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3.

CONSEQUENCES OF ANTIMICROBIAL THERAPY FOR THE COMPOSITION OF THE MICROFLORA OF THE DIGESTIVE TRACT

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EFFECTS OF ANTIMICROBIAL THERAPY UPON DIGESTIVE TRACT MICROFLORA

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INTRODUCTION

Antibiotics may affect the digestive tract microflora in the oropharynx and/or in the intestines, either following incomplete absorption during oral treatment or else by excretion with the bile. They may reach the gut contents and mix with it to establish in the course of several days a steady state concentration. In the oropharyngeal area, the antibiotic concentrations are largely determined by salivary concentrations which may fluctuate with serum levels. Depending on the spectrum of activity of the antibiotic(s) that have reached the digestive tract, sensitive Gram-positive and/or Gram-negative bacteria may suffer of the concentration established. Resistant bacteria on the contrary may flourish under these conditions much better than beforehand and grow out to high numbers. In this process of selective suppression of sensitive bacteria in the oropharynx and/or the gut, either the colonization resistance (CR) associated (*van der Waaij* et al., 1971; *van der Waaij*, 1982) predominantly anaerobic microflora is largely sensitive and suppressed, or not sensitive and largely unchanged. In this last case the aerobic Gram-negative bacteria, which are potentially pathogenic, are generally sensitive and killed (*van der Waaij,* 1982). In the first situation of CR-flora suppression, resistant aerobic Gram-negative bacteria and yeasts may grow out to abnormally high concentrations, a condition commonly described as "overgrowth" (*van der Waaij,* 1979; *Nord* et al., 1984). Such high concentrations of potentially pathogenic bacteria are associated with invasion of the mucous membrane. From there the submucosal tissues and even the mesenteric lymph nodes and spleen are invaded (*van der Waaij* et al., 1972). This event is called "translocation" (*Berg,* 1980).

SELECTIVE SUPPRESSION OF BOWEL FLORA

In case of selective suppression of the aerobic Gram-negatives, the anaerobic fraction of the bowel flora remains largely unaffected (*van der Waaij* et al., 1974). This treatment has been found to reduce Gram-negative infections in the granulocytopenic patient and is named "selective decontamination" (*Sleijfer* et al., 1980). If bacteria replace suppressed flora components during selective decontamination, it normally concerns resident anaerobic species which are resistant to the antibiotic used.

Resident anaerobes do generally not translocate (*Berg* and *Garlington*, 1979). In immunocompromised patients this condition is beneficial as it is associated with a reduction of translocation

in stead of an increase by an overgrowth of Gram-negatives as may occur when the CR-associated flora is largely suppressed by antibiotic therapy (*van der Waaij*, 1982). These phenomena will be discussed in great detail elsewhere in this monograph. Therefore, this overview will be confined to implications of antibiotic therapy for the emergence of resistance in the digestive tract microflora during antibiotic therapy.

FAECAL CARRIAGE OF RESISTANT BACTERIA

Studies which will be quoted hereafter provide further evidence that antibiotic resistance in Gram-negatives may predominantly develop in the intestinal tract and not at the site of infection.

The main anatomical sites at which the occurrence of antibiotic resistant bacteria in the commensal flora have been studied are indeed the intestines, but also the skin and upper respiratory tract. Naturally, attention has focused on the gut because of the large range and number of bacteria present and the association of those bacteria with opportunistic infection such as respiratory tract (*Eickhoff*, 1979) and urinary tract infections (*Ball*, 1986).

Considering the intestines first, there have been a great number of studies but protocols and data analysis vary widely so that direct comparison is difficult. Early classic studies on R-plasmids originate from Japan and mainly concerned the plasmid transfer in the presence of antibiotic treatment (*Watanabe*, 1963).

Although there are a number of studies from the sixties and early seventies of the faecal carriage of antibiotic resistant bacteria in normal individuals, similar comprehensive surveys are not available for the eighties. A study from the early eighties of sensitive enteropathogenic *Escherichia coli* showed that 37% of the isolates obtained, were resistant to ampicillin and 29% to tetracycline (*Gross* et al., 1982). In view of the shift in general practice prescribing habits in the United Kingdom reported, from low ampicillin prescription in 1967 to three fold higher in 1984 while prescription of tetracyclines decreased with about 50% over the same period, such studies would be valuable to confirm changes in resistance rates in relation to prescribing.

Although it is no longer possible to examine urban and rural populations that have not been exposed at some time to therapeutic antibiotics, some studies have attempted to assess the natural occurrence of resistance genes in remote communities that have had little or no contact with antibiotics. The Xhosa communities in South Africa, who avoid Western medicine, were studied in 1973 and 1976. During this time, antibiotic usage increased from zero to moderate usage. During this two year interval, resistance in faecal coliforms increased from 19% to 48% (*Burt* and *Woods*, 1976). A study of an extremely remote island in the Solomon Islands at which modern medicine is shun, revealed only two R-plasmid containing bacteria from 40 samples of faeces and soil, both resistant to tetracycline and streptomycin (*Gardner* et al., 1969). These studies indicate that antibiotic treatment is associated with the emergence of resistant strains in the faecal flora.

ANTIBIOTIC RESISTANCE IN ENTEROBACTERIACEAE FROM HEALTHY VOLUNTEERS

The first survey of the occurrence of antibiotic resistant faecal *E. coli* in normal healthy subjects and animals was reported in 1966 (*Smith* and *Halls*, 1966). The resistance markers studied were relevant. Although only 24 healthy subjects were examined, 62% carried resistant *E. coli*. Approximate analysis of resistance patterns suggested that about 17% were resistant to ampicillin and 70% to tetracycline. The source of the human volunteers was unfortunately not provided.

Again a long time ago in 1966, *Smith* and *Halls* found in 19 of 20 representative *E. coli* from healthy subjects having transferable patterns of drug resistance indicating a high incidence of plasmid-mediated resistance. Concern was expressed in their report and in subsequent studies at the high level of carriage of antibiotic resistant bacteria in apparently normal healthy individuals (*Williams Smith*, 1975; *Leading article*, 1969). Another study in the United Kingdom of urban, rural farming and rural non-farming families by *Linton* et al. (1972) published a few years later confirmed the high incidence of carriage of resistant Enterobacteriaceae. Urban adults had an overall carriage rate of 42% which was considerably lower than the prevalence in children (64%). In contrast rural farming adults carried also in a high percentage (63%) resistant enterobacteria; in children this figure was yet higher (79%). Statistically, the difference in resistance rates between adults and children was significant in both farming and non-farming associated individuals as well as between both groups as a whole. The hypothesis by the author and co-workers was that the difference between children and adults was due to differences in antibiotic use in the two groups. They surveyed the antibiotic prescribing habits of 15 local general practitioners for one week. The highest rate was in school age children, the intermediate in adults and the lowest in children of less than four years of age which led them to the conclusion that antibiotic use could not be the most important factor. *Linton* et al. (1972) however, did not mention the fact that children up to the age of six received a quarter of the prescriptions although they only represent 10% of the total population (*Leading article*, 1974).

It could be argued that the high carriage rates of resistant strains in children are related to their lower standard of personal hygiene and thus their greater chance of acquiring resistant strains. Cross-colonizing of subjects with antibiotic resistant bacteria has been reported both in the absence (*Petrocheilon* et al., 1977) as well as in the presence of antibiotic treatment (*van der Waaij* et al., 1986). Resistant bacteria are not necessarily by themselves good colonizers of the human gut but may persist for a very long time (*Hartley* and *Richmond*, 1975). The explanation for the high carriage rate of resistant Gram-negative enterobacteria in children in comparison to adults may be a combination of: 1. heavy prescribing of oral antibiotics with larger doses for weight in children and 2. a greater chance of cross-contamination in children from siblings and playmates.

The first point may have implicated higher average steady state concentrations in the intestines and, therefore, more often have resulted in suppression of the colonization resistance associated flora in children than in adults. Whether this still holds nowadays in the late eighties is questionable. As reported several years ago about a study in 1971 in the Netherlands (*van der Waaij* et al.,

1986), and as will be reported elsewhere in this monograph, it is likely that in the last two decades inactivation by intestinal contents may have increased particularly as far as ß-lactam antibiotics are concerned. Inactivation of these antibiotics has appeared to he due to ß-lactamases (*Welling* et al., 1987) in the intestinal contents (faeces) and may vary in degree between individuals. Also other antibiotics such as aminoglycosides, polymyxin and quinolones are to a interindividually varying degree inactivated by faecal substances (*Veringa* and *van der Waaij*, 1984).

ANTIBIOTIC RESISTANCE IN ENTEROBACTERIACEAE FROM HOSPITAL PATIENTS

In the late sixties a study of 100 patients admitted to hospital for elective surgery (*Datta*, 1969) was performed. Gram-negative bacilli as well as *E. coli* were studied during hospitalization. A predominance of resistant strains was found amongst *E. coli*, resistant strains being found in 52% of patient's specimens.

Sixty percent of these strains had transferable markers, 17% of patients excreted ampicillin resistant *E. coli* and 34% tetracycline resistant *E. coli*. A comprehensive study by *Moorhouse* (1969) in Dublin in the same period confirmed these figures. A few years later *Shaw* and co-workers (1973) published their findings in comparable groups of 20 to 25 patients who were either not treated or treated with tetracycline or an ampicillin. In this study also an alarming increase of Gram-negative bacteria during hospitalization associated with antibiotic treatment was reported.

R-PLASMID TRANSFER *IN VIVO*

The emergence of resistance among enteric Gram-negative bacilli may exclusively be due to selection of resistant mutants. However, theoretically resistance may spread in hospitals by transfer of resistance plasmids. Whilst all the prevalence studies have usually shown a high incidence of transferability of antibiotic resistance markers to laboratory recipient strains, the relevance of this transfer of R-plasmids amongst members of the resident microflora of the human gut is, however, uncertain (*Lacey*, 1975). A rather recent study by *Platt* and co-workers (1986) , reveals that *in vivo* transfer may occur in hospitalized patients, particularly in *E. coli* during antibiotic treatment. Why other Enterobacteriaceae are not equally able to receive R-plasmids *in vivo* is explained by the authors by giving special emphasis to the fact that the principle habitat of *E. coli* is the human (and animal) gut. In the intestines *E. coli* is known to be predominant to other aerobic Gram-negatives. The Enterobacteriaceae genera other than *E. coli* tend to be minority residents and are also adapted to free-living existence. Plasmid carriage constitutes a biosynthetic burden to Enterobacteriaceae (*Zund* and *Lebek*, 1981). It seems likely that a well-adapted organism like *E. coli* is more readily able to accumulate plasmids than the other enteric Gram-negatives. In the absence of selective pressure, this may confer an energetic disadvantage on the bacteria which carry

R-plasmids. Furthermore, the high spontaneous mutation rate in genera such as *Serratia* (*Platt* and *Sommerville*, 1981) and *Klebsiella* (*Smith*, 1976) may confer sufficient genetic flexibility to reduce the benefits of plasmid carriage.
Finally, anaerobic gut bacteria such as *Bacteroides* spp. are themselves capable of R-plasmid transfer although it seems to be a rare event (*Bawdon* et al., 1982). Although R-plasmid transfer was not seen in the non-antibiotic treated group in a study by *Anderson* and co-workers (1973), there is a reliable report of R-plasmid transfer amongst the bacteria of the faecal flora of a subject who had not received antibiotics for the past 202 days (*Petrocheilon* et al., 1976). Most other reports have involved the administration of antibiotics at or near the time of R-plasmid transfer (*Bawdon* et al., 1982; Lowburry et al., 1969). However, there is one important exception. In patients who have been treated with selective decontamination for Gram-negative infection prophylaxis, the emergence of resistance was not found (*de Vies-Hospers* et al., 1981), despite the fact that acquisition of resistant Gram-negative rods from the environment is apparently not prevented (*Dekker* et al., 1981). Prevention of acquisition of resistant Gram-negatives requires frequent bacteriological monitoring of faeces or the use of a combination of antimicrobials such as colistin (polymyxin) with co-trimoxazole for selective decontamination (*Rozenberg-Arska* et al., 1983).

In summary, if R-plasmid transfer may occur amongst bacteria of the intestinal (faecal) flora of humans not taking antibiotics, transfer is much more likely during antibiotic treatment with a substantial disruption of normal bowel flora and thus of the CR as unavoidable consequence.

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ECOLOGICAL IMPACT OF NARROW SPECTRUM ANTIMICROBIAL AGENTS COMPARED TO BROAD SPECTRUM AGENTS ON THE HUMAN INTESTINAL MICROFLORA

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INTRODUCTION

The administration of antimicrobial agents may have a number of potentially adverse effects in relation to the human intestinal microflora (*Nord* et al., 1986). One is the overgrowth of already present microorganisms such as yeasts which may produce systemic infections in immunocompromised patients and of *Clostridium difficile* which may lead to diarrhoea and/or colitis. A second consequence is the development of antimicrobial resistance and the induction of beta-lactamases among bacteria in the normal microflora. A third effect is the reduction of colonization resistance, i.e. the resistance displayed by the host to implantation of new microorganisms in the normal microflora. Several factors influence the extent to which a given antimicrobial agent will decimate the normal microflora. Predominant among these is the incomplete absorption of orally administered drugs. Poorly absorbed agents can reach the intestine in active form where they destroy susceptible microorganisms and change the ecologic balance. Parenterally administered agents that are secreted in the bile or from the intestinal mucosa also tend to destroy the normal microbial population.

This investigation examined the

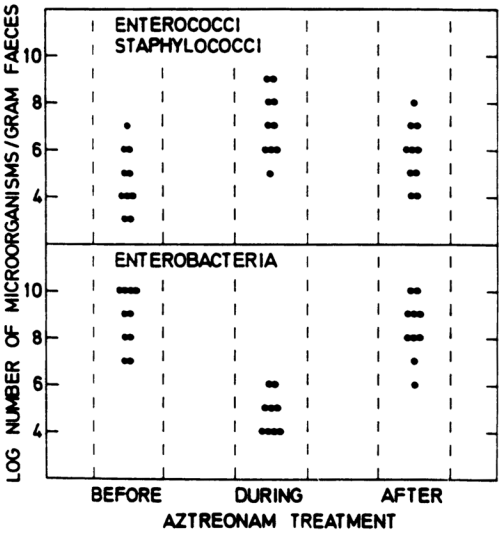

Figure 1: Impact of aztreonam on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

ecological impact of four narrow spectrum antimicrobial agents compared to two broad spectrum antimicrobial agents on the human intestinal microflora.

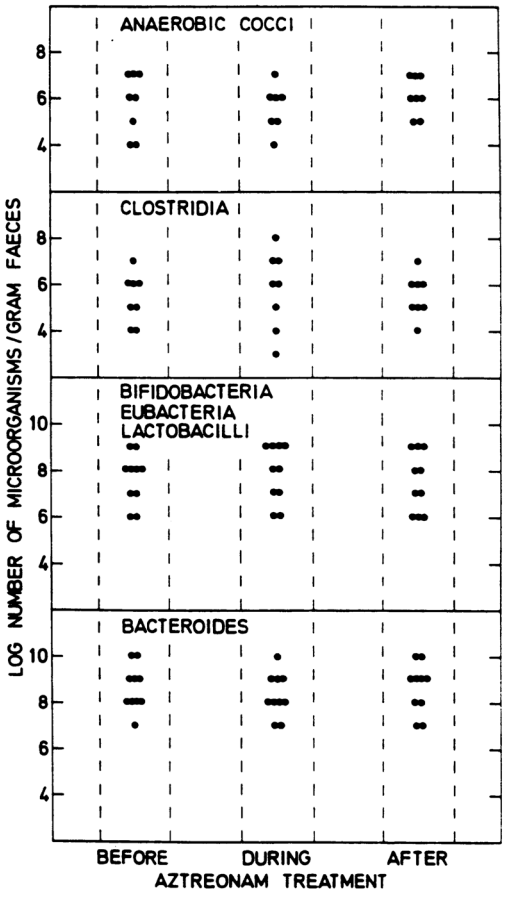

Figure 2: Impact of aztreonam on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

MATERIAL AND METHODS

Patients

Seventy-nine patients, 42 men and 37 women between 21 and 75 years of age (medium age 51 years) with respiratory tract infections, intra-abdominal infections, or urinary tract infections, were included in the study. All patients gave their informed consent to participate in the study which had been approved by the ethical review committees.

Drug administration

Aztreonam

Aztreonam was given intravenously to ten patients in a dose of 1 g b.i.d for 6-12 days.

Cefoperazone

In this group, all patients (n=29) except one received 2 g cefoperazone b.i.d intravenously. One patient with elevated serum creatinine received 1 g b.i.d. The patients were treated for 7 to 14 days.

Clindamycin

Clindamycin was administered perorally to ten patients as 150 mg capsules q.i.d for 7 to 14 days.

Imipenem

Ten patients received 0.5 g imipenem combined with 0.5 g cilastatin q.i.d by intravenous infusion. The treatment period was between 6 and 11 days.

Metronidazole

Metronidazole was given to ten patients by mouth as tablets in a dose of 0.4 g t.i.d for 5-7 days.

Norfloxacin

Ten patients received 200 mg norfloxacin as tablets b.i.d. for 7-9 days.

Sampling procedures

Faecal specimens from all patients were taken before therapy, during therapy and one week to one month after end of therapy. The specimens were collected in sterile plastic containers, immediately frozen and stored at -70°C until they were assayed.

Assay of antimicrobial concentrations in faeces

The concentrations of antimicrobial agents in faeces were determined by the microbiological agar diffusion method; the specimens were processed as previously described by *Kager* et al. (1981).

Microbiological procedures

One gram of the faecal specimen was homogenized in 9 ml prereduced peptone-yeast extract medium. Ten-fold serial dilutions were made to 10^{-8}. Duplicate samples of 0.1 ml of the different dilutions were inoculated onto different non-selective and selective media (*Heimdahl* and *Nord*, 1979). All manipulations of the anaerobic media were carried out in an anaerobic chamber. After incubation, total counts were made on the aerobic and anaerobic blood agar plates and different colonies were isolated and identified as were the colonies found on the selective media.

The microorganisms were identified as described by *Heimdahl* and *Nord* (1979). Enterobacteria were identified biochemically with the API 20E test kit (Analytab Products, N.Y., USA), and oxidative-fermentative, Gram-negative rods with the Oxi-Ferm test kit (Hoffmann-La Roche, N.J., USA).

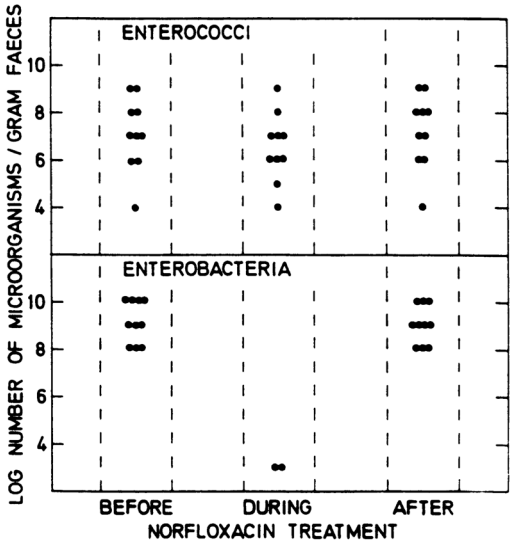

Figure 3: Impact of norfloxacin on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Staphylococci were differentiated by oxidation-fermentation, coagulase and nuclease tests. Streptococci were identified by biochemical and serological tests, and anaerobic bacteria by biochemical tests and gas-liquid chromatography. Yeasts were typed by different cultural and biochemical characteristics.

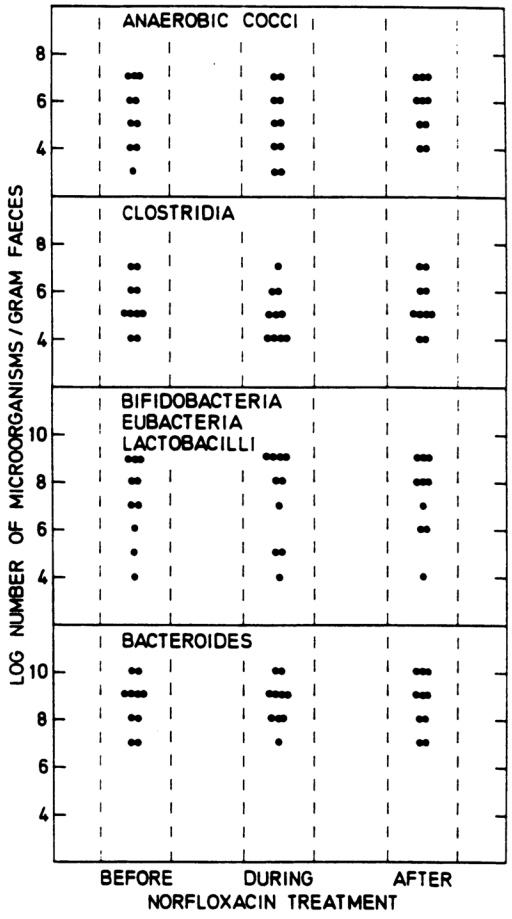

Figure 4: Impact of norfloxacin on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

RESULTS

Impact of narrow spectrum antianaerobic agents on the intestinal microflora

Aztreonam

The impact of aztreonam on the aerobic intestinal microflora is shown in Figure 1. The numbers of enterobacteria were significantly decreased during the treatment while the numbers of Gram-positive cocci - enterococci and staphylococci - increased. At the same period, there were only minor changes in the anaerobic intestinal microflora (Figure 2). The microflora returned to pretreatment levels after the end of therapy.

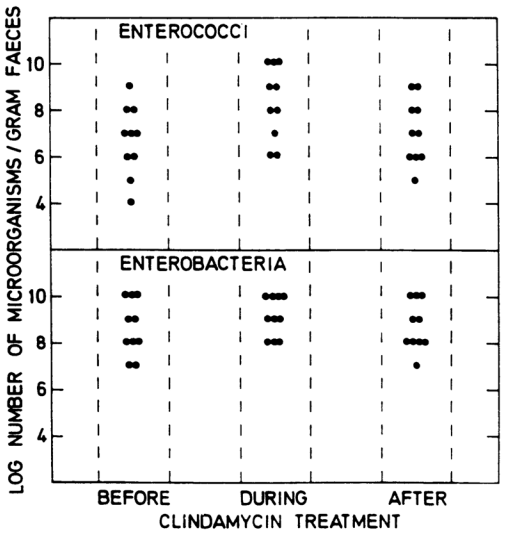

Figure 5: Impact of clindamycin on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

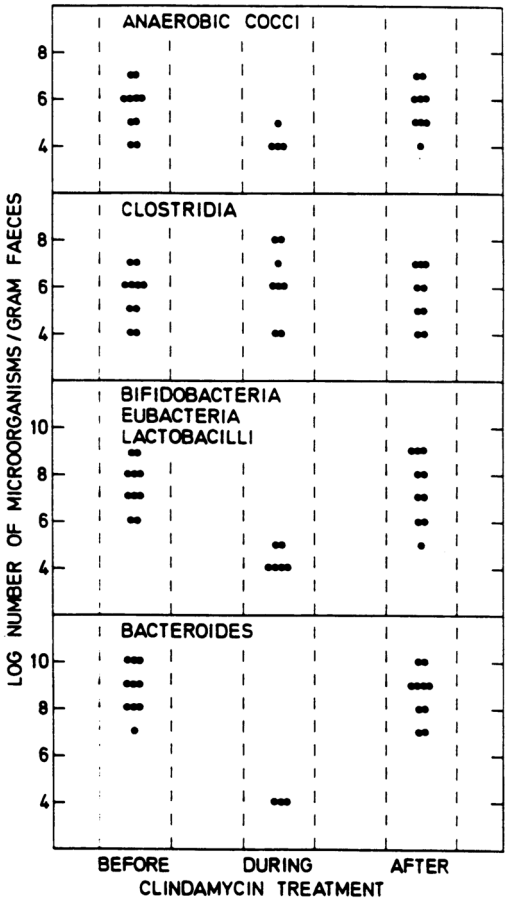

Figure 6: Impact of clindamycin on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

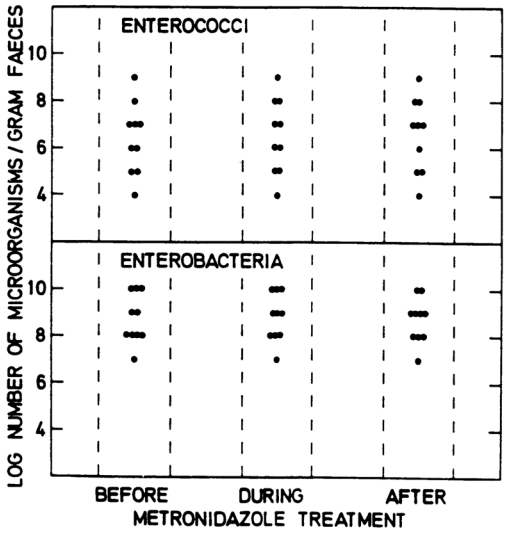

Figure 7: Impact of metronidazole on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

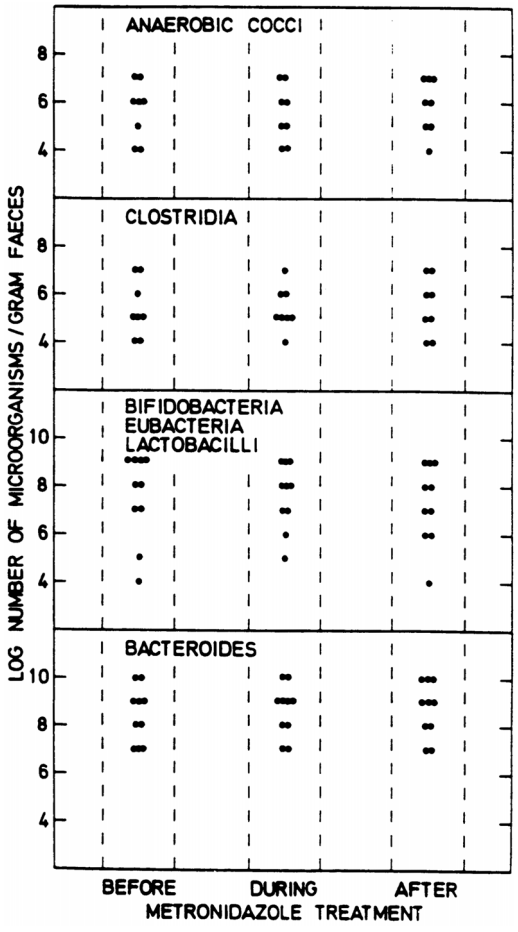

Figure 8: Impact of metronidazole on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Norfloxacin

The aerobic intestinal microflora was considerably affected by norfloxacin treatment (Figure 3). The numbers of enterobacteria were eliminated or strongly suppressed. Minor changes in the numbers of enterococci were noticed. The numbers of enterobacteria returned to normal within one month. Figure 4 shows the effect of norfloxacin on the anaerobic intestinal microflora. Bacteroides, bifidobacteria, lactobacilli, eubacteria, clostridia and Gram-positive cocci were not affected while the numbers of Gram-negative cocci decreased.

Impact of narrow spectrum anti-anaerobic agents on the intestinal microflora

Clindamycin

In the aerobic microflora, the numbers of enterococci slightly increased during the clindamycin treatment period but after one month the numbers were in the same range as before clindamycin treatment (Figure 5). No significant changes in the numbers of enterobacteria were observed during or after the administration of clindamycin. Figure 6 presents the effect of clindamycin on the anaerobic microflora. Pronounced changes occurred during clindamycin treatment. The numbers of anaerobic cocci, Gram-positive and Gram-negative rods decreased markedly and in five patients no anaerobic cocci and bacteroides could be isolated. The numbers of clostridia increased during the treatment period. After one month, the anaerobic microflora was normalized in all patients.

Metronidazole

The impact of metronidazole treatment on the aerobic intestinal microflora is shown in Figure 7. The aerobic microorganisms - enterococci and enterobacteria - were only slightly affected during and after treatment. Only minor changes in the anaerobic microflora occurred at the same period (Figure 8). The microflora normalized in all patients after treatment was terminated.

Impact of broad spectrum anti-aerobic/anti-anaerobic agents on the intestinal microflora

Cefoperazone

Figure 9 shows the effect of cefoperazone on the aerobic intestinal microflora. There was a general decrease in the numbers of aerobic microorganisms during the cefoperazone treatment period. In all patients except one, the numbers of enterobacteria were suppressed to undetectable levels during treatment. The enterococci increased in most patients during and after cefoperazone therapy. In many patients, staphylococci and streptococci decreased to undetectable levels during and after treatment. The numbers of Gram-positive rods were also markedly depressed. The numbers of anaerobic microorganisms were significantly changed (Figure 10). The anaerobic cocci, bacteroides, fusobacteria, bifidobacteria, eubacteria and lactobacilli decreased in many patients to undetectable levels. The numbers of clostridia were not so strongly influenced by cefoperazone therapy as the other anaerobic bacterial groups. In most patients, the intestinal microflora returned to pretreatment levels after one month.

Imipenem

The impact of imipenem therapy on the aerobic intestinal microflora is presented in Figure 11. The numbers of enterobacteria decreased slightly during the treatment period and also the numbers of enterococci were affected to a minor extent. The aerobic flora normalized in all patients after the termination of therapy. The anaerobic intestinal microflora was also slightly affected (Figure 12). There was a minor decrease in the numbers of anaerobic cocci

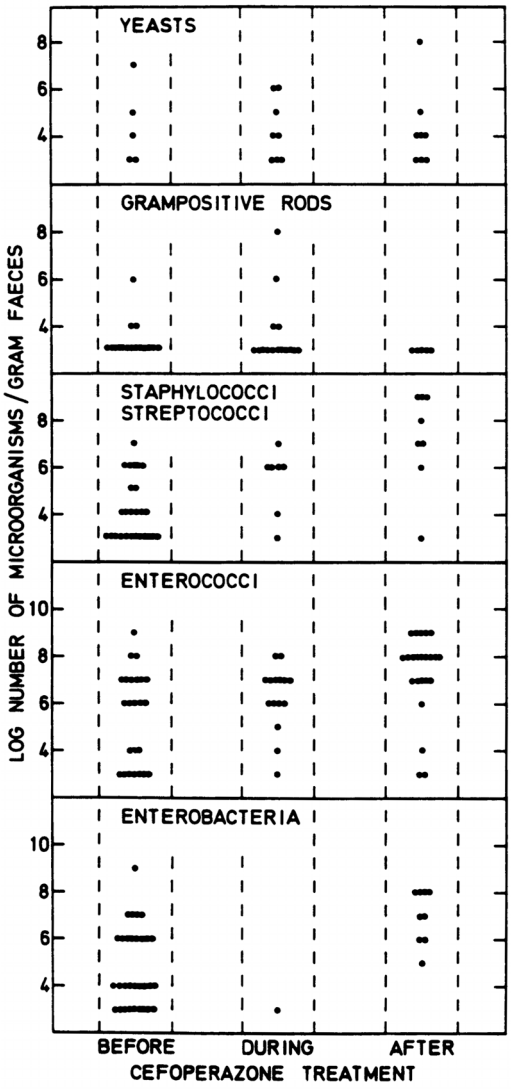

Figure 9: Impact of cefoperazone on the aerobic intestinal microflora in 29 patients. The numbers of microorganisms are given in log numbers per gram faeces.

and bacteroides during the treatment period, while the numbers of Gram-positive rods were not influenced by the imipenem therapy. After treatment the anaerobic microflora returned to normal in all patients.

Concentration of antimicrobial agents in faeces

Table 1 shows the faecal concentrations of aztreonam, norfloxacin, clindamycin, metronidazole, cefoperazone and imipenem before, during and after treatment with respective agent. As can be seen from the table, very high concentrations of cefoperazone were obtained while high norfloxacin concentrations were noticed. Moderate concentrations of aztreonam and clindamycin were demonstrated in faeces. Imipenem and metronidazole could not be detected by the microbiological test.

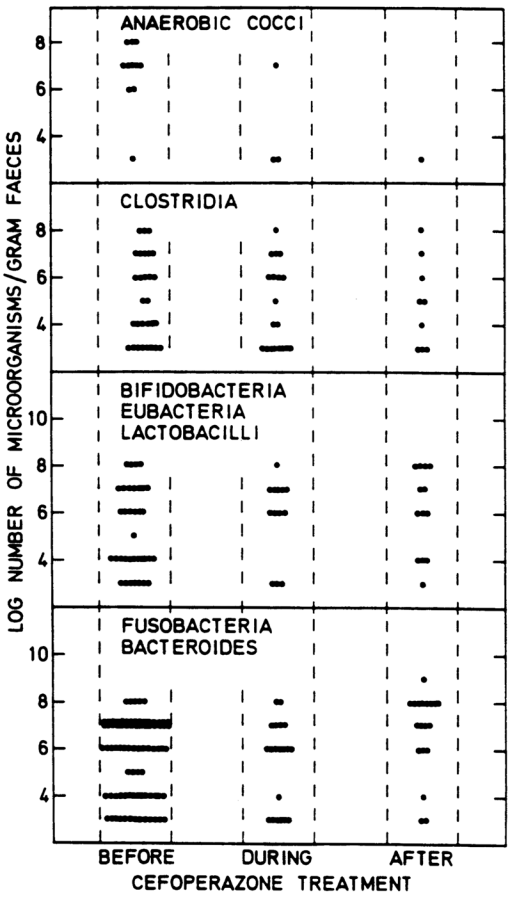

Figure 10: Impact of cefoperazone on the anaerobic intestinal microflora in 29 patients. The numbers of microorganisms are given in log numbers per gram faeces.

DISCUSSION

It has become evident with the introduction of broad spectrum antimicrobial agents that their suppressive activities are directed not only against invading pathogenic microorganisms but also against the host's normal microflora (*Nord* et al., 1986). The changes in the intestinal microflora may result in overgrowth of bacteria and yeasts, proliferation of antimicrobial resistant organisms and increased susceptibility to colonization by new microorganisms (*van der Waaij*, 1982). The knowledge of antimicrobial impacts on the intestinal microflora is especially important in neutropenic and intensive care unit patients in whom the concept of colonization resistance has become a major issue (*Young*, 1989).

In the patients treated with aztreonam, the numbers of enterococci and staphylococci increased. These findings can have clinical implications since enterococcal superinfections during aztreonam treatment have been reported (*Chandrasekar* et al., 1984). The other narrow spectrum antiaerobic agent - norfloxacin - did not cause any significant changes in the aerobic Gram-positive microflora despite high faecal con-

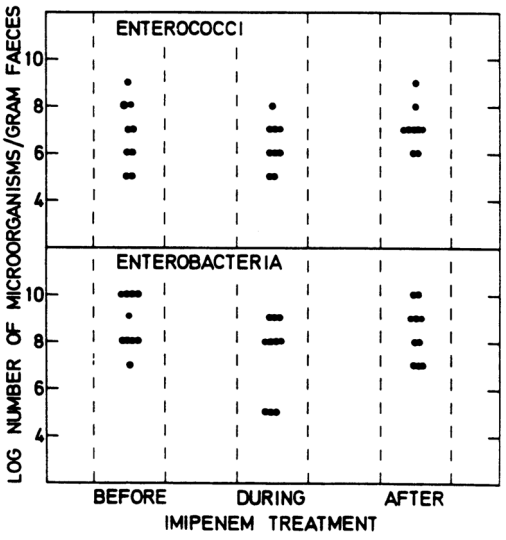

Figure 11: Impact of imipenem on the aerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

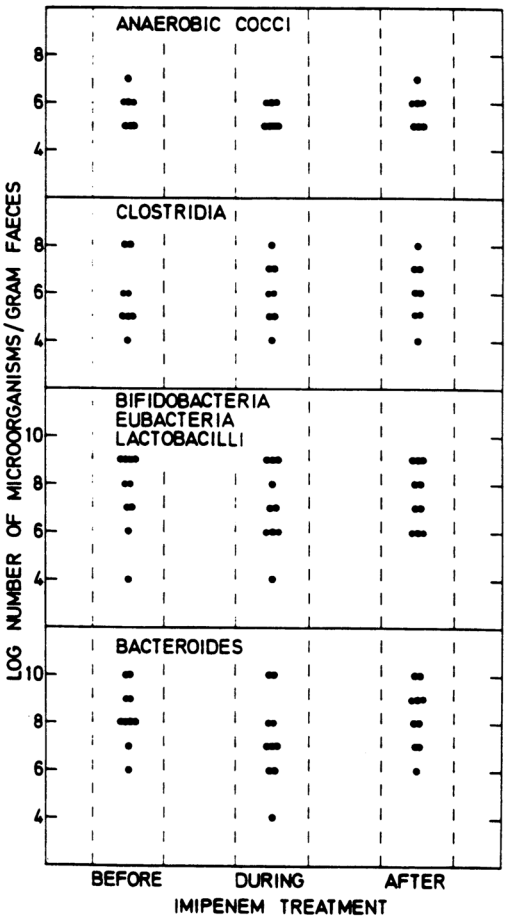

Figure 12: Impact of imipenem on the anaerobic intestinal microflora in 10 patients. The numbers of microorganisms are given in log numbers per gram faeces.

Table 1: Concentrations of aztreonam, norfloxacin, clindamycin, metronidazole, cefoperazone and imipenem, respectively, in faeces in 79 patients (The regimens and dosages are given in Material and Methods)

antimicrobial agent	before treatment		during treatment		after treatment	
	mean value (mg/kg faeces)	range (mg/kg faeces)	mean value (mg/kg faeces)	range (mg/kg faeces)	mean value (mg/kg faeces)	range (mg/kg faeces)
Aztreonam	ND[1]	ND	73	21-88	ND	ND
Norfloxacin	ND	ND	915	305-1900	ND	ND
Clindamycin	ND	ND	110	64-140	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND
Cefoperazone	ND	ND	4300	2100-7800	ND	ND
Imipenem	ND	ND	ND	ND	ND	ND

[1]ND = not detected

centrations. It has recently been shown that norfloxacin binds to faeces which may explain together with an inoculum effect, the paradox of high faecal concentrations of norfloxacin versus the effect on the intestinal microflora (*Edlund* et al., 1988). Thus different antimicrobial agents with narrow antiaerobic spectra can have different ecological impacts on the intestinal microflora.

Clindamycin caused considerable changes in the intestinal microflora due to the high concentration of the agent in the lower intestinal tract. The clinical implication of this finding is well known: *Clostridium difficile* diarrhoea/colitis. Only minor changes in the intestinal microflora occurred in those patients treated with metronidazole. No measurable concentrations of metronidazole could be demonstrated which explains the actual impact on the intestinal microflora. Thus two agents with similar narrow anti-anaerobic spectra can induce different ecological changes in the intestinal microflora.

Cefoperazone and imipenem have broad antimicrobial spectra including both aerobic and anaerobic intestinal microorganisms. However, only cefoperazone treatment was associated with major changes in the intestinal microflora. Cefoperazone is to a large extent excreted unchanged through the bile to the intestine while less than 1% of imipenem is found in the faeces. Thus two broad spectrum antimicrobial agents can have different ecological impacts on the intestinal microflora.

It has often been stated that narrow antimicrobial agents should always be used in preference to broad spectrum antimicrobial agents in order to avoid these ecological problems. This statement is an oversimplification and other factors such as mode of excretion, activity, inactivation and development of resistance must also be considered. These ecological impacts are often difficult to predict when antimicrobial agents are developed, and the clinical studies of new agents should always include an investigation of their effects on the intestinal microflora.

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EFFECT OF ANTIMICROBIAL DRUGS ON THE INTESTINAL MICROFLORA: IMPORTANCE OF PHARMACOKINETIC PROPERTIES OF ANTIBACTERIAL AGENTS

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In order to exert its therapeutic activity, antibacterial agents must reach the site(s) of infection at concentrations that are adequate in order to inhibit the multiplication of the bacteria or - ideally - cause kill of the bacteria in the case of potentially bactericidal agents. At the same time, however, it is desirable that the activity against the microbes in the normal microflora on the skin, mucosal surfaces and, in particular, inside the gastrointestinal canal is left as much as possible undisturbed. The major concern for the intestinal microflora is due to the relatively high number of bacteria there. Thus more than 90% of all the bacteria colonizing the body are found inside the intestines - and 99% of these residing within the colon. Consequently, if we focus on the interaction between an antibacterial drug and the colonic flora, then we know what is happening with nearly all bacteria of any consequence for the well being of the patient. If the colonic flora is left intact, then loose stools and diarrhoea are avoided - and in turn e.g. an occurrence like pseudomembranous diarrhoea due to *Clostridium difficile*.

The importance of pharmacokinetic properties in the context of this conference, i.e. the ability of antibiotics to interfere with the normal microflora (the ecological friendliness of these drugs, as it were) thus boils primarily down to the question of what characteristics are theoretically favourable. In the second event we are concerned with the practical implications, i.e. the ecological favourability of the individual drugs.

It is essential that the concentrations of the antibiotic are below the minimum inhibitory concentrations of the major portion of the normal microflora. This means that the levels should be low in sweat, in saliva and in nasal secretion. The concentrations should also be low inside the intestinal contents; consequently, the antibacterial levels must be low in key secretions like the bile, pancreatic juice and the various secretions of intestinal mucosal glands. These properties can be studied in detail after parenteral administration, although data pertaining to the intestinal glands and pancreatic juice are virtually nil in humans. Biopharmaceutic properties are vital for oral application; this implies that the bioavailability must be high such that one avoids the possibility that most of the dose of a drug is not absorbed and, accordingly, simply transferred to the lower gastrointestinal tract to exert its antibacterial activity there. If a drug is eliminated in high amounts in e.g. the bile, then quantitatively high reabsorption is required in the duodenum and the upper portion of the small intestine in order to achieve low concentrations of the drug in the lower portion of the gastrointestinal tract.

The condition of high bioavailability may, however, not be an important point, since high amounts may be discharged into the faeces. It has, for instance, recently been demonstrated with

ciprofloxacin that this compound, which has a high bioavailability - in the case of ciprofloxacin up to 85% bioavailability has been demonstrated (*Bergan* et al., 1987) - may be eliminated by the mechanism labelled transintestinal elimination (*Rohwedder* et al., 1990). Thereby, the compound is eliminated in significant amounts by passage across the intestinal wall. Transintestinal elimination of ciprofloxacin has been shown to cause very high concentrations of a multiple of up to 100-2000 times the peak serum concentrations inside the colonic contents. In the case of ciprofloxacin, less than 1% of the compound is eliminated in the bile, but 15% by faeces even after intravenous administration (*Rohwedder* et al., 1990).

In cases when large amounts of an antibiotic reach the lower gastrointestinal tract, three mechanisms may explain the lack of a significant interaction with the intestinal microflora. One is binding to intestinal contents. This has been demonstrated to occur for ciprofloxacin (*Edlund* et al., 1988). The second is enzymatic inactivation. This is a possible mechanism explaining why the moderate but potentially sufficient amounts to inhibit portions of the intestinal flora after ampicillin esters like bacampicillin or pivampicillin usually have no deleterious effect on the microflora (*Sjövall* et al., 1986). A third circumstance inhibiting the activity of drugs, which need a high redox potential to act, is anaerobic conditions. Thus the low redox potential of the lower colon will limit the effect of aminoglycosides and fluoroquinolones against aerobic bacteria although they would be inhibited under aerobic conditions.

A series of studies on the interaction between the normal bacterial microflora and antibacterial agents given in therapeutic doses over a period of ca 1 week have shown that some drugs are favourable and some less so to the microbial environment (*Nord*, 1988; *Nord* et al., 1986). Thus three categories of antibacterial agents can be distinguished: Ecologically favourable, ecologically unfavourable, and ecologically uncertain (Table 1)

Characteristic of the Group I substances is that less than 1% of the bacteria of the colonic microflora is modified quantitatively. The explanation is due to a combination of the intrinsic antibacterial activity and the pharmacokinetic properties of the drugs. Thus benzylpenicillin is given parenterally and less than 1% eliminated in the bile and the faecal concentrations are low. This combined with a narrow antibacterial spectrum and enzymatic inactivation due to a high susceptibility to beta-lactamase produced by the *Bacteroides* and other bacteria reduce the concentrations to levels unable to exert any significant influence on the intestinal bacteria.

Table 1: Different groups of antimicrobial agents with regard to their interaction with the normal bacterial microflora

group I ecologically favourable	group II ecologically unfavourable	group III ecologically uncertain
benzylpenicillin	piperacillin and other ureidopenicillins	trimethoprim-sulphonamide
imipenem	cephalosporins (varying degrees)	aminoglycosides
fluorinated quinolones	tetracyclines, also doxycycline	
ampicillin prodrugs like bacampicillin	clindamycin, lincomycin	
metronidazole	erythromycin (and other macrolides)	

Table 2: Relationship between drug excretion in bile and rate of diarrhoeas (*Bergan*, 1986)

Antibacterial agent	Biliary excretion of dose (%)	Rate of diarrhoeas (%)
Ampicillin	<1	10
Benzylpenicillin	<1	5
Cloxacillin	5	15
Carbenicillin	2	8
Cefaclor	4	5
Cefoperazone	70	24
Cefoxitin	<2	<2
Ceftazidime	<1	<2
Ceftriaxone	30	28
Cephalothin	2	4
Cephalexin	<5	11
Chloramphenicol	<1	4
Ciprofloxacin	<5	<2
Clindamycin	10	21
Co-trimoxazole	<1	10
Doxycycline	4-20	12
Erythromycin orally (not microencapsulated)	<5	22
Erythromycin estolate	5	17
Gentamicin	<1	4
Kanamycin	<1	4
Nalidixic acid	-	7
Nitrofurantoin	-	12
Norfloxacin	<1	<2
Ofloxacin	<1	<2
Phenoxymethylpenicillin	<1	5
Rifampicin	25	11
Sulphonamides	<1	8
Tetracyclines	>10	15

Prodrugs of ampicillin like bacampicillin are generally well absorbed. Thus bacampicillin has a bioavailability of 87% (*Bergan*, 1978). Thereby the amount of drug reaching the lower gastrointestinal tract is limited. Biliary concentrations are low and ampicillin is subject to reabsorption. Indeed, the amounts that proceed towards the lower colon would to a considerable extent be enzymatically inactivated. Accordingly, the rate of diarrhoea after bacampicillin is 0.7% compared to 12% after the classic, oral ampicillin (*Bergan*, 1979).

The fluorinated quinolones are reducing the numbers of aerobic Gram-negative rods, but otherwise leave the Gram-positive aerobes virtually unchanged and the anaerobes quantitatively unchanged. This occurs in spite of very considerable faecal quinolone concentrations. However, it appears that the major portion, more than 95% of the ciprofloxacin, is bound to faecal contents and thus not freely available as antibacterially active drug (*Edlund* et al., 1988).

Imipenem is uniquely active and has a broad antibacterial spectrum. Thus it is difficult to predict why the drug is unusually well tolerated and leaves the intestinal microflora virtually unchanged.

It is notable that the compound is eliminated in less than 1% in the bile. However, the drug is not detected in faeces due to enzymatic hydrolysis.

Among the ecologically unfavourable drugs are macrolides and the like, such as erythromycin and clindamycin. Both of these drugs have a relatively narrow antibacterial spectrum. This applies in particular to erythromycin. However, erythromycin is eliminated in high amounts in the bile and is subject to enterohepatic circulation and appears in very high concentrations in faeces (*Josefsson* et al., 1982). Similar considerations apply to clindamycin, which has a high activity against anaerobic bacteria. Both of these antibiotics are mainly without effect on *Clostridium difficile*, which is, consequently, selected by these drugs. The result is frequent loose stools and - in the case of selective overgrowth of the *Cl. difficile* in some patients - development of pseudomembranous colitis due to the activity of clostridial cytotoxins.

Piperacillin and certain cephalosporins are eliminated in considerable amounts in the bile. There seems to be a rough proportional correlation between the amount of the drugs eliminated in the bile, and their antibacterial activity, and the ability of the drugs to induce changes in the intestinal microflora and consequent loose stools and diarrhoea (Table 2).

Some compounds, such as trimethoprim-sulphonamide and aminoglycosides are classified as ecologically uncertain (group III), because these compounds have not yet been well studied in relation to interaction with the normal microflora. Aminoglycosides rarely cause loose stools or pseudomembranous colitis. This obviously is explained by its lack of activity against the anaerobic bacteria which constitute more than 99% of the colonic flora and the fact that aminoglycosides loose their activity at a low redox potential as applies to the colon. Trimethoprim-sulphonamide combinations are known to cause pseudomembranous colitis and are associated with R-factors, so adverse effects on the balance between the constituents of the faecal microflora can be presumed.

Our experience has shown that studies on the quantitative and qualitative effects of antibacterial agents in particular on the colonic microflora are vital and that knowledge of the pharmacokinetic properties of the drugs contributes to explaining why some compounds interact with the normal microflora and others leave it virtually unchanged.

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EFFECT OF ANTIMICROBIAL DRUGS ON THE INTESTINAL MICROFLORA: CRITERIA FOR THEIR USEFULNESS FOR SELECTIVE DECONTAMINATION IN THE NEUTROPENIC PATIENT

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INTRODUCTION

Without prophylactic measures, neutropenic patients have a high chance to develop an infection, increasing with the depth and the duration of granulocytopenia (*Bodey* et al., 1966). Most infections in the granulocytopenic patient are caused by enteric bacteria, mainly Enterobacteriaceae- and Pseudomonadaceae-species (*Schimpff* et al., 1972; *Levine* et al., 1973; *Bodey* et al., 1978). In the past, the most frequently isolated Gram-positive microorganism was *Staphylococcus aureus*. However, there is an increasing number of infections caused by coagulase negative staphylococci and viridans streptococci (*Wade* et al., 1982; *Winston* et al., 1983; *Dekker* et al., 1987; *Kern* et al., 1987; *Peters* et al., 1988). The percentage of infections caused by anaerobes was and is still small, in the order of 1% of the total. As infections in granulocytopenic patients have a high mortality rate, infection prevention is worthwhile to be performed. This has been attempted by either total or selective decontamination. Total decontamination (TD) has the disadvantage of the need of strict isolation of the patient, who in addition should take sterile food and beverages. A disadvantage of TD is the elimination of microorganisms responsible for the Colonization Resistance (CR) (*van der Waaij* et al., 1971; *van der Waaij*, 1982a).

COLONIZATION RESISTANCE AND SELECTIVE DECONTAMINATION

The CR functions as a barrier against exogenous bacteria trying to colonize a certain tract. In the gastrointestinal tract especially the anaerobic bacteria play a major role in maintaining the CR. The majority of the infections in granulocytopenic patients is caused by aerobic bacteria, belonging to the normal gut flora. Elimination of these bacteria without affecting the CR is called selective decontamination of the digestive tract (SD). This method appeared to be efficacious in reduction of the infection frequency in granulocytopenic patients (*Sleijfer* et al., 1980; *Dekker* et al., 1981). Selective decontamination proved to be as efficacious as total decontamination in preventing Gram-negative infections, even without strict isolation procedures (*Kurrle* et al., 1986). SD is cheaper, less laborious and more comfortable for the patient as this method can be performed without strict isolation procedures.

ANTIMICROBIAL AGENTS FOR SELECTIVE DECONTAMINATION

SD can only be performed with antimicrobial agents (AMA) selected on the basis of their effect on the gastrointestinal microflora. An active concentration of the SD-drugs must reach the gastrointestinal tract (the site of action); this amount of SD-drugs must be:

a. not suppressive to the anaerobic CR-related microflora;

b. able to kill one or more of the potentially pathogenic microorganisms (PPMO), most frequently encountered in infections in granulocytopenic patients. These consists of aerobic Gram-negative bacilli as Enterobacteriaceae- and Pseudomonadaceae-species, *Staphylococcus aureus*, yeasts and fungi, coagulase negative staphylococci, and viridans streptococci.

Up to now there are no AMA which are active against coagulase negative staphylococci and viridans streptococci without affecting anaerobes. Yeasts and fungi can be eliminated by the polyenes amphotericin B and nystatin. Both antimycotics are not absorbed after oral administration and have no effect on bacteria.

Elimination of *S. aureus* from the digestive tract appeared to be possible with the absorbable drugs cephradine and co-trimoxazole. Aerobic enteric Gram-negative bacilli except *Proteus*-, *Morganella*-, and *Serratia*-species are highly susceptible to polymyxins, while anaerobes are resistant. Like the polyenes, polymyxin is not absorbed after oral administration. Polymyxin appeared an ideal drug for SD: the effect, elimination of susceptible Gram-negative bacilli is usually reached within three days after oral administration (*de Vries-Hospers* et al., 1981). The only disadvantage of polymyxin is the gap in the antibacterial spectrum. Fortunately, a number of absorbable antibiotics have been shown to be useful for SD. Elimination of Gram-negative bacilli from the digestive tract can be performed by oral administration of co-trimoxazole and nalidixic acid (*de Vries-Hospers* et al., 1981). Later on it was found that in volunteers the same effect could be obtained with the newer quinolones like norfloxacin and ciprofloxacin (*Pecquet* et al., 1986; *de Vries-Hospers* et al., 1987). Administration of these quinolones to neutropenic patients resulted in a reduction of the number of infections caused by Gram-negative bacilli (*Rozenberg-Arska* et al., 1985; *Winston* et al., 1986; *Karp* et al., 1987). Temocillin and aztreonam are β-lactam antibiotics with a small spectrum of activity against aerobic Gram-negative bacilli; both can be used for elimination of these bacteria from the digestive tract (*de Vries-Hospers* et al., 1984; *de Vries-Hospers* et al., 1985).

As mentioned above, anaerobic bacteria are involved in maintaining the CR. The animal experiments especially antimicrobial agents (AMA) with activity against Gram-positive bacteria, such as bacitracin and penicillin, appear to disrupt the CR significantly (*Wiegersma* et al., 1982). On the other hand, AMA like polymyxin, temocillin and aztreonam, which are only active against aerobic Gram-negative bacilli, appeared useful for SD, however, not in all cases: when aztreonam was administered orally to ten volunteers, elimination of aerobic Gram-negative bacilli was reached in eight of them with all three dosages tested (*de Vries-Hospers* et al., 1984). In the two volunteers who could not be decontaminated, aztreonam susceptible Gram-negative bacilli remained present in the faecal cultures during treatment with aztreonam. There was no measurable concentration of aztreonam in the faeces of these volun-

teers. The same phenomenon was found in volunteers who were treated with temocillin intramuscularly (*de Vries-Hospers* et al., 1985).

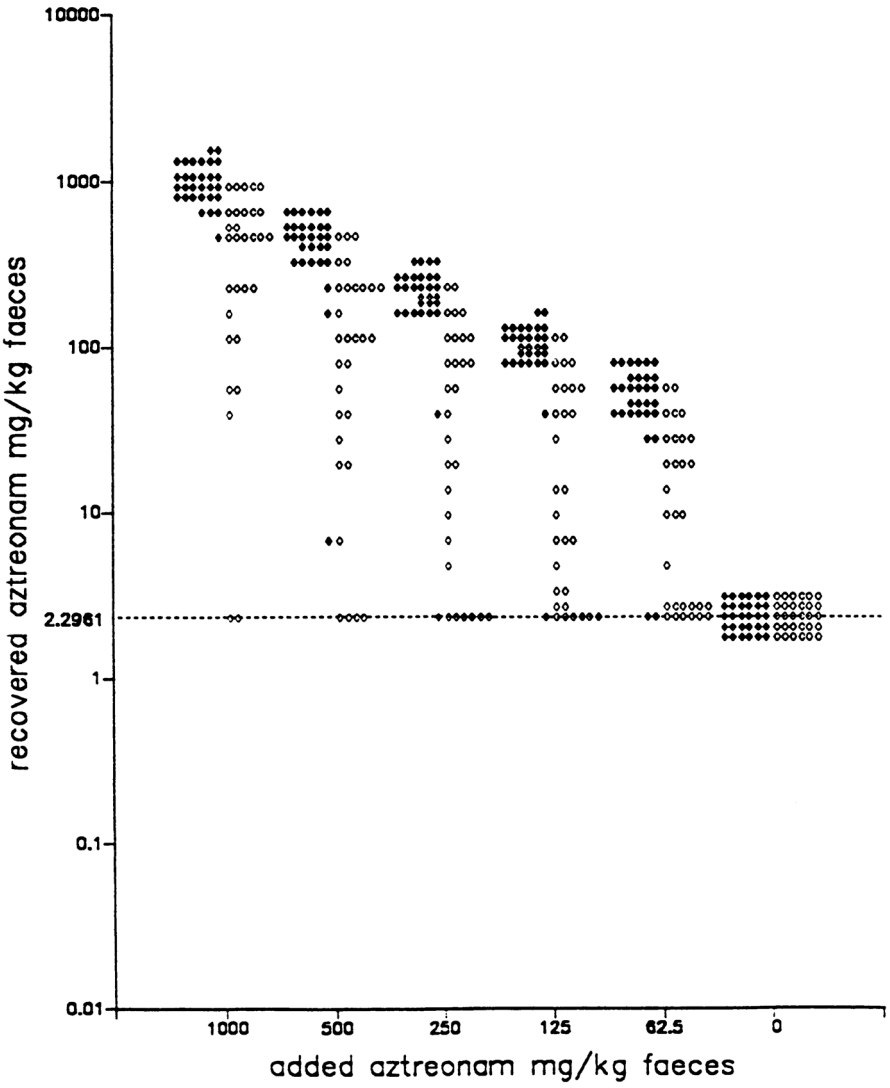

Figure 1: Recovery of microbiological active aztreonam in faecal samples derived from 30 healthy volunteers and mixed with different amounts of aztreonam. The concentration was determined either immediately after mixing (✦) or after 24 hours (✧) of incubation at 37°C.

INFLUENCE OF FAECES ON BIOLOGICAL ACTIVITY OF AZTREONAM AND TEMOCILLIN

To investigate why some of the volunteers treated with aztreonam or temocillin did not respond to antimicrobial treatment, another experiment was performed. Fresh faecal samples were collected from 30 healthy volunteers and

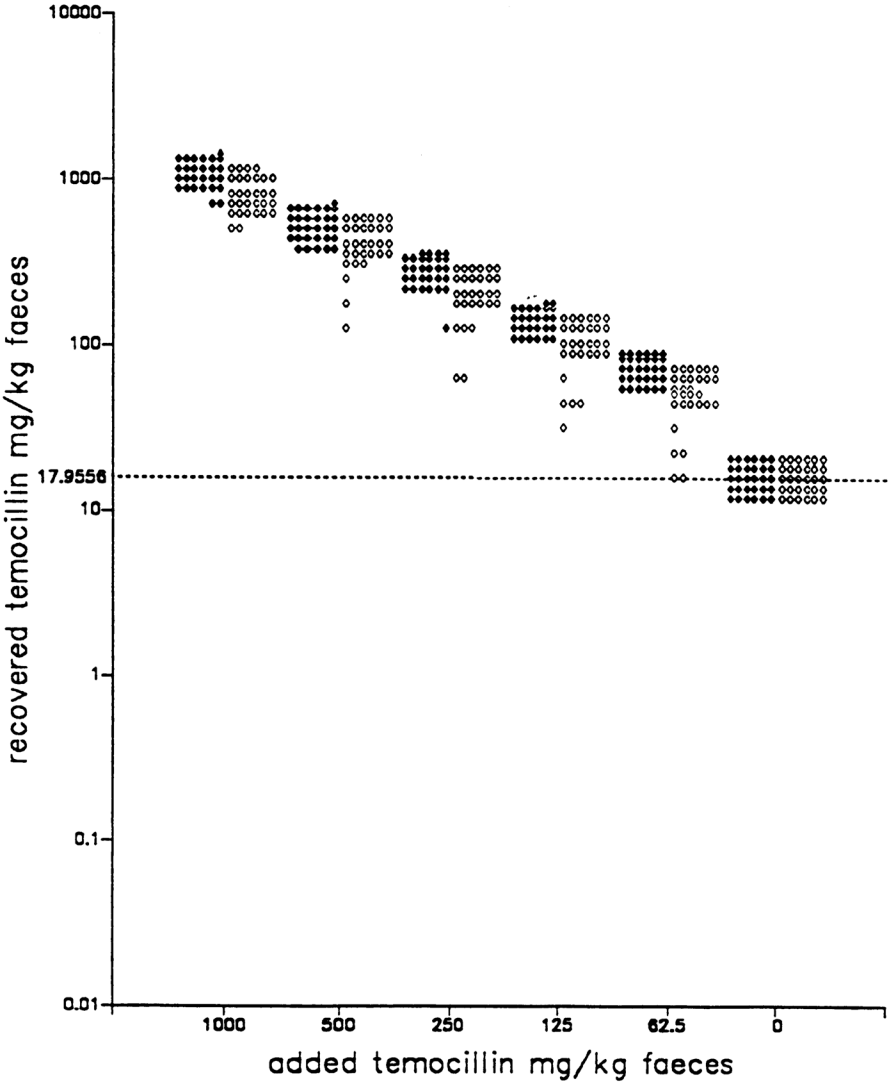

Figure 2: Recovery of microbiological active temocillin in faecal samples derived from 30 healthy volunteers and mixed with different amounts of temocillin. The concentration was determined either immediately after mixing (✦) or after 24 hours (✧) of incubation at 37°C.

mixed with different amounts of the antibiotics: 1000, 500, 250, 125, 62.5 and 0 mg/kg faeces. This mixtures were either immediately centrifuged or after 24 hours of incubation at 37°C. Then the microbiological active amounts of the antibiotics were determined in the faecal supernatants. The results are shown in Figures 1 and 2. It appeared that faeces had more influence on the microbiological activity of aztreonam than on that of temocillin, although there were interindividual differences. In some of the faecal samples, mixed with aztreonam, no microbiological activity was found after 24 hours of incubation with aztreonam (Figure 1). This was also the case in a few samples when the concentration of aztreonam was determined immediately after

mixing. This inactivation of aztreonam and other AMA in the presence of faeces may be due to (ir)reversible binding of antibiotics to faecal material. Another mechanism may be degradation of antibiotics by bacterial enzymes present in faeces, like has been described for aztreonam (*Welling* et al., 1987).

In conclusion: apart from the spectrum of antimicrobial activity and the pharmacokinetic properties of AMA, the influence of faeces on the microbiological activity of an antimicrobial agent must be taken into account, when AMA are screened for their usefulness for SD. When faeces renders AMA inactive, they cannot be used for SD or may be only when applied in relatively high dosages.

To predict the effect of AMA on the CR, and the usefulness for SD, a number of properties of the drug should be known:

a. the spectrum of antimicrobial activity;
b. pharmacokinetic properties;
c. the influence of intestinal contents on the activity of the AMA.

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APPLICATION OF FINDINGS IN EXPERIMENTAL CANCER THERAPY: THE EFFECT OF ANTIBIOTICS WHICH REACH THE DIGESTIVE TRACT IN SUPPRESSIVE CONCENTRATIONS.

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In literature the influence of antibiotics on the immune system is already well documented (*Gillissen*, 1982; *Hoeprich* and *Martin*, 1970; *Mandel*, 1982; *Thong* and *Ferrante*, 1980). Most investigations performed in this field deal only with antimicrobial immune mechanisms. The immune system, however, plays also an important role in the development and spread of neoplastic diseases. Tumour related immunosuppression and immunosuppressive effects of antitumour treatment obviously are responsible for the high incidence of infectious diseases in cancer patients. This, apparently, is the reason for the high frequency and prolonged duration of antimicrobial treatment in patients with malignancies. Accordingly, it seems to be reasonable to speculate that antimicrobial therapy may influence neoplastic processes. Limited information only is available concerning the influence of antibiotics on antitumour immunity. However, there are convincing data about considerable effects of some antimicrobial drugs on cells which are involved in antibacterial and antitumour immunity (*Kasamaki* et al., 19979; *Mandel*, 1982; *Thong* and *Ferrante*, 1980).

The aim of the present study was to investigate the influence of twelve antibiotics (from different pharmacological groups, commonly used in human therapy) on the behaviour of experimental tumours. As a model of experimental neoplastic disease sarcoma L-I and BALB/c-mice were chosen. This tumour arouse spontaneously in the lung

Table 1: Effect of antibiotic pretreatment on local sarcoma L-1 growth and on the survival of mice

Antibiotic used	Tumour weight in g		Survival time in days	
	mean value ± SD	% of control	median	% of control
Control	0.63 ± 0.10	100	39	100
Penicillin G	0.67 ± 0.12	106	37	95
Piperacillin	0.81 ± 0.14	129	35	90
Mezlocillin	0.92 ± 0.13[1]	146	26[1]	67
Cephalotin	0.58 ± 0.09	92	42	108
Cefamandole	0.60 ± 0.11	95	38	97
Cefotaxime	0.56 ± 0.09	89	40	103

[1]$p<0.01$

Table 2: Effect of antibiotic pretreatment on local sarcoma L-1 growth and on the survival of mice

Antibiotic used	Tumour weight in g		Survival time in days	
	mean value ± SD	% of control	median	% of control
Control	0.74 ± 0.12	100	37	100
Gentamicin	0.78 ± 0.16	105	34	92
Amikacin	0.81 ± 0.17	109	32	86
Streptomycin	0.79 ± 0.14	106	34	92
Rifampicin	1.20 ± 0.24[1]	162	29	78
Doxycycline	1.44 ± 0.23[1]	194	26[1]	70
Clindamycin	0.78 ± 0.11	105	38	103

[1]$p<0.01$

of a BALB/c-mouse and was maintained in this strain of mice by subcutaneous transplantation. The characteristics of this tumour were extensively described by *Janik* et al. (1980). For certain studies tumour bearing animals were treated with antimicrobial drugs for ten consecutive days. The dosages of drugs were equivalent to those applied in severe infections in human medicine and they were calculated on a dose per kg body weight basis. *In vitro*, the lowest concentrations of antibiotics added to cell cultures were equivalent to serum concentrations achieved in human medicine after administration of maximal therapeutical dosages.

In the course of investigation it was observed that four out of twelve antibiotics tested significantly modified the

Table 3: Effect of antibiotic pretreatment on artificial metastatic spread after intravenous administration of sarcoma L-1 cells

Antibiotic used	Number of lung colonies	
	mean value ± SD	% of control
Control	32.0 ± 6.3	100
Penicillin G	36.1 ± 5.1	113
Piperacillin	48.9 ± 5.8[1]	160
Mezlocillin	63.4 ± 10.3[1]	198
Cephalotin	29.3 ± 8.9	92
Cefamandole	38.6 ± 10.2	121
Cefotaxime	34.2 ± 8.6	107
Gentamicin	39.2 ± 7.5	122
Amikacin	37.3 ± 6.9	117
Streptomycin	34.6 ± 8.8	108
Rifampicin	58.6 ± 11.2[1]	183
Doxycycline	72.3 ± 12.8[1]	226
Clindamycin	37.1 ± 8.2	116

[1]$p<0.01$

Table 4: Influence of antibiotic treatment on early growth of sarcoma L-1 tumour

Antibiotic used	Tumour weight in g		Survival time in days	
	mean value ± SD	% of control	median	% of control
Control	0.57 ± 0.09	100	35	100
Penicillin G	0.59 ± 0.10	104	34	97
Piperacillin	0.62 ± 0.11	109	36	103
Mezlocillin	0.31 ± 0.06[1]	54	46[1]	131
Cephalotin	0.61 ± 0.12	107	33	94
Cefamandole	0.48 ± 0.11	84	38	109
Cefotaxime	0.51 ± 0.12	89	38	109

[1]p<0.01

growth of transplantable mouse tumour L-I (Table I, 2, 3, 4, 5; Figure l). Mezlocillin, doxycycline and rifampicin could be shown to exert considerable influence on subcutaneous growth of sarcoma L-I tumour and on the number of lung metastases whereas piperacillin exclusively modified the artificial metastatic spread induced by intravenous injection of L-I tumour cells into BALB/c-mice (Table 3).

Administration of two antibiotics (doxycycline and rifampicin) could be shown to increase the tumour growth when the treatment was performed before respectively after tumour cell implantation (Table 2, 5). Both antibiotics did not influence the proliferation of sarcoma L-I cells under *in vitro* conditions (data not presented). *Bassi* et al. (1973) found that rifampicin inhibited the proliferation rate of leukaemia L-1210 cells *in vitro*. However, it is important to mention that these authors used rifampicin concentrations of 100 mcg/ml which were evidently higher than maximal serum concentration that can be achieved *in vivo*. It seems to be most likely that the modulation of tumour growth by rifampicin and doxycycline is rather related to indirect regulatory mechanisms than to direct interaction with the tumour cells. Both, rifampicin and doxycycline could be shown to exert a suppressive effect on cellular immunity which was statisti-

Table 5: Influence of antibiotic treatment on early growth of sarcoma L-1 tumour

Antibiotic used	Tumour weight in g		Survival time in days	
	mean value ± SD	% of control	median	% of control
Control	0.71 ± 0.11	100	37	100
Gentamicin	0.63 ± 0.13	89	38	103
Amikacin	0.68 ± 0.09	96	32	86
Streptomycin	0.66 ± 0.10	93	38	103
Rifampicin	1.06 ± 0.18[1]	149	29	78
Doxycycline	1.21 ± 0.23[1]	170	28	76
Clindamycin	0.73 ± 0.12	103	36	97

[1]p<0.01

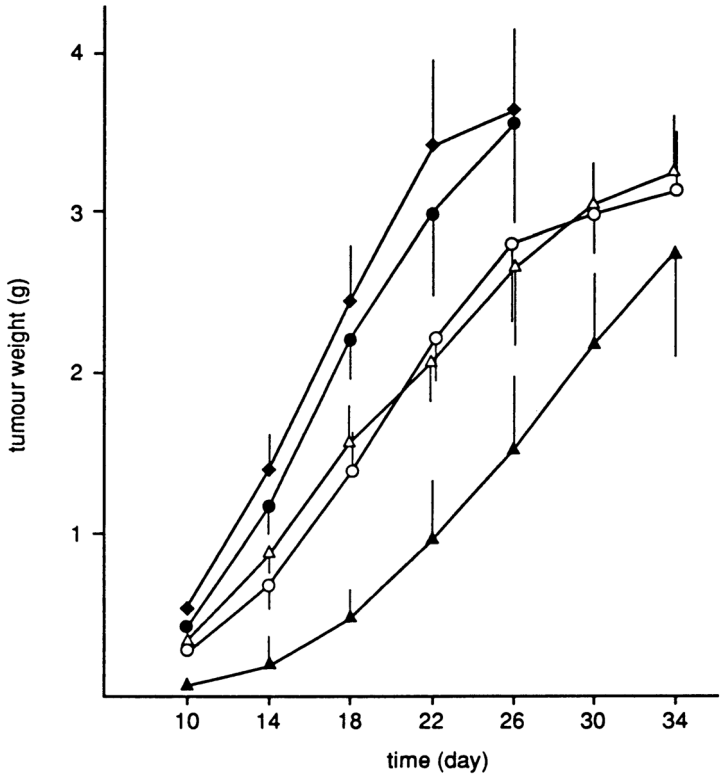

Figure 1: Influence of antibiotic treatment on sarcoma L-1 tumour growth in BALB/c-mice. (❍: control, ▲: mezlocillin, ●: rifampicin, ✦: doxycycline, △: piperacillin)

Table 6: Effect of antibiotics on delayed type skin hypersensitivity to oxazolone

Antibiotic used	Increase in ear thickness (in units of 10^{-3})	
	mean value ± SD	% of control
Control	16.7 ± 1.6	100
Penicillin G	17.2 ± 2.1	103
Piperacillin	10.6 ± 1.4	63
Mezlocillin	6.8 ± 1.7[1]	41
Cephalotin	12.4 ± 1.7	74
Cefamandole	13.1 ± 1.9	78
Cefotaxime	12.1 ± 1.8	72
Gentamicin	12.3 ± 2.2	74
Amikacin	10.8 ± 1.9	65
Streptomycin	16.4 ± 1.8	98
Rifampicin	10.2 ± 2.1	61
Doxycycline	8.8 ± 2.3[1]	53
Clindamycin	17.6 ± 2.2	105

[1]$p<0.01$

Table 7: Delayed type hypersensitivity to oxazolone in mice treated with antibiotics: Recovery (% of control)

Antibiotic used	% of control days after completing the treatment			
	8	12	16	20
Piperacillin	62	65	69	89
Mezlocillin	50[1]	60[1]	65	70
Cephalotin	76	87	97	93
Cefamandole	82	98	95	100
Cefotaxime	74	87	97	102
Gentamicin	89	92	99	96
Amikacin	83	102	99	98
Rifampicin	72	74	69	82
Doxycycline	54[1]	56[1]	58[1]	72

[1]$p<0.01$

cally significant (Table 6, 7, 8). Concerning experimental transplantable tumours the influence of cellular immunity on tumour growth and spread has been extensively proved. In the course of this study it also could be demonstrated that the development of tumour was inhibited in animals which were preimmunized with killed tumour cells (Figure 2). Both, rifampicin and doxycycline administered to preimmunized experimental animals diminished the beneficial immunological effect. BALB/c-mice which were preimmunized with killed sarcoma L-1 cells and submitted to rifampicin and doxycycline treatment showed a similar tumour growth and tumour burden as animals of the nonimmunized control group. Furthermore, in tumour bearing animals

Table 8: Proliferation of spleen lymphocytes from mice treated with antibiotics

Antibiotic used	cpm/10^6 cells (x 10^3) (mean value ± SD)	% of control
Control	28.6 ± 2.9	100
Penicillin G	26.2 ± 2.2	92
Piperacillin	13.2 ± 4.2	46
Mezlocillin	0.063 ± 0.028[1]	0
Cephalotin	16.6 ± 3.1[1]	58
Cefamandole	18.9 ± 2.4	66
Cefotaxime	14.6 ± 2.1[1]	51
Gentamicin	19.6 ± 3.1	68
Amikacin	21.3 ± 2.3	74
Streptomycin	26.3 ± 3.1	92
Rifampicin	10.7 ± 3.4[1]	37
Doxycycline	7.4 ± 2.2[1]	26
Clindamycin	32.8 ± 4.7	115

[1]$p<0.01$

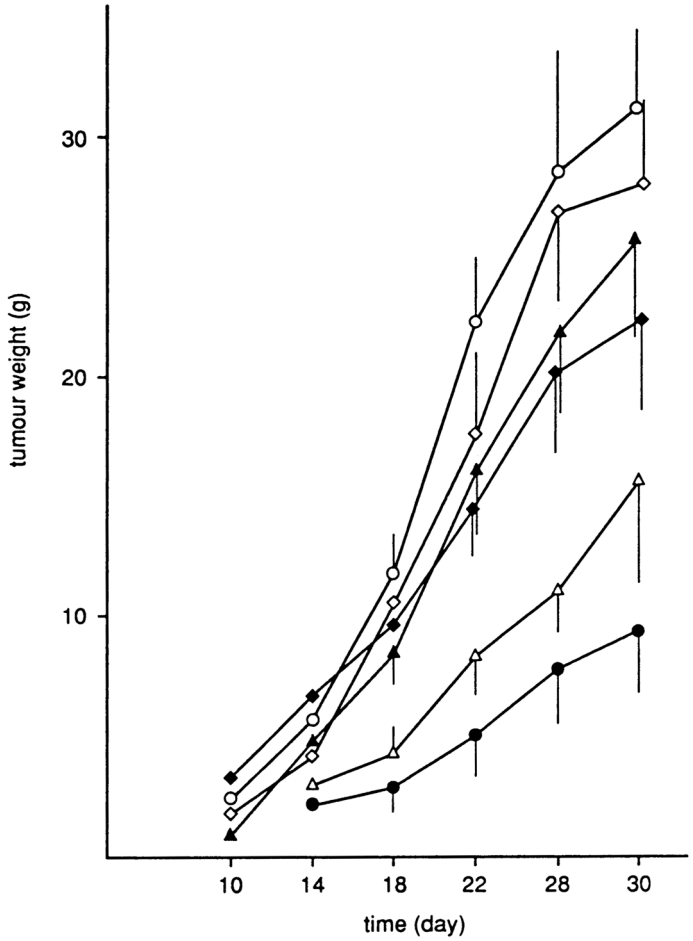

Figure 2: Influence of antibiotic treatment on sarcoma L-1 tumour growth in animals preimmunized with killed tumour cells.
(○: nonimmunized control, ●: immunized, ✧: immunized + doxycycline,
✦: immunized + mezlocillin, ▲: immunized + rifampicin, △: immunized + piperacillin)

treated with rifampicin and doxycycline an inhibition of lymphocyte specific cytotoxicity against sarcoma L-1 cells could be observed (Figure 3). As shown in Figure 5 natural killer (NK)-cell activity was well significantly decreased in animals treated with these antimicrobial drugs. The interpretation of these effects supports the hypothesis that both antibiotics influence tumour growth in an indirect way since, apparently, they influence the host. However, the possibility of a changed expression of tumour cells under the influence of certain antibiotics should also be kept in mind. Rifampicin and doxycycline induced immunosuppression was also observed by other investigators. Recently, *Thong* and *Ferrante* (1980) as well as *Forsgren* and *Banck* (1978) presented evidence that doxycycline inhibited the proliferation rate of mouse lymphocytes *in vitro*. Concerning rifampicine, a suppression of macrophage function was found in addition to the antiproliferative activity for lymphocytes (*Hoeprich* and *Martin*, 1970; *Rook*, 1982). *In vivo*, rifampicin

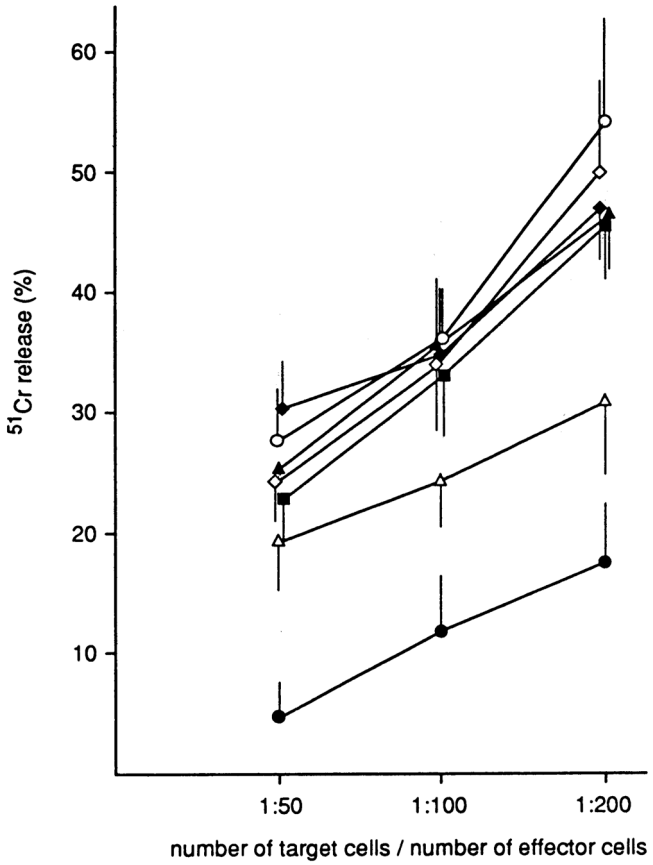

Figure 3: Influence of antibiotic treatment on specific cytotoxicity.
(○: control, ✦: clindamycin, ▲: streptomycin, ✧: gentamicin, ■: amikacin, △: rifampicin, ●: doxycycline)

induced immunosuppression could as well be demonstrated in investigations showing a prolongation of transplant survival in mice (*Serrou*, 1972). Immunosuppressive activity of rifampicin in human therapy has been a matter of discussion, too (*Nessi* et al., 1974).

Interestingly, immunosuppressive effects were not exclusively observed after administration of doxycycline and rifampicin. Inhibition of delayed skin hypersensitivity to oxazolone was also observed after administration of cefotaxime, cefalotin, cefamandol, gentamicin, amikacin, mezlocillin, and piperacillin (Table 6, 7). Furthermore, these antibiotics considerably decreased the proliferation of Con A stimulated lymphocytes (Table 8). However, administration of doxycycline, rifampicin, mezlocillin, and piperacillin induced a more pronounced and longer lasting immunosuppressive effect. These antibiotics (doxycycline, rifampicin, mezlocillin, piperacillin) were able to affect NK-cell activity and the specific cytotoxicity of spleen lymphocytes against sarcoma L-1 cells (Figures 3, 4, 5, 6). Contrary to rifampicin and doxycycline, piperacillin did not influence local tumour growth and the number of spontaneous metastases (Table 4). However, the number of lung metastases increased

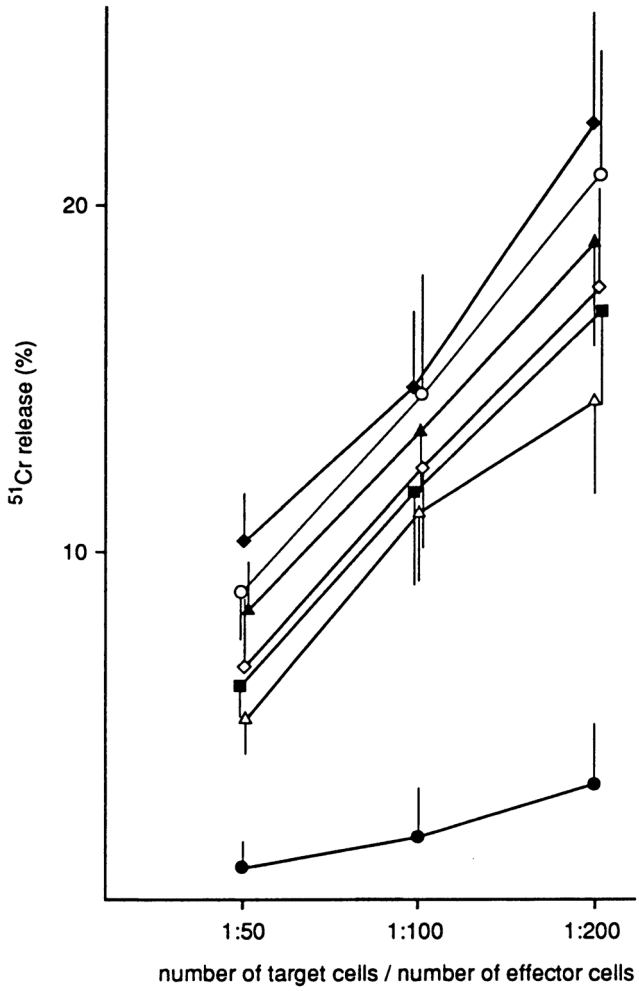

Figure 4: Influence of antibiotic treatment on spleen lymphocytes from tumour bearing animals against autologous tumour cells.
(❍: control, ✦: penicillin G, △: piperacillin, ■: cefotaxime, ✧: cefazolin, ▲: cefamandole, ●: mezlocillin)

when tumour cells were administered intravenously (Table 3).

As previously demonstrated by *Janik* (1977) the function of the immune system plays an important role in the lung colony test. In the delayed type skin hypersensitivity test to oxazolone, piperacillin could be shown to exert a significant immunosuppressive activity. However, its influence on the specific and non specific cytotoxicity was rather less pronounced as compared to rifampicin, doxycyclin, and mezlocillin (Figures 3, 4, 5, 6). These results apparently suggest that the lung colony assay is more sensitive than local tumour growth and spontaneous metastatic spread to check the suppression of antitumour immunity.

The effect of mezlocillin treatment on tumour development and spread in BALB/c-mice offered great variations according to the experimental schedule. Thus, enhancement of tumour growth

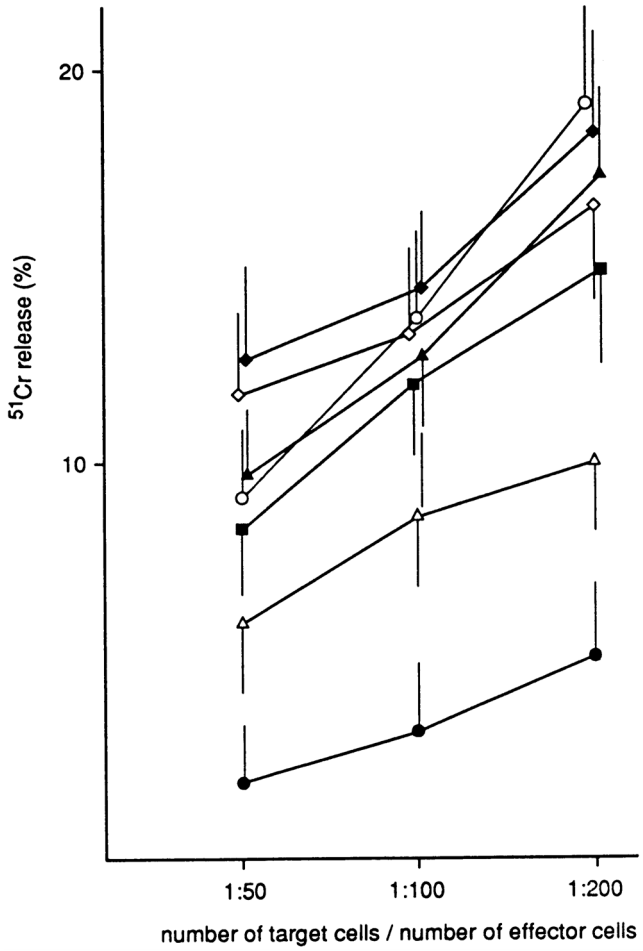

Figure 5: Influence of antibiotic treatment on non specific cytotoxicity.
(❍: control, ✦: clindamycin, ▲: streptomycin, ✧: gentamicin, ■: amikacin, △: rifampicin, ●: doxycycline)

was observed when mezlocillin was administered to animals prior to tumour implantation (Table l). However, application of mezlocillin immediately after tumour implantation manifested a totally opposite effect and resulted in a significant inhibition of tumour growth (Table 4). Enhancement of tumour growth after mezlocillin pretreatment might be the result of immunosuppressive activities of the drug. Administration of the antibiotic after tumour implantation may provide different possibilities and coexistence of at least two phenomena: suppression of host immune system respectively direct effect on tumour cells. Assuming that mezlocillin possesses a cytotoxic activity the resultant effect might be the inhibition of tumour growth. Such a phenomenon can be observed in conventional anticancer chemotherapy. Most of the cytostatic drugs induce a strong immunosuppression and a simultaneous antitumour effect. However, mezlocillin did not affect the growth behaviour of sarcoma L-1 cells *in vitro* when it was added to cell cultures (data not presented). This obviously is in contrast to the hypothesis that mezlocillin possesses cytostatic ac-

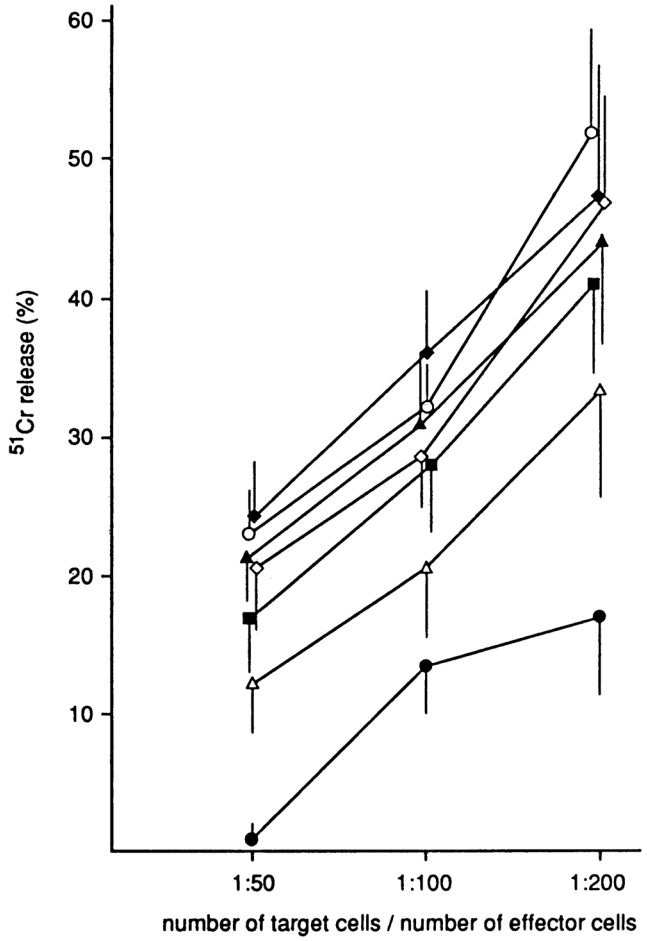

Figure 6: Influence of antibiotic treatment on spleen lymphocytes from C3H mice against YAC-1 cells.
(○: control, ✦: penicillin G, △: piperacillin, ■: cefotaxime, ✧: cephalotin, ▲: cefamandole, ●: mezlocillin)

tivity. It might be possible, however, that metabolites of the antibiotic, which eventually are produced *in vivo*, possess cytotoxic properties. On the other hand, mezlocillin may be able to alter the antigenicity of tumour cells and make them more susceptible to the host defense. However, the latter hypothesis could not be confirmed in experiments on specific antitumour immunity.

All considerations presented so far dealt with the effect of antibiotics on neoplastic tissues or host regulatory mechanisms but completely neglected their main pharmacological activity, the antibacterial effect. Recently, *Wiegersma* et al. (1982) tested twelve antimicrobial drugs and found that mezlocillin only was able to inhibit the growth of aerobic intestinal bacteria in mice considerably. In our studies it could as well be demonstrated that mezlocillin was the most effective antibiotic exerting the greatest influence on the endogenous intestinal flora (Table 9). When the drug was administered subcutaneously it eradicated most of the representative aerobic microorganisms from the diges-

Table 9: Influence of 10 day antibiotic treatment on endogenous intestinal flora and local sarcoma L-1 growth in BALB/c-mice

Antibiotic used	log number of bacteria/g of faeces (mean value ± SD)			tumour weight in g (mean value ± SD)
	Enterobacteriaceae	E. faecalis	S. viridans	
Control	4.9 ± 0.9	5.5 ± 1.2	5.2 ± 1.6	0.71 ± 0.14
Penicillin G	5.4 ± 1.3	3.2 ± 1.7	3.8 ± 1.4	0.74 ± 0.17
Piperacillin	3.5 ± 1.4	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.61 ± 0.16
Mezlocillin	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.35 ± 0.08[1]
Cephalotin	2.9 ± 1.7	4.3 ± 1.1	6.1 ± 1.7	0.68 ± 0.14
Cefamandole	3.3 ± 1.3	6.2 ± 1.4	4.8 ± 1.3	0.73 ± 0.14
Cefotaxime	0.0 ± 0.0[1]	6.2 ± 1.2	6.4 ± 1.8	0.64 ± 0.13
Gentamicin	4.4 ± 0.8	6.1 ± 1.7	5.8 ± 1.3	0.72 ± 0.14
Amikacin	4.3 ± 0.8	5.1 ± 1.1	4.8 ± 0.9	0.70 ± 0.15
Streptomycin	3.6 ± 1.2	4.1 ± 1.2	3.9 ± 1.6	0.65 ± 0.12
Rifampicin	4.6 ± 1.0	5.0 ± 1.4	4.5 ± 1.7	1.12 ± 0.20[1]
Doxycycline	3.7 ± 1.3	3.9 ± 1.3	4.2 ± 1.4	1.13 ± 0.22[1]
Clindamycin	5.3 ± 1.1	5.7 ± 0.9	6.1 ± 1.9	0.66 ± 0.14

[1]$p<0.01$

tive tract of mice. Three days of treatment with mezlocillin were sufficient to eliminate the endogenous intestinal microflora. Considering the antitumour effect, the observation that 3 days of experimental therapy with mezlocillin was comparable to 10 days of treatment seems to be interesting (Table 10).

Concerning pharmacology, the lack of correlation between cumulative doses of mezlocillin and intensity of antitumour effect seems to be an additional argument which supports the hypothesis that apparently the drug indirectly influences tumour behaviour. Experiments on the pharmacokinetics of mezlocillin in mice proved that subcutaneous injections of the antimicrobial drug caused significant serum concentrations which were measurable for a short period of time only. However, high concentrations of the drug per-

Table 10: Effect of mezlocillin on endogenous intestinal flora and local sarcoma L-1 growth according to duration of treatment

Experimental group	log number of bacteria/g of faeces (mean value ± SD)			tumour weight in g (mean value ± SD)
	Enterobacteriaceae	E. faecalis	S. viridans	
Control	5.1 ± 1.1	5.7 ± 1.7	4.9 ± 1.4	0.66 ± 0.13
Mezlocillin				
10 days	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.33 ± 0.07[1]
7 days	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.31 ± 0.09[1]
3 days	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.38 ± 0.10[1]

[1]$p<0.01$

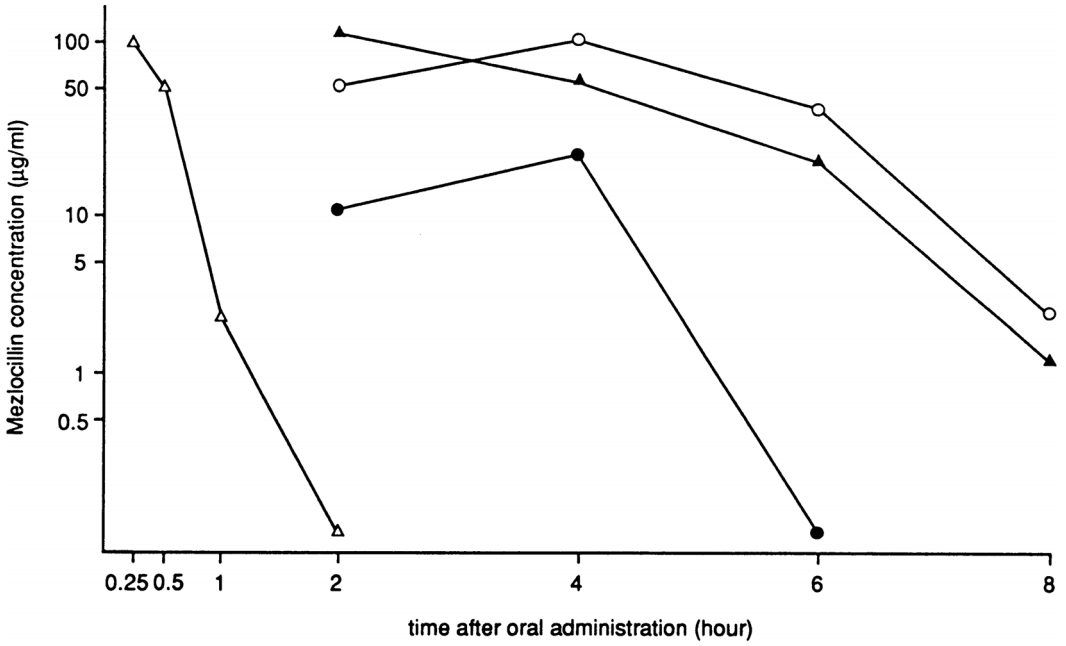

Figure 7: Concentration of mexlocillin in mouse serum and coecum contents after subcutaneous and peroral administration of the drug.
△: serum concentration (dose: 150 mg/kg body weight s.c.)
▲: coecum concentration (dose: 150 mg/kg body weight s.c.)
❍: coecum concentration (dose: 75 mg/kg body weight p.o.)
●: coecum concentration (dose: 25 mg/kg body weight p.o.)

sisted in guts of BALB/c-mice for about 6 hours (Figure 7). In another set of experiments tumour bearing animals were orally treated with mezlocillin (Table 11). The fact that no measurable serum concentrations of the drug could be detected after oral administration supported the assumption that there is no direct interaction between mezlocillin and tumour cells. Seven days of treatment with mezlocillin caused eradication of most species of the endogenous intestinal microflora in BALB/c-mice with the exception of aerobic Gram-positive bacteria and anaerobic propionibacteria. A total recovery of the aerobic and anaerobic gastrointestinal microflora could be verified 4-7 days after terminating mezlocillin treatment (Table 12, 13). Interestingly, oral administration of mezlocillin resulting in elimination of the endogenous intestinal microflora (Table 11) as well exerted a considerable antitumour effect.

The character of the assumed relationship between endogenous intestinal microflora and antineoplastic activity still is unclear. Recently, it was postulated that biologically active components of microbial cells might be liberated due to rapid killing respectively metabolic activities of the microorganisms. Furthermore it was shown that antitumour effect could also be related to elevated levels of endotoxin produced by *E. coli*. This lipopolysaccharide (LPS) is well known for its ability to trigger antitumour activity by means of inducing tumour necrosis factor (TNF) secretion (*Kabir* et al., 1978). Evaluation

Table 11: Effect of mezlocillin administered subcutaneously or orally on endogenous intestinal flora and local sarcoma L-1 growth

Experimental group	log number of bacteria/g of faeces (mean value ± SD)			tumour weight in g (mean value ± SD)
	Enterobacteriaceae	E. faecalis	S. viridans	
Control	4.3 ± 0.7	5.1 ± 1.3	4.6 ± 0.8	0.85 ± 0.17
Mezlocillin 300 mg/kg/day s.c.	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.43 ± 0.10[1]
Mezlocillin 150 mg/kg/day p.o.	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.0 ± 0.0[1]	0.33 ± 0.07[1]

[1]$p<0.01$

of LPS levels in coecum contents during mezlocillin treatment, however, showed a decrease of this immuno-active substance at the time when the antitumour effect obviously was apparent (data not presented). Moreover, gastrointestinal tract decontamination with mezlocillin in BALB/c-mice resulted in a significant reduction of peritoneal macrophage function in antitumour activity assays (Figure 8).

From all antibiotics tested only mezlocillin induced a considerable inhibition of Gram-negative anaerobic growth in the digestive tract. Metronidazole is well known for its excellent activity towards Gram-negative anaerobes, however, it proved to be inactive towards Gram-positive anaerobes and most aerobes. In the further course of investigation both antimicrobial drugs (mezlocillin and metronidazole) were compared with respect to their potential influence on cellular and humoral immunity and experimental tumour growth. In confirmation of previous studies mezlocillin treatment of BALB/c-mice for 7 consecutive days resulted in significant suppression of the specific cellular and humoral immune response and in significant inhi-bition of local sarcoma L-1 tumour

Table 12: Effect of a 7 days oral mezlocillin treatment on aerobic intestinal microflora of BALB/c-mice

	log number of bacteria/g of faeces (mean value ± SD)				
		time after finishing mezlocillin-treatment			
organism found	before treatment	24 h	4 days	7 days	11 days
E. coli	6.38 ± 0.43	0	8.60 ± 0.45	9.44 ± 0.43	7.00 ± 0.76
E. faecalis	6.20 ± 0.43	0	9.40 ± 0.79	8.56 ± 0.57	6.78 ± 0.43
Bacillus sp.	4.66 ± 0.50	0	0	5.83 ± 0.46	5.53 ± 0.34
Coag.-neg. staphylococci	5.34 ± 0.29	0	7.53 ± 0.38	6.80 ± 0.30	6.98 ± 0.40
α-haemolytic streptococci	6.60 ± 0.44	0	0	7.25 ± 0.21	7.23 ± 0.35
Gram-positive bacteria	5.44 ± 0.44	9.10 ± 0.40	8.05 ± 0.35	7.15 ± 0.21	5.66 ± 0.31

SD: standard deviation

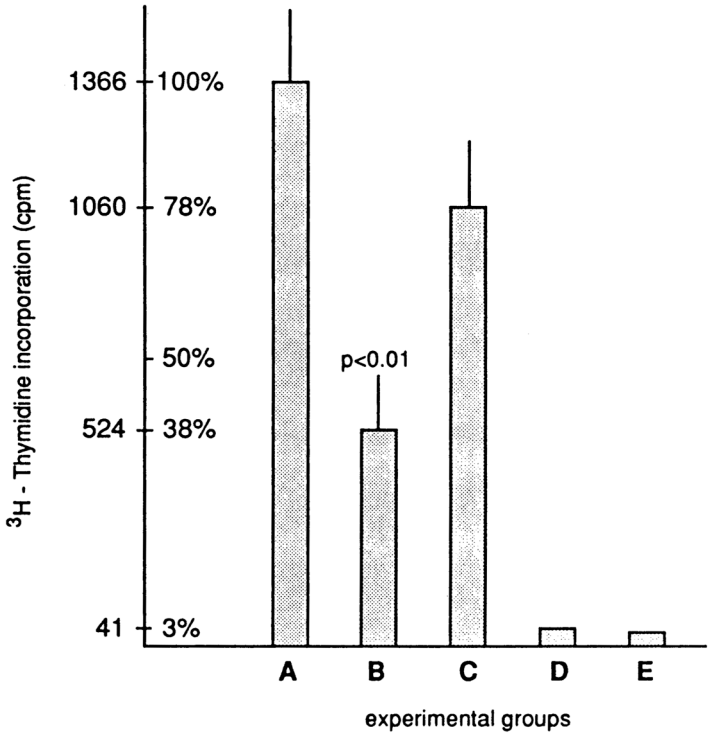

Figure 8: ^{3}H-thymidine incorporation in sarcoma L-1 cells (**A**), in sarcoma L-1 cells and control macrophages (**B**), in sarcoma L-1 cells and macrophages from decontaminated mice (**C**), in control macrophages (**D**), and in macrophages from decontaminated mice (**E**).

growth. Oral administration of metronidazole for 7 consecutive days as well resulted in suppression of humoral IgG response and inhibition of local tumour growth. However, metronidazole treatment did not influence the specific cellular immunity (Table 14, 15). Altogether we can assume that other (so far un-

Table 13: Effect of a 7 days oral mezlocillin treatment on anaerobic intestinal microflora of BALB/c-mice

	log number of bacteria/g of faeces (mean value ± SD)				
		time after finishing mezlocillin-treatment			
organism found	before treatment	24 h	4 days	7 days	11 days
Bacteroides sp.	8.62 ± 0.50	0	0	9.00 ± 0.37	8.12 ± 0.67
Bifidobacterium sp.	9.23 ± 0.28	0	0	8.97 ± 0.21	9.42 ± 0.53
Clostridium sp.	8.29 ± 0.34	0	9.46 ± 0.57	9.09 ± 0.25	8.10 ± 0.71
Eubacterium sp.	9.00 ± 0.25	0	0	10.02 ± 0.28	9.45 ± 0.55
Lactobacillus sp.	8.25 ± 0.64	0	9.45 ± 0.12	9.64 ± 0.22	9.37 ± 0.47
Propionibacterium sp.	8.21 ± 0.76	4.57 ± 0.50	nt	nt	8.62 ± 0.71

SD: standard deviation
nt: not examined

Table 14: Effect of a 7 days mezlocillin-therapy (300 mg/kg b.w. daily s.c.) and reverse consequences of a stimulation with a mixed *Bacteroides*-vaccine (B.V.) in BALB/c-mice

	Delayed skin hypersensitivity to oxazolone increase in ear-thickness (in units of 10^{-3} cm) (mean ± SD)	Humoral IgG-response Number of IPFC/10^8 spleen cells (x 10^3) (mean ± SD)	Local sarcoma L-1 tumour weight in mg (mean ± SD)
Control	14.0 ± 2.0 = 100%	9.8 ± 1.8 = 100%	429 ± 106 = 100%
Mezlocillin	8.2 ± 2.4[1] = 58%	2.0 ± 1.5[1] = 20%	145 ± 73[1] = 34%
Mezlocillin + B.V (1 mg i.p. on 1st day)	12.3 ± 2.9 = 88%	5.1 ± 1.3[1] = 53%	355 ± 140 = 83%
Mezlocillin + B.V (2 mg p.o. on 2nd day)	12.4 ± 2.1 = 89%	8.8 ± 2.9 = 82%	290 ± 86[2] = 68%

SD: standard deviation
IPFC: indirect plaque-forming cells
i.p.: intraperitoneally
p.o.: perorally
[1]$p<0.05$
[2]$p<0.01$

known) mechanisms than those related to Gram-negative anaerobic microorganisms might be involved in these phenomena.

Oral or intraperitoneal administration of heat killed vaccine (mixed from 9 *Bacteroides* species isolated from faeces of healthy non-treated BALB/c-mice) could - at least partially - reverse the mezlocillin and metronidazole-induced

Table 15: Effect of a 7 days metronidazole-therapy (30 mg/kg b.w. daily p.o.) and reverse consequences of a stimulation with a mixed *Bacteroides*-vaccine (B.V.) in BALB/c-mice

	Delayed skin hypersensitivity to oxazolone increase in ear-thickness (in units of 10^{-3} cm) (mean ± SD)	Humoral IgG-response Number of IPFC/10^8 spleen cells (x 10^3) (mean ± SD)	Local sarcoma L-1 tumour weight in mg (mean ± SD)
Control	14.6 ± 2.5 = 100%	5.8 ± 1.2 = 100%	570 ± 130 = 100%
Metronidazole	15.9 ± 2.9 = 110%	3.2 ± 1.2[1] = 55%	290 ± 84[1] = 51%
Metronidazole + B.V (1 mg i.p. on 1st day)	15.2 ± 2.1 = 104%	4.3 ± 1.1 = 74%	488 ± 159 = 84%
Metronidazole + B.V (2 mg p.o. on 2nd day)	14.4 ± 1.6 = 98%	5.9 ± 1.5 = 102%	524 ± 125 = 92%

SD: standard deviation
IPFC: indirect plaque-forming cells
i.p.: intraperitoneally
p.o.: perorally
[1]$p<0.05$

effects (Table 14, 15).

Investigations of *Abrams* et al. (1963) have shown that the presence of the endogenous intestinal microflora is necessary for the normal proliferation of the intestinal epithelium. *Crabbe* et al. (1968) found that the function of local immunological structures of the digestive tract was dependent on the presence of intestinal bacteria. These findings, however, deal with local phenomena in the digestive tract. With respect to the data presented it should be considered that the presence of the physiological (gastrointestinal) microflora may also be important for proliferative activities in other organs and in neoplastic tissue.

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SPECIFIC INACTIVATION OF ANTIMICROBIAL AGENTS AND ITS INTERINDIVIDUAL DIFFERENCES

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INTRODUCTION

ß-Lactamases are enzymes of bacterial origin which hydrolyse the C-N bond in the ß-lactam ring of a penicillin or a cephalosporin. The effect of these enzymes was first observed by *Abraham* and *Chain* (1940), immediately after the first report on the clinical use of benzylpenicillin (*Chain* et al., 1940). For an adequate antibiotic therapy it is important to know the resistance of a bacterial species against ß-lactam antibiotics. Similarly, the resistance of the bacterial flora of an individual may be of importance in this respect. At present many ß-lactamases from a large number of different strains of bacteria have been purified and characterized (*Bush*, 1989). The gastrointestinal tract is the largest source of bacteria in humans. Some 400 distinct species of bacteria can normally be isolated from the faeces (*Holdeman* et al.,1976). Some of these bacteria may produce ß-lactamases and release them into the intestinal contents, thereby interfering with the activity of antibiotics that reach the lower intestinal tract. In the present study, the effect of faecal enzyme preparations on 4 antibiotics was studied i.e. benzylpenicillin (penicillin G), cefotaxime, aztreonam and carumonam.

Benzylpenicillin, the first discovered penicillin, is effective *in vitro* against Gram-positive cocci and some Gram-negative bacteria. It is also active against a number of anaerobic microorganisms.

Cefotaxime is a third-generation cephalosporin with a high intrinsic activity and a broad spectrum (*Heymes* et al., 1977; *Drasar* et al., 1978; *Neu* et al., 1979). Its spectrum of activity includes, in addition to *Haemophilus influenzae* and the Enterobacteriaceae, some *Pseudomonas aeruginosa* and *Bacteroides fragilis* strains.

Aztreonam (SQ 26,776) is a synthetic monobactam that is minimally (<1%) absorbed after oral administration (*Swabb* et al., 1983). Its poor intestinal absorption and the selective activity against Gram-negative bacilli (*Sykes* et al., 1982) render this drug particularly suitable for selective decontamination (*de Vries-Hospers* et al., 1984) of immunocompromised patients.

Carumonam (Ro 17-2301; AMA-1080) is also a synthetic monobactam. *In vitro* it has been shown to be active against many, predominantly Gram-negative, aerobic rods, primarily members of the families Enterobacteriaceae, Neisseriaceae; *Haemophilus* spp., and *Pseudomonas aeruginosa*. It is not active or only weakly active against Gram-positive and anaerobic bacteria. It originates from sulfazecin, an N-sulfonated monocyclic ß-lactam antibiotic first discovered in the culture broth of *Pseudomonas acidophila* sp. nov. (*Imada* et al., 1985). Modification of this compound resulted in carumonam, an antibiotic with a high antibacterial activity (*Kishimoto* et al., 1983).

These 4 antibiotics were incubated with faecal enzyme preparations from

12 healthy human volunteers and the remaining amount of antibiotic was quantitated by reversed-phase high-performance liquid chromatography (HPLC) .

MATERIALS AND METHODS

Faecal samples

Faecal samples were collected from 12 healthy adult volunteers (5 females, 7 males). During a period of 24 months samples were collected at months 0, 6, 14 and 24. The volunteers did not receive antimicrobial drugs, at least not two weeks prior to the collection of the faecal samples. From volunteer 9, faecal samples were also collected during a shorter period of time (2 weeks). Faecal samples were stored at -20°C.

Faecal enzyme preparations

Faecal enzyme preparations were prepared as described by *Welling* et al. (1987). 0.5 g of faeces was suspended and homogenized in 1.5 ml of demineralized water containing 0.1% (w/v) Triton X-100. The suspensions were centrifuged for 10 min (9000 x g). The supernatants were centrifuged for 60 min at 100,000 x g (50Ti rotor, Beckman L5-65 ultracentrifuge). The supernatants were dialyzed against phosphate-buffered saline, pH 7.2. The retentate was used as enzyme preparation.

Antimicrobial agents

Benzylpenicillin (sodium salt) was from Gist-Brocades NV, Delft, The Netherlands. Cefotaxime (sodium salt) was from Roussel B.V., Hoevelaken, The Netherlands. Aztreonam (SQ 26,776) and its inactive open ring form (SQ 26,992) were a gift from the Squibb Institute for Medical Research, Princeton, NJ, USA. Carumonam (Ro 17-2301/010) was a gift from Hoffmann-La Roche BV, Mijdrecht, The Netherlands.

Enzymatic inactivation

An amount of 25 μl of antibiotic (1 mg/ml phosphate-buffered saline, pH 7.2 [PBS]) was incubated for 20 h at 37°C with 200 μl faecal enzyme preparation. As controls were incubated, 25 μl PBS and 200 μl enzyme preparation, to account for the background from the faecal enzyme preparation and 25 μl antibiotic solution (1 mg/ml PBS) and 200 μl PBS to account for any nonenzymatic degradation of the antibiotic. The incubation was terminated by putting the samples on ice. Aliquots of 50 μl of the incubation mixture were analyzed in duplicate by HPLC. Peak heights were proportional to concentration and the percentage inactivation was calculated by considering the peak height obtained after HPLC of the incubation mixture with antibiotic and without faecal enzyme preparation as 0% enzymatic inactivation.

High-performance liquid chromatography

The chromatography system consisted of a Waters M 6000A pump, a Rheodyne 7125 injector and a Pye-Unicam LC-UV detector. The reversed-phase (C18) column used for all HPLC assays was a Nucleosil 10 C-18 column (250 x 4.6 mm) from Nacherey-Nagel, Düren, Germany) equipped with a guard column containing the same material.

Elution conditions were as follows: the column was eluted at a flow-rate of 1.5 ml/min for benzylpenicillin with 15 mM sodium phosphate pH 7.2 and methanol in a ratio of 70 : 30 (v/v). The absorbance was monitored at 214 nm;

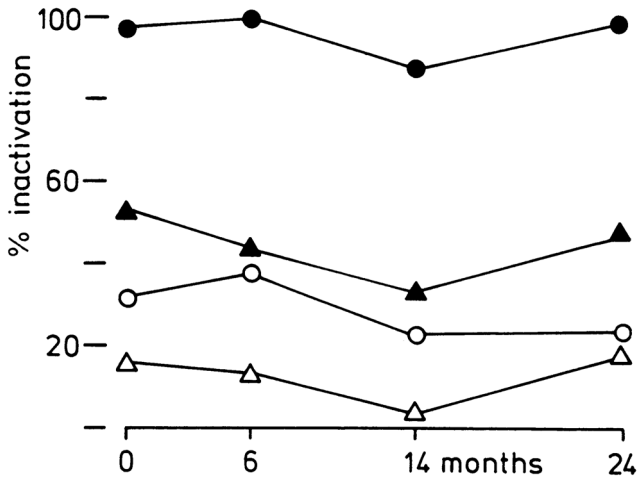

Figure 1: Percentage inactivation of cefotaxime (●), benzylpenicillin (▲), aztreonam (○), carumonam (△) after 20 h incubation at 37°C by faecal enzyme preparations from 12 healthy human volunteers at month 0, 6, 14, 24.

cefotaxime with 7 mM phosphoric acid and methanol in a ratio 60 : 40 (v/v). The absorbance was monitored at 254 nm; aztreonam and carumonam with 5 mM tetrabutylammoniumhydrogensulphate (adjusted to pH 3.0 with 1 M K_2HPO_4) and methanol in a ratio of 70 : 30 (v/v). The absorbance was monitored at 293 nm.

RESULTS

Inactivation of benzylpenicillin, cefotaxime, aztreonam and carumonam was investigated with an HPLC-assay. The principle of this assay is that the antibiotic is incubated with a faecal enzyme preparation. In addition appropriate control samples are similarly treated and the disappearance of the antibiotic can be monitored by reversed-phase HPLC. In order to obtain information on the variability of the inactivation of the antibiotics by faecal enzyme preparations, it was studied over a period of 24 months at month 0, 6, 14 and 24. The average percentage inactivation after 20 h of incubation at 37°C determined with faecal enzyme preparations from 12 volunteers, ranged from 97 to 13%. Cefotaxime was inactivated for 97% (median value 98%), benzylpenicillin for 44% (median value 30%), aztreonam for 28% (median value 19%) and carumonam for 13% (median value 11%). In Figure 1 the average values of inactivation at month 0, 6, 14 and 24 are shown. This figure also shows that in this group of volunteers the extent of inactivation of a particular antibiotic is relatively constant. This may be entirely different within an individual (see Figure 2 as an example). Cefotaxime inactivation remains at a high relatively constant level. Carumonam is hardly inactivated, but differences in inactivation from 37% (month 0) to 4% (month 14) may occur. The variability in inactivation of benzylpenicillin and aztreonam is larger. The faecal enzyme preparation of this volunteer inactivated benzylpenicillin for 100% at month 0 and 14, but

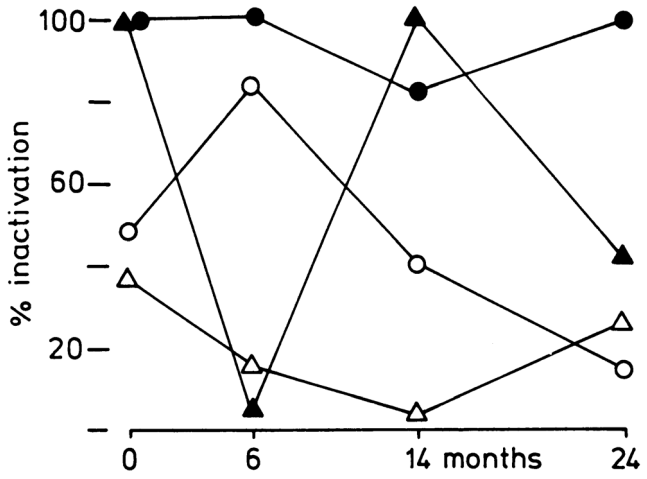

Figure 2: Percentage inactivation of cefotaxime (●), benzylpenicillin (▲), aztreonam (○), carumonam (△) after 20 h incubation at 37°C by faecal enzyme preparations from one volunteer at month 0, 6, 14, 24.

for only 5% at month 6 and at month 24, 42% inactivation was measured. Aztreonam inactivation ranged from 84% at month 6 to 15% at month 24. Similar variability in inactivation was observed with the other faecal enzyme preparations except for those which hardly inactivated a particular antibiotic at all. This prompted us to investigate this inactivation also during a shorter period of time. Faecal enzyme preparations from 3 volunteers were used to determine the inactivation of aztreonam over a period of 14 days (*Welling* and *Groen*, 1989). Figure 3 shows the inactivation of aztreonam by the faecal enzyme preparations of one volunteer. For example at day 9 we found hardly any inactivation (7%) and then a rapid increase in inactivation to 69% at day 12. The inactivation of aztreonam by faecal enzymes from 2 other volunteers showed fluctuations from 0 to 15% (day 1 and 3, respectively) and 43 to 22% (day 7 and 9, respectively). The results of the long and the short study period show that inactivation cannot be predicted.

DISCUSSION

Our first study on the effect of faecal enzymes on antibiotics was initiated by the results of *de Vries-Hospers* et al. (1984). Aztreonam was orally administered to 10 volunteers in order to eliminate selectively the potentially pathogenic Gram-negative bacteria. During this selective decontamination with aztreonam faecal counts of Gram-negative bacilli decreased in most volunteers. In the faeces of two volunteers in whom Gram-negative bacilli persisted, aztreonam was not detectable. *Ehret* et al. (1987), who conducted a similar study with 8 volunteers, found 64 to 876 mg of aztreonam/kg in the faeces of 6 volunteers, while the remaining two had low levels or no aztreonam at all in the faeces after oral administration of 300 mg/day. These results indicated that aztreonam can be inactivated by faecal material. We showed that this inactiva-

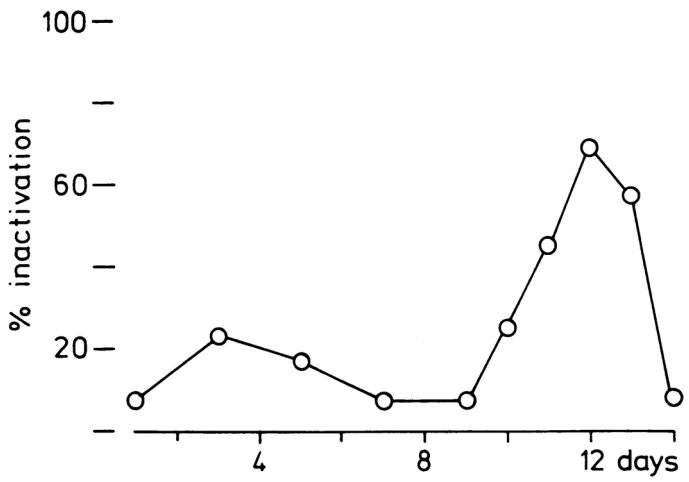

Figure 3: Percentage inactivation of aztreonam (○) after 20 h incubation at 37°C by faecal enzyme preparations from one volunteer over 14 days.

tion is most probably due to ß-lactamase activity and that this enzyme activity could be inhibited by clavulanic acid (*Welling* et al., 1987) .

Although *Swabb* et al. (1983) regard degradation unlikely, others (*Livermore* and *Williams*, 1981; *Phillips* et al., 1981) have also reported on inactivation of aztreonam by ß-lactamases. In a next study (*Welling* and *Groen*, 1989) individual differences in inactivation of aztreonam were monitored over a longer period of time (24 months, at month 0, 6 and 24) and during 14 days. Considerable inter- and intra-individual differences were found. The conclusion from that study was that aztreonam inactivation cannot be predicted and that it may be worthwhile to determine prior and during selective decontamination with aztreonam the extent of inactivation.

This type of inactivation by faecal ß-lactamases will most likely not be limited to one ß-lactam antibiotic but probably is a widespread phenomenon. This prompted us to study the effect of faecal enzyme preparations on a number of other ß-lactam antibiotics. Surprisingly, the third generation cephalosporin cefotaxime was most rapidly inactivated by all faecal enzyme preparations. This could be due to ß-lactamases produced by the anaerobic component of the bacterial flora. The 4 antibiotics were inactivated to a different extent but when the inactivation percentages of the 12 volunteers are averaged, at a fairly constant level at 0, 6, 14 and 24 months (see Figure 1). This suggests a stability of the bacterial flora which is only meaningful when this group of volunteers is considered as a population. Examination of the inactivation of each antibiotic by individual enzyme preparations at month 0, 6, 14 and 24 provides an entirely different picture (see Figure 2). For example, benzylpenicillin may be inactivated for 100% at one particular sampling time (month 0) but not at all at another sampling time (month 6). The same is true for shorter intervals (Figure 3). At one particular day aztreonam was hardly inactivated (day 9) and a few days later (day 12) it was inactivated for 69% by a faecal enzyme preparation of the same subject.

Examination of the individual inactivation data also reveals that when carumonam is inactivated for more than 20% by a particular faecal enzyme prepara-

tion, penicillin is also inactivated (for 20% or more) by the same enzyme preparation from 8 out of 8 volunteers and aztreonam (for 20% or more) by those from 7 out of 8 volunteers. This type of inactivation could be due to different enzymes or to one enzyme with different substrate affinities since the three antibiotics generally were not inactivated to the same degree. Although this pattern of inactivation was most frequently found, we also observed 84% inactivation of aztreonam while benzylpenicillin was hardly inactivated (5%).

Several authors have reported on penicillin and cephalosporin resistance in *Bacteroides* sp. (*Anderson* and *Sykes*, 1973; *Britz* and *Wilkinson*, 1978; *Del Bene* and *Farrar*, 1973; *Garrod*, 1955; *Olsson* et al., 1976; *Pinkus* et al., 1968; *Richmond* and *Sykes*, 1973). Quantitatively, the *Bacteroides fragilis*-group is predominant in the human faecal flora (*Meijer-Severs* and *van Santen*, 1986). They may represent therefore together with other anaerobic species in the intestine, a large potential source of antibiotic-inactivating enzymes. *Meijer-Severs* and *van Santen* (1986) found considerable interindividual differences in *Bacteroides* cultural counts (between 8.83 and 10.24) although the total anaerobic cultural counts showed only one log difference. According to *Simon* and *Gorbach* (1984), the composition of the bacterial flora is relatively stable in single subjects over longer periods of time, while the metabolic activity measured by determination of bacterial enzymatic activity may show marked changes. We have found that the enzymatic activity may be considerably different inter- and intra-individually and that it may change with time. This could be due to dietary modulations of the composition of the bacterial flora. This variable bacterial population in the colon may produce a number of different ß-lactamases or, depending on the composition of the flora, variations in the concentration of one type of enzyme.

The practical consequences of these findings are different for different antibiotics. When the antibiotic is intended for selective decontamination of the intestinal tract (aztreonam and carumonam) it may be worthwhile to know to which extent it will presumably be inactivated by the faecal enzymes prior and during selective decontamination. When the antibiotic has a broad spectrum and is not intended for selective decontamination (benzylpenicillin, cefotaxime) a fraction of the antibiotic may reach the lower intestinal tract through the biliary canal after parenteral administration (benzylpenicillin, cefotaxime). It is epidemiologically important when the antibiotic is then inactivated by bacterial enzymes. Then disturbance of the bacterial flora is prevented and therewith a barrier is maintained against colonization (colonization resistance, *van der Waaij*, 1982) by potentially pathogenic microorganisms from the environment.

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NON-SPECIFIC INACTIVATION OF ANTIMICROBIAL AGENTS

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INTRODUCTION

The potential of an antimicrobial agent to disturb the normal intestinal microflora is related to its *in vitro* properties, its route of administration, dose and pharmacokinetic properties. Specific as well as non-specific inactivation of the antimicrobial agent may also affect the extent to which the intestinal microflora is altered.

The present study concerns non-specific inactivation of norfloxacin in the gastrointestinal tract. Previous studies on the new quinolones have shown that administration of these agents in therapeutic doses results in very high concentrations in faeces (Table 1). If these figures are compared with the MIC_{90} of the new quinolones against some common intestinal microorganisms, the MIC_{90} for both aerobic and anaerobic intestinal microorganisms are with few exceptions far below these levels.

Earlier studies by our research group and other groups have shown that administration of the new quinolones causes selective ecological changes in the gastrointestinal microflora. Several investigations including healthy volunteers as well as different categories of patients, show uniform results. The aerobic Gram-negative microorganisms are eliminated or strongly suppressed during administration, while the aerobic Gram-positive and the anaerobic micro-

Table 1: Concentrations of new quinolones in faeces after various doses

Quinolone	Dose (mg/day)	Faecal concentration Range respectively mean value (mg/kg)	Reference
Norfloxacin	400x2	120-1400	Meckenstock et al. 1985
Norfloxacin	400x2	2271	Pecquet et al. 1986
Norfloxacin	400x2	1756	Boerema et al. 1986
Norfloxacin	200x2	303-1906	Edlund et al. 1987
Norfloxacin	200x2	125-1000	Maschmeyer et al. 1988
Norfloxacin	400x2	250-1000	Maschmeyer et al. 1988
Ciprofloxacin	500x2	185-2220	Brumfitt et al. 1984
Ciprofloxacin	250x2	1600-6400	Maschmeyer et al. 1988
Ciprofloxacin	500x2	1200-6400	Maschmeyer et al. 1988
Enoxacin	400x2	100-500	Edlund et al. 1987
Ofloxacin	200x2	327	Pecquet et al. 1987
Pefloxacin	400x2	645	Janin et al. 1987

flora are almost unaffected (*Edlund* and *Nord*, 1988). Thus, despite high levels of new quinolones in faeces, the main part of the intestinal microflora remains unaffected.

Different theories have been raised to explain the discrepancies of the very high concentrations of quinolones in faeces and the relatively sparse effect on the gastrointestinal microflora. *Lewin* and colleagues (1989) have suggested that oxygen is required for the bactericidal activity of the new quinolones. They found that lack of oxygen results in a bacteriostatic activity of ciprofloxacin and ofloxacin against *Escherichia coli* and *Staphylococcus aureus* in contrast to the bactericidal activity exhibited under aerobic conditions. The milieu of the intestines is mainly anaerobic so this theory might partly explain the phenomenon. An inoculum effect on norfloxacin activity against anaerobic strains has been reported by *Goldstein* and colleagues (1987), while other groups have failed to find any major inoculum effects. A third theory is that quinolones have the ability to bind to microorganisms or to other faecal components, resulting in only a minor part of the agent being free in the intestines to exert the antimicrobial effect. Reversible binding to faeces has earlier been reported for other antimicrobial agents (*Hazenberg* et al., 1985; *Hazenberg* et al., 1986; *van Saene* et al., 1985). Thus gentamicin, tobramycin, polymyxin B and neomycin have been shown to bind to the solid part of faeces.

Uptake of new quinolones into bacterial cells and their interactions with cell membranes has been and is currently studied by several researchers (*Bedard* et al., 1987; *Chapman* and *Georgopapadakou*, 1988; *Cohen* et al., 1988; *Bedard* and *Bryan*, 1989). Binding of quinolones to bacterial cells might in part reflect the mechanism of action. The mode of action of the quinolones is not yet completely understood.

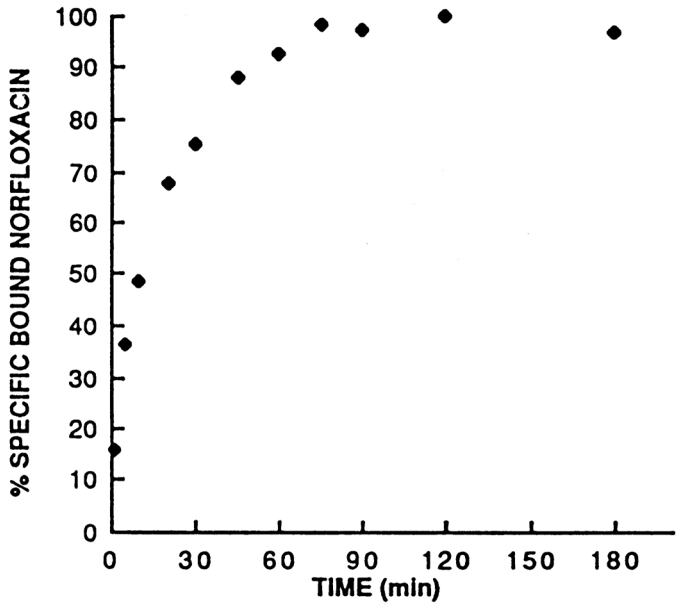

Figure 1: Association of [^{14}C]norfloxacin to faeces at 37°C. The results are expressed as percent of maximum specific binding.

The aim of the present study was to analyze the binding between quinolones such as norfloxacin and faecal material.

MATERIAL, METHODS AND RESULTS

The model used for the binding experiments was ligand-receptor interactions with radioactive labelled norfloxacin as ligand, and diluted faecal suspensions as receptor. Binding to norfloxacin to faeces was measured by using a membrane filter technique. The reaction mixture contained either a fixed concentration of [^{14}C]norfloxacin (1.5 μM) together with various concentrations of faecal suspensions, or varying concentrations of [^{14}C]norfloxacin (0.1-500 μM) and a fixed amount of faecal suspension (5.3 g/l). Specific binding was determined by the difference between total radioactivity found in the absence or presence of excess of unlabelled norfloxacin (560 μM). After binding at 37°C for various times during end over end rotation, the reaction mixtures were filtered under vacuum. The filters were washed and then counted for radioactivity in a liquid scintillation spectrometer.

The association of norfloxacin to faeces is shown in Figure 1. The specific binding of norfloxacin increases with time and reaches a plateau after 90 min incubation at 37°C. According to these results, 120 min incubation at 37°C was used to reach a maximum binding in all subsequent experiments.

Binding of norfloxacin was shown to be reversible and temperature dependent. Norfloxacin and faeces were incubated as described earlier to reach a maximum binding. The samples were then centrifuged and washed three times at 4°C, and the resuspended samples were further incubated for up to 240 min. Figure 2 shows dissociation of norfloxacin from faeces at different

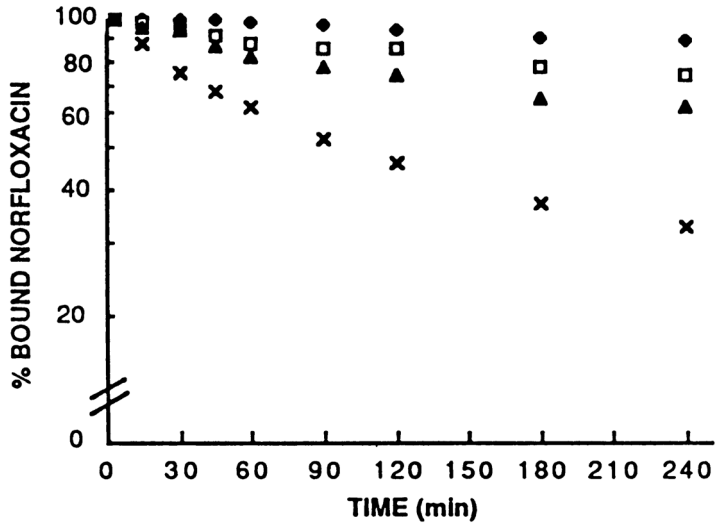

Figure 2: Dissociation of 1.5 μM [^{14}C]norfloxacin bound to faeces at various temperatures. The results are expressed as the percentage of the specific binding found at the time zero (logarithmic scale). Symbols: u, 4°C; o, 20°C; s, 37°C; x, 37°C + 560 μM norfloxacin.

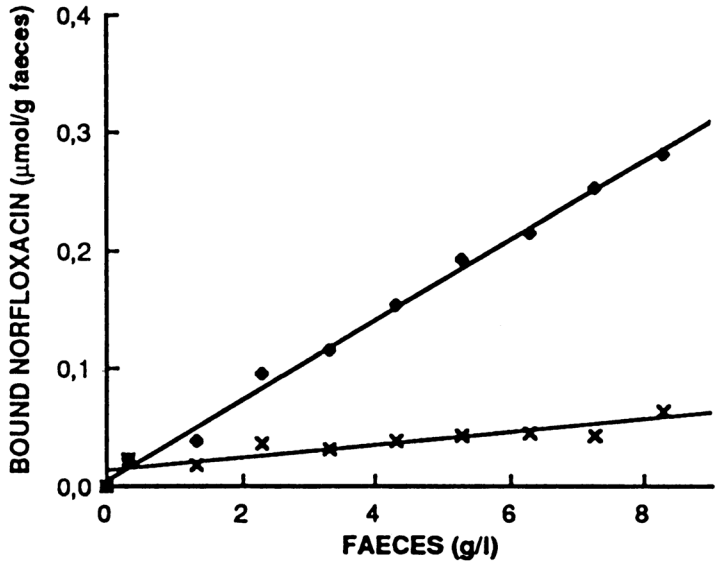

Figure 3: Binding of 1.5μM [^{14}C]norfloxacin to an increasing concentration of faeces. Symbols: u, specific binding; x, non-specific binding.

temperatures. After 240 min incubation at 37°C, 60% and 35% were dissociated in the presence and absence, respectively, of unlabelled norfloxacin. At

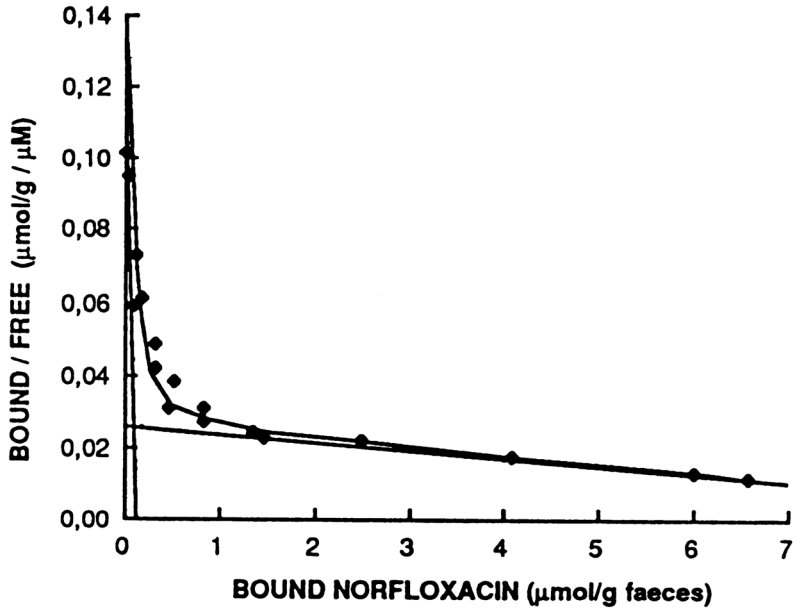

Figure 4: Scatchard plot for [^{14}C]norfloxacin binding to faeces (F1). The asymptotes as well as the curve combining the experimental data points were drawn by the computer program SAS PROC NLIN and represent the best fit.

Table 2: Numerical estimation of dissociation constants, K_D, and maximum binding capacities, B_{max}, for binding of quinolones to different faecal samples and to a suspension of *B. fragilis*. All assays were made in triplicate

Antimicrobial agent	Faeces/ bacterial suspension	K_{D1} (μM)	K_{D2}	B_{max1} (μmol/g dry weight)	B_{max2}
[^{14}C]norfloxacin	F1	1.0	450	0.74	72.4
[^{14}C]norfloxacin	F2	0.6	830	0.27	153.0
[^{14}C]norfloxacin	F3	1.4	450	0.59	63.3
ciprofloxacin	F1	3.4	860	-*	-
enoxacin	F1	2.4	920	-	-
ofloxacin	F1	2.3	860	-	-
pefloxacin	F1	2.9	530	-	-
norfloxacin	F1	1.4	550	-	-
[^{14}C]norfloxacin	*B. fragilis*	2.4	1100	0.96	121

*B_{max}-values for unlabelled quinolones could not be determined due to assay by an indirect competitive method

4°C, only 10% of the bound norfloxacin was dissociated after 240 min.

The binding of norfloxacin to faeces was found to be a linear function of faeces concentration. Figure 3 shows binding of labelled norfloxacin to increasing concentrations of faecal suspensions after 120 min incubation at 37°C.

The ability of increasing concentrations of [^{14}C]norfloxacin (0.1-500 μM) to bind to faeces (5.3 g/l) was also assayed. Scatchard plot of the data (Figure 4) was non-linear, which means that more than one binding class is involved. According to this Scatchard plot and by calculating with a non-linear regression computer program, two different binding classes were found, one with high affinity and low capacity (= class 1) and one more unspecific binding class with low affinity and high capacity (= class 2).

The equilibrium dissociation constants K_D and the total number of binding sites B_{max} for each of the binding classes were determined by the computer program. This assay was also performed for two additional faecal samples, F 2 and F 3. Table 2 shows the numerical estimates of the parameters K_{D1}, K_{D2}, B_{max1} and B_{max2}. Binding of unlabelled ciprofloxacin, enoxacin, pefloxacin and norfloxacin to faeces was determined by an indirect method using their ability to compete with 1.5 μM [^{14}C]norfloxacin binding to faeces. The K_D values for these quinolones are in the same range as those for labelled norfloxacin obtained by the direct method described earlier, which supports the accuracy of the methods used (Table 2). In order to find out if the quinolones bind to the bacterial or non-bacterial fraction of faeces, the binding between labelled norfloxacin and a suspension of *Bacteroides fragilis* was studied. Two classes of binding were found in accordance with

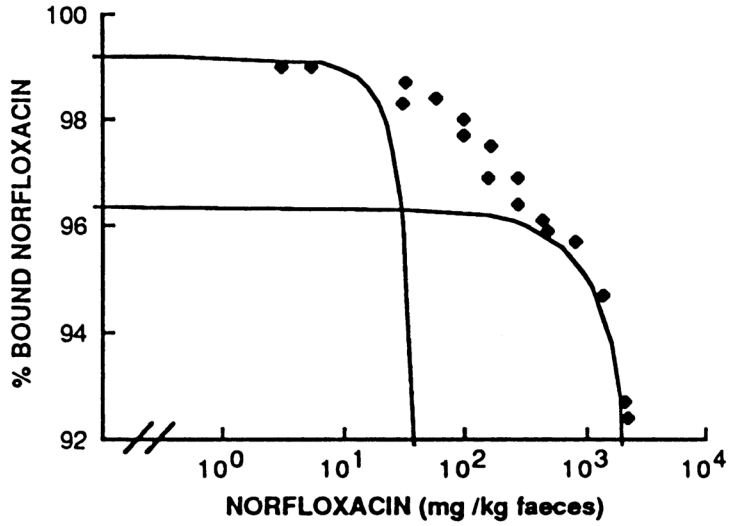

Figure 5: Fraction of bound norfloxacin related to total norfloxacin concentration in 100% faeces (extrapolated values). The experimental data points and the predicted curves for each binding class derive from studies on F1.

the findings of norfloxacin binding to the whole faecal fraction (Table 2). These results imply that norfloxacin binds mainly to the bacterial fraction of faeces.

The K_D and B_{max} values from the assay of F 1 were used to generate a curve relating fraction of bound drug to total norfloxacin concentration extrapolating to undiluted faeces. This may give a hint of the clinical situation. Figure 5 shows the predicted curves for the two binding classes and the experimental data points. At a norfloxacin concentration of 1000 mg/kg faeces, which is common in clinical situations, more than 95% of the drug is bound to faeces, and thus only 50 mg/kg is available as free drug. Furthermore, with such a high K_{D2} value the low affinity binding may be underestimated because of fast dissociation which may occur during filtration and washing procedures.

The MBC values of norfloxacin against *Enterococcus faecium* were studied in the presence of increasing concentrations of *B. fragilis* cells. *B.*

Table 3: Effect of various concentrations of *B. fragilis* on minimum bactericidal concentration of norfloxacin against *E. faecium.*

Bacteroides fragilis dry weight (g/l)	MBC (mg/l)
0	8
1.0	8
2.0	16
4.0	64
8.0	256

fragilis was used in this assay since it is one of the predominating microorganisms in the normal intestinal microflora. The assay was performed under aerobic conditions so that *B. fragilis* cells were unable to multiply but were present for binding to norfloxacin. The MBC values were strongly affected by the presence of *B. fragilis* cells as shown in Table 3. These results imply that norfloxacin binds to the *B. fragilis* cells and that only a minor fraction is free to exhibit bactericidal effect on *E. faecium*. In 100% faeces, the dry weight of bacteria is approximately 10-fold greater than the dry weight of *B. fragilis* used in this assay.

CONCLUSIONS

The present study shows that binding to faeces may act as a non-specific inactivation of norfloxacin. The results can be summarized as follows:

The specific binding of norfloxacin is reversible, saturated after 90 min incubation at 37°C and increases linear with faecal concentrations. Scatchard plots and non-linear regression computer analyses revealed two different binding classes; one primary specific binding and one secondary more unspecific binding. These results suggest that binding of norfloxacin to faeces, preferably to the bacterial fraction, may explain the paradox of the high faecal concentrations of norfloxacin versus the actual effect on the normal intestinal microflora.

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SELECTIVE INTESTINAL DECONTAMINATION IN DIFFERENT CLINICAL SITUATIONS

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INTRODUCTION

Infection continues to be a major problem in immunocompromised patient. Patients may run an increased risk of infections, basically for two reasons:

1. colonization at abnormal sites and/or in abnormal high numbers due to decreased colonization resistance (CR); i.e. the digestive tract may form a major source for both bacterial (Gram-negative and Gram-positive) and fungal infections,
2. decreased defense capacity:
   a. at the first line of defense: skin and mucosal lining,
   b. at the second line of defense: immune system.

The immunological system is very complex, however it is clinically useful to classify roughly host defense mechanisms into two separate systems: T cell dependent and a T cell independent immunity. Defects in the defense capacity of one of these systems increase the risk of infection by a specific group of microorganisms (*Peterson*, 1984).

Microorganisms which require T cell dependent mechanisms to control their intracellular multiplication are viruses - (herpesviruses) HIV, fungi, parasites (*Toxoplasma*, *Pneumocystis carinii*) and bacteria (mycobacteria, *Legionella*, *Listeria* and *Salmonella*). Although defects in the T cell independent system can be divided into defects in humoral defense system and defects in the polymorpholeucocyte system, defects in both systems are characterized by infections caused by aerobic bacteria like *Staphylococcus aureus* and other Gram-positive cocci, *Haemophilus influenzae* and aerobic Gram-negative rods (*Wade* and Schimpff, 1988).

These considerations will influence which antimicrobial agents will be used in different clinical situation as prophylaxis and empirical therapy.

A uniform regimen for prophylaxis to infections caused by T cell dependent microorganisms is difficult due to the heterogeneous aspects of the causative agents. Although some results have been obtained with the administration of prophylactic antimicrobials of these group of infections nowadays it will be impossible to cover in a rather easy way all these microorganisms. Prophylaxis for infections caused by T cell independent microorganisms on the other hand is much simpler.

COLONIZATION RESISTANCE AND SELECTIVE INTESTINAL DECONTAMINATION

Many of the T cell independent infections are caused by aerobic bacteria and yeasts which are generally regarded

as components of the normal microflora of the oropharynx or intestines.

Total bowel suppression to eradicate these microorganisms from the gastrointestinal tract (G.I. tract) is efficiently in reducing the number of severe infections, only when highly protective isolation measurements are employed (*Levine* et al., 1973; *Dietrich* et al., 1977).

Aerobic Gram-negative bacteria and staphylococci are the most common pathogens in the hospitalized immunocompromised patient. Disturbances of the integrity and microecology of the G.I. tract and skin play an important role in the pathogenesis of these microorganisms. In the normal situation these bacteria are a minority in the G.I. tract.

Van der Waaij (1974) demonstrated in animal studies that the inoculum necessary to colonize the digestive tract of conventional mice with bacteria was much higher than in germfree mice.

Suppression of growth of colonizing and newly ingested aerobic Gram-negative bacteria and yeasts is due to the hostile environment provided by the - predominantly anaerobic oropharyngeal and intestinal microflora; a flora selected and maintained by the host organism (*van der Waaij* et al., 1982). The host organisms contributes in this respect in two ways: 1. by selection of the bacteria which are "permitted" to colonize the mucosal surfaces of the G.I. tract immunologically (*van der Waaij*, 1988) and 2. by removing unwanted bacteria together with the by peristaltic movements. This combined action of host and resident microflora is called "colonization resistance" (CR) (*van der Waaij* and *de Vries*, 1974).

When the resident microflora of the G.I. tract is suppressed by antimicrobial agents, "bacterial of fungal overgrowth" may develop, either in the oropharynx, and the intestines. As a consequence of this overgrowth their is a good chance that invasion of sufficient severity occurs to cause an infection (*Tancrede* and *Andremont*, 1985).

This information leads to the obvious conclusion that infections in the high risk patient could be prevented if the existing colonization(s) at any of these sites in the digestive tract can be stopped and from then on, be prevented for the duration of the condition(s) of increased risk for infection.

By means of selective decontamination (SDD) the potentially pathogenic microorganisms (ppmo), in this case aerobic Gram-negative rods, *S. aureus* and yeasts are eliminated from their main reservoir the G.I. tract, in the meantime leaving the relatively harmless indigenous anaerobic flora intact.

So for SDD antimicrobial agents are used, which are not suppressive to the C.R. associated microflora but effectively eradicate these ppmo from the G.I. tract.

USE OF SDD IN DIFFERENT CLINICAL SITUATIONS

As cited above the eradication of the ppmo in the G.I. tract by means of SDD may be useful in clinical situations characterized by a relatively high incidence of infections with T cell independent infections, especially if this condition of a decreased T cell independent immunological system is temporary. The different clinical situations will be discussed here after.

1. Patients with chemotherapy for malignancies causing bone marrow suppression

Granulocytopenia secondary to cancer chemotherapy accounts for the

largest proportion of neutropenic patients in the hospital especially if neutropenia is lasting for more than ten days. This is plausible since chemotherapy causes in addition to severe granulocytopenia mucosal lesions.

Aerobic Gram-negative bacilli are the most common pathogens in this setting causing severe and often life threatening infections (*Wade* and *Schimpff*, 1988). In 1977 the antibacterial prophylactic effect of co-trimoxazole (TMP-SMZ) on Gram-negative infections was discovered in a study on the prevention of *Pneumocystis carinii* infections in leukemic children (*Hughes* et al., 1977). Since that time several studies have been undertaken that confirmed the prophylactic effect of TMP-SMZ. (*Gurwith* et al., 1979; *Kaufman* et al., 1983; Sleijffer et al., 1980; Dekker et al., 1981).

Dekker et al. (1981) showed that the use of TMP-SMZ can lead to an increased colonization of the G.I. tract with TMP-SMZ resistant Gram-negative rods during treatment of leukemic patients. The problem of the acquisition of resistant Gram-negatives was solved by the additional administration of polymixines (*Rozenberg-Arska* et al., 1983). *Candida* colonization and infections seem to be provocated by TMP-SMZ, like by many other antimicrobials. The addition of an oral antifungal drug like amphotericin B strongly reduced the incidence of *Candida* colonization and infection (Ezdinli et al., 1979; Dekker et al., 1981).

Since 1977, several studies have been published which showed the effect of SDD in neutropenic patients. In these studies different prophylactic regimens have been used. These studies have documented the fact that the application of SDD-antimicrobials, single or in combination, has significantly reduced the incidence of infections in patients with severe granulocytopenia (*Wade* et al., 1981; *Dekker* et al., 1987; *Karp* et al., 1987). One of the questions is whether a regimen including absorbable drugs like TMP-SMZ or quinolones, are superior -partly due to their systemic effect - to a regimen of non-absorbable drugs alone.

The EORTC Gnotobiotic Project Group investigated the combination colistin and neomycin versus colistin and TMP-SMZ. It appeared that the combination of colistin neomycin was less effective in preventing bacterial infection than did the colistin and TMP-SMZ combination (*Kurrle* et al., 1986).

The last years special attention has been paid to use of the new quinolones for SDD. Especially ciprofloxacin seems a promising drug for the prevention of infection in patients with granulocytopenia.

Advantages of ciprofloxacin versus TMP-SMZ were the good compliance, less adverse reactions and the absence of colonization with resistant Enterobacteriaceae (*Dekker* et al., 1987). On the other hand, TMP-SMZ has the advantage that if -besides granulocytopenia- an accompanying T cell defect exists, it protects the host against infections with organisms such as *Pneumocystis carinii*.

2. Patients in ICU units

The severely ill patients entering an intensive care unit (ICU) are prone to infections. This emphasises especially patients with long-lasting surgery and trauma patients (*Craven* et al., 1986; *Fife* and *Kraus*, 1988). There is a general agreement that injury brings about a suppression of host response defenses, and that this suppression is dose related (*Munster*, 1984). There is less agreement about which areas of immune response are the most important, and this may differ in different clinical situations.

The most common site of infection in mechanically ventilated patients is the respiratory tract. The mechanism of mechanical cleansing of the trachea and larynx by ciliar movement, which normally prevents colonization by ppmo, is interfered by the presence of an intratracheal cannula (*Johanson* et al., 1972). The use of antacids, to avoid stress ulcers and bleeding results in bacterial growth in the gastric juice. Retrograde pharyngeal colonization by organisms from the stomach may then under these circumstances contribute to the pathogenesis of nosocomial pneumonia (*Driks* et al., 1987; *Tryba*, 1987).

The second most common site of infection is the urinary tract, due to indwelling urinary catheters. Because many of these infections are caused by aerobic Gram-negative bacteria and staphylococci SDD may be beneficial in these group of patients.

Several studies have been performed to investigate this beneficial effect of SDD for respiratory tract infections. The first study by S*toutenbeek* et al. (1984) showed that there was remarkable reduction in hospital acquired pneumonia in the SDD treated patients compared to a historical control group, but additional systemic antibiotic prophylaxis was necessary to prevent early endogenous infections.

Although they clearly showed a reduction of the oropharyngeal colonization by ppmo, pneumonia and other infections by SDD treatment combined with systemic prophylaxis, there was no significant difference in the length of stay in the ICU nor on the mortality. Also other prospective studies with SDD in ICU settings did not show a difference in total mortality (*Unertl* et al., 1987; *Kerver* et al., 1987; *Ledingham* et al. 1988).

In conclusion it can be said that SDD in ICU patients, particularly in multiple trauma patients, may have benefit but needs further prospective investigation.

3. Transplantation patients

Transplantation patients are at increased risk for infection because of the immunosuppressive treatment given to prevent rejection of the transplant. All patients consequently have T cell suppression and are therefore at risk for T cell dependent infections. Within this category there are considerable differences in the incidence of an infection.

Most severely compromised transplant patients are those with allogeneic bone marrow (BM) transplantation (*Bortin* et al., 1983). They require strong immune suppression in order to mitigate or prevent graft versus host disease. Strong immune suppression involves increase risk for all T cell dependent infections enlisted above. However, the onset of such infections occurs usually not before the end of the first month after transplantation. By this time, the graft has repopulated the bone marrow and normal granulocyte counts may occur. In the first two weeks, however, severe granulocytopenia may exist as well as residual mucosal damage due to chemotherapy/irradiation for conditioning of the graft. This condition make these patients initially comparable to patients undergoing remission induction therapy for acute leukemia. SDD has been reported to be successful in infection prevention in BM-transplant patients (*Schmeiser* et al., 1988).

Of the remaining transplant patients, those with an implanted organ that is normally involved in the defense to infection, the liver, are the second in line regarding increased risk (*Kusne* et al., 1988).

Firstly the difficult operative procedure combined with abnormal bleeding often results in intra-abdominal haematoma.

Secondly it may take two to three weeks in these patients, before the Kupffer cells repopulate the terminal venules of the portal circulation and before the bile flow has restored. Regarding the latter, the patient is in general at lower risk if during transplantation an end to end anastomosis has been possible (the normal physiological situation) than if the gall bladder is connected to an ileum loop (Rough-Y anastomosis). In this case an open connection exists between the small intestines (jejunum) and the gall bladder; a condition which facilitates bacterial spread from the intestinal lumen into the bile duct system of the liver. An end to end anastomosis could be particularly important for the reduction of an infection risk in patients who have pretransplant enhanced PPMO-colonization in their small bowel. SDD prophylaxis has been reported to be successful in reducing Gram-negative infection (*Wiessner* et al., 1988).

4. Patients with extensive burns

Infection is supposed to be the primary cause of death in severely burned patients who survive the first 72 hours (*Monafo*, 1979; *Kagan* et al., 1985). Although bacteraemia mostly results from infected wounds, the principle infection leading to death is lower respiratory tract infection (*Goodwin* and *Yurt*, 1986; *Sittig* and *Deitch*, 1988). When there is beside the burnwound also lungdamage as a result of smoke inhalation, mortality due to pneumonia can be extremely high. Severely burned patients -like other trauma patients- suffer of an immunosuppression of nearly all components of the immune system. (*Munster*, 1984).

Several authors have investigated the close relationship between the microflora of the burnwound and the microflora of the G.I. tract (*Burke* et al., 1977; *Jarett* et al., 1978; *van Saene* and *Nicolai*, 1979; *Brook* and *Randolph*, 1981; *Manson* et al., 1990). SDD by means of aztreonam in experimentally burned mice decreased wound colonization during a 20 day period. *Jarrett* et al. (1978) using a non-absorbable regimen with erythromycin, neomycin and nystatin together with high hygienic policies showed a reduction in the colonization and infection rate of the patients compared with a control group.

Until now, only non-randomized studies have been undertaken which seem to show a beneficial effect of SDD with regard to the degree of bacterial colonization as well as to infectious complications (*Manson* et al., 1987).

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