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SCIENCE CHAIRMAN'S ADDRESS

Close encounters of the microbial kind

By Stephen Denyer, BPharm, PhD, FRPharmS

In his address at the British Pharmaceutical Conference on 24 September, the Science Chairman, Professor Stephen Denyer spoke about how collaborative research in microbiology seeks to keep pace with the microbial evolution that has led to the advent of widespread antibiotic resistance. He said that finding new approaches to the diagnosis, prevention and cure of infection will require a clearer understanding of microbial virulence and behaviour at both the molecular and host-interaction level

During my scientific career I have been privileged to work with colleagues from a great many disciplines — close encounters of the manifold kind, you might say. These collaborations have offered insights into biochemistry, biomaterials, food microbiology, genetics, immunology, medicine, phytochemistry, synthetic chemistry and surface analysis: applied microbiology is truly a science of the borderlands (to quote Professor Max Sussman's presidential address at the Society for Applied Bacteriology in 1997). Of course, such diversity did, initially, complicate my choice of subject for this scientific address. But a dispassionate look showed clearly a thread that has connected much of my research to these disciplines: an attempt to understand how to control contamination and infection.

In a conference that has ageing as a major theme, you might be forgiven for thinking that age-related diseases are by far the major cause of morbidity and mortality. This may be true in the developed world but globally 45 per cent of all deaths, and 65 per cent of early childhood deaths, are from infectious disease. Indeed, in 2001 just three diseases killed 5.4 million people worldwide: malaria, where 90 per cent of the one million deaths were in Africa; tuberculosis (TB) with 1.9 million dead, almost all in the developing countries where multiple-drug resistance is spreading; and HIV/AIDS with 1.8 million of the 2.5 million deaths concentrated in sub-Saharan Africa. Even in our more privileged environment we see, almost daily, unpredictable challenges from *Escherichia coli* 0157:H7, *Legionella pneumophila*, *Listeria monocytogenes*, *Salmonella* spp, *Campylobacter* spp, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis*.

Population growth, poor public infrastructure, modern travel, and changes in agricultural and food production, ecology and climate all contribute to the emergence or re-emergence of infectious disease. Much human effort has been dedicated to alleviation of this suffering, particularly in the development of powerful antibiotics, where pharmacy has had a major role to play. Unfortunately the discovery of new, commercially useful, antibiotic agents has declined significantly in recent years. In my address today, I would like to use some of my own research interests in microbiology to help illustrate aspects of this global effort

aimed at understanding and controlling microbial infection.

EARLIEST ENCOUNTERS

The microbial world constitutes three principal classes: the virus, the bacterium and the fungus (moulds and yeasts). Although only clearly identified as specific entities over the past 325 years or so,¹ their influence has been recognised for millennia. Thus, food maturation and beverage fermentation processes fall into antiquity, decomposition and spoilage were controlled by desiccation in ancient Egypt where also honey was first used as an antiseptic for wounds, and the antimicrobial potential of wine and plant extracts have been variously reported in the Bible, Koran and other religious works. Fur-

ther, in ancient Greece cobwebs were employed to cleanse wounds and, in the 9th and 10th centuries AD, Arab doctors of the Baghdad school were using the curative properties of mould appearing on farinaceous food. Biological weapons have been a feature of war since the catapulting of plague-ridden corpses into besieged fortresses and the despoiling of water courses by animal carcasses and human excreta.

The micro-organism, more particularly from my perspective and interest the bacterium, is a truly fascinating entity. We are surrounded by a microbial flora; if unchecked, a single staphylococcal bacterium would grow to cover the land surface of our planet to a depth of 2m within 48 hours (according to a calculation by Peter Gilbert and Michael Brown during a train journey from Birmingham to London). With replication that fast, evolution is happening in front of our eyes. Harnessed for good, this characteristic is highly beneficial offering unparalleled biotechnological opportunities; more frequently, it offers a continuous challenge to the ingenuity, resilience and resourcefulness of the human race.

Science Chairman



Professor Stephen Denyer has, since 1991, been head of the University of Brighton school of pharmacy and biomolecular sciences and professor of pharmaceutical and applied microbiology. His research interests include microbial pathogenicity, rapid microbiological methods, mechanisms of antibacterial action and biocompatible biomaterials. He has served for a number of years on the Royal Pharmaceutical Society's Science Committee and Conference Committee. He is a member of the Committee on Safety of Medicines subcommittee on chemistry, pharmacy and standards, and the Department of Health's Standing Pharmaceutical Advisory Committee. He is a member and former chairman of the editorial board of the *Journal of Pharmacy and Pharmacology*.

FIRST CONTACT: DIAGNOSIS

For over 100 years, culture has been the mainstay of microbiological identification; its strongest feature is confirmation of viability and its weakest, the need for sustained incubation periods. In our modern world, advances in diagnostic microbiology, including antibody detection, specific enzyme assays and molecular genetic techniques, offer rapid determination of food-borne contamination and infectious disease; their adaptation to the detection of bioterrorism is hotly pursued.

Unfortunately, these technologies are often unavailable, unreliable or too costly for laboratories in the developing nations, where rapid, accurate diagnosis could transform aspects of health care. There is no need more urgent than in the diagnosis of tuberculosis (Figure 1, top), where culture often takes six to eight weeks to give a result and the traditional microscope slide method (sputum smear) is only 30–70 per cent accurate² (Figure 1, bottom). Every undiagnosed and untreated (or improperly treated) individual with active infection will serve to propagate the disease.

Collaborative work with the late Professor Gordon Stewart of the University of

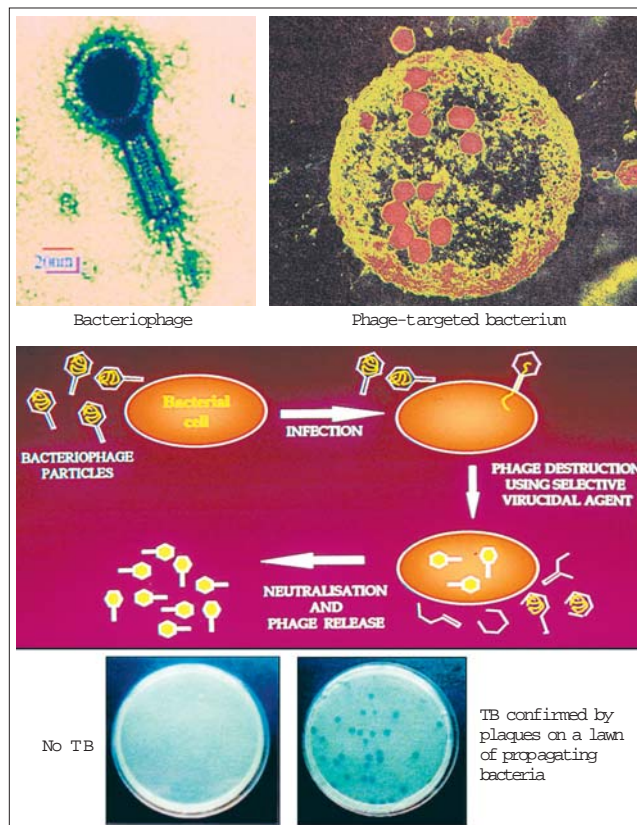
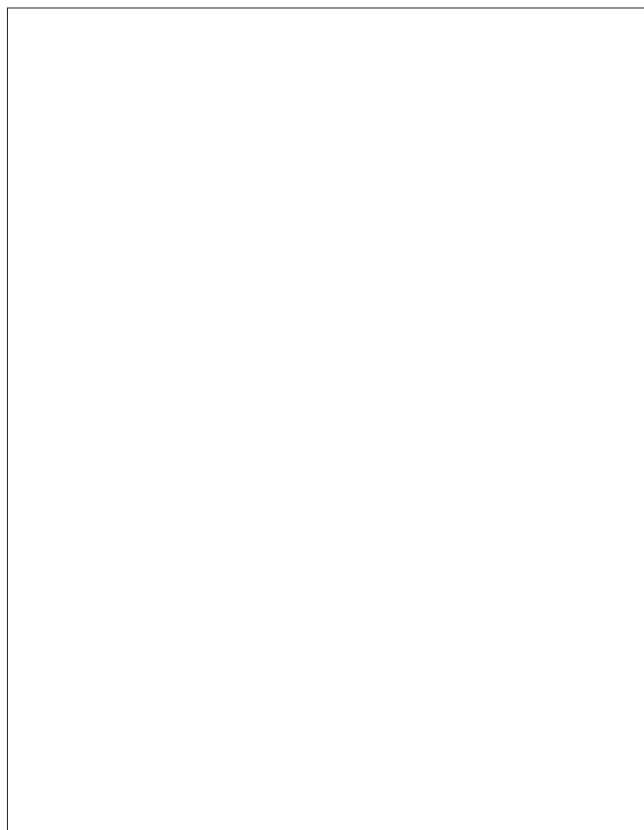


Figure 2. Phage interaction technology. Top, colour-enhanced images of an individual bacteriophage and phage interaction with a target bacterial cell; bottom, the principles of phage amplification where target bacteria can be rapidly identified and enumerated following phage infection

Nottingham has led to a laboratory method for TB diagnosis tailored to the needs of the developing world. This technique, which employs rapidly multiplying bacterial viruses (bacteriophage) to infect and “label” *Mycobacterium tuberculosis* in sputum (Figure 2, top), subsequently amplifies the bacteriophage signal in a lawn of propagating bacteria creating plaques from bacterial lysis (Figure 2, bottom); these plaques can be counted manually and a result obtained in 24 to 48 hours.^{3,4} In recent large-scale South African trials, this method showed a specificity of 99 per cent, and sensitivity of 72.5 per cent with positive and negative predictive values of 0.91 and 0.96, respectively, when compared with the gold standard of culture — and in one-twentieth of the time! Other studies have shown that the method may largely solve the problem of early detection of non-pulmonary TB where the normally low bacterial burden in specimens has previously meant culture was the only option.

Since the TB organism must be viable to become infected by the bacteriophage the test can be readily adapted to antibiotic sensitivity testing.⁵ In another national centre trial, this method has found clinical utility offering an overall accuracy of 97 per cent enabling more effective treatment selection earlier (with a consequent reduction in resistance risk).

I am pleased to be part of a Commonwealth Pharmaceutical Association initiative planned for selected African countries where local pharmacists will assist in the prevention and treatment of HIV-related tuberculosis.

CLOSEST ENCOUNTERS: MICROBIAL ATTACHMENT

Only in laboratory media do we regularly find free-floating planktonic bacteria. In nature their natural condition is to bind to surfaces, and this is particularly true of their association with the human host (Table 1).

Surface attachment and subsequent consolidation of bacterial microcolonies as a biofilm under an extracellular biopolymer (slime) layer offers significant survival advantages to the micro-organism, not least the creation of its own microenvironment under a protective coating. This is particularly noticeable in infections related to medical devices such as indwelling catheters, cardiac valves, pacemakers, knee and hip prostheses, and contact lenses. Such infections are notoriously difficult to treat, with effective doses of antibiotics often exceeding safe levels.

Sometimes these agents, if used sub-optimally, can alter bacterial surfaces suffi-

ciently to encourage further bacterial adhesion to devices and subsequent biofilm formation, as we showed in work with Dr Mark Wilcox from Sheffield and Professor Roger Finch and Professor Paul Williams from Nottingham. For example, exposure to one-quarter of the growth inhibitory concentration of vancomycin frequently led to increased adherent growth of coagulase-negative staphylococci to silicone rubber.⁶ The propensity to adhere is governed by surface chemistry, that for the medical device is influenced by conditioning molecules from the biological environment while the microbial surface composition is extremely plastic, being strongly affected by growth phase and nutrient conditions.

Staphylococcus epidermidis is an emergent pathogen in device-related infection, and particularly catheter colonisation in continuous ambulatory peritoneal dialysis (CAPD) where, on average, a patient will suffer one episode of peritonitis every 24 months.⁷ A fruitful collaboration with Professor Martyn Davies and Professor Paul Williams from Nottingham University showed how growth in patient dialysate radically altered the surface chemistry, determined by X-ray photoelectron spectroscopy, and the protein profile, determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of clinical isolates.⁸ This capacity to change surface character is particularly important for an organism struggling to adapt to a constantly changing environment such as the dialysed peritoneum.

More recently, using an *in vitro* model of CAPD peritonitis⁹ developed at Brighton

TABLE 1: TYPICAL EXAMPLES OF MICROBIAL COLONISATION OF THE HUMAN HOST IN HEALTH AND DISEASE

Association	Consequence
<i>Health</i>	
Digestive tract	Commensal organisms
Nasal flora	Commensal organisms
Skin	Commensal organisms
<i>Disease</i>	
Pulmonary	Cystic fibrosis
Urological	Urinary tract infection
Dental	Tooth decay
Digestive tract	Gastroenteritis

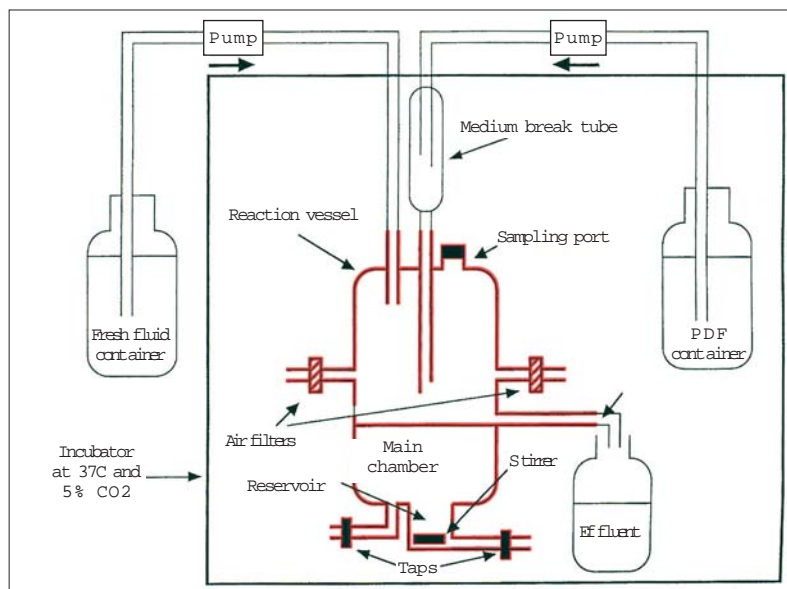


Figure 3. In vitro model of peritonitis in continuous ambulatory peritoneal dialysis

(Figure 3), Dr Geoff Hanlon, Janet Brant and I have shown that the staphylococcal surface never stops changing.¹⁰ During dialysis the fluid composition changes, particularly when the fluid is exchanged, altering growth rates which elicits a complete transformation in bacterial surface hydrophobicity (Figure 4). Through this mechanism, the organism can switch from a "swarming" variant capable of colonising fresh surfaces to a consolidated protected biofilm, resistant to flushing out and to the harmful effects of fresh fluid. This phenotypic variation is typical of the adaptive behaviour of bacteria which effectively regulates virulence.

PREVENTING INFECTION, RESISTING CURE

Antibiotics have been our main weapon against infection since their first extensive use by the British and American armies during the 1939–45 war. Worldwide, this reliance has gradually led to over-use, under-regulation, and inappropriate application with the consequent emergence of resistance. The ability of bacteria to exchange genes coding for antibiotic resistance has guaranteed the presentation of multiresistant strains to challenge even our most powerful agents.

Recent epidemiological surveys place the United Kingdom top in Europe for antibiotic-resistant *Staphylococcus aureus*, with 46.1 per cent of isolates resistant, compared to Greece at 38.6 per cent, Belgium at 20 per cent and Sweden, Iceland and Denmark below 3 per cent. Hospital reservoirs have led to nosocomial infection with virulent multi-resistant bacteria such as MRSA and vancomycin-resistant *Enterococcus* (VRE). Inadequate treatment of TB in the developing world has raised the spectre of multidrug-resistant tuberculosis emerging as a threat in the UK.

With increasing antibiotic

resistance, attention is now focused on the role of disinfection in controlling the spread of multiple resistance. It is a salutary lesson to realise that cross-resistance between selected disinfectant agents and antibiotics has been recognised¹¹ (Table 2).

Particular concern arises over the prodigal use of triclosan, found in everything from paints and children's toys to food preparation utensils, and its possible association with mechanisms of resistance relevant to antibiotics. A poster at this conference from the school of pharmacy at Brighton draws attention to potential cross-resistance with tetracycline.¹² There is anxiety too over possible cross-resistance with the antitubercular drug isoniazid¹³

FIGHTING BACK: CONTROLLING INFECTION

A variety of approaches are under investigation to combat the threat of infection. Currently much research effort is being placed on directly tackling the resistance mechanisms elaborated by pathogens.¹⁴ Novel target options are emerging as we better understand the control of gene expression in pathogenic organisms.¹⁵ Tricking the bacterium into an autocidal (suicidal) response, or encouraging accumulation of a biocide concealed as a nutrient are approaches being explored for disinfectant

TABLE 2: SOME EXAMPLES OF POSSIBLE DISINFECTANT:ANTIBIOTIC CROSS-RESISTANCE

Disinfectant	Genetic locus	Antibiotic
Benzalkonium chloride	<i>qacA-E</i>	Aminoglycosides, oxacillin, tetracycline, trimethoprim, sulphonomides
Chlorhexidine	Plasmid pSAJ1	Aminoglycosides, trimethoprim
Pine oil	<i>marA</i>	Ampicillin, chloramphenicol, nalidixic acid, tetracycline
QACs	Plasmid pSK1	Aminoglycosides, trimethoprim
Triclosan	<i>acrAB, marA, soxS</i>	Ciprofloxacin, tetracycline
Triclosan	<i>inbA</i>	Isoniazid

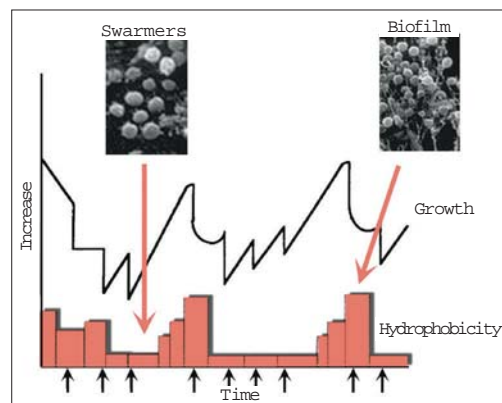


Figure 4. *Staphylococcal* behaviour in CAPD peritonitis. Bacterial regrowth is synchronised with fluid exchanges (indicated by arrows). Overnight, when there is no fluid change, bacteria continue to grow and their surface becomes more hydrophobic. Hydrophobic bacteria attach to surfaces resisting wash-out (right-hand inset) while more hydrophilic variants (left-hand inset) migrate to colonise fresh areas

agents.¹⁶ In the latter approach, chemically combining a phenolic disinfectant with a nutrient substrate (galactose) tricks the bacterium into transporting the entire molecule into the cell. The chemical link is subsequently cleaved intracellularly and the biocide released to elicit its action at target, thereby overcoming any external protective penetration barriers.

An understanding of microbial adhesion offers new opportunities to purpose-design medical devices less prone to colonisation. Professor Andrew Lloyd, Dr Matteo Santin and I, in collaboration with colleagues in the UK and mainland Europe, have been experimenting with hydrophilic coatings either sterically hindering microbial approach or transforming molecular associations to reduce adhesion.^{17,18} In the most successful systems, it has been possible also to control the host protein conditioning of surfaces, further limiting the possibility of microbial associations with indwelling biomaterials.¹⁹

An entirely different approach, originally advocated in the treatment of dysentery, typhoid and cholera between the world wars but largely dropped with the advent of antibiotics, employs bacteriophages to control infection. Recently popularised as a concept by publicity emerging from their use in Georgia under the auspices of the Tbilisi Institute, there have been examples of bacteriophage therapy in the UK.²⁰

At Brighton, again with Geoff Hanlon, we have explored the use of *Pseudomonas aeruginosa* biofilm, a severe, though thankfully relatively rare, complication of medical device infection and also a complicating feature of cystic fibrosis. Astonishingly, it appears that the bacteriophage acquires an enzyme from its original bacterial host which it subsequently uses to liquefy biofilm exopolysaccharide, allowing penetration to underlying target bacteria. The bacteriophage then infects the *Pseudomonas aeruginosa*, propagates intracellularly and causes the bacterial cell to lyse,

releasing infective particles to continue the disinfection process²¹ — a self-propagating antibiotic that can eradicate over 90 per cent of the biofilm (Figure 5)! This approach is, of course, self-limiting, since the bacteriophage will multiply only as long as sensitive bacteria remain. We are to continue this work under the auspices of a Royal Pharmaceutical Society research studentship: the target organism will be *Acinetobacter*, a multiple-resistant nosocomial coloniser of severely-burned patients.

Finally, the potential for phage use in bioremediation cannot go unremarked. Investigations are under way to use bacteriophage to clear *Campylobacter* from broiler-house chickens instead of the use of feed-additive antibiotics.

OVERVIEW

Microbes and man have always existed in uneasy balance. With the advent of widespread antibiotic resistance the power of microbial adaptation has been fully revealed, placing in stark relief the fragile nature of this balance. I hope I have shown, in some small way, how collaborative research in microbiology seeks to keep pace with microbial evolution. It is clear that we must meet the challenge of understanding microbial virulence and behaviour, at both the molecular and host-interaction level, in order to establish new approaches to infection diag-

nosis, prevention and cure. Pharmaceutical and medical microbiology are an integral part of the pharmacist's training and can lead us to make important contributions in scientific research and health care practice.

Sir William Bate Hardy once said "You know, this applied science is just as interesting as pure science, and what's more it's a damn sight more difficult." He was recognising a truth, that science needs to be applied successfully to deliver greatest benefit. Pharmacy *is* science in application, whether at the laboratory bench, the dispensary counter or the patient bedside. The pharmaceutical sciences, in all their diversity, are thus the bedrock of our reputation and authority. In academia this reputation has probably never stood higher with UK schools of pharmacy collectively gathering some of the highest accolades in teaching and research excellence. The 2002 British Pharmaceutical Conference is a celebration of our application of pharmaceutical science to the practice of pharmacy.

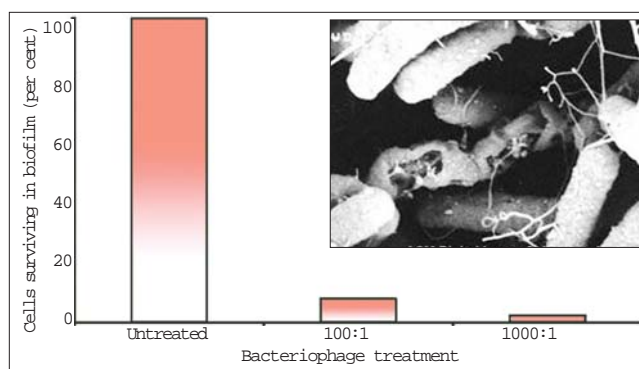


Figure 5. Effect of bacteriophage on the survival of *Pseudomonas aeruginosa* biofilm. Bacteriophage treatment is represented by the ratio of phage to bacteria. The centre of the inset shows typical bacterial cell wall damage following phage-induced lysis

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