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Medical microbiology enters the 21st century

Bernard Dixon BSc, PhD, DSc, CBiol, FIBiol

Science writer and Consultant
Ruislip Manor, Middlesex, UK

Any assessment of the future of medical microbiology must begin with its two major challenges today. Firstly, a whole package of measures is going to be required to combat antibiotic resistance. These include the re-examination of organisms which could not hitherto be grown *in vitro*, to see if they produce effective antimicrobials; the genetic engineering of bacteria and fungi to synthesise hybrid molecules[1], and alternative strategies such as competitive inhibition of microbial adhesion[2] and perhaps also interference with quorum sensing. But the greatest hope comes from bioinformatics and the new targets for attack which are now being revealed with the sequencing of successive pathogens[3].

The second immediate challenge is that posed by "new" and resurgent infections. This compels us to recognise anew that microorganisms will exploit any opportunity to cause disease, from changes in agricultural practice to the introduction of immunosuppressive drugs in organ transplantation. Much more conscious effort is required to anticipate such consequences and thus cope with them. Fortunately, there are signs of this happening in the case of global warming, which will allow mosquitoes and other vectors to extend their breeding grounds[4].

One of the ironies of medical microbiology today is that, at the very time when molecular genetics is transforming vaccine manufacture, immunisation remains an under-used method of combating infection. For example, nearly four million children die each year from diseases (such as measles, hepatitis B, rubella and *Haemophilus influenzae* b infections) which are preventable by existing vaccines.

It is, however, the wide variety of emerging prophylactics that illustrates the full scale of the transformation likely in the years ahead. Vaccines against no less than 75 different infectious diseases are currently at various stages of research and development[5]. For the first time since the advent of medical science, we can be confident that immunisation will prove possible against most, if not all, human pathogens of major significance.

One of the most heartening developments during recent years has been the discovery that certain new vaccines, previously validated in industrialised countries, are also effective in parts of the world where poor nutrition might have rendered them less powerful. Thus trials in The Gambia of a novel *Hib* vaccine (made by linking outer capsular polysaccharide to a powerful immunogen) indicate that it will substantially reduce childhood deaths due to meningitis and pneumonia in developing countries[6].

Newly sequenced genomes are also revealing hitherto unrecognised targets of immunisation. The earliest returns may well come from *Mycobacterium tuberculosis* – through the incorporation of new proteins in BCG, for example, or the construction of novel attenuated strains by altering base sequences involved in virulence.

Prospects for immunisation against cancers are also escalating rapidly. Four major examples are carcinoma of the uterine cervix, hepatocellular carcinoma, stomach cancer and nasopharyngeal carcinoma, which may be preventable by vaccines against human papillomavirus, Epstein Barr virus, *Helicobacter pylori* and hepatitis B virus respectively. One early sign of success is the dramatic decline in hepatocellular carcinoma in children since Taiwan established universal immunisation against hepatitis B[7].

But can increasing numbers of potentially life-saving immunogens be incorporated into immunisation schedules that are already quite full? One possible answer is to extend routine immunisation beyond the infant years where it is concentrated at present. Thus 9–12 year old children might

Also in this issue:

be targeted for immunisation against sexually transmitted diseases, such as gonorrhoea, which were hitherto not preventable in this way.

Controlled-release products may also ease the pressure on immunisation schedules. Vaccines delivered slowly or intermittently over months or even years could supplant the conventional system of initial doses followed by boosters later in infancy[8].

We also require much greater understanding of microbial evolution and the horizontal movement of genes. The potential of natural recombinants to cause widespread illness and fatalities was underlined by the acute anxieties about the severe cases of flu acquired by human patients from chickens in Hong Kong in 1997. Although there seemed to be no person-to-person transmission on this occasion, the episode reawakened fears that a pandemic strain could arise in this way and disrupt human society as profoundly as did the pandemic of 1918–19.

A thousand years from now, the great 20th century story of magic bullets could well be a footnote of history. The advent of modern drugs certainly had a substantial impact on infections such as tuberculosis and diphtheria. Yet this was less spectacular than as is often imagined. Over a much longer period, improvements in nutrition and sanitation were far more effective in reducing the toll of communicable disease. That lesson, obscured by the antibiotic revolution, needs to be re-learned as we place increasing emphasis not on the treatment of infections but on their prevention by immunisation and other measures.

Those who favour an ecological view of infectious disease also point to recent "low-technology" innovations such as the development of probiotics, living microorganisms given to animals to protect them against pathogens. Bacteria used in this way can, for example, protect mice against Salmonella infections[9] and control diarrhoea in pigs[10]. Emerging human applications include the use of bacteria and yeasts to combat intestinal and vaginal infections[11].

One plausible prediction for the future is, of course, that the world will be increasingly sterile. Poliomyelitis, measles and other viruses will follow smallpox virus into extinction, while the remaining major pathogens are reliably controlled by the super-drugs of tomorrow.

An alternative scenario is one in which bacterial resistance has finally defeated both scientific ingenuity and the massive resources of the pharmaceutical industry. This could allow the great plagues of history to return and determine the course of human affairs again, just as they did in the distant past.

But if we look forward from an ecological perspective, and consider long- rather than short-term solutions to the problems posed by communicable disease, we may see instead a third possibility. This is of a world characterised by harmonious coexistence between humans and microorganisms, to the lasting benefit of both. "Give us this day our daily germs" – the paradoxical title of a recent paper by two medical bacteriologists[12] – may prove to have been a truly prophetic sentiment.

REFERENCES

1. Marsden, A.F.A., Wilkinson, B., Cortes, J. *et al.* (1998). Engineering broader specificity into an antibiotic-producing polyketide synthase. *Science* **279:** 199–202.
2. Kelly, C.G., Younson, J.S., Hikmat, B.Y. *et al.* (1999). A synthetic peptide adhesion epitope as a novel antimicrobial agent. *Nature Biotechnology* **17:** 42–47.
3. Jenks, P.J. (1998). Microbial genome sequencing – beyond the double helix. *British Medical Journal* **317:** 1568–71.
4. McMichael, A.J. and Haines, A. (1997). Global climate change: the potential effects on health. *British Medical Journal* **315:** 805–809.
5. Katz, S.L. (1997). Future vaccines and a global perspective. *The Lancet* **350:** 1767–1770.
6. Mulholland, K., Hilton, S., Adegbola, R. *et al.* (1997). Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate for prevention of pneumonia and meningitis in Gambian infants. *The Lancet*, **349:** 1191–1197.
7. Chang, M.-H., Chen, C.-J., Lai, M.-S. *et al.* (1997). Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. *New England Journal of Medicine*, **336:** 1855–1859.
8. Sasiak, A.B. and Sesardic, D. (1997). Controlled release vaccines. *Journal of Medical Microbiology*, **46:** 975–977.
9. Silva, A.M., Bambirra, E.A., Oliveira, A.L., *et al.* (1999). Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* subsp. *typhimurium* in conventional and gnotobiotic mice. *Journal of Applied Microbiology*, **86:** 331–336.
10. Zani, J.L., Weykamp da Cruz, F., Freitas dos Santos, A. *et al.* (1998). Effect of probiotic CenBiot on the control of diarrhoea and feed efficiency in pigs. *Journal of Applied Microbiology* **84:** 68–71.
11. Elmer, G.W., Surawicz, C.M. and McFarland, L.V. (1996). Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. *Journal of the American Medical Association* **275:** 870–876.
12. Rook, G.A.W. and Stanford, J.L. (1998). Give us this day our daily germs. *Immunology Today* **19:** 113–116.

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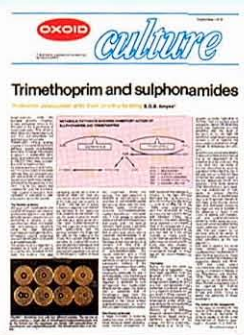
Mike Smith, Chief Executive Officer, Oxoid Ltd

In September 1978, when Culture was first published, Oxoid was an independent company within the Brooke Bond Group with operations in two countries, the UK and Germany. Twenty one years later, we are, once again, an independent business, but with our own organisations in 13 countries. Oxoid's growth is attributable to its reputation amongst the community of microbiologists for service and quality. For many of our customers and partners we are the bacteriology reference point.

It was to enhance our reputation for scientific excellence that Eric Bridson and Joe Ridge, Euromed Communications, first conceived of *Culture*. They were determined that the academic content would be of the highest quality and that it would be a 'must read' for all microbiologists. This concept has been continued to the present day, most notably by David Post who has been responsible for the journal in recent years.



First issue of Culture

A typical print run is more than 20,000 copies and there is always a steady demand for previous editions. Thus it would seem that from the customer's perspective there is a requirement for this type of journal, but how should Oxoid view it? As we strive to become more efficient, should we still invest hard-earned money in such a publication?

I believe that in a crowded and mature market-place the need to differentiate the company by virtue of its excellent technical service becomes ever more important. The expertise displayed by our Sales teams, our Technical Support teams and our literature sets us apart from most other companies.

Thus, as the product offerings of the various competitors converge, *Culture* will be even more important to the positioning of Oxoid. Therefore I would like to wish it a very successful 21st anniversary edition and hope that at least some of us will be here for the 50th.

Approaches for the discovery of new antibacterial agents

Ian Chopra PhD, DSc

Director of the Antimicrobial Research Centre,
University of Leeds, UK

Introduction

The ability to treat bacterial infections with chemotherapeutic agents , introduced with the discovery of penicillin and Prontosil in the 1930s (**Figure 1**), represents one of the most important medical achievements of the twentieth century. Indeed, the rapid advances made in the discovery of new antibiotics and other antibacterial agents during the so-called "golden" period between 1940 and the mid 1960s (**Figure 1**) led to widespread optimism that bacterial infections could be completely conquered. This period of optimism is captured by the famous remark made in 1969 by the US Surgeon General who testified to Congress that "the time has come to close the book on infectious disease".[1]

However, even the from the very earliest period of the antibiotic era the potential for the emergence of drug resistant bacteria has been recognised.[2] Unfortunately, selection of organisms resistant to antibacterial agents has continued to the present day and the next millennium has arrived with the dramatic emergence of resistance to antibacterial agents in all significant bacterial pathogens.[3-5] Furthermore, bacteria multiply-resistant to virtually all chemotherapeutically useful antibacterial agents have been identified among clinical isolates of some bacterial species.[3]

The latest twist to this increasingly alarming situation has been the recent isolation of low-level glycopeptide-resistant, methicillin-resistant strains of *Staphylococcus aureus* (so-called "VISA" strains) in various parts of the world.[6] As noted by Cookson[6] the emergence of VISA strains may herald the appearance of high-level vancomycin-resistant strains of *S.aureus*. Such an event would have serious consequences for the control of nosocomial staphylococcal infections.

Although sensible measures to limit antibiotic usage to valid therapeutic indications and to reduce the spread of resistant organisms are of value in limiting the emergence of resistant organisms, the resistance problem has required, and continues to require, renewed effort by the pharmaceutical industry to create products that will prevent or treat infections caused by antibiotic-resistant pathogens.

Two principal drug discovery approaches have been employed to date:

a) The expansion of known drug classes to include organisms resistant to earlier members of the class,

b) Identification of novel agents active against previously unexploited targets.

Since the mid-1970s industrial approaches to the

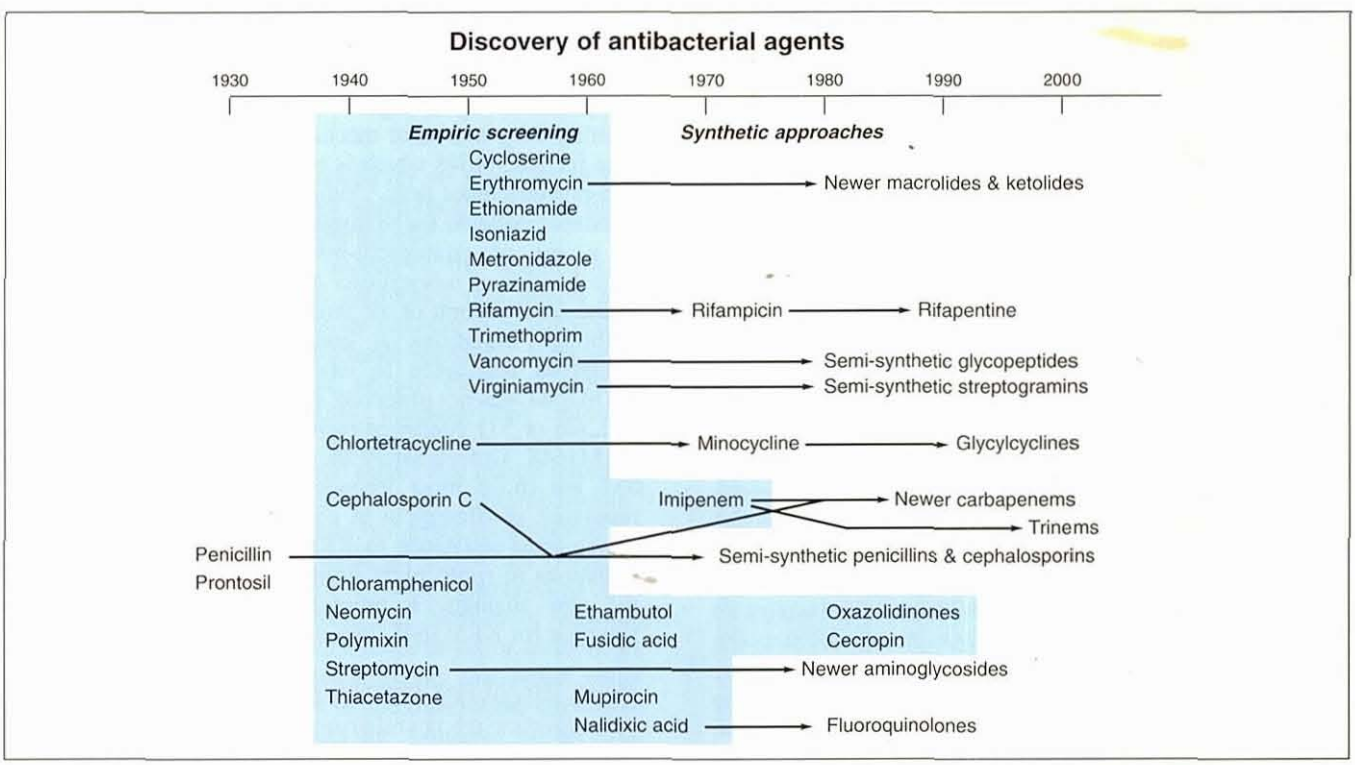


Figure 1. The discovery of antibacterial agents. Empiric screening has been based upon the identification of antibacterial agents by their ability to inhibit bacterial growth. Synthetic approaches comprise chemical modification of existing drug classes to improve their properties e.g. circumvention of resistance mechanisms to earlier members of the class. Only representative antibacterial agents are indicated.

development of new antibacterial agents have been dominated by the first paradigm, namely expansion of known drug classes (**Figure 1**). Indeed, in recent years, the oxazolidinones and cyclic peptides (exemplified by the discovery of cecropin) represent the only new classes of antibacterial agents under development (**Figure 1**). This article considers the two broad approaches for the discovery of new antibacterial agents and concludes that the former strategy is no longer sufficient to meet the clinical demands of the next century. Since the emphasis for future research will be the discovery of novel agents active against new molecular targets, it will be important to pursue approaches that minimise the emergence of bacterial resistance to newly discovered agents. This article also addresses this issue.

Expansion of known drug classes

Expansion of known drug classes has been the principal strategy adopted by the pharmaceutical industry to combat the emergence of bacterial resistance to antibacterial agents. Essentially, this strategy depends on the synthesis of repertoires of new analogues related to known antibacterial agents to create structural modifications that circumvent the resistance phenotype.

Early examples of this approach include development of semi-synthetic penicillins such as methicillin and the isoxazolyl penicillins which were developed on the basis of their stability to staphylococcal penicillinases.[4,7] More recent examples include the third generation cephalosporins, exemplified by agents such as cefotaxime (introduced in 1981) and ceftazidime (introduced in 1985), which were developed on the basis of their stability to the TEM-1 and SHV-1 β-lactamases which are broadly dispersed amongst clinical isolates of Gram-negative bacteria.[7]

The strategy has continued to the present day. For example the glycylcyclines (pre-clinical) represent a new class of tetracycline analogues which exhibit antibacterial activity against bacteria expressing efflux-based resistance to older tetracyclines which is attributable to failure of the efflux proteins to recognise and export the new analogues.[4] Modification of the target site for antibacterial action also results in resistance to a number of agents including the tetracyclines , macrolides, β-lactams, glycopeptides and rifampicin. The chemical synthesis of analogues of these antibiotic classes has yielded new derivatives that bind to the refractory targets. These agents, which are at various stages in the Research and Development process, include the glycylcyclines, the ketolides, various carbapenems, N-alkyl-substituted glycopeptides and the rifampicin analogue KRM1648.[4,8,9] (also,see **Figure 1**).

Limitations of introducing analogues of existing drug classes

Expansion of existing drug classes to meet the clinical challenges imposed by resistant organisms has undoubtedly led to the introduction of a number of clinically successful agents. However, this approach can now only be considered at best a temporary solution to the problem of resistance. Unfortunately, the existence of resistance mechanisms to earlier members of the drug class often provides the organisms with a head-start for mutational adaptation by which expression of resistance to the newest member of the class also rapidly emerges. Notable examples include the relatively limited number of amino acid changes in the TEM-1 and SHV-1 β-lactamases required to confer resistance to the third generation cephalosporins[7] and the

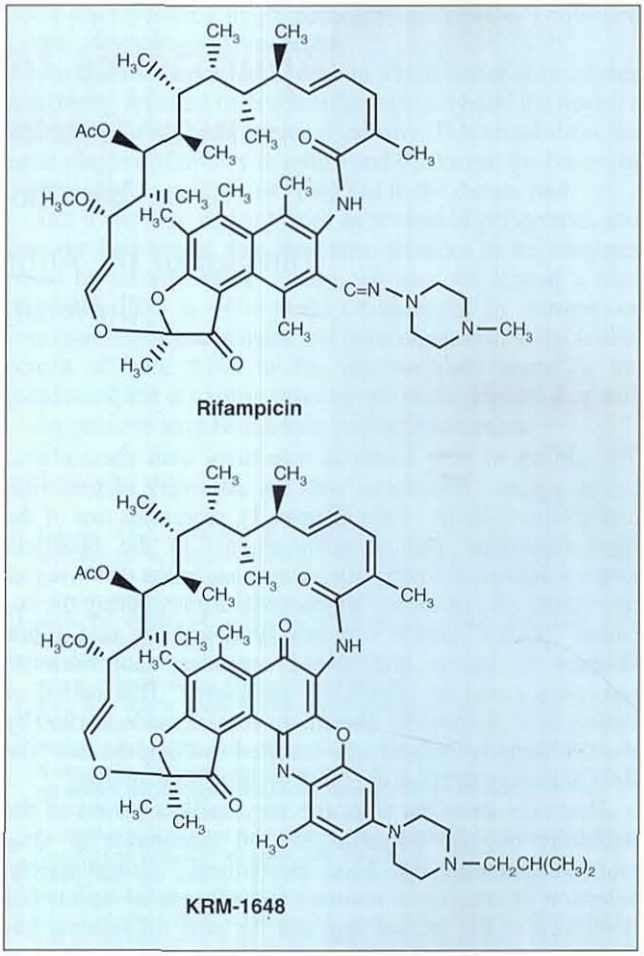


Figure 2. The structures of rifampicin and KRM1648.

observation, at least under laboratory conditions, that single-site mutations in a gene encoding a tetracycline efflux pump confer resistance to members of the new glycylcycline group of antibiotics.[10]

Apart from the possibility that new mutations will arise in existing resistance genes to confer resistance to new drug variants, the introduction of new analogues may already be compromised by a high level of cross-resistance to an existing drug resistance mechanism. This appears to be the case for KRM1648 which is being developed for use as an anti-mycobacterial drug.[9] Yang *et al* [9] examined the cross-resistance patterns for rifampicin and KRM1648 (**Figure 2**) in a set of rifampicin-resistant clinical isolates of *Mycobacterium tuberculosis* containing defined mutations in the *rpoB* subunit of the target enzyme RNA polymerase. Although mutations at codons 514,516,518 and 529 conferred resistance to rifampicin, cross-resistance to KRM1648 was not observed. However, mutations at codons 513,526 or 531 conferred resistance to both rifampicin and KRM1648. Unfortunately the mutations in *rpoB* at codon 531 are those most frequently (>60%) responsible for resistance to rifampicin in clinical isolates. The extent to which cross-resistance to KRM1648 mediated by existing mutations in *rpoB* might limit the clinical effectiveness of the new analogue is presently unknown. However, the situation for KRM1648 does not look promising.

Drug discovery strategies to minimise the emergence of resistance

If expansion of existing drug classes can no longer be relied upon to produce future generations of antibacterial agents, what

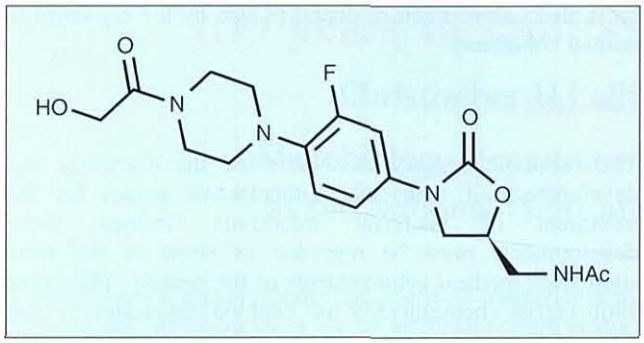
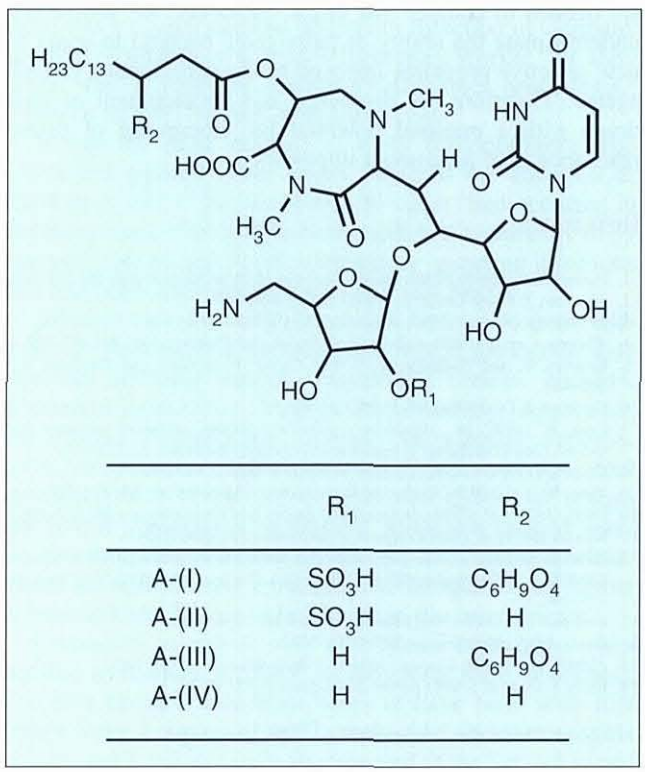


Figure 3. The structure of eperezolid , a member of the oxazolidinone class of antibacterial agents.



	R_1	R_2
A-(I)	SO_3H	$C_6H_9O_4$
A-(II)	SO_3H	H
A-(III)	H	$C_6H_9O_4$
A-(IV)	H	H

Figure 4. Structures of four types of liposidomycins.

strategies should now be employed for the discovery of new agents and are there approaches which could minimise future problems of resistance to the new agents?

A minimum requirement should be the search for, or design of, structurally novel antibiotics that inhibit new molecular targets. Such agents are unlikely to be susceptible to existing mechanisms of bacterial resistance because of their structural novelty and unique mode of action.[4] Examples of new inhibitors meeting this minimal requirement are eperezolid (**Figure 3**) a member of the oxazolidinone class of synthetic agents that inhibit initiation of bacterial protein synthesis[8], and the liposidomycins[11] (**Figure 4**), a group of naturally occurring inhibitors of the translocase I reaction of bacterial peptidoglycan synthesis. These compounds have unique structures unrelated to other drugs that are already in clinical use. Novelty of structure does indeed translate into unique mode of action as there are no clinically useful antibacterial agents that target either initiation of protein synthesis or the translocase I reaction. Furthermore, interference with the synthesis of bacterial peptidoglycan presents a particularly attractive target because this polymer is essential for bacterial survival and its absence from mammalian cells offers prospects for the development of drugs with highly selective activity against bacteria.

The search for new drugs can be further refined by introducing even more stringent criteria to reduce the potential for emergence of resistance. One approach is not only to seek novel drugs, but also to find drugs that simultaneously inhibit more than one new molecular target. Simultaneous inhibition of more than one target renders the emergence of resistance less likely since mutations are required in all targets to confer resistance to the drug. This approach is most likely to be successful with groups of essential bacterial enzymes that are mechanistically related where it may be possible to design or screen for a single inhibitor of more than one member of the enzyme class. Examples of related enzyme systems that might be amenable to multiple blockade with a single agent are the mycobacterial enzymes involved in the biosynthesis of the mycolic acid and arabinogalactan cell envelope components of these organisms, and the tRNA synthetases and muramyl peptide ligases which are potential broad-spectrum drug targets.

The muramyl peptide ligases encoded by *murD, murE* and *murF* (**Figure 5**), which are involved in the early stages of peptidoglycan synthesis represent particularly attractive targets for the development of an antibacterial drug with multiple blockade properties.[12] Each of these enzymes is mechanistically related (i.e. they are all non-ribosomal ATP-dependent peptide synthases), they have an essential role in peptidoglycan synthesis (i.e. they are lethal targets), and

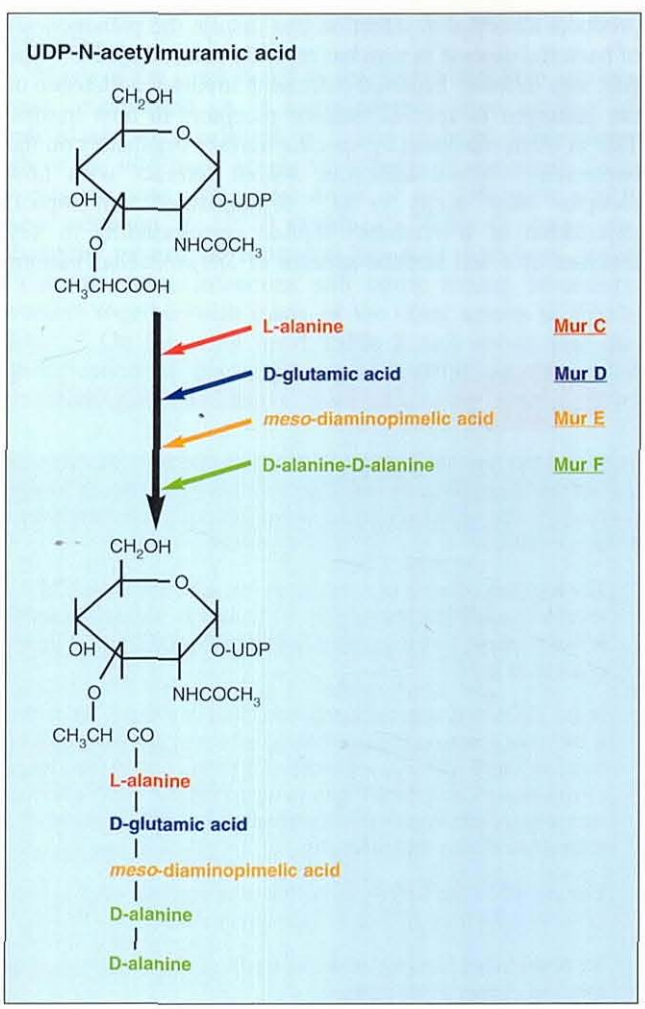


Figure 5. Sequence of addition of amino acids to UDP-N-acetylmuramic acid during the early stages of bacterial peptidoglycan synthesis. The amino acids are added sequentially under the direction of specific muramyl peptide ligases encoded by the *murC-murF* genes.

they utilise D-amino acids or meso-diaminopimelic acid which, having no eukaryotic counterpart, provides good prospects for discovering a selective bacterial inhibitor.

Furthermore, as the enzymes are soluble proteins, this has already allowed acquisition of structural data on some enzymes[13] and offers good prospects for structural analysis of the remaining proteins. Acquisition of structural data permits application of structure-based inhibitor design, a powerful molecular method for drug design.[14]

Another approach that may minimise problems of resistance is to seek inhibitors of bacterial targets that are not only novel in their own right but are involved in the disease process itself.[4] The genes encoding such products are intimately involved in the disease process and are to be distinguished from the so-called "housekeeping" genes which are required for general growth and survival of the bacterium. New molecular biological approaches such as *in vivo* expression technology (IVET),[4,15] signature-tagged mutagenesis (STM)[4,15] and proteomics[15] are being utilised to discover and characterise bacterial genes involved in infection. The value of developing drugs targeted to infection genes would be selective removal of pathogens at the site of infection only. Since the entire bacterial cell population would not be killed (i.e. only those organisms at the sites of infection would be affected) the selective pressure to develop resistance would not be severe.[4]

Drug discovery processes based on targeting infection mechanisms are still in their infancy, and it is, of course, difficult to predict the likely success of this strategy. However, proof of the concept that interference with gene products involved in infection can disrupt the pathogenesis of bacterial disease in man has recently been obtained.[16] The first step in many bacterial infections involves adherence of the pathogen to specific surface receptors in host tissues. This is often mediated by specific surface organelles on the bacterium, termed adhesins, which interact with host receptor sites. Kelly *et al*[16] demonstrated that topical application of a synthetic peptide corresponding to key residues of a cell surface adhesin of *Streptococcus mutans* prevented colonisation of dental plaque by the organism in human volunteers.

Conclusions

The twentieth century has witnessed the discovery and development of many chemotherapeutic agents for the treatment of bacterial infections. Indeed, these developments must be regarded as some of the most significant medical achievements of the century. The era of antibacterial chemotherapy has enabled physicians to treat infection rather than simply offer palliative care. Unfortunately, as we enter the new millennium many of our existing antibacterial agents are under threat from the widespread emergence of bacterial resistance. New agents are needed to counter this threat. However, we should not underestimate the ability of pathogenic bacteria to adapt to new selective pressures imposed by the introduction of new agents. Therefore the discovery and development of new drugs with a minimal potential for emergence of future resistance is of paramount importance.

References

1. Cassell, G.H. (1997) FEMS *Immunology and Medical Microbiology* **18:** 271–274.
2. Abraham, E.P. and Chain, E. (1940) *Nature* **146:** 837.
3. Armstrong, D. *et al.* (1995) *Microbial Drug Resistance* **1:** 1–4.
4. Chopra, I. *et al.* (1997) *Antimicrobial Agents and Chemotherapy* **41:** 497–503.
5. Baquero, F. and Blazquez, J. (1997) *Trends in Ecology and Evolution* **12:** 482–487.
6. Cookson, B.D. (1998) *Culture* **19**(1): 1–5.
7. Bush, K. (1997) In : *Antibiotic resistance : origins, evolution, selection and spread.* Ciba Foundation Symposium 207. Wiley, Chichester, pp 152–166.
8. Chopra, I (1998) *Current Opinion in Microbiology* **1:** 495–501.
9. Yang, B. *et al.* (1998) *Journal of Antimicrobial Chemotherapy* **42:** 621–628.
10. Guay, G.G. *et al.* (1994) *Antimicrobial Agents and Chemotherapy* **38:** 857–860.
11. Kimura K-I. *et al.* (1998) *Journal of Antibiotics* **51:** 1099–1104.
12. Faraci, W.S. (1992) In: *Emerging targets in antibacterial and antifungal chemotherapy.* Sutcliffe J. & Georgopapadakou, N.H. (eds.). Chapman and Hall, New York, pp 176–204.
13. Bertrand, J.A. *et al.* (1997) *EMBO Journal* **16:** 3416–3425.
14. Kuntz, I.D. (1992) *Science* **257:** 1078–1082.
15. Allsop, A.E. (1998) *Current Opinion in Biotechnology* **9:** 637–642.
16. Kelly, C.G. *et al.* (1999) *Nature Biotechnology* **17:** 42–47.

100 years of laboratory-acquired infections

Christopher H Collins, MBE, DSc., FRCPath

Microbiology Department, Imperial College of Medicine
at the National Heart and Lung Institute, London, UK

It was inevitable that no sooner had scientists learned how to grow pathogenic microbes in and on artificial culture media, than some of them became infected with the bacteria they handled. Two cases of laboratory-associated typhoid fever occurred in German laboratories in 1885, only five years after the causative organism had been identified. In 1893 there was a further typhoid laboratory infection in Germany and one of tetanus in a French laboratory worker.[1] In 1889 there was a case of laboratory-acquired brucellosis,[2] followed in 1903 by one of blastomycosis.[3]

It was the large number of early bacteriologists who contracted typhoid fever which attracted the attention of Kisskalt in 1915.[4] He noted that 50 cases had occurred in Germany since 1895 and he later published a summary of 59 cases of typhoid and 24 other laboratory- acquired infections that had been reported between 1915 and 1928.[5]

Between the two world wars, laboratory infections with most of the identified bacterial and fungal pathogens were reported, including anthrax, brucellosis, cholera, glanders, tularaemia, tuberculosis, leptospirosis, diphtheria (cutaneous), gonorrhoea (cutaneous), plague, shigellosis, syphilis, coccidiomycosis, histoplasmosis, blastomycosis and sporotrichosis.[6] Investigations into the agents of typhus (*Rickettsia prowazekii*) and Rocky Mountain spotted fever (*R. rickettsii*) led to fatalities i.e. Ricketts and Prowazek whose names are remembered in the name of the agent of the former disease and McClintock, who died from the latter disease.

Laboratory infections with viral agents came later, when suitable techniques for their investigation were developed. The first of these infections seem to have been with Rift Valley fever, Louping ill and Lymphocytic choriomeningitis viruses, but it was not until after the end of the Second World War, in the heyday of virology, that many more agents were incriminated in laboratory-acquired infections, especially during research into their identity. Indeed, the first indications that some newly-discovered viruses (e.g. certain arthropod-borne agents) were human pathogens became obvious when the investigators themselves became infected.[6]

During World War 2 the possibility of using pathogenic microorganisms as weapons was considered and there were several laboratory-associated infections which occurred as a result of both offensive and defensive investigations.

Eric Bridson

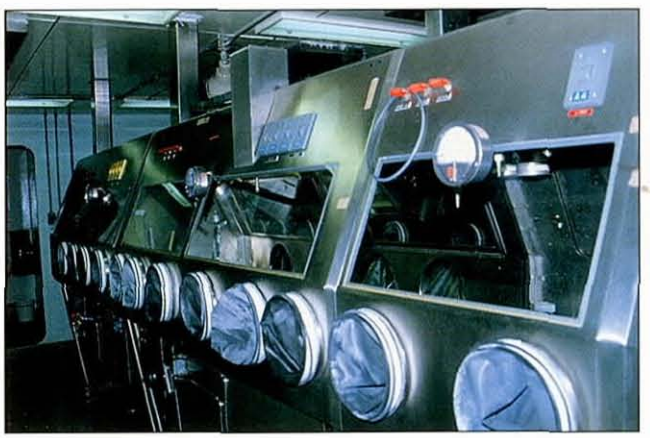


Figure 1. Safety cabinets in CDC Atlanta laboratories.

Inevitably, the information was "classified" and was not made available until many years after hostilities had ceased. A series of surveys by Sulkin and Pike,[7-9] beginning in 1951 and continuing until 1976,[6] mostly by postal questionnaires, indicated that, globally, between 1925 and 1976 4039 laboratory-acquired infections had been reported. To date, figures obtained by extrapolation from published reports suggest that the figure is now about 5,000 but it is recognised that many more remain unreported for various reasons.

Changing patterns

The analyses of Pike[8] show that changes could be detected in the incidence of infections caused by the different classes of microorganisms. Between 1925 and 1974 the numbers caused by bacterial agents declined while those associated with viruses increased. There was a peak of infections by rickettsial agents between 1945 and 1954, while those caused by fungi rose steadily between 1925 and 1974. These changes reflect the interests of research microbiologists and the development of new techniques that facilitated their infections.

Many of the cases noted in the Sulkin and Pike surveys were institutional i.e. a comparatively large number of individuals were infected in a few research laboratories where the infectious agents were being investigated. Such events are now rare, as are some of the infections which were common in 1978[6] (**Table 1**). **Table 2** shows the changing pattern of laboratory-acquired infections since 1978, but some infections still occur among laboratory workers together with many of the other agents in Pike's lists.[6,7-9] On the other hand **Table 2** also shows that the identification of the agents of almost all the "new" or emergent diseases, notably those caused by viruses, have

Table 1. The most common agents of laboratory-acquired infections

1925–1976*	1977–1998§
Brucella spp.	Hepatitis B virus
Coxiella burnetii	*Brucella* spp.
Hepatitis B virus	*Mycobacterium tuberculosis*
Salmonella typhi	*Salmonella typhi*
Francisella tularensis	*Shigella* spp.
Mycobacterium tuberculosis	*Coxiella burnetii*
Dermatophyte fungi	Human immunodeficiency virus
Venezuelan equine encephalitis virus	
Chlamydia psittaci	
Coccidioides immitis	

* Data from Pike[8]
§ Data from Collins and Kennedy[6]

Table 2. The changing pattern of laboratory acquired infections since 1978.

No new reports of laboratory-acquired infections	New and emergent agents associated with laboratory-acquired infections
Clostridium tetani	*Bordetella pertussis*
Erysipelothrix rhusiopathiae	*Campylobacter jejuni*
Francisella tularensis	*Escherichia coli* O157
Mycobacterium leprae	*Helicobacter pylori*
Pasteurella multocida	Hantaviruses
Treponema pallidum	Human immunodeficiency virus
Vibrio cholerae	Parvovirus 19
Yersinia pestis	Sabia virus
Chlamydia psittaci	Vaccinia (recombinant)
Rickettsia orientalis	*Cryptosporidium*
Rickettsia prowazeki	
Rickettsia rickettsii	

been followed by laboratory-associated infections.[6] The outbreaks of hantavirus and verocytotoxigenic *Escherichia coli* infections in the general populations were followed by reports of laboratory-associated infections (for references see Collins and Kennedy[6]). The latest of these new agents is the Sabia virus, identified in South America in 1994, which in 1995, infected a laboratory worker who worked with it in North America.[10]

There have been other changes in the patterns of laboratory-acquired infections. Between 1942 and 1957 it became obvious that many of the infections, where the source or cause was not immediately obvious, were the result of the inhalation of airborne microorganisms ("aerosols") released during accepted microbiological procedures.[6] Dr John Richardson of the CDC Atlanta (pers. comm.) referred to this period, when most of the work was done, as the "frightening fifteen years" (**Table 3**). Improvements in techniques and the gradual introduction of microbiological safety cabinets have reduced the incidence of these infections (See **Figures 1, 2** and **3** showing the Category 4 laboratory at CDC Atlanta). The lower incidence of airborne infections among laboratory workers has not been paralleled, however, by a reduction in the numbers of infections resulting from other exposures. Infections due to ingestion still occur[6] and mouth pipetting still persists in many laboratories.[6,11] Those associated with mucocutaneous and percutaneous exposure, especially following needlestick injuries, have increased.[6,12,13]

There were also changes in the distribution of infections among different laboratory activities. In Pike's 1976 survey[8] most infections occurred in research establishments. Since then most seem to have occurred in clinical laboratories, sometimes attributable to breaches in good technique and sometimes because the infectious agent was not suspected in the clinical sample.[6] There has, of course, also been an increase in the numbers of these laboratories.

Preventive measures

Kisskalt[4,5] found that most of the cases of laboratory-acquired typhoid fever were the result of aspiration of cultures during mouth pipetting and recommended the use of what we now term rubber teats. The first worker who published general recommendations on the safe handling of pathogens was Winfried Fricke[14] of the University of Jena,

Table 3. The age of aerosols, 1942-1957. Procedures shown to generate infected air-borne particles

Flaming loops	Plating cultures
Pipetting	Pouring
Centifuging	Homogenising
Shaking	Subculturing
Tissue grinding	Opening screw-capped bottles
Using syringes and needles	Opening ampoules
Animal inoculation	Animal autopsy

For references see Collins and Kennedy[6]

who published a booklet in 1919 on "*Precautionary Measures for Bacteriological and Serological Work for Physicians, Students, Laboratory Assistants and Bacteriology Courses.*" This contained, in 70 pages, much advice on general, as well as bacteriological safety, with no less than 41 drawings. Like Kissalt, he was concerned about the hazards of mouth pipetting and listed 25 references to devices that obviated this practice. He was probably the first bacteriologist to suspect that infections were not only acquired by ingestion, inoculation and contact but also by the inhalation of airborne infectious particles. The booklet, which had almost disappeared from the literature, did not attract much attention, until it was mentioned by Briggs Phillips in 1965.[1] A few copies were recovered in 1984 by the good offices of the publishers but even then an English language review passed unremarked.[15]

During the immediate post-war years little attention was paid to safety in microbiology but from 1952 there were several publications, many of them relating to work carried out in the United States during the "classified information" period. Many of these publications revealed that microorganisms are released, as aerosols, during a wide variety of microbiological procedures. The US was not alone, however, in contributing to an understanding of how infections were acquired in the laboratory and how they could be prevented. In the UK important contributions were made by Darlow[16,17] and his colleagues at the (former) Microbiological Research Establishment, Porton Down. Unfortunately, little official notice was taken in clinical laboratories where the general view seems to have been that these problems occurred only in research establishments.

The recognition that many infections among laboratory workers were acquired by the inhalation of infected aerosols stimulated research into their containment in what are now known as microbiological safety cabinets. The early models

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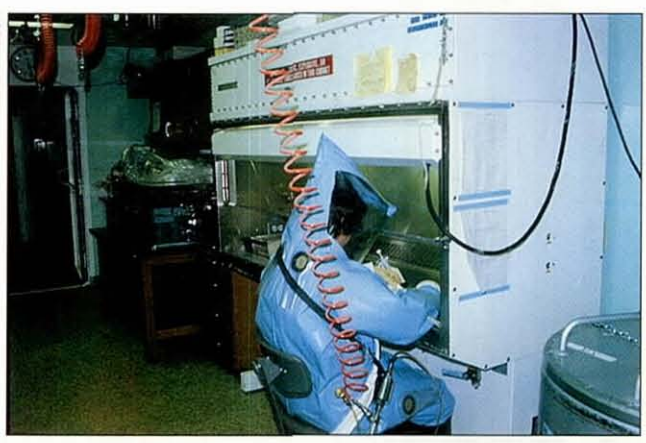

Figure 2. Category 4 laboratory at CDC Atlanta.

were not particularly successful and some actually enhanced the hazard. By the early 1970s, however, studies on the behaviour of air-borne particles and their entrapment led to improved standards for their manufacture, installation and performance such as those of the British Standards Institution.[18] Lack of instruction in their use, unfortunately, has often led to misunderstandings among users about the kind of protection they offer.

Awareness and training

The various outbreaks, in recent years, of resurgent and emergent diseases with associated infections among laboratory workers, have stimulated some interest, albeit often temporary but they have resulted in official (and other) publications (for references see Collins and Kennedy.[6]) Unfortunately, much of the good advice, recommendations and even some of the legal requirements, have not always reached the bench worker. The modern trend towards the "management" of laboratory safety, which usually entails no more than lip service paid to the accumulation of official literature, has clearly failed to reduce the numbers of breaches in safe procedures. The answer to the problem posed by laboratory-acquired infections must be good and safe microbiological techniques; which regrettably is neither taught, nor required in qualifying examinations.

REFERENCES

1. Phillips, G.B. (1965). Safety in the chemical laboratory. XIII. Microbiological hazards in the laboratory — Part 1 — Control. *J Chem Educ* **42:** A43–A46.
2. Birt, C., Lamb, C. (1899). Mediterranean or Malta fever. *Lancet* **1:** 701–710.
3. Evans N. (1903). A clinical report of a case of blastomycosis of the skin from accidental inoculation. *JAMA* **40:** 1772–1775.
4. Kisskalt K. (1915). Laboratoriumsinfektionen mit Typhusbazillen. *Z Hyg Infektionskr* **50:** 145–162.
5. Kisskalt K. (1929). Laboratoriumsinfektionen mit Typhusbazillen und anderen Bakterien. *Arch Hyg Bakteriol* **101:** 136–160.
6. Collins, C.H., Kennedy, D.A. (1999). Laboratory-acquired Infections. 4th ed. Oxford, Butterworth-Heinemann.

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Figure 3. Worker in "space-suit" in Category 4 laboratory at CDC Atlanta.

7. Sulkin, S.E., Pike, R.M. (1951). Laboratory-acquired infections. *JAMA* **147:** 1740–1745.
8. Pike, R.M. (1976). Laboratory-associated infections. Summary and analysis of 3291 cases. *Hlth Lab Sci* **13:** 105–114.
9. Pike, R.M. (1978). Past and present hazards of working with infectious hazards. *Arch Path Lab Med* **102:** 333–336.
10. Ryder, R.W., Gandsman, E.J. (1995). Laboratory-acquired Sabia virus infection. *N Eng J Med* **333:** 1716–1718.
11. Evans, M.R., Henderson, D.K., Bennett, J.E. (1990). Potential for laboratory exposure: biohazardous agents found in blood. *Amer J Publ Hlth* **80:** 423–427.
12. Abiteboul, D. (1997). Blood exposure data in Europe. In Collins C.H., Kennedy D.A. (Eds.) *Occupational Blood-borne Infections.* Wallingford, CAB International, pp. 59–74.
13. Jagger, J., Bentley, M. (1997). Clinical laboratories: percutaneous exposure. In Collins CH, Kennedy DA. (Eds.) *Occupational Blood-borne Infections.* Wallingford, CAB International, pp. 91–198.
14. Fricke, W. (1919). *Schutzmassnahmen bei bakteriologischem und serologischem Arbeiten.* Jena, Gustav Fischer.
15. Collins, C.H. (1984). In perspective: laboratory-associated infections. *Abst Hyg Commun Dis* **59:** R1–R15
16. Darlow, H.M. (1969). *Safety in the Microbiology Laboratory.* In Norris J.R., Ribbons D.W. (Eds). *Methods in Microbiology* 1. London, Academic Press.
17. Darlow, H.M. (1972). Safety in the Microbiology Laboratory: An Introduction. In Shapton, D.A, Board, R.G. (Eds.) *Safety in Microbiology.* London, Academic Press, 1–1919.
18. BS 5726 Specification for microbiological safety cabinets. Part 1-4. London, British Standards Institution, 1979. (superseded by BS 5726, 1992).

Extremophiles – desert cyanobacteria

Terence J. Painter, BSc, MA, PhD

Institute of Biotechnology,
Norwegian University of Science and Technology,
Trondheim, Norway

Introduction

It is estimated that life began on Earth about 3.5 x 10^9 years ago when the planet was still a very hostile environment. Self-reproducing probiotic cells evolved into the prokaryotes which successfully filled every niche on Earth. In postulating how life could have arisen and continued in such harsh conditions, it is helpful to look for evidence in contemporary micro-organisms that can multiply at the limits of survival – the extremophiles. Examples of such organisms are the crypto-endolithic cyanobacteria, photosynthetic bacteria living inside rocks in both hot and cold deserts.[1] Prominent among these are filamentous species (e.g. *Nostoc calcicola*[2-4]) which can fix nitrogen autonomously (non-symbiotically).[5]

Endolithic protection

The Atacama desert in Chile is one of the driest places on Earth. If it rains at all, it is only two or three times a century. The only source of water is atmospheric humidity with a little dew on colder nights. In such conditions, cyanobacteria can only survive by boring into limestone, just a few millimetres below the surface of the rock. Both coccoid species, e.g. *Gloeocapsa* (**Figure 1**) and the filamentous species can bore tunnels into limestone. In sandstone that consists of quartz crystals embedded in a matrix of calcite, scanning electron micrographs show that the latter component is dissolved selectively (**Figure 2**). The depth of tunnelling is critical; too deep and the level of light is too low for photosynthesis, too shallow and the high light intensity will inhibit photosynthesis. The presence of nutrients in limestone, from its origin in plankton and coral, plus the shielding effect from excessive solar radiation, aid the survival value of this environment. Additionally, limestone and sandstone are porous and hygroscopic; hence they can store water absorbed from dew or directly from the atmosphere, and the slow loss of water by evaporation provides a more stable temperature than the surrounding sand.[1]

Biogenic rock formation

Calcium carbonate also serves cyanobacteria well in other extreme environments where there is no exogenic limestone in which to hide. Many semi-arid regions contain alkaline, salt-rich lagoons, ponds and river beds that gradually dry out in the long, dry seasons. Cyanobacteria thrive in the shrinking mud despite the intense solar radiation. They do this by constructing their own 'parasol'. The hair-like threads of cells (trichomes) are surrounded by a gelatinous sheath within which shiny micro-crystals of calcite are reversibly precipitated. These act similarly to self-regulating sunglasses but the density of the precipitate is controlled by the level of photosynthesis rather than the light intensity as such.[5] A likely control mechanism, based upon the selective uptake of bicarbonate ions during active photosynthesis, has been proposed.[4] Another factor that would control the density of the precipitate and the opacity of the sheath is the humidity of the atmosphere; this is because the sheath material is hygroscopic, and it expands as it takes up water from the cooler night air.[1,4]

After long periods of total desiccation and high temperatures most (but seldom all) of the cells have died, and the precipitation of calcite in the sheaths is especially

Figure 1. Excavation in crystalline limestone by coccoid cyanobacteria (probably *Gloeocapsa* spp.) in the Negev desert, Israel.
(Photo: E. Imre Friedmann)

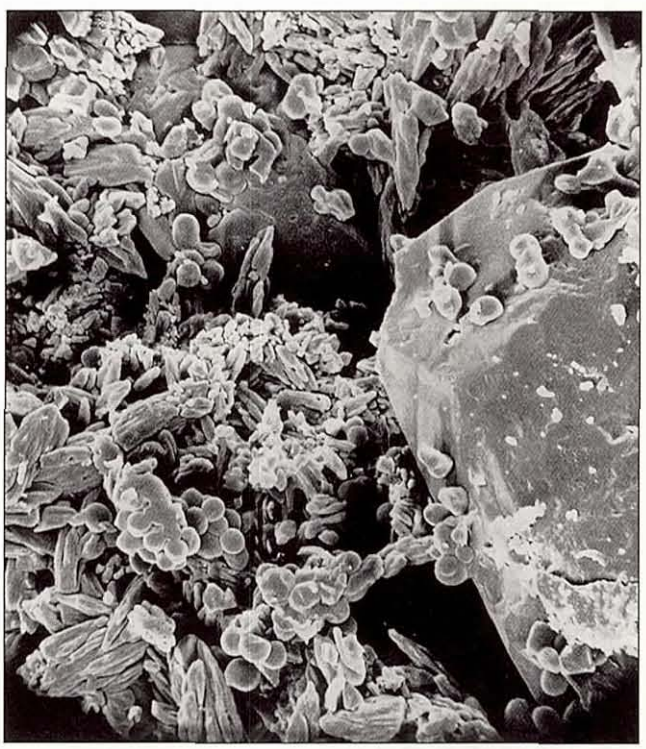

Figure 2. Scanning electron micrograph of coccoid cyanobacterial cells in Nubian sandstone, Negev desert. The small calcite crystals which cement the larger grains of quartz together are visibly eroded by the adhering cells.
(Photo: E. Imre Friedmann)

Figure 3. Fossil stromatolite, 1.3 x 10^9 years old, from the Glacier National Park, Montana, USA.
(Photo: J. William Schopf)

heavy. The sheath decays and petrification follows, forming stromatolite fossils. These are essentially a biogenic form of calcite. Stromatolites up to 3.5 x 10^9 years old have been found, showing that cyanobacteria were among the first photosynthetic prokaryotes.[6] **Figure 3** shows a younger stromatolite about 1.3 x 10^9 years old. The characteristic laminations record periods of rapid growth by surviving viable cells when water was abundant, followed by long periods of desiccation. Modern stromatolites are still being formed and can be seen in warm marine waters such as Shark Bay, Western Australia and Freshwater Creek, Andros Island (Bahamas).[5]

Sheath proteoglycans as biochelators

The dissolution and redeposition of calcium carbonate is normally a question of pH fluctuation between 5 and 7. Desert soils, however, are almost always alkaline, and the pH rarely falls below 7. Cyanobacteria grow optimally at pH 7.5 – 9.5 and some will grow even at pH 10.2; hence pH variation alone cannot explain the tunnelling in limestone or the accumulation of calcite in stromatolites. The fact that cyanobacteria can grow even on volcanic ash, which is totally lacking in both nitrogen and organic matter suggests that they can abstract essential elements from insoluble minerals by producing their own 'biochelator' instead of relying upon humic acids produced by saprogenesis. Indeed, the chelatory function is built into the structure of a complex proteoglycan which comprises most of the gelatinous sheath material that surrounds the trichomes. This biopolymer consists of a protein core to which polysaccharidic chains are linked chemically. Cation-selective binding-sites occur in both the protein (aspartic and glutamic acid) and the glycan (hexuronic acid).[3]

The biological success of this chelatory jelly can be ascribed to its more obvious advantages, i.e., it is insoluble in water and will stay around the trichomes; it is transparent and allows photosynthesis; and it is as permeable to small molecules and ions as is a solution. A less obvious advantage is that it aids in the excavation of rock. The jelly adheres to the rock surface, dries to a horny layer, and shrinks. Such is its adhesiveness, enhanced by its powerful chelatory ability for the rock minerals, that, as it shrinks, it tears off thin, lamellar plates or scales. This process depends upon the diurnal changes in the humidity of the atmosphere which in the desert take place at sunrise and sunset.

Desert reclamation and bio-mining

If cyanobacteria could be cultivated in the desert on a very large scale, their sheath proteoglycans could act in a similar way to humus in the soil. They require only light for energy, and most filamentous forms can fix nitrogen without associated legumes. Most significantly, they grow so much faster than legumes or other forms of 'green manure' that they could be usefully applied to the soil even when the rainy season lasts for only a few weeks.[7] Such a large-scale cultivation of filamentous cyanobacteria has so far been confined to natural lagoons or very large artificial ponds. In the USA alone, hundreds of tons of *Spirulina platensis* are produced annually for use as a dietary protein and vitamin

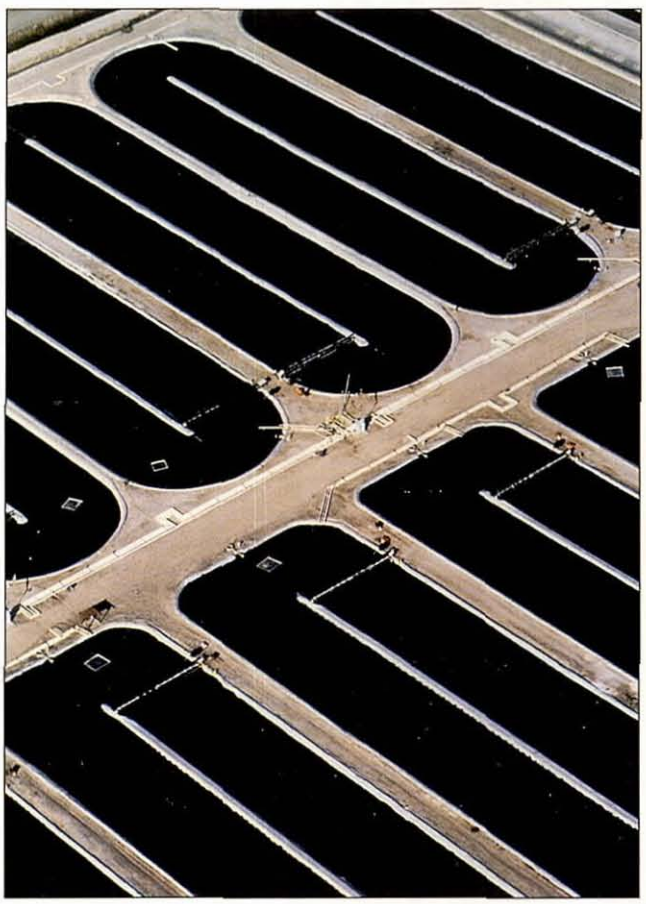

Figure 4. Large-scale cultivation of filamentous cyanobacteria (*Spirulina platensis*) at Calipatria in the desert region of southern California, USA. Courtesy of Earthrise Farms. (Aerial photo: Jay S. Simon)

supplement (**Figure 4**).

Another promising application of the large-scale cultivation of certain cyanobacteria is the upgrading of manganese and iron ores. This possibility arises because some species accumulate the hydroxides of iron and/or manganese in their sheaths, just as others accumulate calcite.[8] 'Desert varnish' is a description of polished brown or black rocks in the desert.[9] The varnish is a thin layer of the oxides of iron and manganese (Fe_2O_3, Fe_3O_4 and MnO_2). It is extracted from rock dust blown by wind on to rock surfaces that have been colonised by certain cyanobacteria.[9] It is estimated that the content of iron and manganese in these biofilms represents an enrichment factor of up to 10,000 compared to their content in the original rock.[10] This remarkable phenomenon may represent an alternative to tunnelling under conditions of intense solar radiation (**Figure 5**).

Conclusion

It is difficult to overemphasize the role of cyanobacteria in the evolution of life on Earth. A period of 2.5 x 10^9 years, ending with the Phanerozoic 550 million years ago, has been described as 'The Age of Cyanobacteria'.[6] It was the photosynthetic activity of these organisms during that period which produced free oxygen in the atmosphere, raising it from zero to 2% v/v, forming a protective ozone layer, and substantially reducing the high levels of atmospheric carbon dioxide. These changes allowed the evolution of eukaryotic cells, leading in turn to multicellular plants and animals. Perhaps in the present anxieties about rising carbon dioxide levels and the 'greenhouse' effect, a new look should be given to putting the cyanobacteria back to work.

Acknowledgements

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Figure 5. *Desert varnish. These basaltic rocks in the Gobi desert (Mongolia)* look as if they have been blackened by fire, but their dark, greyish-brown coating contains the oxides of manganese and iron.
(Photo: E. Imre Friedmann)

References

1. Friedmann, E.I. and Galun, M. (1974) *Desert Algae, Lichens and Fungi.* In: *Desert Biology.* Brown, G.E. (ed). Academic Press, New York.
2. Painter, T.J. (1993). *Carbohydrate Polymers,* **20:** 77–86.
3. Painter, T.J. (1995). *Carbohydrate Polymers,* **26:** 231–233.
4. Painter, T.J. (1996). *Biologist,* **43:** 154–157.
5. Carr, N.G. and Whitton, B.A. (1982). *The Biology of Cyanobacteria.* Blackwell Scientific Publications, Oxford.
6. Schopf, J.W. and Klein, C. (1992). *The Protozoic Biosphere.* Cambridge University Press, New York.
7. Shields, L.M. and Durrell, L.W. (1964). *Botanical Review,* **30:** 92–128.
8. Mann, N.H. and Carr, N.G. (1992). *Photosynthetic Procaryotes.* Plenum Press, New York.
9. Krumbein, W.E. and Jens, K. (1981). *Oecologia,* **50:** 25–38.
10. Metting, B. (1991). In: *Semi-arid Lands and Deserts.* Skujins, J. (ed). Marcel Dekker, Inc., New York.