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Drug resistance and antiviral agents
It might seem premature to discuss the possible emergence of drug resistant virus mutants when the practice of antiviral chemotherapy is merely in its infancy. Yet with each of the three compounds in clinical use, methisazone, idoxuridine and amantadine, drug resistant viruses have been selected with relative ease, at least in the laboratory (Appleyard & Way, 1966; Renis & Buthala, 1965; Oxford, Logan & Potter, 1970). At present, of course, very little data is available about the emergence of drug resistant viruses in man. In the case of isatin-3-thiosemicarbazone, drug dependent mutants of vaccinia virus have also been obtained. We are presented with the distinct possibility of a repetition in the virology world of the tremendous problems now being encountered of bacterial resistance.

Many of the properties of these drug resistant viruses are similar to those found in drug resistant bacteria. Thus, influenza viruses resistant to 1-aminoadamantane hydrochloride exhibit cross resistance to the structurally related compounds alpha-methyl-1-adamantanemethylamine and 2-amino adamantane sulphate. Furthermore, ‘transfer’ of drug resistance has been documented recently with influenza as we shall discuss below. With influenza virus the drug resistance problem is heightened by the process of genetic recombination or reassortment. The influenza virus genome is uniquely in the form of discrete fragments of RNA, a transcript of each piece acting as a monocistronic message and coding for a particular virus protein. If two influenza viruses with widely varying biological characteristics co-infect a cell, then the virus progeny may be mixed, possessing some of the properties of both parents. Using this laboratory model of genetic reassortment, it has now been shown that resistance to amantadine can be transferred from one strain of influenza to another (Appleyard & Maber, 1975). Influenza has successfully survived as the last pandemic virus infection of man and this must be attributed in part to the biological adaptability of the virus including for example antigenic ‘drift’ and ‘shift’ whereby the virus side-steps the problem of an otherwise growing population of immune persons. The problem is being encountered at present as we attempt to control influenza by immunoprophylaxis and one can only anticipate similar problems in the future with any widespread use of chemoprophylaxis or chemotherapy.

On a more hopeful note, all the drug resistant and drug dependent virus mutants are providing useful information on the mode of action of antiviral compounds. In this context it should be emphasized that although we know that methisazone inhibits a post-translation process in the vaccinia virus infected cell, and that amantadine inhibits influenza virus penetration or uncoating, yet the precise point of inhibition at the molecular level remains to be discovered. The successful selection of more effective antivirals in the future may depend on a precise understanding of the mode of action of the present series of compounds. Random screening, although used with great success in the search for antibacterials, has not produced potent and selective inhibitors of viruses. Finally, in a recent paper, Herrmann (1976) has proposed that only true antiviral agents with a real selective inhibitory effect on some virus specific process are able to allow the selection of drug resistant virus mutants. Compounds which appear to inhibit virus replication, but which may not result in the production of drug resistant mutants are considered to inhibit virus replication non-specifically by interfering with some cell associated function. A possible daunting practical problem of the future is being turned at present to the advantage of the molecular virologist and
may indeed lead to the selection of better antiviral agents.

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Cerebral abscess
The basic principles upon which the successful treatment of cerebral abscess is dependent can be summarized as follows. The possibility of the diagnosis must be considered, the lesion localized and aspirated together with, where appropriate, other surgical treatment including that of the primary source of the infection and bactericidal chemotherapeutic agents appropriate to the causative microorganisms must be administered. As almost every species of human bacteria have been found in cerebral abscesses the bacteriological studies must be meticulous. Where the infection is associated with metastatic spread from the lungs and other distal foci the micro-organisms encountered are usually Staph. aureus, various types of streptococci, enterobacteria, Pseudomonas aeruginosa, and occasionally Fusobacteria and anaerobic streptococci. Similar organisms are encountered in abscesses complicating compound fractures of the skull, penetrating injuries, fractures of the base of the skull involving the air sinuses or local infections of the scalp.

A completely different bacteriological picture is encountered in cerebral abscesses associated with local spread from chronic suppuration in the middle ear, paranasal air sinuses and to some extent congenital cyanotic heart disease. Such abscesses almost invariably have a mixed bacterial flora consisting of enterobacteria, especially Proteus spp. and various types of obligate anaerobes such as Bacteroides fragilis, B. melaninogenicus, Fusobacterium necrophorum, Fusobacterium spp. and anaerobic or microaerophilic streptococci. Frequently several species of anaerobes are present. The frequency with which anaerobes are encountered in such infections is by no means universally recognized. In a review of 200 cases of supratentorial abscess, 113 of which were associated with an E.N.T. source (Garfield, 1969), no mention is made of Fusobacterium or Bacteroides. Similarly Shaw & Russell (1975) reviewing 47 cases of cerebellar abscess, 93% of which were secondary to otogenic disease, do not mention these two genera. Heineman & Braude (1963) in a review of 16 papers made the pertinent observation that those series reporting low percentages of sterile culture contained the highest percentage of anaerobes and vice versa. This bacteriological hiatus may be partly responsible for the observation in a leading article in the British Medical Journal (1975) that brain abscesses carry almost the same mortality now as that reported by Sir William Macewen in 1893.

One of the possible reasons for the apparent failure of some workers to isolate anaerobes from brain abscesses is the overgrowth of co-existing aerobes, particularly Proteus spp. It is therefore invaluable to use a medium, such as agar with incorporated nalidixic acid at a final concentration of 50 mg/l (to be published), which selectively inhibits Gram-negative aerobes.

With the exception of B. fragilis the anaerobes mentioned above are usually highly sensitive to penicillin (Finegold, Harada & Miller, 1967). Clindamycin is active against all these species (Bartlett, Sutter & Finegold, 1972; Martin, Gardner & Washington, 1972) although resistance to this antibiotic appears to be emerging (Goldring, Scott, McNaught & Gillespie, 1975) and we ourselves have encountered two strains of B. fragilis from cerebral abscesses which were sensitive to clindamycin only at 5 and 10 mg/l, respectively. An alternative to clindamycin in this situation is chloramphenicol, but its potential toxicity is a hazard, particularly in view of the necessity for prolonged treatment. Metronidazole has recently been shown to be extremely effective in the treatment of three patients with otogenic brain abscesses (Ingham, Selkon, So & Weiser, 1975) and we have also used it successfully to treat a patient with meningitis associated with chronic middle ear...
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infection from which anaerobic organisms were isolated (unpublished). Co-existing aerobes were of course treated with appropriate bactericidal chemotherapeutic agents in all four cases. The efficacy of metronidazole is probably due to its rapid bactericidal action (Ralph & Kirby, 1975) and the high concentrations attained in the abscess cavities, which exceeded blood levels in the two cases studied by us (unpublished data). Clearly empirical chemotherapy must be commenced before the results of bacterial culture and sensitivity testing are available. The agents employed will vary in different centres; currently in this hospital the combination of gentamicin, ampicillin and metronidazole is used for this purpose. No reliable indication of the minimun duration of chemotherapy can be deduced from the various published series because in many instances the bacteriological information is limited. The practice in this hospital is to continue appropriate chemotherapy for 4 to 8 weeks.

The leading article in the British Medical Journal (1975) concluded that to think of the possibility of brain abscess is half way to the diagnosis of a very dangerous disease; perhaps to consider anaerobic bacteria is half way to the treatment.

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References


Gentamicin-resistant Staphylococcus aureus

Although Gram-negative bacilli resistant to gentamicin are now frequently encountered (Greene, Moody, Schimpff, Young & Wernik, 1973; Shafi & Datta, 1975; Meyer, Lewis, Halter & White, 1976), gentamicin resistance in Staphylococcus aureus has been very rare. However, two outbreaks of infection in hospitals caused by gentamicin-resistant strains of Staph. aureus have occurred recently (Speller et al., 1976; Bint, George, Healing, Wise & Davies, 1976). In both of these outbreaks the strains were resistant to many other antibiotics including neomycin, streptomycin, kanamycin and tobramycin. There was a strong suggestion that previous administration of antibiotics to which the strains were resistant, especially gentamicin and tobramycin, was associated with colonization and infection. In particular, topical use of gentamicin seems to be associated with the appearance and spread of gentamicin-resistant Staph. aureus (Bint et al., 1976; Warren & Roberts, 1976). It is important at this early stage to assess the significance of these reports and to produce a strategy to preserve the value of gentamicin and tobramycin.

The limited amount of evidence available from studies of gentamicin-resistant strains of Staph. aureus suggests that the resistance is plasmid borne. In three studies (Speller et al., 1976; Bint et al., 1976; Porthouse, Brown, Graeme Smith & Rogers, 1976) the resistance to gentamicin and tobramycin was mediated by drug-inactivating enzymes. Genes coding for drug-inactivating enzymes are nearly always carried on plasmids. Soussy, Bouanchaud, Fouace, Dublanchet & Duval (1975) studied a gentamicin-resistant strain of Staph. aureus and by sedimentation analysis of DNA
demonstrated a plasmid locus for the resistance. Transduction of gentamicin resistance from a resistant Staph. aureus to a sensitive strain has been demonstrated (Bint et al., 1976). It is therefore likely that spread of gentamicin resistance to sensitive strains of Staph. aureus could occur by bacteriophage-mediated transduction.

The appearance of resistance to gentamicin in Staph. aureus after a decade of use of the antibiotic is very similar to the delay in appearance of neomycin-resistant strains which did not appear for 9 years after its introduction (Lacey, 1975). Extensive dissemination of neomycin-resistant strains often followed topical use (Lowbury, Babb, Brown & Collins, 1964; Alder & Gillespie, 1967). When neomycin-resistant strains first appeared, the strains were closely related on phage-typing evidence. This does not appear to be true of the recently isolated gentamicin-resistant staphylococci. The strains isolated during the two outbreaks (Speller et al., 1976; Bint et al., 1976) are different judging by their phage-typing patterns (Dr V. G. Alder, personal communication). It seems probable therefore that transduction to new strains of staphylococci is already taking place and that these strains will appear in other hospitals in Great Britain.

The reason for the delay of many years in the appearance of both neomycin and gentamicin resistance in Staph. aureus can only be speculative. It is probable (Lacey, 1975) that the neomycin resistance gene was an extreme rarity in the staphylococcal population. When a plasmid bearing the gene, small enough for inter-cell transduction, occurred, neomycin resistance spread to a wide variety of strains. Staph. aureus normally colonizes the skin and nose and it is at these sites that contact between the bacteria and antibiotics is most important; both selection of resistant strains and transduction of resistance between strains may occur (Lacey, 1971).

The most important step in the prevention of widespread resistance to gentamicin in Staph. aureus would be the severe restriction, or even prohibition, of the use of topical preparations of gentamicin. It is to be hoped that topical preparations of tobramycin and amikacin will not be marketed. More use could be made of disinfectants such as chlorhexidine, povidone-iodine and quinolines, and dyes such as magenta paint for treating superficial skin infections. The systemic use of gentamicin, tobramycin and amikacin should also be reduced by more rational prescribing; the wider use of antibiotic policies designed to minimize the use of these antibiotics would be helpful.

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References


The relevance of urine and serum antibacterial activity to the treatment of urinary tract infections

There is much evidence that successful chemotherapy of uncomplicated urinary tract infections...
infections depends on the concentration and efficacy of antibiotics in the urine rather than in the blood. Drugs such as nitrofurantoin, nalidixic acid and oxolinic acid are often effective although they do not reach antibacterial levels in the blood. Urinary infections by Gram-negative organisms that would have been scored as resistant by conventional disk tests have been successfully treated with antibiotics such as oral penicillin G (Stamey, Govan & Palmer, 1965; Hulbert, 1972), penicillin V (Stamey et al., 1965; Gower, Marshall & Dash, 1975), and tetracyclines (Hoffman, 1970; Stamey et al., 1974; Musher, Minuth, Thorsteinsson & Holmes, 1975). Clinical studies with carfecillin, a new orally administered ester of carbenicillin, have shown that it will often eradicate urinary infections due to Pseudomonas even though blood levels are much lower than the inhibitory concentrations of carbenicillin (Wilkinson, Reeves, Wise & Allen, 1975). These observations do not, of course, imply that these antibiotics should necessarily be chosen for such infections.

Even in patients with chronic urinary infections where there may be appreciable tissue involvement, the urinary concentration of antibiotics seems to be more important than blood concentrations. In a prospective study of 84 patients with chronic or recurrent infections, McCabe & Jackson (1965) found that antimicrobial activity in the serum did not separate the cures from the failures, but that inhibitory activity in the urine was directly related to the cure of infection. These results suggest that back diffusion of antibiotics occurs from concentrated urinary solutions into infected tissues. In a prospective study of cancer patients with urinary infection, although serum antibacterial levels influenced the outcome of treatment, there was a better correlation between response to treatment and concentrations of drug in urine. In this study, infection was cured in at least 90% of patients whose urine was bacteriostatic at a dilution of 1 in 4 or more (Klastersky, Didier, Swings & Weerts, 1974).

The antibiotic content of some disks used for routine sensitivity testing has been shown to be too low to predict the activity of the antibiotics at the concentrations reached in urine. Thus 10 out of 26 urinary Enterobacteriaceae which appeared resistant to disks containing 25 µg of amoxycillin were rapidly killed by urine from patients receiving therapeutic doses of this agent; similar results were obtained with ampicillin (Anderson, Warner & Forshaw, 1975). Again, species of Micrococcaceae appeared resistant to mecillinam when tested with disks containing 10 µg of antibiotic. Nevertheless, urine from volunteers receiving therapeutic doses of pivmecillinam (a mecillinam ester intended for oral therapy) was bacteriostatic or cidal for 34 out of 35 Micrococcaceae isolated from patients with urinary infection. In fact, the concentrations of mecillinam in urine were generally much higher than the minimum inhibitory concentrations of this antibiotic for Micrococcaceae, and results from testing with a 50 µg disk is likely to predict the outcome of treatment (Anderson, Adams, Wilson & Shepherd, 1976). The ease for a change in laboratory sensitivity test methods is summarized by Stamey et al. (1974) who conclude: "The cure of urinary infection depends upon antimicrobial concentrations in urine rather than serum and testing for antimicrobial sensitivity based on urinary levels should be available in clinical practice." To follow this advice, clinical laboratories should, perhaps, report categories of sensitivity similar to those suggested by Ericsson & Sherris (1971).

Recognition of the importance of urinary antibacterial activity should encourage studies of treatment with smaller doses of some drugs. This would reduce the tendency to cause proliferation of resistant bacteria in the bowel and other sites and may also minimize some side-effects and reduce interference with the commensal flora.

A further source of error in the prediction of the outcome of treatment in the diagnostic laboratory is that urine usually differs in pH, osmolality, and constituents, from laboratory media. These differences may not be important for the assessment of the penicillins or cephalosporins but are critical for some other agents. There are, for example, many reports of the enhancement of the effect of sulphonamides by urea (e.g. Neter & Clark, 1944). Cotrimoxazole may be bactericidal and its components synergistic in thymine and thymidine free media (e.g. Bushby, 1973), but are not so in urine (Lewis, Anderson & Lacey, 1974; Anderson, Lacey, Lewis & Sellin, 1974). Urine has an inhibitory action upon aminoglycosides that could be important in patients with renal failure. The mean inhibitory and bactericidal concentrations of gentamicin for Pseudomonas were 30 to 40 times higher in urine than in trypticase soy broth (Minuth, Musher & Thorsteinsson, 1976). Light incula
used in laboratory sensitivity tests do not always provide a good model of urinary infection where large numbers of organisms are sometimes present. Thus, disk sensitivity tests may give an over-optimistic view of the sensitivity of ampicillin resistant strains of *Escherichia coli* to cephalosporins (Greenwood & O'Grady, 1976). Laboratory methods currently used to determine bacterial susceptibility to antibiotics in the urinary tract may not, therefore, reflect the behaviour of organisms in the patient. Surveys of the sensitivity of urinary pathogens based on antibiotic disk or other tests in conventional laboratory media should be interpreted with some reserve.

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References


Antibiotics in bone

There are a number of technical problems in determining antibiotic concentrations in bone and therefore considerable variation in the reported antibiotic concentrations when the results of studies of different workers are compared. This applies to a number of antibiotics. Antibiotic concentrations in bone are related to the time and route of administration and the rapidity with which the antibiotic is given. Other factors include the microbiological assay method used and the site from which bone is taken. Some workers study normal (uninfected) bone, whilst others examine infected bone taken from patients with osteomyelitis (Hierholzer, Rehn, Knothe & Masterson, 1974). These points must be clarified when reporting the results of bone level studies.
Several antibiotics have been reported to penetrate bone well. These are lincomycin (Grady & Stern, 1965; Vacek, Hejzlar & Pavlansky, 1967; Norden & Kennedy, 1971; Beavis, Beavis, Parsons & Paddock, 1975; Parsons, Beavis, Paddock & Hossak, 1976), clindamycin (Panzar, Brown, Epstein, Lipson, Mahaffey & Atkinson, 1972; Vacek, Hejzlar, Slavik & Pavlansky, 1972; Nicholas, Meyers, Levy & Hirschman, 1975), sodium fusidate (Hierholzer, Knothe & Rehn, 1970; Chater, Flynn & Wilson, 1972) and gentamicin (Rosin, Rosin & Kramer, 1974). Some of the cephalosporins penetrate bone well. These include cephaloridine (Hughes, Benson, Dash & Field, 1975), cephalexin (Gump & Lipson, 1968; Norden & Kennedy, 1971; Hierholzer, Lienzenmeier, Kleinig & Hoerster, 1974), cephadrine (Parsons, Beavis, Paddock & Hossack, 1976) and cephalizin (Hierholzer, Lienzenmeier, Kleinig & Hoerster, 1974; Kondo, 1974). Although the isoxazolylpenicillins and ampicillin are widely used in the treatment of osteomyelitis, there are few data about their bone penetration (Kramer & Weuta, 1972; Koleczn, Nelson, McHenry, Gavan & Penovich, 1974) and this merits further study.

We have measured the concentration of lincomycin, cephradine and cephalizin in normal bone taken from patients undergoing prosthetic joint replacement for osteoarthritis of the hip, a procedure performed by most orthopaedic surgeons which has given many elderly patients a new lease of life. Whilst the operation is usually successful, infective complications occur in 2 to 6% of patients (Benson & Hughes, 1975). An infected prosthesis is a disaster in an elderly debilitated patient since it requires re-operation. Thus, any measure that reduces the incidence of postoperative infection should be considered. Some believe that the infections are airborne and occur when the head of the femur is removed and the prosthesis is inserted. Three common organisms isolated from the atmosphere are Staph. epidermis (42%), Corynebacterium (30%) and Strep. viridans (10%) (Fitzgerald, Peterson, Washington, Van Scoy & Coventry, 1973). Some surgeons (Charnley & Eftekhar, 1969) have reduced their infection rate by operating in an operating theatre where the air is changed frequently or other sophisticated flow systems are present and they make sure that other surgeons do not use the same theatre. Since this is not practical in most units, parenteral antibiotics are often given when the head of the femur is removed.

Although many microbiologists object to prophylactic antibiotics several studies have shown a reduced incidence of postoperative infections of the prosthesis (Fitzgerald, Petersen, Washington, Van Scoy & Coventry, 1973; Pavel, Smith, Ballard & Larsen, 1974; Wilson, Aglietti & Salvati, 1974) after empirical prophylactic parenteral antibiotics. There is now a need to evaluate critically these regimes and if confirmed to select the best route and mode of administration to achieve therapeutically effective concentrations of the correct antibiotics in bone in patients undergoing this procedure. Organisms that have caused prosthetic infections include Staph. aureus, Strep. viridans, several Gram-negative organisms (e.g. Pseudomonas aeruginosa and Klebsiella), Aspergillus and Corynebacterium. Thus, a wide range of antibiotics may be necessary for prophylaxis and treatment in patients with infected prostheses. Recent interest has focussed on the bone concentrations of lincomycin, clindamycin (which is primarily anti-staphylococcal) and the cephalosporins.

Therapeutically effective concentrations of lincomycin against penicillinase producing Staph. aureus followed intramuscular injection of 0.5 g 6 h preoperatively. Cephazolin, which is highly protein bound, gave bone concentrations well above the M.B.C. for Staph. aureus and those Gram-negative organisms that cause postoperative prosthetic infections while cephradine produced the lowest bone concentrations of those antibiotics that we studied (Parsons et al, 1976).

The concentration of antibiotic in bone is measured by several methods. Many workers attempt to extract all the bone by crushing bone fragments with a pestle and mortar. Unfortunately, the bone cannot be dissolved in strong acid since this would inactivate antibiotic within bone and prevent its detection by microbiological assay. An alternative method is to agitate small bone fragments at the optimum pH in the appropriate buffer. After regular agitation for the optimum time the concentration of antibiotic in the buffer is measured. A criticism of this technique is that there is no way of knowing whether all the antibiotic is extracted. Even if the buffer is changed and bone fragments are agitated until no further antibiotic can be detected, there is no guarantee that all the available drug has been leached out of the bone. It is important...
to estimate antibiotic concentrations in duplicate in adjacent bone samples to obtain an idea of antibiotic distribution within bone. This also cross checks the accuracy of the assay.

A good correlation between the results of the grinding and agitation assay methods indicates that the laboratory methods are reliable but wide variations between the results of the two methods does not necessarily reflect badly on the technician performing the assay. The range of the results is a valuable guide to the location of the antibiotic. Low concentrations of antibiotic eluted in the buffer may indicate that the drug is tightly bound to tissue proteins within the bone. Thus, simultaneous assay by the crushing and agitation methods should always be performed to establish whether drug distribution is intravascular and/or extravascular. Duplicate assays delineate those antibiotics (e.g. cephradine) that are firmly bound to tissue proteins within bone and are therefore not readily available to exert their antibacterial effect (Parsons, Beavis, Paddock & Hossack, 1976) and those antibiotics, e.g. lincomycin (Parsons, Beavis, Paddock & Hossack, 1976) and cephaloridine (Parsons, Beavis, David & Paddock, 1976) that are loosely bound and readily eluted from bone.

Although the concentrations after intramuscular lincomycin (600 mg 6-hourly preoperatively) provided therapeutically effective concentrations well above the M.I.C. against Staph. aureus in the majority of patients (Parsons, Beavis, Paddock & Hossack, 1976), two of the twelve patients studied developed pseudomembranous colitis (Parsons, Salfield & Beavis, 1976). We therefore decided to study cephradine and cephaloridine to see if these broad spectrum cephalosporins were suitable alternatives to lincomycin.

Intramuscular cephradine gave low levels of the antibiotic in bone, whilst the concentrations after an intravenous bolus of 2 g of cephaloridine gave concentrations well above the M.B.C. for staphylococci. On the present evidence, the prophylactic antibiotic of choice in total hip replacement seems to be cephaloridine, but there is a real need for objective data on the bone concentrations of the isoazolylpenicillins.

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