REVIEW ARTICLE

Antibiotic therapeutics in laboratory animals

T. H. Morris

Department of Laboratory Animal Science, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK

Summary

Information on antibiotic therapeutics in laboratory species, especially in rodents and rabbits, is reviewed. A number of areas are considered: interference by antibiotics with an experiment, antibiotic toxicity, routes of administration, effects of formulation on bioavailability, antibiotic prophylaxis, use of combinations of antibiotics, misuse of antibiotics, regulatory approval for antibiotic use in animals, sources of information on antibiotic indications and dose, and extrapolation of dose information from other species.

Keywords Antibiotics; laboratory animals; therapy

The purpose of this review is to highlight some specific questions and problems concerning the use of antibiotics in the common laboratory species. Although dogs, cats, horses and domestic species are used in research, they will not be discussed here as there is a large volume of information on the clinical use of antibiotics in these species, and as many of these antibiotics are approved by regulatory authorities for use in these species, clear indications for their rational use are provided (National Office of Animal Health 1992, VPD 1991).

This review deals principally with rodents and rabbits since they make up over 90% of the animals used in research. There is no uniform source of information on rational use of antibiotics comparable to that available for the common companion and domesticated species. The situation is summarized well by Latt (1976) '. . . dosages of therapeutic agents for laboratory animals are scattered throughout the scientific literature or are extrapolated from dosages recommended for other species'.

Other non-mammalian species are used as laboratory animals in smaller quantities, for example birds and poikilotherms such as reptiles, fish and amphibians and these species will not be discussed as there can be major differences in pharmacokinetics. An introduction to antibiotic therapeutics for these species can be found in specialized medical texts (BSAVA, Jacobsen *et al.* 1991).

A number of areas will be considered: interference by antibiotics with an experiment, antibiotic toxicity, routes of administration, effects of formulation on bioavailability, antibiotic prophylaxis, use of combinations of antibiotics, misuse of antibiotics, regulatory approval for antibiotic use in animals, sources of information on antibiotic indications and dose, and extrapolation of dose information from other species.

Interference by antibiotics with experimental studies

Bacterial infections in animals used for experimental studies are undesirable. At the most basic level the morbidity produced by an infection increases animal discomfort and experimental variation. More specific interactions can also occur, for example, studies on enteric pathogens or enteric diseases such as malabsorption may be compromised by concurrent enteric bacterial infection and the associated

Accepted 17 May 1994

pathology. However, it should also be recognized that use of an antibiotic to 'solve' the problem of a concurrent bacterial infection may itself interfere with the experiment. The adverse effects of the aminoglycosides on renal function or the fluoroquinolones on juvenile cartilage formation may produce relatively obvious problems. However, the effects may be less overt. If a compound is under investigation, and concurrently an antibiotic with pharmacokinetic disposition by the same mechanism is also administered to the animal, then the kinetics of this compound under investigation may be affected. As a specific example the concurrent administration of chloramphenicol prolonged the duration of xylazine-ketamine anaesthesia in rats but not dogs (Nossmann *et al.* 1990). Concurrent administration of chloramphenicol to dogs and cats undergoing pentobarbital anaesthesia significantly increased the duration of anaesthesia (Adams & Dixit 1970). These results suggest a competitive interaction at the level of biotransformation. Chloramphenicol may competitively enhance the activity of bioinactivated compounds and decrease the activity of bioactivated compounds metabolized through the same family of cytochromes required for the metabolism of many drugs (Burns & Conney 1965). Direct drug interactions may also occur, fluoroquinolones compete for gamma-aminobutyric acid (GABA) receptor sites in the central nervous system, and thus their use would be contraindicated in certain neurological studies (Bahri & Blouin 1991). It is also clear from the examples cited above that interfering effects differ between species, making prediction more difficult. Studies of antimicrobial efficacy may be compromised where toxic antibiotics are used, such as clindamycin in the rabbit (Sande & Johnson 1975) without concurrent controls treated with antibiotic but no bacterial challenge. The confounding effects of antibiotics can be even more subtle. When bacitracin, gentamicin and the antifungal nystatin were given to rats to reduce their intestinal microflora, caecocolonic motility was altered and there was increased faecal excretion of dry matter and water (Cherbut *et al.* 1991). In a study that models a more common clinical situation, intestinal motility was altered when amoxycillin-clavunate was given to human patients (Caron *et al.* 1991). These changes could influence not only absorption and excretion of a test compound, depending on the sites of drug absorption, but also studies of the intestinal tract itself. Antibiotic use should therefore be very critically reviewed if given during the course of an experimental study.

Antibiotic toxicity

In all species even drugs with a high therapeutic index can cause toxicity at very high doses or when the usual dose is not adjusted to take account of the age and condition of the animal (Table 1). Studies with new-born mice and guineapigs show that a variety of liver enzyme systems are essentially absent at birth and only begin to appear at the end of the first week of life (Jondorf *et al.* 1958). Renal excretion mechanisms such as glomerular filtration and proximal tubular secretion may be absent at birth and develop over days or weeks depending on species (Prescott & Baggott 1988). In adult life impairment of renal or hepatic function, whether by disease or experimental manipulation, may alter the required antibiotic dose. As an example Table 1 documents the adjustments on dosage required when using tetracycline in animals with renal dysfunction. It may even be important to consider the animals's diurnal rhythm (Heinze *et al.* 1992). Mice are nocturnally active animals, and barbiturate sleep time can be up to twice as long when given during the day rather than at night (Davis 1962). Biotransformation of some antibiotics may be by hepatic mechanisms similar to pentobarbitone. It is possible that doses for a particular antibiotic might be extrapolated from animals active during the day to the mouse. When these agents are administered to a mouse during the light phase they

Table 1 Modification of antibiotic dosage with reduced renal or hepatobiliary function

System affected	Antibiotic affected	Effect on dose
Hepatic function reduced	Erythromycin, chloramphenicol, metronidazole, clindamycin, lincomycin	Reduce dose
Biliary obstruction	Ampicillin, fluoroquinolones	Normally excreted in the bile, biliary obstruction may reduce access to site of infection
Renal function reduced	Erythromycin, chloramphenicol, doxycycline	Give usual dose
Renal function reduced	Most penicillins and cephalosporins, clindamycin, lincomycin, trimethorpim, sulphonamides	Consider minor dose reduction
Renal function reduced	Aminoglycosides, carbenicillin, ticarcillin, vancomycin, metronidazole, fluoroquinolones	Consider major dose reduction
Renal function reduced	Tetracyclines, cephaloridine, nitrofurantoin, polymixin	Avoid using these antibiotics

Adapted from Prescott & Baggot 1988, Sande 1990b

could be metabolized to a different extent than if given during the active dark phase, leading to differences in antibiotic plasma concentrations. This potential for differences in plasma concentrations and even ineffective therapy would be increased if extrapolation of dose quantity or frequency was also inaccurate (see section below 'Extrapolation of antibiotic dose information between species'). In addition, antibacterials that are eliminated via the kidney may have blood levels that vary depending on the time of day of administration. For example sulphonamides given during the active phase in chickens and calves are eliminated twice as fast compared to when given during the animal's resting phase (Heinze *et al.* 1992). There is also evidence of strain differences within laboratory species with respect to the toxic effects of antibiotics. Tobramycin is more toxic in Fischer rats than in Sprague-Dawley rats (Reinhard *et al.* 1991).

Many species have adverse reactions to particular antibiotics at doses that are normally safe therapeutic doses in other species. In rodents and rabbits this is one of the most important considerations in antibiotic therapeutics (Tables 2 and 3). It should be emphasized that the single most important mechanism of antibiotic toxicity in rodents and rabbits is the secondary effects from the disruption of the normal enteric flora. In the guineapig and hamster the specific cause of death is often the toxin produced by overgrowing *Clostridium difficile* (Richard 1990, Fekety 1986, Manning *et al.* 1984). In one study in guineapigs, aureomycin caused overgrowth of *Listeria monocytogenes* which then led to septicaemia and widely distributed necrotic lesions (Roine *et al.* 1953). In the rabbit the toxins produced by *C. perfringens* and *C. spiroforme* have been implicated in lincomycin (Rehg & Pakes 1982) and clindamycin (Katz *et al.* 1978) induced enteritis.

The hamster is particularly sensitive to toxigenic *C. difficile* overgrowth caused by a very wide range of antibiotics (Bartlett *et al.* 1978, Fekety 1986, Fekety *et al.* 1979) and it has been suggested that treatment of hamsters with any antibiotics should be undertaken with caution since mortality is high (Richard 1990). Tetracycline, metronidazole and to a lesser extent chloramphenicol are relatively poor inducers of enterocolitis in hamsters (see Table 2) but the high incidence of renal dysfunction in older hamsters (Hubbard & Schmidt 1987) may even make tetracycline administration hazardous (see Table 1).

Apart from severe enterocolitis caused by other antibiotics even the 'classical' guineapig toxicity to penicillin most probably is not due to 'allergy' (Anon 1992). Newborn and germ-free guineapigs are not susceptible to penicillin toxicity (Manning *et al.* 1984), so deaths in normal animals after penicillin may simply be another example

of antibiotic-induced enterocolitis. Toxicity may be related to overgrowth of toxins from Gram-negative bacteria (De Somer *et al.* 1955), or *C. difficile* cytotoxin (Lowe *et al.* 1980), which have been isolated from caecal contents of guineapigs which died after penicillin treatment. The incidence of toxic effects may depend on whether such organisms are present in a particular animal's flora. Thus guineapigs may accurately predict what happens in man when antibiotics are given at too high a dose for too long. However, in another study (Roine *et al.* 1953) penicillin included in the diet at a rate of 50 mg/kg did not cause fatalities or weight loss. There do also remain reports of specific penicillin hypersensitivity in guineapigs (Hoar 1976).

Many other antibiotics are also toxic in the guineapig, (see Table 2), but certain cephalosporins at certain dose rates, cephaloridine 12.5 mg intramuscularly for 14 days (Dixon 1986), and cefazolin 100 mg/kg intramuscularly twice a day (Fritz *et al.* 1987) have been shown not to produce mortality.

Streptomycin is reported to be toxic to mice at doses of 3–6 mg/kg (Galloway 1968, Harkness & Wagner 1989a). However massive single doses of streptomycin, 500 mg per animal, or 50 mg per animal initially followed by approximately 4 mg did not appear primarily to cause toxicity (Bonhoff *et al.* 1954). These higher doses did render animals more susceptible to salmonella infection, but the mechanism is unclear. Anaerobic bacteria are recognized as very important in limiting the colonization of the digestive tract by potential pathogens such as salmonella (Wiegersma *et al.* 1982, Schaedler & Orcutt 1983) but streptomycin is inactive against anaerobes.

Antibiotic toxicity in the gerbil has received little attention, but in view of the susceptibility to enterocolitis of some other rodents, caution should be exercized. In contrast, as summarized in a review by Richard (1990), the rat seems to tolerate a wide range of antibiotics at therapeutic dose rates. As a specific example lincomycin is toxic at relatively low doses in guineapigs, hamsters (see Table 2) and rabbits (see Table 3), but 300 mg/kg orally to rats for 30 days was reported as non-toxic (Gray *et al.* 1964). There has been little stimulus for detailed studies on the mechanisms of this relative tolerance in the rat and mouse. Few clinical problems are seen when many antibiotics are used in rat and mice, and the hamster is an excellent model for antibiotic-induced colitis in man (Fekety 1986). One clue to this tolerance may be found in studies by Dabard *et al.* (1979), which suggest mice and rats are relatively resistant to the effects of clostridia. After inoculation of pregnant animals with *Clostridium difficile*, *C. tertium* and *C. perfringens*, large numbers of these bacteria were found in the guts of their offspring. In rats and mice this caused no apparent problems, whilst in hares and rabbits fatal enteritis occurred.

A wide range of antibiotics have been reported as toxic in the rabbit (Table 3). It is clear that lincomycin and clindamycin are particularly dangerous. Toxicity of erythromycin, spectinomycin and minocycline is relatively milder, and is manifested as depressed growth rates. The situation with penicillins and cephalosporins is less clear. Ampicillin has been shown to cause serious enteritis with mortality in several studies. The acute toxicity of penicillin is known, but where penicillin has been claimed to contribute to enteritis other antibiotics that cause enteritis, lincomycin (Thilsted 1981) and ampicillin (Rehg & Lu 1981), have also been fed. However the combination of antibiotics can cause mortality, for example aureomycin/sulphamethazine/penicillin, where individual agents did not (Hagen 1967). Indications that penicillin and cephalexin have less potential to cause enteritis than ampicillin come from quantitative faecal bacteriological studies (Schröder *et al.* 1982). They reported the antibiotic-induced depression in lactobacilli numbers, and the rise in coliform bacteria and clostridial numbers is less with penicillin and cephalexin than with ampicillin. There do

Table 2 Toxic doses of antibiotics in rodents

Antibiotic	Mouse	Rat	Guineapig	Hamster
Penicillin			5000 IU IP: 60% mortality, 10000 IU PO 20% mortality[13] 1000000 IU IM, two doses in 24 hours[§§]. $^{7}/_{8}$ died[17]	100 mg PO, 600 mg SC: 100% mortality within 5 days[21]
Procaine	0.3 mg/kg: 90% mortality[14]		0.4 mg/kg[15], 125 mg/kg: 100% with convulsions[16]	
Ampicillin			8 mg/kg SC tid for 5 days: 20% mortality by day 8[18]	5 mg[†] PO tid 5 days: 90% mortality,
Cephalosporins			Cefazolin, 100 mg[§] IM qid 5 days: $^{3}/_{12}$ died[9]	Cephalexin, 5 mg[‡] PO tid 5 days: 90% mortality, Cefoxitin 10 mg[†] IM tid 5 days: 100% mortality, Cephalothin 20 mg[†] IM tid 5 days: 80% mortality[8,22]
Carbenicillin				100 mg/kg PO: $^{9}/_{10}$ animal died within 8 eight days[21]
Ticarcillin				100 mg/kg PO $^{10}/_{10}$ animal died within 6 eight days[21]
Lincomycin			30 mg/kg SC on alternate days, most animals died 5–14 days after treatment started[7]	>10 mg/kg SC: $^{20}/_{24}$ animals died with enteritis[20]
Clindamycin			75 mg/kg IP od: 100% mortality in 6–8 days[3]	3 mg[†] PO tid 5 days: 100% mortality
Streptomycin	6 mg/kg IM: acutely 100% mortality[14]		60 mg per animal[‡] once PO: 100% mortality in 3–6 days[2]	Acutely lethal at 'therapeutic' dose rates[19]
Gentamicin				1 mg[†] PO tid 5 days: 100% mortality[8]
Neomycin				285 mg/kg PO: death within 5 days[21]
Chloramphenicol				10 mg[‡] PO tid for 5 days: 20% mortality[8], $\geqslant$300 mg/kg PO: enteritis[21]

Erythromycin			oral ≥ 33 mg/kg for 3 days: 40% mortality, 33 mg/kg IP: 100% mortality[11]	5 mg† PO tid 5 days: 100% mortality[8] 30–200 mg/kg IP: 100% mortality[11]
Aureomycin			5 mg/kg PO: 100% mortality[5], 8/9 died when included at 100μg/kg diet[10]	
Tetracycline	150 mg/kg[5,4]	[4]	50 mg/kg in diet[5,4]	100 mg/kg PO: majority of animals died within 3–4 days[21,4]
Chlortetracycline			20 mg/animal‡ PO: mortality, % not given[2]	
Vancomycin				5 mg† PO tid 5 days: 90% mortality[8]
Bacitracin			20001U/animal, 80% mortality[12]	
Spiramycin	acute oral LD_{50} 3.13 g/kg[1]	acute oral LD_{50} 4.85 g/kg[1]	acute oral LD_{50} 3.5 g/kg[1], chronic oral LD_{50} with enteritis 0.25 g/kg[1]	
Trimethoprim-sulphamethoxazole				33 mg trimethoprim, 167 mg sulphamethoxazole/kg PO: 6/20 animals died[21]

[1]Boyd & Price-Jones (1960), [2]Eyssen *et al.* (1957), [3] Knoop (1979), [4]Potential toxicity when genitourinary disease present (see Table 1), [5]Roine *et al.* (1953), [6]Cited by Richard (1990), [7]Grey *et al.* (1964), [8]Bartlett *et al.* (1978), [9]Fritz *et al.* (1987), [10]Roine & Ettala (1952), [11]Kaipainen & Faine (1954), [12]Farrar *et al.* (1966), [13]De Somer *et al.* (1955), [14]Galloway (1968), [15]Cited by Richard (1990) and Galloway (1968): Procaine may be toxic in its own right as a component of procaine pencillin, and [16]Richard & Kueter (1946) report the guineapig is more sensitive to procaine than mice or hamsters, [17]Lowe *et al.* (1980), [18]Young *et al.* (1987), [19]Killby & Silverman (1967), [20]Small (1968), [21]Fekety *et al.* (1979), [22]Ebright *et al.* (1981) reported on toxicity of 8 cephalosporins,

PO = *Per os*
IM = Intramuscular
SC = Subcutaneous
IU = International units

† = Per 70–90 g hamster
‡ = Per 250–400 g guineapig
§ = Per 650–750 g guineapig
§§ = Per 250–300 g guineapig

od = once a day
tid = three times a day
qid = four times a day

Table 3 Adverse effects of antibiotic treatment in rabbits[1]

Antibiotic	Toxic dose	Toxic effects
Ampicillin	25 mg/kg IM for 2 days	Fatal enteritis[13]
	5 mg/kg IM for 2 days	Weight loss[13]
	40 mg/kg for 4 days	40% fatal enteritis over next 2 weeks[14]
	10 mg/kg PO for 6 days	50% fatal enteritis over next month[15]
	8 mg/kg bid SC	Enteritis, previously also had penicillin[8]
	>5 mg/kg PO antibiotic treated water for 3 days	Fatal enteritis in 7/11 rabbits[12]
Penicillin	LD_{50} 5.25 g/kg PO	Both acute and chronic toxicity (enteritis)[7]
Cephalexin	200 mg rabbit† for 7 days	Diarrhoea[11]
Lincomycin	100 mg PO single dose in 1.5–2.0 kg rabbits	66% mortality with enteritis[3]
	24 mg/kg PO antibiotic treated water	90% mortality with enteritis[6]
	30 mg/day PO in 2.0–2.5 kg rabbits	100% mortality with enteritis by 3 days[5]
	1.3 mg/ adult rabbit in feed for 3 days	30/130 rabbits died with enteritis[9]
	0.2 mg/kg IM for 2 days	33% mortality in 2 days[13]
Clindamycin	15 mg/kg PO for 3 days	100% mortality with enteritis[4]
	5 mg/kg PO for 2 days	50% mortality with enteritis within 72 hours[13]
	Single IV dose of 30 mg/kg	4/6 rabbits had fatal enteritis 12–14 days after treatment[16]
Tylosin	100 mg/rabbit† for 7 days	Diarrhoea[11]
Erythromycin	3 g/l in drinking water for 7 days†	Diarrhoea[11]
Spectinomycin	1 g/l in drinking water for 7 days†	Diarrhoea[11]
Vancomycin	75 mg/kg IV	Acute toxicity with 100% mortality[10]
Minocycline	30 mg/kg IM for 3 days	Reduction in growth rate[18]
Spiramycin	acute oral LD_{50} 4.85 g/kg	Nervous signs[17]

[1]Data adapted from Laval (1990), [2]Boyd (1960), [3]Rehg & Pakes (1982), [4]Katz *et al.* (1978), [5]Fesce *et al.* (1977), [6]Maiers & Mason (1984), [7]Boyd (1960), [8]Rehg & Lu (1981), [9]Thilstead (1981), [10]Nicolau *et al.* (1993), [11]Schröder *et al.* (1982), [12]Milhaud *et al.* (1976), [13]Licois (1980), [14] Morisse (1978), [15]Schatzmann *et al.* (1977), [16]Lipman *et al.* (1992), [17]Boyd & Price-Jones (1960), [18]cited by Laval (1990)

PO = *Per os*
IM = Intramuscular
SC = Subcutaneous
IV = Intravenous
‡ = *rabbits were 8–10 weeks old*

not appear to be any detailed reports in the literature on adverse effects of amoxycillin. Although its use in rabbits is usually proscribed in product information, this may be at least partly based on potential for toxicity. Manufacturers of amoxycillin do hear anecdotal reports of its use without apparent complications in rabbits (Hoare C, personal communication). However caution with amoxycillin is perhaps justified as shown by a recent single case investigated by the author. A 4 kg rabbit was given 75 mg of an injectable long acting amoxycillin preparation. Two days later the animal was found with circulatory collapse and diarrhoea. At necropsy the caecum was enlarged, contained fluid faeces and its mucosa was haemorrhagic. Histological examination of the caecum showed haemorrhage, mucosal ulceration and heterophil invasion of the lamina propria and surface epithelium, similar to lesions reported in clindamycin-induced enteritis (Katz *et al.* 1978). Examination of caecal contents revealed a toxigenic strain of *C. difficile* to be present and ELISA (Launch Diagnostics) showed *C. difficile* enterotoxin was present. *C. difficile* toxin has been reported in lincomycin-associated colitis in rabbits (Rehg & Pakes 1982). In

addition, all the aerobic bacteria isolated were coliforms, similar to the situation following ampicillin administration (Schröder *et al.* 1982).

As enterocolitis is such a problem it should be noted that it has been prevented by oral administration of antibiotics that are not absorbed across the intestinal tract. Gentamicin (80 μg/ml) and polymixin B (50μg/ml) in the drinking water (Kaiser *et al.* 1992) have been reported as effective in the guineapig. In hamsters bacitracin at 3 mg/ml drinking water, or specifically when tetracycline (given at 500 mg/ml drinking water) is in use concurrent administration of 250 mg/l of the non-absorbed sulphonamide sulphaguanidine, are reported as effective (Richard 1990). In the rabbit fatal enteritis normally caused by ampicillin, (20 mg/kg for 3 days) in the rabbit was avoided by concurrent administration of gentamicin (10 mg/kg/day) (Escoula *et al.* 1981), and enteritis caused by lincomycin (30 mg/day orally for 3 days in 2.0–2.5 kg rabbits) was prevented by gentamicin (30 mg/day orally) (Fesce *et al.* 1977). Another approach is to use ion exchange resins to bind the clostridial toxins, and this has been shown to have a beneficial effect in the clindamycin-induced enteroxaemia in rabbits (Lipman *et al.* 1992) and hamsters (Taylor & Bartlett 1980).

The fluoroquinolones are potentially useful agents in veterinary medicine, as they are broad spectrum, bactericidal, orally active and their is little cross-resistance developed to other classes of antibiotics (Bahri & Blouin 1991). These properties alone make them of interest in laboratory animal medicine, but the relative lack of incidence of induced enterocolitis at clinical dose rates is noteworthy. Recently Rolf (1993) showed that enrofloxacin, (22 mg/kg) given to guineapigs orally for 6 days, produced no sign of enterocolitis, in contrast to the enterocolitis he found only after a single injection of penicillin (60 000iu). The reported dose range for enrofloxacin for rabbits, guineapigs and hamsters is in the range 5–10 mg/kg (Dorrestein 1992). In rabbits 25 mg/kg enrofloxacin was given for 12 days as part of reproductive toxicity assessment without adverse effects (Althreuther 1992). The no-effect dose levels for rats and mice were 165 mg/kg and 550 mg/kg for 13 weeks administration (Althreuther 1992).

Routes of administration

a) Oral administration

Relative to many other agents (see section above on 'Antibiotic toxicity'), tetracyclines are often considered by clinicians to be useful and relatively safe broad spectrum antibiotics for use in laboratory animals. Many authors (see section on drug doses below) quote dose rates for oral administration of tetracyclines. However, whilst these drugs may be safe in rats and mice (Dabard *et al.* 1979), hamsters (Bartlett *et al.* 1978, McNeil *et al.* 1986), gerbils (based on the author's experience of colony medication to control *Bacillus piliformis*) and rabbits (Percy & Black 1988) both aureomycin (Roine & Ettala 1952), oxytetracycline (Roine *et al.* 1953) and chlortetracycline (Eyssen *et al.* 1957) are toxic to guinepigs at therapeutic dose rates. In addition in some of the species where tetracyclines are safe, rabbits and rats, recent reports have shown that the oral route is of little use, and their effectiveness in other rodents, where systemic absorption is required, should be questioned. Administration of tetracycline in the drinking water of rabbits at concentrations up to 1600 mg/l produced low to undetectable serum levels, and water intake was reduced at the highest drug concentration (Percy & Black 1988). Even higher drinking water concentrations of 4 g/l tetracycline were given to rats and again water intake was reduced and there was no tetracycline detectable in the serum (Porter *et al.* 1985). The therapeutic effectiveness of oral tetracyclines in preventing hepatic necrosis caused by Tyzzer's disease in rodents (Harkness & Wagner 1989b) should be viewed in the knowledge that where present *B. piliformis* is normally a resident of the

intestinal tract and that tetracyclines are probably not absorbed and are therefore likely to remain in the intestinal tract at high concentrations.

Although amoxycillin is bactericidal, has a broad antibacterial spectrum, is readily available in formulations suitable for addition to drinking water and as liquid for direct oral administration, and is rapidly and well absorbed orally (Mizen *et al.* 1981, Palmer *et al.* 1976) toxicity in some rodent species may limit the use to rats and mice and possibly rabbits (but see section above on 'Antibiotic toxicity'). The same concerns apply to the less well absorbed penicillins.

Oral formulations of cephalosporins are available both as veterinary and human liquids for direct oral administration. Their use has been suggested in several reviews (Flecknell 1983, McKellar 1989) however a critical review of the primary data summarized in Tables 2 and 3, suggested actual or potential toxicity of some cephalosporins in hamsters, guineapigs and rabbits, despite anecdotal reports of 'safe' clinical use. Cephalosporin use is more likely to be safe in rats and mice, as many reports of use in experimental infections, for example by Glauser and Bernard (1982), describe prolonged use of these drugs.

Oral presentations of trimethoprim/sulphonamides appear to be both relatively safe and suitable for a broad range of infections (Richard 1990, Laval 1990). Most formulations tend to be tablets, paediatric human products or veterinary products formulated as pastes for administration to large animals. None of these presentations is amenable to use as mass medication in the drinking water, although water soluble poultry formulations could be utilized.

Chloramphenicol is available in oral presentations and can be used in guineapigs. Given orally at doses between 30 and 60 mg/kg for 6 days it caused no overt toxicity (Eyssen *et al.* 1957). However, to prevent drug resistance, chloramphenicol drug data sheets often recommended that its use be restricted to situations where clinical experience and laboratory testing indicate no other antibiotic can be used (National Office of Animal Health 1992). Alternatives in guineapigs include potentiated sulphonamides and quinolones.

Fluoroquinolone antibiotics are potentially useful as broad spectrum orally active antibiotics. Initial studies of oral absorption of norfloxacin (dissolved in solvents normally only used in experimental situations) in mice, rats and rhesus monkeys indicated effective blood levels could be achieved (Gilfillan *et al.* 1984). Pharmacokinetic studies on enrofloxacin in rabbits have shown 5 mg/kg twice a day orally gives effective tissue levels (Broome *et al.* 1991). Dorrestein (1992) has summarized the oral dose for rabbits, guineapigs and hamsters as 5–10 mg/kg, and the empirical drinking water concentration at 100 mg/l.

b) Parenteral administration

One of the most interesting aspects of parenteral drug administration is what has been termed 'the injection site dogma' (Marshall & Palmer 1980). This dogma holds that peak serum concentrations are greatest, and time of onset of this peak most rapid when a drug is given intravenously, and that intramuscular administration gives greater peak concentration and more rapid onset of peak serum concentration than subcutaneous administration. Marshall and Palmer (1980) using calves and ten Voorde *et al.* (1990) using dogs, injected ampicillin and amoxycillin subcutaneously and intramuscularly and found that this dogma was not always true. There was often no major difference in bioavailability, especially with amoxycillin. Similar findings were reported for long acting oxytetracycline in the rat (Curl *et al.* 1988). Interestingly the site of subcutaneous injection was often more important, the quantity of drugs absorbed over a fixed period of time was significantly less when ampicillin was given over the neck compared to the thorax (ten Voorde *et al.* 1990). These findings have some impact in small laboratory animals. Injection volumes may be relatively large, as some

rodent and rabbit doses are relatively higher than in the larger companion animals (see section below on 'Extrapolation of antibiotic dose information between species') for which preparations are formulated. The muscle mass available for intramuscular injection is small, so the risk of trauma, nerve damage, intermuscular injection and pain on injection is greater. However, the example given above may not hold for all antibiotics. The half-life of cefazolin in the guineapig is reduced by half when the drug is given by intramuscular compared to subcutaneous injection (Kaiser *et al.* 1992), which may reflect different absorption kinetics. Intraperitoneal administration may also give higher drug levels than subcutaneous administration. This was shown in a study with oxytetracycline in rats, although therapeutic drug concentrations were achieved *via* both routes and the irritant effect of oxytetracycline was more marked *via* the intraperitoneal route (Porter *et al.* 1985). Subcutaneous drug administration in rabbits and rodents technically is often easier and safer and the questioning of the injection site dogma does suggest that bioavailability via this route may not be dissimilar to intramuscular injection.

Effect of drug formulation

Formulation, (salt form, solvents and excipients) affects therapeutics of a particular product by altering bioavailability (the rate and extent of absorption of the same drug in the same dosage form). Bioinequivalence (defined as differences in bioavailability between the same drug dosage form, for example 2 brands of chloramphenicol tablets) between oral formulations of oxytetracycline, chloramphenicol (up to 100% differences in plasma concentrations) and penicillins (<X10 differences in peak concentrations) have been reported in dogs (Watson 1992). The use of long acting preparations of oxytetracycline in the rabbit has been recommended by Laval (1990), they are routinely used by the author in common marmosets, and these preparations may maintain effective oxytetracycline concentrations in the rat for at least 72 h (Curl *et al.* 1988). However, different formulations of long acting oxytetracycline differ markedly in the degree of tissue irritation they produce. Those preparations that are most irritant can cause visible distress to the animal, tissue damage, prolonged tissue residues and the lowest and most delayed peak concentrations (Nouws *et al.* 1990). Therefore care must always be exercised when transferring between different brands and in particular human generic presentations of antibiotics. A particular caution is necesssary with different trimethoprim-potentiated sulphonamide brands. They may contain different sulphonamides. For example, some veterinary brands contain sulfadiazine and in the rabbit the half life of this sulphonamide is only about 1 h, compared to 5–10 h in other species (DuSouich *et al.* 1978). Thus, depending on the brand, the effective drug may be only trimethoprim.

Antibiotic prophylaxis

This is an important use and misuse of antibiotics and Sande *et al.*'s (1990a) classification of indications for prophylactic antibiotic use serve as a basis for discussion:-

1. *Prophylaxis to protect healthy animals from infection.* Examples in veterinary medicine would be the well-recognized use of long acting oxytetracycline to protect sheep exposed to pasteurella from infection (Frazer 1991). In laboratory animal medicine prophylaxis might include the use of oral tetracycline to prevent outbreaks of Tyzzer's disease (Harkness & Wagner 1989b). The line between use and misuse is often not clear. In some cases reduction of experimental stress might also prevent infection, but these changes may make it impossible to use the animals for that experimental procedure.
2. *Prevention of secondary bacterial infection.* Examples would include prevention of bacterial infections in

immunocompromised animals or secondary to viral disease (Walsh *et al.* 1988). Again whilst depopulation, decontamination and rederivation, or the use of isolators may solve many of these problems, they may not be feasible or economical, especially in the short term.

3. *Prophylaxis prior to invasive procedures in animals with previously implanted devices.* Antibiotics are used to prevent colonization of a previously implanted device (e.g. catheters), following the bacteraemia produced by minor procedures (e.g. teeth cleaning or endoscopic intestinal biopsy). The same considerations that apply to the timing of antibiotic administration at surgery apply here.
4. *Prophylaxis at surgery.* The use of antibiotics to prevent infection may be 'the single most frequently abused principle of (veterinary) surgery' (Rosin 1988). If correct aseptic technique is followed there is usually no need for prophylactic antibiotics. However, perioperative wound contamination may be inevitable. In many cases, even when sterile packs are prepared for rodent surgery (McNeal *et al.* 1988), surgical procedures are performed on multiple animals using the same instruments. In addition, animal movement and interference with the wound postoperatively also predisposes to infection. In reality many rodent procedures are probably performed even without sterile technique or antibiotic prophylaxis. This may be a result of the commonly held premise that rats are less susceptible to perioperative infection than other animals. Recently this concept was critically reviewed by Waynforth (1989). There was some evidence that rats may eliminate bacteria more efficiently. The principal factor in establishing wound infection from wound contamination is however the concentration of bacteria (Goldschmidt 1972, Kaiser *et al.* 1992). Waynforth (1989) cited the claim that the short procedures with small incisional sites that are common in rodent surgery might reduce the incidence or severity of infections that would otherwise be expected from investigators with limited surgical skills and a reduced recognition of the potential for infection. The argument would be that this low incidence may be related to low bacterial numbers as these incisions are smaller compared to those in larger animals. Whilst it may be true that the total number of bacteria per wound might be less in rodents compared to larger animals it could also be argued that the concentration of potential contaminants, principally the animal's and operator's skin flora and unsterile equipment (Kaiser 1990), remains the same. Unfortunately there are no well designed studies that directly compare infection rates between species, using bacterial strains of the same pathogenicity, similar inoculum volumes, concentrations and sites, and with similar co-factors such as foreign material. These difficulties are demonstrated by comparing methods in detail in studies by Goldschmidt (1972) using rats, by Kaiser *et al.* (1992) using guineapigs and by Elek & Conen (1958) using human volunteers. It therefore remains quite possible that the effects of wound contamination in rodents are real but also not easy to recognize. In a recent study craniotomy or laparotomy wounds in rats were deliberately infected during surgery (Bradfield *et al.* 1992). Despite the fact that the rats showed no clinical signs of infection, there were significant changes in measures of behaviour, serum biochemical and haematological parameters and in the histological appearance of the wounds. Aseptic technique should be used as standard but there does seem to be a role for the rational use of perioperative antibiotics where aseptic technique cannot be absolutely assured or inadvertant breaks in aseptic technique occurs.

The consensus regarding perioperative antibiotic use in the human (Wenzel 1992) and veterinary medical (Rosin 1988) literature

is that antibiotic use is recommended for clean-contaminated surgery and when chronic implantation of foreign material takes place (Walsh *et al.* 1988). When wound contamination occurs during surgery it takes several hours for the bacteria to progress to a logarithmic phase of growth, this is illustrated with data from an experimental study in the rat in Fig 1. It is therefore vital that antibiotics, if they are used, should be administered *pre-operatively* to allow peak blood levels to be achieved *at the time of wound contamination* before significant bacterial proliferation takes place. The choice of antibiotic depends on the species, surgical procedure and local knowledge on antibiotic resistance, but it is likely that the chosen antibiotic needs to be given at least 1–2 h preoperatively or intravenously at the onset of surgery (Wenzel 1992). In addition there is clear evidence that there is no value in continuing antibiotics longer than 24 h postoperatively (Weersink *et al.* 1991, Haven *et al.* 1992), unless clinical signs suggest an infectious process resistant to the prophylactic regimen.

Antibiotics that have neuromuscular blocking properties (e.g. aminoglycosides, polypeptide antibiotics and possibly tetracyclines), should be used with great care when neuromuscular blocking agents are used as part of the anaesthetic protocol (Jones 1992).

Use of antibiotic combinations

The indications for use of combinations of antibiotics include production of a synergistic effect, prevention of resistance and efficacy in polymicrobial infections (Fantin & Carbon 1992). However it is essential that these combinations are critically reviewed (English & Prescott 1983, Hirsch *et al.* 1990). In general this review has not occurred with rodents and rabbits and in addition the penicillins and aminoglycosides commonly used may cause toxicity (Tables 2 and 3). Some information on possible candidates for usable combinations can be gained from studies using rodents as animal models for this application (Fantin & Carbon 1992, Mizen *et al.* 1991).

Misuse of antibiotics

It is worthwhile to highlight some of the common misuses of antibiotics (Sande *et al.* 1990a) to enable critical review of antibiotic usage in rabbits and rodents.

1. *Treatment of untreatable infections.* This simply means using antibiotics in situations where they could never have any effect on the infectious agent, for example viral infections. This misuse should be clearly distinguished from acceptable use to treat a confirmed secondary bacterial infection.
2. *Therapy of non-specific pyrexia.* Bacterial infections are not the only causes of pyrexia, they also include pain, postoperative pyrexia, metabolic disorders etc.
3. *Improper dosage.* With smaller laboratory animals the main problem may be under-dosage, (see section below on 'Extrapolation of antibiotic dose information between species'), particularly if dosages are extrapolated on a mg/kg basis.
4. *Absence of surgical drainage.* When there is purulent exudate there may simply be inadequate antibiotic penetration without surgical drainage. An example here would be pasteurella abscesses in rabbits.
5. *Lack of adequate bacteriological information (e.g. sensitivity and blood levels).* In individual cases this may mean relying solely on past experience and clinical judgement to select an antibiotic. Even when species-specific bacteriology is not available or is pending it may help to use simple tests such as examination of smears for bacterial morphology and Gram staining to select a more suitable antibiotic. The increasing use of antibiotics in an unstructured manner contributes to the development of drug resistant bacteria. Even in veterinary medicine the development of strict policies to prevent nosocomial infections (defined as those acquired in animal holding or treatment facilities) and to define antibiotic usage has been suggested

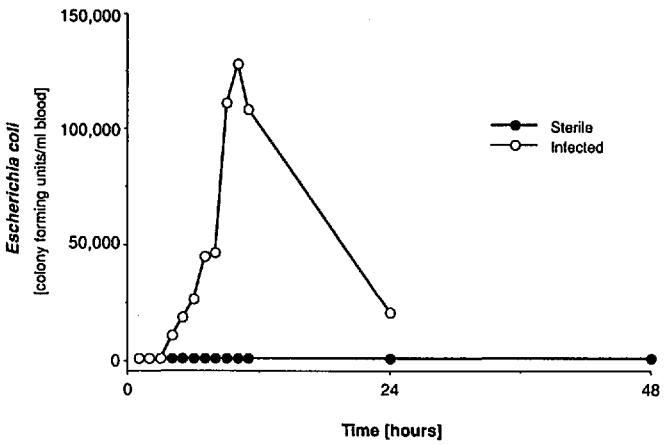

Fig 1 Numbers of bacteria in rat peripheral blood after introduction of a bacteria laden fibrin clot into the abdominal cavity *via* a laparotomy (De Marsh P & Smith E, personal communication)

(Murtaugh & Mason 1989). An indication of the potential of different antibiotics to produce resistant bacteria by disturbing the resident flora is given in Table 4, although there may be species differences (Wiegersma *et al.* 1982).

Regulatory approval for use of antibiotics in laboratory animals

When treating the common domestic animals many of the factors mentioned above are taken into account and combined with the dose obtained from the product's labelling. This option is usually not available when treating laboratory species. It has been suggested that the pharmacokinetic data obtained from rodents or rabbits in the early stages of a drug's development be submitted to the regulatory authorities and this would provide dose information at little cost. There are at least two problems with this approach. First, many of these studies may not be at therapeutic dose rates and the formulations of the drug are often not comparable (Gilfillan *et al.* 1984). Second, the regulatory authorities require considerably more data than just this information. In a large market such as the United States, for example (Kilgore R, personal communication, Guest 1993) the pharmacokinetic data merely provides the initial data for a dose titration infection study in the target species, and this must be followed by a second well-controlled infection study. If required, for example if indications are sought for administration to rabbits reared for food, the most costly parts of the process are the safety studies, consisting of chronic toxicity testing at 3 dose rates and an acute toxicity test, these costs alone can exceed ECU 100 000. Nevertheless in certain markets it is possible that the increasing numbers of small mammals being kept as pets may encourage the development of products with specific labelling suitable for laboratory species. In France for example a recent survey showed 7.6 million households (6% of the total) possessed rodents, compared to 33% with a dog and 22% with a cat, and this has encouraged pharmaceutical companies to investigate this market (Anon 1993).

Table 4 Effects of antibiotics on host microflora and colonization resistance

No adverse effects	Cephalosporins[1,2], aminoglycosides[1], trimethoprim[1,2], sulphonamides[1,2], erythromycin[1,2], doxycycline[1,2], parental penicillins[1]
Moderate effects	Amoxycillin[1,2], tetracycline[1], chloramphenicol[1]
Significant effects	Ampicillin[1], cloxacillin[1,2], metronidazole[1], furazolidone[1], penicillin V[2]

Data from: [1]Jones (1986), [2]Wiegersma *et al.* (1982).

Sources of information in drug addiction and dose

Apart from antibiotic doses listed as treatment of specific diseases in clinical texts, there are a number of reviews that can act as a guide for clinicians. A selection of some of the most useful is given below. A comprehensive referenced list of doses for rats is given by Kruckenberg (1979), this list includes antibiotics, although only one-third of the citations refer to primary studies on the antibiotic. Indications, appropriate antibiotics and their doses for rodents are reviewed by Richard (1990), for rabbits by Laval (1990) and Mureau (1988) and for rodents and rabbits by Jacobsen *et al.* (1991). Doses for rabbits, rodents and other species are also given by Latt (1976), Flecknell (1983), Harkness & Wagner (1989a) and McKellar (1989).

A *Formulary for Laboratory Animals* by C. Terrance Hawk and Steven L. Leary which will contain fully referenced doses is planned to be published by Iowa State University Press, Ames, Iowa in May 1995.

Extrapolation of antibiotic dose information between species

In the majority of situations an antibiotic dose cannot be found for a particular laboratory species, or the basis on which the published dose was obtained is unclear. In particular there is often no convenient way to discover whether the data is obtained or extrapolated from primary studies on the same species or if it was extrapolated or estimated from doses in other species. An understanding of the basic principles for the extrapolation of doses between species would therefore be useful.

There are two general approaches to inter-species scaling, either using an allometric or a physiological approach (Mordenti 1986, Mizen & Woodnutt 1988).

Allometry is the study of the proportion changes correlated in variations in size of either the total organism or the part under consideration (Boxenbaum 1984). It has been found that over 100 highly diverse biological parameters and many pharmacokinetic parameters are linearly related to body size (Calabrese 1991), 3 indicators have been found useful in interspecies scaling: body weight, body surface area and physiological time (Table 5).

For body weight the mathematical principle that underlies this allometric relationship is described by the equation:

$$\text{Log } P = \text{Log } a + b.\text{Log } W, \text{ which can be simplified to; } P = aW^b$$

Where P = parameter of interest, W = weight, with a and b as constants. This formula is described and illustrated by Kirkwood (1983). The constant b has been found for many parameters to be approximately 0.75 (Kirkwood 1983, Calabrese 1991). The constant a fixes the value of P when W = 1 kg (Kirkwood 1983, Mordenti 1986). A special case may exist for very small mammals with a weight between 2.5 and 100 g, where the constant b may be lower at 0.5 (Bartels 1982). These models can be used to clarify and predict relationships between dose and body weight between different species (Kirkwood & Merriam 1990). How in practice can results such as those in Table 6, which are calculated from actual data, be derived when dose information for a particular species is not available? An example is shown in Table 7 to clarify the process. It is immediately apparent that in many cases the dose extrapolated on a $mg/kg^{0.75}$ basis, especially for smaller animals such as rodents, is relatively higher than if it were extrapolated from the dose for a larger animal such as a companion animal on a mg/kg basis. The advantages of the subcutaneous route of injection with this relatively higher dose volume is therefore even more obvious.

Body surface area has been used instead of body weight to derive allometric comparisons. The use of this indicator is perhaps best known in medicine for antineoplastic drugs, and an example using 3 species of laboratory rodents and man is shown in Table 8. Identical sulphadiazine blood levels were achieved in human patients when doses were calculated on a body surface area basis (Calabrese 1991).

Table 5 Representative body surface area to body weight rate ratios for various species

Species	Body weight (kg)	Surface area (m^2)	Km factor[a]	Dose equivalent (kg^{-1})
Man, adult	60	1.6	37.5	1
Man, child	20	0.8	25	1.5
Mouse	0.02	0.0006	3	12.5
Rat	0.15	0.025	6	6.3
Cat	3	0.24	12.5	3
Dog	16	0.65	24.5	1.5
Sheep/goat	50	1.1	45.5	0.8
Pig	75	1.5	50	0.75
Cow	150	2.4	62.5	0.6
Cow	500	5.0	100	0.4
Pony	280	4.4	63.5	0.6
Horse	350	4.0	87.5	0.4
Horse	650	5.9	110	0.3

[a]To express a mg/kg dose in any given species as an equivalent mg/m^2 dose, multiply the dose by the appropriate Km factor. E. g. in the cat 10 mg/kg is equivalent to $10\ mg/kg \times 12.5 = 125\ mg/m^2$.
Setting the dose equivalent for man (adult, 60 kg) as 1, we may obtain the dose equivalent kg^{-1} (in relation to man) dividing the Km factor for man by the Km factor of any species given in the table.
Data from Van Miert (1989), reproduced with permission

Table 6 Comparison of streptomycin/dihydrostreptomycin[1] dose rates between species on a mg/kg and $mg/kg^{0.75}$ basis

Species	Weight (kg)	Suggested dose mg/kg	Suggested dose $mg/kg^{0.75}$
Horses and cattle	500	6.94	32.50
Calves, pigs and sheep	100	11.36	35.00
Piglets	10	25.00	45.00
Dogs and cats	3	75.00	32.50

[1]a mixture of 125 mg/ml of each drug, total 250 mg/ml active agents.
Data adapted from Kirkwood (1983)

Table 7 Worked example to extrapolate oral dose of amoxycillin from dog to squirrel monkeys

1. Dose in dog = 10 mg/kg (National Office of Animal Health 1992)
   Total dose for 10 kg dog = 100 mg
   Dose for a 10 kg dog expressed in $mg/kg^{0.75} = 17.8\ mg/kg^{0.75}$
2. For a 750 g squirrel monkey
   Total dose = $weight^{0.75} \times$ dose in $mg/kg^{0.75} = 0.80 \times 17.8\ mg/kg^{0.75} = 14.34$ mg
   (Total dose if extrapolation had been on a mg/kg basis would be 7.5 mg)
   Converting this total dose of 14.34 mg for a 750 g squirrel monkey to mg/kg:-
   Dose mg/kg = 19.12 mg/kg
3. Comparison to actual pharmacokinetic data (Mizen *et al.* 1981)
   Peak blood level for dog given oral dose of 10 mg/kg amoxycillin = 6.1 μg/ml
   Area under the curve for dog given oral dose of 10 mg/kg amoxycillin = 15.3 μg.h/ml
   Peak blood level for squirrel monkey given oral dose of 25 mg/kg amoxycillin = 13.0 μg/ml
   Area under the curve for squirrel monkey given oral dose of 25 mg/kg amoxycillin = 29.5 μg.h/ml

Body surface area, calculated using the allometric equation:-

$$\text{Surface area} = 11.7 \times \text{Weight}^{0.66}$$

was used to extrapolate doses of antibiotics from man to rhesus monkeys (Kelly *et al.* 1992). However, the surface area method has not been used to any great extent because of controversy over whether it is fundamentally more inaccurate than body weight (Calabrese 1991). An example is the difficulty in measuring surface area in an individual (Van Miert 1989). However, Van Miert (1989) has correlated the product of body surface area and body weight and produced a more accurate basis for

Table 8 Comparison of the mg/kg and mg/m² dose of the antineoplastic drug mechlorethamine in man and 3 laboratory rodents

Species	Weight (kg)	Surface area (m^2)	Total dose (mg)	Total dose (mg/kg)	Total dose (mg/m^2)	Total dose (mg/m2)*
Mouse	0.018	0.0075	0.072	4.0	9.6	12
Hamster	0.050	0.0137	0.15	3.0	10.9	—
Rat	0.25	0.045	0.5	2.0	11.1	12
Man	70.0	1.85	21–28.0	0.3–0.4	11.3–15.1	11.2

Data from Pinkel (1958), except * from Van Miert (1989), see Table 5, this dose was obtained by using the Km factor multiplied by the dose in mg/kg

interspecies comparison, see Table 5. Many chronological parameters, such as life span, number of heartbeats per minute and gestation period also are related by the basic allometric equation described above, but in this case the constant b averages approximately 0.25 (Kirkwood 1983). This means that as body size decreases these parameters greatly increase and this is well illustrated in Fig 2. The implication of this is that instead of raising the dose rate in proportion to the dose, the dose frequency can be increased with $time^{0.25}$. However in very small rodents this may be impractical as the dose frequency becomes too high (Fig 2).

The allometric approaches described above are inherently simple and approximate, indeed they have been described as a 'black box' approach, because no attempt is made to determine organ distribution or make physiological assumptions (Mordenti 1985 & 1986).

In contrast the physiological approach is more complex. Blood flow to eliminating organs, tissue and fluid volumes, drug concentrations, protein and enzyme binding are measured in one species. Conceptual models are drawn to describe the presence and movement of drugs and metabolites within body compartments. Mass balance equations are then drawn for the sum of the processes occurring in each compartment and solved simultaneously. Once pharmacokinetics are defined predictions for other species are obtained by substituting the original biochemical, anatomical and physiological data with data from the new species and recalculating the equations to produce extrapolated pharmacokinetic data (Mordenti 1985 & 1986, Baggot 1992).

It is obvious that basic allometry is the most simple method of species scaling. It is possible to develop more complex allometric models to derive more detailed pharmacokinetic data (Boxenbaum 1984) and overcome the limitations of the simple $mg/kg^{0.75}$ approach. However, certain conditions have been suggested for successful use of allometry.

1. Compounds are renally excreted
2. There are no interspecies differences in metabolism
3. Protein binding is low
4. Pharmacokinetics are first order
5. Confirmatory data is obtained from a wide range of species and body weights (Mizen & Woodnutt 1988)

Unfortunately these conditions are not always all met, and in particular species differences in metabolism are not uncommon, such as trimethoprim (Baggot 1992), sulphadiazine (De Souich *et al.* 1978) or novel antibiotics (Smith *et al.* 1973).

The physiological approach is the method of choice where the details of drug distribution are important and where there is strong protein binding and extensive metabolism. However, the methodology is more complex and costly (Ritschel *et al.* 1992), less commonly used and usually calculated using computer programs.

Conclusions

Antibiotics may interfere with an experimental protocol, either by a direct interaction or by influencing metabolism or pharmacokinetics of compounds under

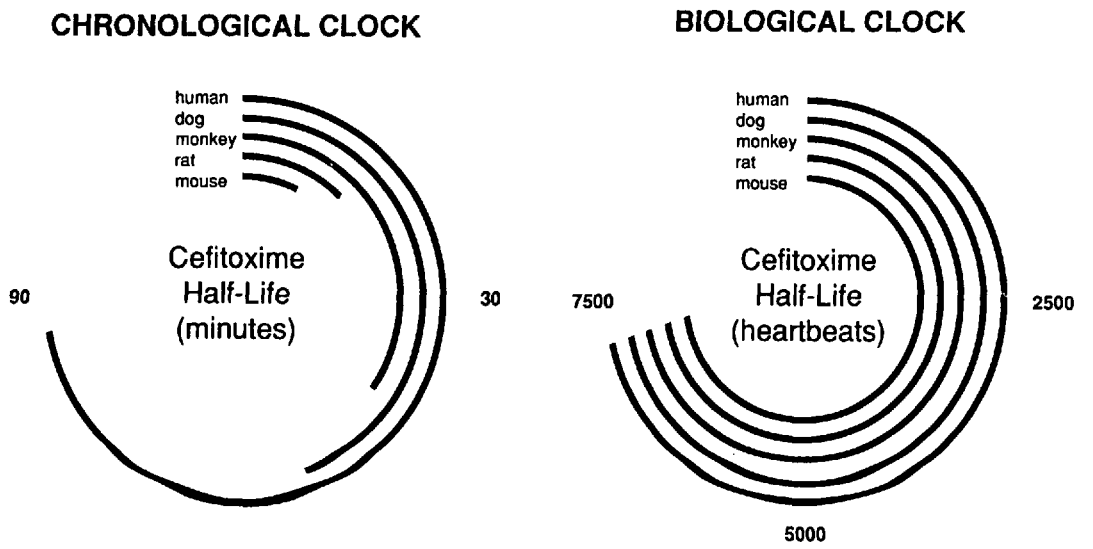

Fig 2 ***Ceftizoxime half-life in various mammals depends on the reference system used to denote time.*** **(a) Chronological clock for ceftizoxime half-life based on chronological time. Half-lifes are reported in minutes (b) Biological clock for ceftizoxime half-life based on physiological time. Half-lifes are reported in heartbeats. From Mordenti (1985) with permission. ©1985 American Society for Microbiology**

investigation. The incidence of this is probably underestimated and antibiotic use must be critically reviewed. It is clear that the principal side effect of antibiotics in rodents and rabbits is induced enterocolitis. The resistance of rats and mice to this phenomenon is also remarkable. More consideration should be given to the route of antibiotic administration to rodents and rabbits. Suitable agents and presentations do exist for effective oral administration, but these do not include common tetracyclines, and as an alternative fluoroquinolones show promise as effective and safe broad spectrum agents. Bearing in mind the small muscle mass of rodents and rabbits and the relatively large injection volume, compared to larger species, coupled with evidence that the subcutaneous route may be as effective as intramuscular injection the former route should always be considered. The indications for antibiotics should also be critically reviewed, as in other areas they are probably overused. In particular prophylaxis at surgery may be less than optimal as rational guidelines are still not being followed. A consideration of the basic principles of veterinary pharmacology and the factors summarized above will help rationalize the use of antibiotics in laboratory species.

The lack of controlled studies on antibiotic therapeutics in laboratory animals, together with relatively little extrapolation from primary data that is available, means that many 'doses' are based on clinical response and lack of overt side effects. It is possible that as small mammals become more popular as pets specific products, with dosage information based on controlled studies, may become available. However a complete range of suitably labelled products for all species will never be available. Where justified the use of scaling with doses extrapolated from other species, particularly as it is practical using allometric principles, should result in more realistic dose regimens.

Acknowledgments I would like to thank A. Gisby, R. Kilgore, L. Mizen, G. Palmer & L. Rolf for their assistance and P. Flecknell, H. Hughes, M. Landi, L. Rolf and B. Waynforth for critical review.

References

Adams RA, Dixit BN (1970) Prolongation of pentobarbitol anaesthesia by chloramphenicol in dogs and cats. *Journal of the American Veterinary Medical Association* **156**, 902–6

Altreuther P (1992) Safety and tolerance of enrofloxacin in dogs and cats. In: *Proceedings of the 1st Internations Baytril Symposium* Bonn: Bayer AG, pp 15–19

Anon (1992) Animal rights lobby stung by the exposure of penicillin myth. *Research Defence Society Newsletter* **April** 11

Anon (1993) New class of pet opens market opportunities in France. *Animal Pharm* no. **284**, 5–6

Baggot JD (1992) Clinical pharmacokinetics in veterinary medicine. *Clinical Pharmacokinetics* **22**, 254–73

Bahri LE, Blouin A (1991) Fluoroquinolones: A new family of antimicrobials. *The Compendium of Continuing Education for the Practicing Veterinarian* **13**, 1429–33

Bartels H (1982) Metabolic rate of mammals equals the 0.75 power of their body weight. *Experimental Biological Medicine* **7**, 1–11

Bartlett JG, Chang TW, Moon N, Onderdonk AB (1978) Antibiotic induced lethal enterocolitis in hamsters: Studies with eleven agents and evidence to support the pathogenic role of toxin producing bacteria. *American Journal of Veterinary Research* **39**, 1525–30

Bohnhoff M, Drake BL, Miller CL (1954) Effect of streptomycin on susceptibility of intestinal tract to experimental salmonella infection. *Proceedings of the Society for Experimental Biology and Medicine* **86**, 132–7

Boxenbaum H (1984) Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm. *Drug Metabolism Reviews* **15**, 1071–121

Boyd CE (1960) The acute oral toxicity of benzylpenicillin potassium in the rabbit. *Antibiotics and Chemotherapy* **10**, 376–84

Boyd EM, Price-Jones MA (1960) The comparative acute oral toxicity of spiramycin adipate in mice, rats, guineapigs and rabbits. *Antibiotics and Chemotherapy* **10**, 273–84

Bradfield JF, Schachtman TR, McLaughlin RM, Steffen EK (1992) Behavioural and physiological effects of inapparent wound infection in rats. *Laboratory Animal Science* **42**, 572–8

Broome RL, Brooks DL, Babish JG, Copeland DD, Conzelman GM (1991) Pharmacokinetic properties of enrofloxacin in rabbits. *American Journal of Veterinary Research* **52**, 1835–41

Burns JJ, Conney AH (1965) Enzyme stimulation and inhibition in the metabolism of drugs. *Proceedings of the Royal Society of Medicine* **58**, 955–60

BSAVA (Publication dates vary) *Manuals of: Reptiles, Ornamental Fish, Parrots, Budgerigars and other Psittacine Birds, Exotic Pets.* Cheltenham UK: British Small Animal Veterinary Association

Calabrese EJ (1991) Scaling: An attempt to find a common denominator. In: *Principles of Animal Extrapolation* Chelsea, Michigan: Lewis, pp 499–527

Caron I, Ducrotte P, Lerebours E, Colin R, Humberts G, Denis P (1991) Effect of amoxycillin-clavulanate combination on the motility of the small intestine in human beings. *Antimicrobial Agents and Chemotherapy* **35**, 1085–8

Cherbut C, Ferre JP, Corpet DE, Ruckebusch Y, Delfort-Laval J (1991) Alterations of intestinal microflora by antibiotics: Effects on fecal excretion, transit time and colonic motility in rats. *Digestive Diseases and Science* **36**, 1729–34

Curl JL, Curl JS, Harrison JK (1988) Pharmacokinetics of long acting oxytetracycline in the laboratory rat. *Laboratory Animal Science* **38**, 430–4

Dabard J, DuBois R, Martinet L, Ducluzeau R (1979) Experimental reproduction of neonatal diarrhoea in young gnotobiotic hares simultaneously associated with *Clostridium difficile* and other clostridium strains. *Infection and Immunity* **24**, 7–11

Davis WM (1962) Day-night periodicity in pentobarbital response of mice and the influence of socio-psychological conditions. *Experimentia* **XVIII/515**, 235–7

De Somer P, Van de Voorde H, Eyssen H, Van Dijck P (1955) A study on penicillin toxicity in guinea pigs. *Antibiotics and Chemotherapy* **5**, 463–9

Dixon LW (1986) Antibiotic toxicosis in the guinea pig. *Texas Veterinary Medical Journal* **48**, 31

Dorrestein GM (1992) Enrofloxacin in pet avian and exotic animal therapy. In: *Proceedings of the 1st International Baytril Symposium* Bonn: Bayer AG, pp 63–70

DuSouich P, McLean AJ, Lalka D, Jenkins B, Haegele DK, McNay JL (1978) Sulfadiazine handling in the rabbit. 1. Pseudosaturation of N-Acetyltransferase. *Journal of Pharmacology and Experimental Therapeutics* **207**, 221–35

Ebright JR, Fekety R, Silva J, Wilson KH (1981) Evaluation of eight cephalosporins in hamster colitis model. *Antimicrobial Agents and Chemotherapy* **19**, 980–6

Elek AD, Conen PE (1958) The virulence of *Staphylococcus pyogenes* for man. A study of the problems of wound infection. *British Journal of Experimental Pathology* **38**, 573–86

English PB, Prescott CW (1983) Antimicrobial chemotherapy in the dog. II: Some practical considerations. *Journal of Small Animal Practice* **24**, 371–83

Escoula L, Canguilhem R, Larrieu G, More J (1981) Sur la sensibilité du lapin a l'association anpicillin-gentamicine. *Annales Recherches Vétérinaire* **12**, 11–7

Eyssen H, De Somer P, van Dijck (1957) Further studies on antibiotic toxicity in guinea pigs. *Antibiotics and Chemotherapy* 7, 55–61

Fantin B, Carbon C (1992) In vivo antibiotic synergism: Contribution of animal models. *Antimicrobial Agents and Chemotherapy* **36**, 907–12

Farrar WE, Kent T H, Elliott VB (1966) Lethal gram-negative bacterial superinfection in guinea pigs given bacitracin. *Journal of Bacteriology* **92**, 496–501

Fekety R, Silva J, Toshniwal R *et al.* (1979) Antibiotic associated colitis: Effects of antibiotics on *Clostridium difficile* and the disease in hamsters. *Review of Infectious Diseases* **1**, 386–96

Fekety R (1986) Animal models of antibiotic-induced colitis. In: *Experimental Models in Antimicrobial Chemotherapy Volume 2* New York: Academic Press, pp 61–72

Fesce A, Ceccarelli A, Fresce E, Balsari A (1977) Ecophylaxis: Preventive treatment with gentamicin of rabbit lincomycin-associated diarrhoea. *Folia Veterinari Latino* 7, 225–42

Flecknell P (1983) Retraint, anaesthesia and treatment of children's pets. *In Practice* **May**, 85–95

Fraser CM (ed) (1991) Septacemic pasteurellosis of sheep. In: *The Merck Veterinary Manual* Rahway NJ: Merck & Co, p 401

Fritz PE, Hurst WJ, White WJ, Lang CM (1987) Pharmacokinetics of cefazolin in guineapigs. *Laboratory Animal Science* **37**, 646–51

Galloway JH (1968) Antibiotic toxicity in white mice. *Laboratory Animal Care* **18**, 421–5

Gilfillan EC, Pelak BA, Bland JA, Malatesta PF, Gadebusch HH (1984) Pharmacokinetic studies of norfloxacin in laboratory animals. *Chemotherapy* **30**, 288–96

Glauser MP, Bonard M (1982) Treatment of ascending *Escherichia coli* pyelonephritis with Ceftriaxone alone and in combination with gentamicin. *Chemotherapy* **28**, 410–6

Goldschmit F (1972) Reproducible topical staphylococcal infection in rats. *Applied Microbiology* **23**, 130–4

Gray JE, Purmalis A, Feenstra ES (1964) Animal toxicity studies of a new antibiotic lincomycin. *Toxicology and Applied Pharmacology* **6**, 476–96

Guest GB (1993) Using proper drugs and using them poorly. *Agri-Practice* **14**, 20–4

Hagen KW (1967) Effect of antibiotic-sulfonamide therapy on certain microorganisms in the nasal turbinates of domestic rabbits. *Laboratory Animal Care* **17**, 77–80

Harkness JE, Wagner JE (1989a) Clinical Procedures. In: *The Biology and Medicine of Rabbits and Rodents 3rd edition*. Philadelphia: Lea & Febiger, pp 55–84

Harkness JE, Wagner JE (1989b) Tyzzer's disease. In: *The Biology and Medicine of Rabbits and Rodents 3rd edition*. Philadelphia: Lea & Febiger, pp 198–200

Haven ML, Wichtel JJ, Bristol DG, Fetrow JF, Spears JW (1992) Effects of antibiotic prophylaxis on post operative complications after rumenotomy in cattle. *Journal of the American Veterinary Medical Association* **200**, 1332–5

Heinze W, Kruger S, Schröder A (1992) Chronological influences on the action of drugs. *Monatsheffe für Veterinarmedizin* **47**, 643–51

Hirsch DC, Jang SS, Biberstein EL (1990) Lack of supportive susceptibility data for use of ampicillin together with trimothoprim-sulfonamide as a broad-spectrum antimicrobial treatment of bacterial disease in dogs. *Journal of the American Veterinary Medical Association* **197**, 594–6

Hoar R (1976) Toxicology and Teratology. In: *The Biology of the Guinea-pig* (Wagner J, Manning P, eds). New York: Academic Press, p 273

Hubbard G B, Schmidt RE (1987) Noninfectious diseases. In: *Laboratory Hamsters* (Van Hoosier G, McPherson C, eds). New York: Academic Press, pp 169–72

Jacobsen E, Kollias GV, Peters LJ (1991) Dosages for antibiotics and parasitacides used in exotic animals. In: *The Compendium Collection of Continuing Education for the Practicing Veterinarian* (Johnston DE, ed). Trenton NJ: Veterinary Learning Systems, pp 202–9

Jondorf WR, Maickel RP, Brodie BB (1958) Inability of newborn mice and guinea-pigs to metabolise drugs. *Biochemical Pharmacology* **1**, 352–4

Jones RL (1986) Control of nosocomial infection. In: *Current Veterinary Therapy IX* (Kirk RW, ed). Philadephia: WB Saunders, pp 19–24

Jones RS (1992) Muscle relaxants in canine anaesthesia 2: Clinical applications. *Journal of Small Animal Practice* **33**, 423–9

Kaiser AB (1990) Post operative infections and antimicrobial prophylaxis. In: *Principles and Practices of Infectious Disease* 3rd edition (Mandell EL, Douglas RG, Bennet JE, eds). New York: Churchill Livingstone, pp 2245–57

Kaiser AB, Kernodle DS, Parker RA (1992) Low-Inoculum model of surgical wound infection. *Journal of Infectious Diseases* **166**, 393–9

KaipainenWJ, Faine S (1954 Toxicity of erythromycin. *Nature* **174**, 969–70

Katz L, LaMont JT, Trier JS, Sannenblick EB, Rothman SW, Broitman SA, Rieth S (1978) Experimental clindamycin associated colitis in rabbits. *Gastroenterology* **74**, 246–52

Kelly DJ, Chulay JD, Mikesell P, Friedlander AM (1992) Serum concentrations of penicillin, doxycycline and ciprofloxacin during prolonged therapy in rhesus monkeys. *Journal of Infectious Diseases* **166**, 1184–7

Killby VA, Silverman PH (1967) Toxicity of antibiotics in laboratory rodents. *Science* **156**, 264

Kirkwood JK (1983) Influence of body size on animals on health and disease. *Veterinary Record* **113**, 287–90

Kirkwood JK, Merriam J (1990) Variation in plasma half life of gentamicin between species in relation to bodyweight and taxonomy. *Research in Veterinary Science* **49**, 160–5

Knoop FC (1979) Clindamycin associated enterocolitis in guinea pigs: Evidence for a bacterial toxin. *Infection and Immunity* **23**, 31–3

Kruckenberg SM (1979) Drugs and Dosages. In: *The Laboratory Rat Vol. 1* (Baker H, Lindsey J, Weisbroth SH, eds). New York: Academic Press, pp 413–21

Latt RH (1976) Drug dosages for Laboratory Animals. In: *Handbook of Laboratory Animal Science Vol III* (Melby EC, Altman NH, eds). Vol III, Cleveland, Ohio: CRC Press, pp 561–9

Laval A (1990) Choix de l'anti-infectieus chez le lapin d'agrément. *Recueil de Médicine Vétérinaire* **166**, 375–9

Licois D (1980) Action toxique de certains antibiotiques chez le lapin. *Recueil de Médicine Vétérinaire* **156**, 915–9

Lipman NS, Weischedel AK, Connors MJ, Olson DA, Taylor NS (1992) Utilisation of cholestryramine resin as a preventative treatment for antibiotic (clindamycin) induced enterotoxaemia in the rabbit. *Laboratory Animals* **26**, 1–8

Lowe BR, Fox JG, Bartlett JG (1980) *Clostridium difficile* associated cecitis in guinea pigs exposed to penicillin. *American Journal of Veterinary Research* **41**, 1277–9

Maiers JD, Mason SJ (1984) Lincomycin-associated enterocolitis in rabbits. *Journal of the American Veterinary Medical Association* **185**, 670–1

Manning PJ, Wagner JF, Harkness J E (1984) Biology and diseases of guinea pigs. In: *Laboratory Animal Medicine* (Fox J, Cohen B, Loew F, eds). New York: Academic Press, p 173

Marshall AB, Palmer GH (1980) Injection sites and drug bioavailability. In: *Trends in Veterinary Pharmacology and Toxicology, Proceedings of the 1st European Congress on Veterinary Pharmacology and Toxicology* (van Miert A, Frens J, van der Kreek F, eds). Amsterdam: Elsevier, pp 54–60

McKellar QA (1989) Drug dosages for small mammals. *In Practice* **March**, 57–61

McNeal J, Brown MJ, Bennet BY (1988) Sterile drape packs for survival surgery in small animals. *Laboratory Animal Science* **38**, 517–18 Abstract p 33

McNeil PE, Al-Mashat PR, Bradley RA, Payne AP (1986) Control of an outbreak of wet tail in a closed colony of hamsters. *(Mesocricetus auratus). Veterinary Record* **199**, 272–3

Milhaud G, Renault L, Vaissarie J, Maire CI (1976) Sensibilité du lapin a l'ampicillin. *Recueil de Médicine Vétérinaire* **152**, 843–7

Mizen L, Bhandari K, Sayer J, Catherall E (1981) Pharmacokinetics and distribution of Augmentin (amoxycillin/clavulanic acid) in laboratory animals. *Drugs under Experimental Clinical Research* **VII**, 263–7

Mizen L, Woodnutt G (1988) A critique of animal pharmacokinetics. *Journal of Antimicrobial Chemotherapy* **21**, 273–80

Mordenti J (1985) Forecasting cephalosporin and monobactam antibiotic half-lives in humans from data collected in laboratory animals. *Antimicrobial Agents & Chemotherapy* **27**, 887–91

Mordenti J (1986) Man versus beast: Pharmacokinetic scaling in mammals. *Journal of Pharmaceutical Sciences* **75**, 1028–40

Morisse J-P (1978) Induction d'une enterite de type colibacillaire chez le lapin. *Recueil de Médicine Vétérinaire* **129**, 625–32

Mureau (1988) L'antibiothérapie chez le lapin. *Cuniculture* **15**, 75–9

Murtaugh RJ, Mason GD (1989) Antibiotic pressure and nosocomial disease. *Veterinary Clinics of North America: Small Animal Practice* **19**, 1259–74

National Office of Animal Health (1992) *Compendium of Data Sheets for Veterinary Products*. London: Datapharm Publications

Nicolau DP, Freeman CD, Nightingae CH, Quintiliani R (1993) Pharmacokinetics of minocycline and vancomycin in rabbits. *Laboratory Animal Science* **43** 222–5

Nossman BC, Amouzadeh HR, Sangiah S (1990) Effects of chloramphenicol, cimetidine and phenobarbital on and tolerance to xylazine-ketamine anaesthesia in dogs. *Veterinary and Human Toxicology* **32**, 216–9

Nouws JFM, Smulders A, Rappalini M (1990) A comparative study on irritation and residue aspects of five oxytetracycline formulations administered intramuscularly to calves, pigs and sheep. *The Veterinary Quarterly* **12**, 129–38

Palmer GH, Buswell JF, Dowrik JS, Yeoman GH (1976) Amoxycillin: A new veterinary penicillin. *Veterinary Record* **99**, 84–5

Percy DH, Black WD (1988) Pharmacokinetics of tetracycline in the domestic rabbit following intravenous or oral administration. *Canadian Journal of Veterinary Research* **52**, 5–11

Pinkel D (1958) The use of body surface area as a criteria of drug dosage in cancer chemotherapy. *Cancer Research* **18**, 853–6

Porter WP, Bitar YS, Strandberg JD, Charache PC (1985) A comparison of subcutaneous and intraperitoneal oxytetracycline injection methods for control of infectious disease in rats. *Laboratory Animals* **19**, 3–6

Porter WP, Bitar YS, Strandberg JD, Charache PC (1985) Absence of therapeutic blood concentrations of tetracycline in rats after administration in drinking water. *Laboratory Animal Science* **35**, 71–5

Prescott JF, Baggot JD (1988) *Antimicrobial Therapy in Veterinary Medicine*. Oxford: Blackwell Scientific Publications

Rehg JE (1980) Cecal toxin(s) from guinea pigs with clindamycin associated colitis, neutralised by *Clostridium sordelli* antitoxin. *Infection and Immunity* **27**, 387–90

Rehg JE, Pakes SP (1982) Implications of *Clostridium difficile* and *Clostridium perfringens* iota toxins in experimental lincomycin-associated colitis in rabbits. *Laboratory Animal Science* **32**, 253–7

Rehg, JE, Lu Y-S (1981) *Clostridium difficile* colitis in a rabbit following antibiotic therapy for pasteurellosis. *Journal of the American Veterinary Medical Association* **179**, 1296–7

Reinhard MK, Hottendorf GH, Powell ED (1991) Differences in the sensitivity of Fischer and Sprague-Dawley rats to aminoglycoside toxicity. *Toxicological Pathology* **19**, 66–71

Richard Y (1990) Choix de l'anti-infectieus chez les rongeurs. *Recueil de Médicine Vétérinaire* **166**, 367–73

Richards RK, Kueter KE (1946) Competitive inhibition of procaine convulsions in guinea pigs. *Journal of Pharmacology and Experimental Therapeutics* **87**, 42–52

Ritschel WA, Vachharajani NN, Johnson RD, Hussain AS (1992) The allometric approach for interspecies scaling of pharmacokinetic parameters. *Comparative Biochemistry and Physiology* **103C**, 249–53

Roine P, Ettala T (1952) Toxicity of aureomycin to guinea pigs. *Nature* **169**, 1014

Roine P, Ettala T, Raitio A (1953) Effects of aureomycin and some other antibiotics on the guinea pig. *Suomen Kemistikehti* **26**, 17–22

Rolf LL (1993) Lack of toxicity of high oral doses of enrofloxacin in the guinea pig (*Cavia porcellus*). *Contemporary Topics in Laboratory Animal Science* **34**, abstract PO26

Rosin E (1988) The timing of antibiotic administration for antimicrobial prophylaxis in surgery. *Veterinary Surgery* **17**, 181

Sande MA, Johnson ML (1975) Antimicrobial therapy of experimental endocarditis caused by *Staphylococcus aureus*. *Journal of Infectious Diseases* **131**, 367–75

Sande MA, Kapusnik-Unere JE, Mandell GL (1990a) Antimicrobial agents: General considerations. In: *The Pharmacological Basis of Therapeutics 8th ed.* (Gilman AG, Rall TW, Nies AS, Taylor P, eds). New York: Pergamon Press, pp 1041–4

Sande MA, Kapusnik-Unere JE, Mandell Gl (1990b) Antimicrobial agents: General considerations. In: *The Pharmacological Basis of Therapeutics 8th ed.* Gilman AG, Rall TW, Nies AS, Taylor P, eds). New York: Pergamon Press, p 1035

Schaedler RW, Orcutt RP (1983) Gastrointestinal microflora. In: *The Mouse in Biomedical Research Vol III* (Foster H, Small D, Fox J, eds). New York. Academic Press, p 339

Schatzmann HJ, Tscharner C, Tschabold M (1977) Schädliche wirkung einer oralen behandlung von kaninchen mit ampicillin. *Schweizer Archive für Tierheikunde* **119**, 149–53

Smith CS, Poutsaika JW, Schreider EC (1973) Problems in predicting drug effects across species lines. *The Journal of International Medical Research* **1**, 489–503

Schröder C, Matthes S, Löliger H ch. (1982) Untersuchungen über die verträglichkiet oraler antibiotikamedikation beim kaninchen. *Kleintierpraxis* **27**, 221–68

Small JD (1968) Fatal enterocolitis in hamsters given lincomycin hydrochloride. *Laboratory Animal Care* **18**, 411–20

Taylor NS, Bartlett JG (1980) Binding of *Clostridium difficile* cytotoxin and vancomycin by anion-exchange resins. *Journal of Infectious Diseases* **141**, 92–7

Thilstead JP (1981) Fatal diaorrhoea in rabbits resulting from the feeding of antibiotic contaminated feed. *Journal of the American Veterinary Medical Association* **179**, 360–1

Van Miert AS (1989) Extrapolation of pharmacological and toxicological data based on metabolic weight. *Archives of Experimental Veterinary Medicine, Leipzig* **43** Suppl., 481–8

ten Voorde G, Broeze J, Hartman EG, van Gogh H (1990) The influence of the injection site on the bioavailability of ampicillin and amoxycillin in beagles. *The Veterinary Quarterly* **12**, 73–9

VPD (1991) *Veterinary Pharmaceuticals and Biologicals*. Lenexa, Kansas: Veterinary Medicine Publishing Company

Walsh TJ, Bacher J, Pizzo PA (1988) Chronic silastic central venous catheterisation for induction, maintenance and support of persistent granulocytopenia in rabbits. *Laboratory Animal Science* **38**, 467–71

Watson ADJ (1992) Bioavailability and bioequivalence of drug formulations in small animals. *Journal of Veterinary Pharmacology and Therapeutics* **15**, 151–9

Waynforth B (1989) Standards of surgery for rodents: Do we need to change? *Scandinavian Journal of Laboratory Animal Science* **16** (Suppl. 1), 43–6

Weersink A, Visser M, Vos A *et al.* (1991) Amoxycillin-clavulanate prophylaxis against wound infections after clean contaminated surgery. *European Journal of Surgery* **57**, 271–5

Wenzel RP (1992) Preoperative antibiotic prophylaxis. *New England Journal of Medicine* **326**, 337–9

Wiegersma N, Jansen G, van de Waaiji D (1982) Effect of twelve antimicrobial drugs on the colonisation resistance of the digestive tract of mice and on endogenous potentially pathogenic bacteria. *Journal of Hygiene (Cambridge)* **88**, 221–30

Young JD, Hurst WJ, White WJ, Lang C Max (1987) An evaluation of ampicillin pharmacokinetics and toxicity in guinea pigs. *Laboratory Animal Science* **37**, 652–6 [with *ERRATA* **38**, 345]