lot of information on their websites about the nature of the career. I also attended the Science, Engineering and Technology Careers Fair where lots of patent firms send representatives. If you'd like to know more, drop me an email to ehealey@jakemp.com.

SNAPSHOT Research Image Competition

This issue's winners are...

Dr Sonia Muliyil and Dr Clémence Levet, Dunn School of Pathology

The winners of this issue's SNAPSHOT competition are Drs. Sonia Muliyil and Clémence Levet! The winning image, titled 'Optical Illusion' was generated by creating mirror images of the *Drosophila* larval brain stained for an endoplasmic reticulum (ER) marker (green), Lamin (red) and DAPI (blue) acquired using high resolution imaging techiques.

Sonia Muliyil completed her PhD in Cell and Developmental Biology, studying the complex cross talk between mitochondrial remodelling, stresses and apoptotic signals, using a model for wound healing. She has been awarded both Human Frontier Science Program (HFSP) and European Molecular Biology Organisation (EMBO) fellowships for research carried out in the Freeman lab at the Dunn School of Pathology.

Clémence Levet performed her PhD in Bertrand Mollereau's group in the Laboratory of Molecular Biology of the Cell in Lyon, France. She studied the role of ER stress and autophagy in the regulation of neurodegeneration in the *Drosophila* eye. In 2014, she joined the Freeman lab as a post-doc/lab manager.

Research in the Freeman lab focusses on understanding the mechanisms underlying the control of signalling by members of the rhomboid-like superfamily of proteins, using an array of model organisms. One of the projects in the lab is aimed at understanding the cellular and physiological role of iRhoms (a class of inactive rhomboids), which are highly conserved through evolution.

In *Drosophila,* an iRhom 'pseudoprotease' was found to be highly enriched in neurons and shown to inhibit epidermal growth factor (EGF) signalling by driving EGF ligands through a process known as ER-associated degradation (ERAD). High resolution imaging of neurons in the *Drosophila* brain and retina through development as well as in adulthood is an integral part of this project and is helping to uncover the functional relevance of elevated neuronal expression of the *Drosophila* iRhom, and to investigate its molecular role in ERAD.

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SNAPSHOT
Research Image Competition

Win a £50 book voucher kindly provided by Oxford University Press!

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of the Hilary 2017 issue of *Phenotype*.

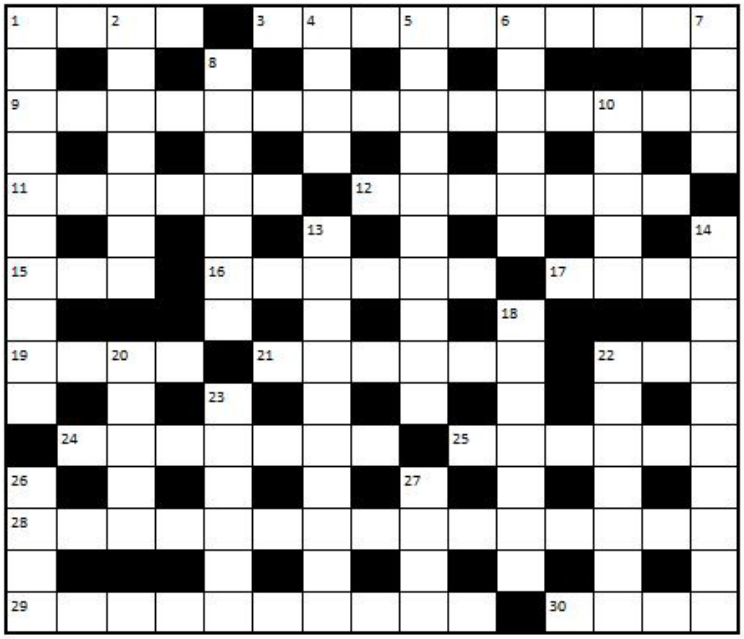
To enter, send images to rebecca.hancock@linacre.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

PHENOTYPE crossword

Fish challenges you to this 10th cryptic crossword for *Phenotype* on the theme of antibiotics. Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to heather.booth@st-annes.ox.ac.uk. Entries received before Friday 2nd December will be entered into a prize draw to win one of the four books reviewed in this issue.

The winner of the cross-word competition will recieve their choice of one of the books reviewed on pages 28 to 29, kindly provided by

WILEY-BLACKWELL



ACROSS

1. Shackle copper who's very loud (4)
3. Wager a 50 on performance of morning class of **14**-like **5**s (4-6)
9. Game piece carries a certain danger? (2,7,2,4)
11. Head Secretaries in society work together (6)
12. A degree (90 degrees? 180 degrees?) in primary study of infected cyst (7)
15. Start up stealing cars, initially? (1,1,1)
16. A state getting nothing right, for example, is nothing new (6)
17. A simple lake? (4)
19. Measure length of bird (excluding head) (4)
21. Every second Latino child embraced martial art (6)
22. A piece of winter equipment that backwards types left out (3)
24. One from Cambridge permits delivery of 14 by this method (7)
25. Tag out if holding large rodent, . . . (6)
28. . . . Cat, infant or loon makes one standoffish (15)
29. Idiot messages in Lorem Ipsum, for example (5,5)
30. Like a family? (4)

DOWN

1, 26. Clavicle broken in two and ulna fractured; I'd take calcium and 14 centrally - with this, to overcome 5,13? (10,4)
2. Discoverer of 14 loudly removes heart of small rodent (7)
4. New First Lady turns up in uniform (4)
5,13. A problem with 14: it incites bacteria so new forms . . . (10,10)
6. . . . can alter one's emotional response? (6)
7, 27. Tie up to Spooner's dodgy pole (4,4)
8. A painful back could even be treated with spray (7)
10. Children subject to release (5)
13. see **5**
14. Revolutionary drug from mould in nice pill? (10)
18. She crystallised **14** using nuclear anodes in a mercury/potassium/indium setting (7)
20. 19: a new developer of **14** (5)
22. Putin's leading Kremlin to build a satellite (7)
23. Developer of 14 will abscond while holding half of **16** (7)
26. see **1**
27. see **7**

Answers to the crossword from Issue 24, Trinity 2016:

Across: 1, 24. Untranslated regions **7,28.** Open reading frame, **8.** Introns, **10.** Grin, **11,20 down.** Polyadenylation signal, **13.** see 23 down, **14.** SOHCAHTOA, **17.** Ergonomic, **19.** Ado, **21.** Biomineralises, **22.** Toss, **24.** see 1 across, **27.** Mate, **28.** see 7 across.
Down: 1. Uni, **2.** Totally, **3.** Nun, **4.** Tar, **5.** Donation, **6.** Fellow, **9.** Ovals, **10.** Glycaemia, **11.** Power, **12.** Enhancers, **15.** Promoter, **18.** Cling, **19.** Adenoma, **20.** see 11 across, **23, 13 down.** Sea way, **25.** Egg, **26.** See