The changing face of chemotherapy

W. Brumfitt and J.M.T. Hamilton-Miller

Department of Medical Microbiology, The Royal Free Hospital School of Medicine, Pond Street, Hampstead, London NW3 2QG, UK.

Summary: The historical development of antibiotics has been summarized. Three distinct phases are discernible. The first (from historical times to about 1900) involved mostly folk remedies. The second (1900–c. 1940) was ushered in by Paul Ehrlich’s development of the concept of ‘selective toxicity’ and saw the establishment of arsenicals and sulphonamides. The third, lasting to the present day, started with the exploitation of the pioneering studies of Fleming, Dubos and Waksman on antibiotic production by soil fungi. This latest phase has continued with the improvement of natural products by the skills of the medicinal chemist.

The properties and evolution of three major groups of antibiotics, penicillins, cephalosporins and aminoglycosides are fully described.

Finally, pathways of possible future evolution of antibiotics are outlined.

Introduction: from pre-history to the 20th century

The idea of using natural or artificial substances to prevent or reverse ‘putrefaction of wounds’ is a very old one, and has been known since the start of history. Thus, the ancient Egyptians used myrrh (from Commiphora spp.) and frankincense (from Boswellia spp.), the Assyrians anticipated Paul Ehrlich by several centuries by introducing arsenic. The use of mercury, pioneered by the Arabs for syphilis and championed by Paracelsus, lasted from the 16th to the early 20th century. Moses was advised to treat leprosy with hyssop (Leviticus, Ch. 14) and the Good Samaritan disinfected the wounds of his assaulted fellow traveller by pouring in oil and wine (Luke, Ch. 11). The Romans dressed wounds with spider’s webs dipped in honey and also observed that keeping drinking water in silver vessels prevented the spread of dysentery through their troops. Finally, mouldy bread often yields a growth of Penicillium spp. Thus it could be speculated that the practice of applying mouldy bread to wounds was effective by virtue of antibiotic production.

Plants were a rich source of antimicrobial substances. Beside the examples already mentioned, cinchona bark was first used in Peru to combat malaria, and ipecacuanha was found to be effective for amoebic dysentery. The famous British herbalist Culpepper (1826) lists many plants which are active in infections – to mention just two: Carduus benedictur (the holy thistle) cures ‘quartan agues’, and lovage ‘resisteth poison and infection’. A modern herbal also gives many plant remedies – for example an extract of the coneflower (Echinacea sp.) is a specific for abscesses. It is interesting to note that such extracts have been shown to antagonize hyaluronidase. Also, at least one major pharmaceutical company is exploring the flora found in Madagascar for pharmacological activity (including antimicrobial properties).

Before the germ theory had been properly formulated it had been realised intuitively for centuries that many diseases were passed from person to person and that this chain could be broken by the use of appropriate chemicals. Thus, simple antiseptics such as ‘hydrated lime’ (hypochlorite), tincture of iodine and phenol were in wide use by the mid-19th century.

Ehrlich can justly be called ‘The Father of Chemotherapy’, and his researches on vital dyes such as methylene blue and trypan red and then with arsenicals prepared the way for the antibiotic era which was formally ushered in by Domagk and his colleagues with the discovery that Prontosil rubrum had antibacterial properties in vivo.

Synthetic compounds: the 1930s, the dawn of rational chemotherapy

Antifolate agents

The chemotherapeutic era, as generally recognized,
can be said to have started with the introduction of the sulphonamides into clinical use in 1935. These were outstandingly successful at first in the treatment of meningitis, streptococcal infections and infections of the urinary tract. Staphylococcal infections, however, were found rather poorly, due to the neutralization of the antibacterial activity of the sulphonamides by pus (see below).

The active principle of Prontosil rubrum was found to be sulphanilamide. Due to its relatively low intrinsic antimicrobial activity (some 40 times less than modern sulphonamides), large doses of this compound had to be used, with undesirable side effects. This provided a stimulus for chemists to try to improve upon the existing molecule. These endeavours were successful in a remarkably short time, maximally active sulphonamides such as sulphadiazine and sulphathiazole having been patented by 1942. This speed was due to the fact that it had been shown that the antimicrobial activity of a sulphonamide depended upon its ionization characteristics. This allowed rational synthetic programmes to be undertaken. Although the intrinsic activity of sulphadiazine could not be improved upon, further modifications in the sulphonamide molecule have resulted in pharmacokinetically superior compounds such as sulfamethopyridazine. The latter has a half-life of about 100 hours and can, therefore, be given once a week.

In this way were born the discipline of medicinal chemistry and the concept of quantitative structure-activity relationships (QSAR), both of which have played and will continue to play an enormously important role in the development of improved antibiotics.

Sulphonamides were shown to exert their antibacterial activity by inhibiting the biosynthesis of bacterial folate. Acquired resistance to these compounds, which started to emerge soon after their introduction, was due to alteration in the target enzyme. The bacteriostatic action of sulphonamides is reversed in the presence of substances such as p-aminobenzoate and thymidine, which may be found in pus.

Research into sulphonamides also produced the anti-leprosy drug diaminodiphenyl sulphone (Dapsone).

Sulphonamide fell increasingly out of favour from the late 1940s, due to increasing resistance problems and the availability of broader spectrum antibiotics.

Other synthetic anti-folate antimicrobial compounds

The anti-malarial pyrimethamine and the antibacterial trimethoprim, both inhibitors of microbial folate biosynthesis, arose from an area of research entirely different from that which involved the sulphonamides. Both were found incidentally during the research programme by Burroughs Wellcome into anti-cancer agents which would act by inhibiting human folate metabolism. Trimethoprim is a remarkable compound in two respects. First, it has a unique mode of action amongst the antimicrobial agents by inhibiting an enzyme (dihydrofolate reductase) present in both bacterial and human cells. Second, the differential toxicity between human and bacterial cells is explained by small chemical differences in the structure of human and bacterial dihydrofolate reductase so that the affinity of trimethoprim is about 60,000 times greater for the bacterial enzyme. Thus, trimethoprim in therapeutic doses has negligible toxicity for host cells. Trimethoprim has enjoyed very wide usage worldwide in combination with sulphamethoxazole as co-trimoxazole (Septrin and Bactrim). However, there is now no doubt that the great antibacterial activity and wide therapeutic success of co-trimoxazole is due almost entirely to the trimethoprim component. Further, many of the arguments in favour of the fixed combination, although probably put forward in good faith, are specious. In particular the claims for synergy by combining sulphonamide and trimethoprim and also prevention of resistance to trimethoprim by the combination have not withstood scientific investigation. Unhappily, trimethoprim is an example of an excellent drug the value of which has now become eroded by increasing resistance. This is not only widespread in hospitals where resistance was due to an R factor but has subsequently spread into the community. To make matters worse because of integration of the R factor into the bacterial chromosome a stable resistance results. In the United Kingdom resistance includes strains of Staphylococcus aureus especially those resistant to methicillin (MRSA). The latter organisms are proving to be a major therapeutic problem since so few antibiotics are active against them.

Other synthetic compounds

It is clear from the above that antimicrobial chemotherapy started by the exploitation of synthetic molecules. It is notable, however, that following the arsenicals and anti-folate compounds, future advances in general mainstream chemotherapy have been made almost exclusively through natural products and their chemical modification. On the other hand, some notable contributions have been made in specific areas of chemotherapy by totally synthetic compounds. These include the treatment of
tuberculosis where \( p \)-aminosalicylate, isoniazid, ethambutol, pyrazinamide and ethionamide are still used. Other examples are the versatile nitro compounds, of which nitrofurantoin, after 30 years, remains invaluable in the treatment of urinary infections, the nitro-imidazoles (metronidazole, tinidazole) widely used both therapeutically and prophylactically when anaerobes are pathogens, and nitrothiazoles in schistosomiasis and other fluke infections. It is interesting to note that nitrofurantoin has a safety record which is better documented than any other product used for the treatment of urinary infections.

Nalidixic acid and the related 4-quinolones will be discussed in a later section.

The 1940s: antibiotics from the soil

Fleming's discovery of penicillin, reported in 1928, did not turn into a finding of clinical relevance until the mid-1940s, when the problems involved in producing and purifying large amounts of the antibiotic had been solved due to the efforts of Florey and his colleagues, in collaboration with several pharmaceutical companies in the USA. However, Fleming's observation stimulated others to search for antibiotics in soil organisms. Dubos discovered tyrothricin – a mixture of the peptide antibiotics gramicidin and tyrocidin (produced by \textit{Bacillus brevis}) in 1939, and Waksman reported the isolation of streptomycin (from \textit{Streptomyces griseus}) in 1944. While tyrothricin was too toxic for parenteral use, streptomycin revolutionized the treatment of tuberculosis, and helped Waksman win the Nobel Prize. In the period between 1947 and 1957 chloramphenicol (1947), polymyxins (1947), chlorotetracycline (1948), neomycin (1949), oxytetracycline (1950), erythromycin (1952), cephalosporin C (1955), vancomycin (1956), novobiocin (1956), rifampicin B (1957) and kanamycin (1957) – to name but a few – were reported. Some of these substances are still in use and will be referred to later.

The 1960s – the decade of the penicillins

The isolation of the penicillin ‘nucleus’ (6APA) in 1959 at Beecham Research Laboratories paved the way for the semi-synthesis and marketing of a very large number of penicillins. Some of the more successful ones marketed in the 1960s have been: ampicillin, amoxycillin, methicillin, flucloxacillin, carbenicillin and ticarcillin, followed later by mezlocillin, azlocillin, pipercillin and mecillinam. These compounds made a great impact initially on both Gram-positive and Gram-negative pathogens, but resistance (generally caused by the spread of R-factor mediated \( \beta \)-lactamas) emerged in Gram-negative strains, particularly in the hospital population, severely curtailing their usefulness. Compounds such as ampicillin and flucloxacillin remain very useful today for most purposes in the domiciliary situation, and in hospitals flucloxacillin remains effective for the majority of Gram-positive infections. However, the selection of bacterial resistance continues relentlessly, and it is predictable that strains such as MRSA, JK diphtheroids and enterococci will become more widespread (especially in compromised patients), further reducing the usefulness of penicillins. Even the recent development of inhibitors of \( \beta \)-lactamas (such as clavulanic acid and sulbactam) will probably only partially alleviate this decline.

Aztreonam is an example of a new class of \( \beta \)-lactam, namely the monobactams. This is an example of a new antibiotic which is remarkable in having a range of activity restricted to the Gram-negative organisms. Apart from its activity against a number of important Gram-negative organisms the decreased likelihood of disturbing other commensal flora may prove to be an advantage.

The 1970s – the decade of the cephalosporins

The first commercially successful semisynthetic cephalosporin (cephalothin) was made in 1962, following the isolation of the ‘nucleus’ 7ACA from cephalosporin C (this proved to be much more difficult that the analogous process in the penicillin series). However, for the first few years cephalosporins were perceived only as more expensive substitutes for penicillins. It was not until penicillin-resistant bacterial strains became increasingly common that the cephalosporins' intrinsic stability to \( \beta \)-lactamas and the fact that this stability could be increased greatly in a rational and predictable manner caused an increase in their use, especially in the treatment of nosocomial infections caused by Gram-negative bacteria. The advent of the so-called 'third generation' cephalosporins such as ceftazidime which combine very high intrinsic activity, great \( \beta \)-lactamase stability and activity against \textit{Pseudomonas aeruginosa} must represent the peak of achievement in this class of compound. These latest cephalosporins seem set to replace aminoglycosides as drugs of choice in the treatment of acute sepsis of unknown aetiology, and also seem likely to be effective alone in the primary treatment of infection, manifested by fever and leukopenia, in immunocompromised patients. It is interesting to note that the early cephalosporins (cephaloridine
and cephalothin) had a powerful action against Gram-positive bacteria but much less against anaerobes, certain Gram-negative bacteria and none against some important nosocomial strains, such as pseudomonads. By contrast, the latest compounds (so-called third generation cephalosporins) are highly active against Gram-negative bacteria – including the pseudomonads (especially Ps. aeruginosa) and are also more active against anaerobes. However, this greater activity is gained at the expense of remarkably decreased activity against the Gram-positive bacteria. It is not widely appreciated that no cephalosporin currently available is active against Streptococcus faecalis. Thus, superinfection with this organism is a well known complication of cephalosporin therapy.

The aminoglycosides

As mentioned above, streptomycin revolutionized the treatment of tuberculosis in the 1950s, but the ease with which bacteria acquired resistance and a high propensity for ototoxicity and nephrotoxicity soon severely limited the use of this antibiotic for more general use. Neomycin proved too toxic for parenteral use, and kanamycin, although enjoying a very wide market, never really became established as an aminoglycoside of first choice due to its deficiencies against resistant hospital pathogens. The gentamicin complex which was introduced in 1964, on the other hand, rapidly found favour for the treatment of acute sepsis, due to its broad antibacterial spectrum. This included the much feared pathogen Ps. aeruginosa which until then could only be treated by the polymyxins, the value of which was limited by their toxicity – especially nephro- and neurotoxicity. Early clinical use at too large a dose gave gentamicin the reputation of being highly ototoxic and nephrotoxic, whereas when properly used – i.e. dosage controlled by determining peak and trough levels in individual patients – the incidence of toxicity is acceptably low. One problem about such monitoring is the increased time and therefore cost which results from the need to obtain blood samples in order to carry out estimations at frequent intervals.

Increasing resistance among Gram-negative bacilli during the 1970s led to intensive investigation of the cause of such resistance. The result of these studies was the discovery of the large family of aminoglycoside-inactivating enzymes. Once the biochemical mechanisms of action of these enzymes had been made clear it was possible to modify the antibiotic molecules to render them insusceptible to enzymic attack. This process, involving close cooperation between microbiologist, biochemist and medicinal chemist, was somewhat analogous to that which occurred in respect of the β-lactam antibiotics and the enzymes which inactivate them, the β-lactamas (see above). In the case of the aminoglycosides the result of these collaborative efforts included amikacin (a derivative of kanamycin A in which the inactivating enzymes' target sites were protected). By contrast dibekacin (a kanamycin B product not available in the United Kingdom) is a product where the enzymes' targets have been removed completely, thus solving the problem in a different way.

Amikacin has been in clinical use for some 12 years, and resistance emergence so far has been small. This may be because, due to its high cost, usage of amikacin has been relatively small. On the other hand, there is increasing published evidence that for this antibiotic resistance emergence may not be connected with total use. For whatever reason, amikacin can still be regarded as an antibiotic with a future, whereas the other aminoglycosides seem to be drugs of which is in decline.

Other groups of antibiotics which have benefited from chemical modification

The rifamycins were isolated from Streptomyces mediterranei but owe their current clinic therapeutic importance entirely to the skill of the medicinal chemist. Of the original naturally occurring compounds rifamycin B had some antimicrobial activity but this was insufficient for clinical use. The early modifications, rifamycin SV and rifamide, created little interest outside Italy, as they were of quite low antibacterial activity, were somewhat erratically absorbed and had unfamiliar pharmacokinetic profiles (due to excretion being almost entirely in the bile). However, a later product, rifampicin, has become extremely important in the treatment of tuberculosis, and is increasingly being realised to be an antibiotic of great potential for the management of infections caused by MRSA and other recalcitrant Gram-positive species.

Lincomycin is another natural product whose activity has been successfully increased by chemical means, in the form of clindamycin. Lincomycin has had a somewhat chequered history, having been introduced first as an antistaphylococcal agent. Lincomycin and clindamycin enjoyed a resurgence of popularity in the early 1970s due to their broad anti-anaerobic spectrum as well as good activity against Gram-positive cocci, but since that time their association with antibiotic-induced colitis has reduced their usage substantially.
The macrolides are a large group of natural products of which only erythromycin and, to a much lesser extent, spiramycin and oleandomycin have made any significant clinical impact in the UK. Bioavailability has always been a problem with erythromycin, and chemical modifications – albeit of a relatively minor nature – have enabled this to be improved. Thus, the stearate and particularly a recent development roxithromycin are better absorbed by mouth than the naturally occurring free base. Intramuscular (ethylsuccinate) and intravenous (lactobionate) preparations are also available. Another ester, the estolate, is now used with caution because of reports of hepatotoxicity. It has apparently not been possible to alter the antimicrobial spectrum of the macrolides by chemical modification. These compounds are still important, particularly in certain respiratory infections such as Legionnaires disease and mycoplasma pneumonia. In campylobacter enteritis, erythromycin is the drug of choice. For other indications erythromycins are usually ‘second line’ antibiotics but are often used as a substitute for benzylpenicillin when the patient is ‘penicillin sensitive’.

In the tetracycline series radical changes have been made in both the antibacterial and pharmacokinetic properties. Beneficial characteristics have been introduced into this series by increasing the lipophilicity. Minocycline and doxycycline, which are semi-synthetic compounds, are the two best examples: the former is active against many tetracycline-resistant staphylococci while the latter allows a once-a-day regimen due to its prolonged half-life.

In the past 5 years there has been a remarkable change in the properties and chemotherapeutic potential of the nalidixic acid group. The first clinically useful compound in this totally synthetic series, nalidixic acid, has been available since the early 1960s. This was used purely as a urinary anti-infective. It has a spectrum limited to the common Gram-negative pathogens, and has to be given in a 6-hourly schedule in order to prevent resistance emerging. Early attempts to improve on these properties met with only modest success – cinoxacin, rosoxacin, piromidic acid and pipemidic acid have been marketed but have found only a small therapeutic niche. However, a later phase of chemical modification in this series has had a spectacular result, as the fluorinated 4-quinolone family has emerged.

The synthesis of these compounds probably represents the most exciting development in antimicrobial chemotherapy since the isolation of 6APA. Already several have been marketed such as norfloxacin, ciprofloxacin, enoxacin, ofloxacin and pefloxacin and many more are under investigation.

This group of compounds is very intrinsically active against a wide range of Gram-negative rods (including Ps. aeruginosa), and have substantial activity against Gram-positive species as well. Long half-lives mean that a twice daily schedule is practicable. Their unique mode of action – on DNA gyrase – suggests that resistance emergence may not be common. The quinolones are concentrated in the tissues due to their lipophilicity, and so the only moderate blood levels that are attained must not be taken to imply (as would be the case for some other antibiotics) poor clinical performance. Indeed, clinical trials in many indications show excellent results. It is too soon to be dogmatic as to the place of the quinolones in the treatment of infectious diseases in general, but the first indications are certainly favourable, and have done nothing to reduce the initial promise of these drugs. A fuller assessment can be made in a few years time. Meanwhile, we consider it important that the quinolones be reserved for serious infections or the treatment of multi-resistant strains. It is to be hoped that use in general practice would be severely curtailed – otherwise the problem of resistance may emerge.

Drugs which have not been modified

Included here are substances such as vancomycin and fusidic acid. These are interesting in that the appearance of new problems, especially the MRSA, have resulted in these drugs being used much more commonly. In the glycopeptide field other drugs similar to vancomycin but apparently with better pharmacokinetic properties and less toxicity have appeared. Of these the one where investigation is most advanced is teicoplanin. This should be available for general use by the summer of 1988.

Examples of changes in the morbidity and mortality of infectious diseases unrelated to antimicrobial chemotherapy

While not underestimating the impact of antimicrobial agents on the control of infectious diseases remarkable changes which are unrelated to these substances have been observed. The increasing availability of international statistics makes accurate analysis of these changes possible.

Early in the 20th century tuberculosis was a universal scourge well deserving the epithet bestowed upon it by Bunyan of the ‘Captain of the Men of Death’. However, a remarkable decline in the incidence of tuberculosis had already been noted before the discovery of anti-tuberculosis
drugs and, for that matter, before the discovery of the causal organism which was identified by Koch in 1882.

Subsequently important factors in the control of the disease were improvement in housing, nutrition and sanitation together with the knowledge that tuberculosis was a transmissible disease.

However, antibiotics played an important part in the treatment of tuberculosis. The advent of anti-tuberculosis drugs, notably streptomycin (1944), para-amino salicylic acid (1946) and isoniazid (1952) revolutionized the treatment of all forms of tuberculosis. From the patient's point of view the outlook of uncertainty, or of despair, was replaced by one of confident optimism. Long periods of bed rest are now unnecessary and although appropriate surgical treatment is of great importance in certain cases, it is true to say that efficient chemotherapy leaves only the occasional case where it is required.

The discovery of new anti-tuberculosis agents especially those which are bactericidal and penetrate cells have resulted in advances in treatment. These include the reduction of duration of treatment (which initially was two years and is now three months), and oral treatment allowing therapy at home with obvious cost benefits.

Problems still remain, for example non-compliance by patients may lead to the emergence of resistant organisms. Also lack of awareness of the danger of tuberculosis led to a reduction in BCG vaccination of 'at risk' subjects and consequently of herd immunity.

Tuberculosis is an example of a serious infection where antimicrobial drugs only play a part in its control. However, a more striking example is the global eradication of smallpox which has been achieved without the help of specific antiviral agents. In contrast, an example of persistence of a transmissible disease, in spite of the availability of effective chemotherapeutic agents, is malaria.

A change in virulence of an organism (as opposed to host resistance) may also cause a decrease in morbidity and mortality in infectious disease. A remarkable example of this is rheumatic fever: this is well known to be associated with streptococcal sore throat which is therefore an essential precursor of the disease. However, evidence from mortality rates and from necropsy studies on children leaves little doubt that in the economically developed countries of the temperate zone, rheumatic fever in its more serious and easily recognized forms has decreased progressively in incidence since at least the beginning of the twentieth century. This process of decline underwent acceleration after 1930 and has since continued, but foci of higher incidence persist in certain urban areas of the United States and Britain. Thus, as recently as 1942 recruiting to the American Air Force was almost halted because of epidemic streptococcal sore throat followed by an incidence of rheumatic fever which was as high as 4 per cent. Only a decade later a number of reports concerning patients with streptococcal sore throat in the USA and in this country have shown an incidence of rheumatic fever of 0.1 per cent or less. The reason for a change to the benign nature of such infections remains unclear. However, circumstantial evidence strongly indicates that a decrease in the virulence of Group A β-haemolytic streptococci for humans is an important factor.

The fall in incidence of rheumatic fever following infection by β-haemolytic streptococcus Group A has not been brought about by the availability of antibiotics. Although it was shown that by treating a streptococcal sore throat with a 10-day course of benzylpenicillin, which eliminates the streptococci, rheumatic fever was prevented, it is now known that even in the absence of antibiotic treatment rheumatic fever rarely complicates streptococcal pharyngitis. This is an example of change in the virulence of an organism which is unrelated to the use of antibiotics.

Future prospects

The last 50 years have seen a rapidly evolving picture in terms of available antibiotics, and many groups have experienced rises and falls. Examples of 'antibiotics of today' are β-lactams (cephalosporins, cephemycins, monobactams and β-lactamase inhibitors), aminoglycosides, quinolones and rifampicin. 'Antibiotics of yesterday' must include streptomycin and sulphonamides. Of great interest is the question: which will be the 'antibiotics of tomorrow'? While one can only speculate on this topic, there are several directions which can be seen as distinct possibilities for the future evolutionary pattern of antibiotic development. These include:

(a) The revival of 'old' antibiotics in response to new needs. We see definite prospects for the renaissance of a compound related to novobiocin in response to the appearance of MRSA. The nitrofurans also have the advantage of very rare resistance acquisition. Nitrofurantoin is still valuable in the treatment of urinary infections.

(b) Renewed search for new natural products. It had been thought that resources capable of producing new natural antibiotics were exhausted, following the long phase of no further discoveries after gentamicin. However, the finding of such compounds as the monobactams and teicoplanin showed that this view was erroneous. Large poten-
tial sources of new natural products remain relatively untapped – e.g., the Plant Kingdom.

(c) Production of new chemical entities. To avoid cross-resistance, it would seem logical to concentrate on molecules whose chemical structure differs completely from those of existing antibiotics rather than to continue to modify existing molecules. Examples of this approach already exist, for instance in paldimycin and the Du Pont series of oxazolidinones.

(d) The production of antibiotics with highly specific ultra-narrow antibacterial spectra should be developed to complement the existing very broad spectrum compounds. The new class of antibiotics would be used once the invading pathogen had been accurately identified. There are already examples of narrow spectrum compounds in use, notably cefsulodin against *Ps. aeruginosa*.

It will be an instructive and perhaps humbling exercise to re-read this article in the year 2000, and to see how many predictions have proved true.

Bibliography


The changing face of chemotherapy.

W. Brumfitt and J. M. Hamilton-Miller

Postgrad Med J 1988 64: 552-558
doi: 10.1136/pgmj.64.753.552

Updated information and services can be found at:
http://pmj.bmj.com/content/64/753/552

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/