Old Herborn University Seminar Monograph 3:
Consequences of antimicrobial therapy for the composition of the microflora of the digestive tract.
Editors: Carl Erik Nord, Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Institute for Microbiology and Biochemistry, Herborn-Dill, Germany: 31-46 (1993).

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

K. ROSZKOWSKI[1], J. BEUTH[2], H.L. KO[2], W. ROSZKOWSKI[1], J. JELJASZEWICZ[3], and G. PULVERER[2]

[1]National Research Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; [2]Institute of Medical Microbiology and Hygiene, University of Cologne, Cologne, Germany; and [3]National Institute of Hygiene, Warsaw, Poland

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

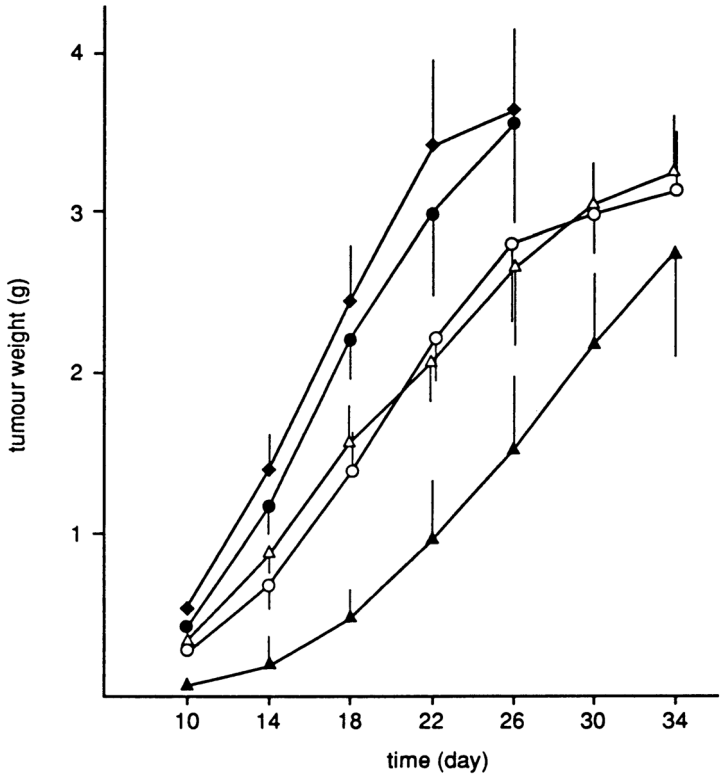

Figure 1: Influence of antibiotic treatment on sarcoma L-1 tumour growth in BALB/c-mice. (❍: control, ▲: mezlocillin, ●: rifampicin, ✦: doxycycline, △: piperacillin)

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]$p<0.01$

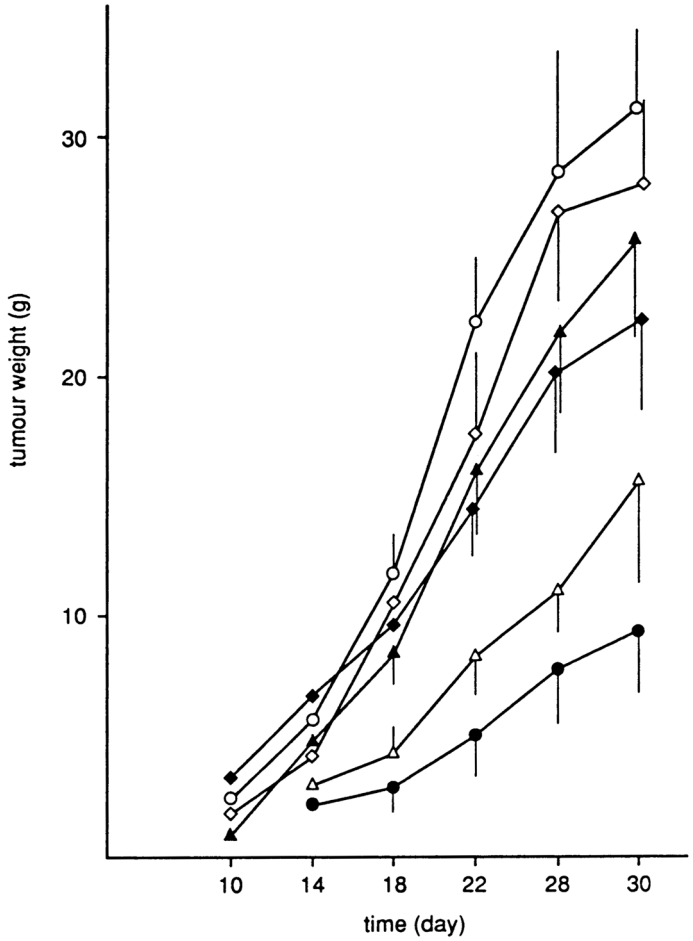

Figure 2: Influence of antibiotic treatment on sarcoma L-1 tumour growth in animals preimmunized with killed tumour cells.
(○: nonimmunized control, ●: immunized, ✧: immunized + doxycycline,
✦: immunized + mezlocillin, ▲: immunized + rifampicin, △: immunized + piperacillin)

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

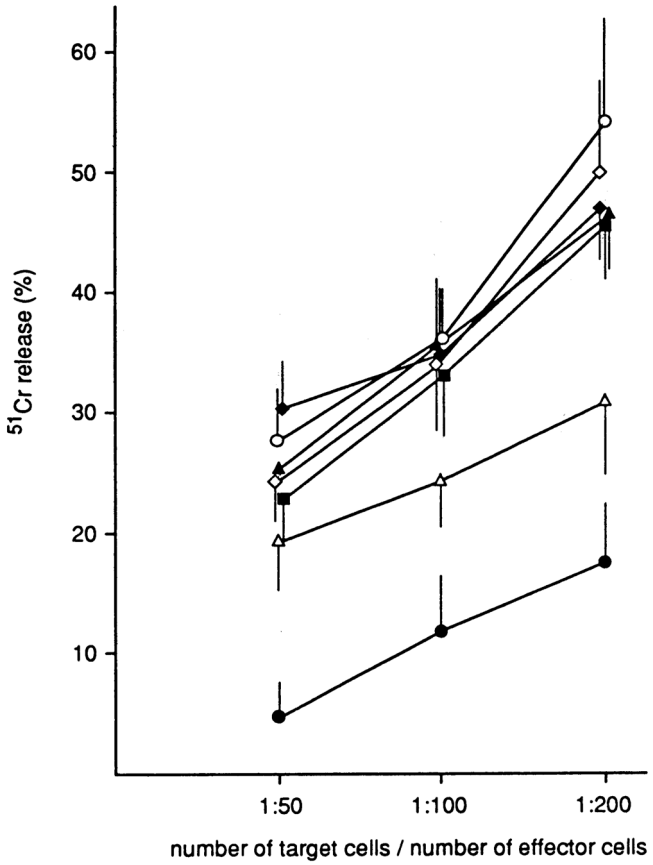

Figure 3: Influence of antibiotic treatment on specific cytotoxicity.
(○: control, ✦: clindamycin, ▲: streptomycin, ✧: gentamicin, ■: amikacin, △: rifampicin, ●: doxycycline)

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

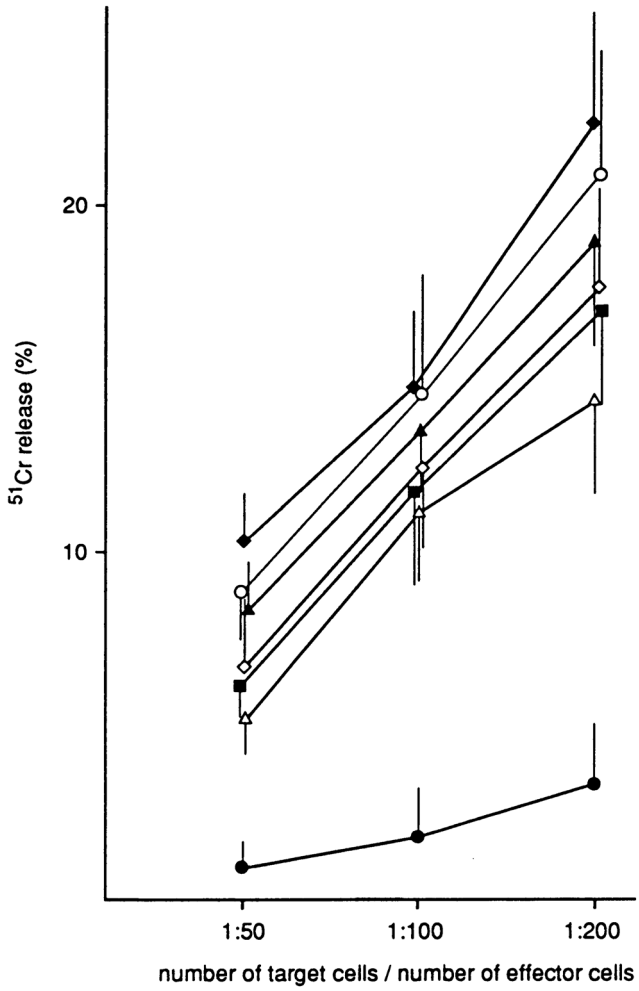

Figure 4: Influence of antibiotic treatment on spleen lymphocytes from tumour bearing animals against autologous tumour cells.
(❍: control, ✦: penicillin G, △: piperacillin, ■: cefotaxime, ✧: cefazolin, ▲: cefamandole, ●: mezlocillin)

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

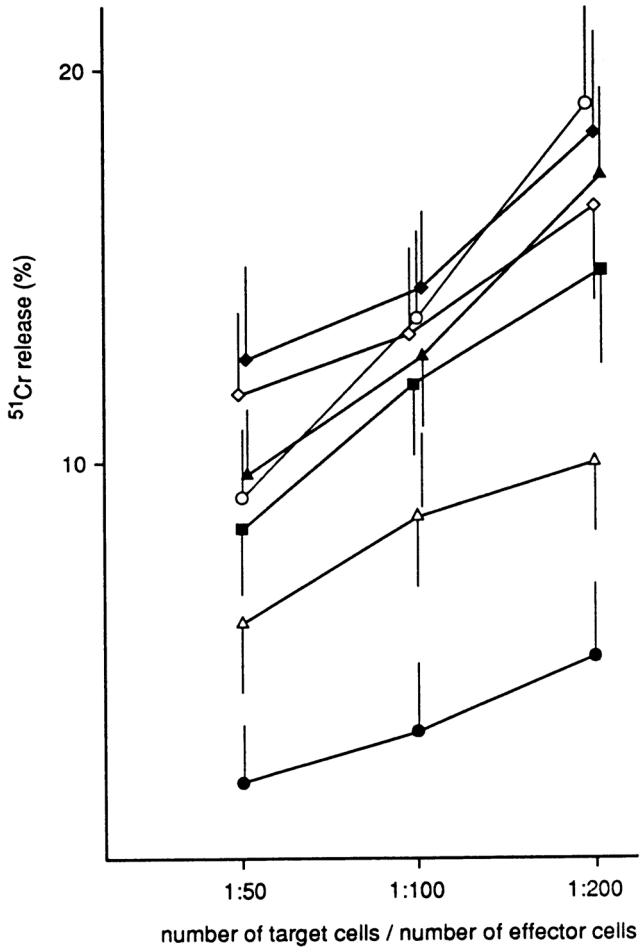

Figure 5: Influence of antibiotic treatment on non specific cytotoxicity.
(❍: control, ✦: clindamycin, ▲: streptomycin, ✧: gentamicin, ■: amikacin, △: rifampicin, ●: doxycycline)

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-

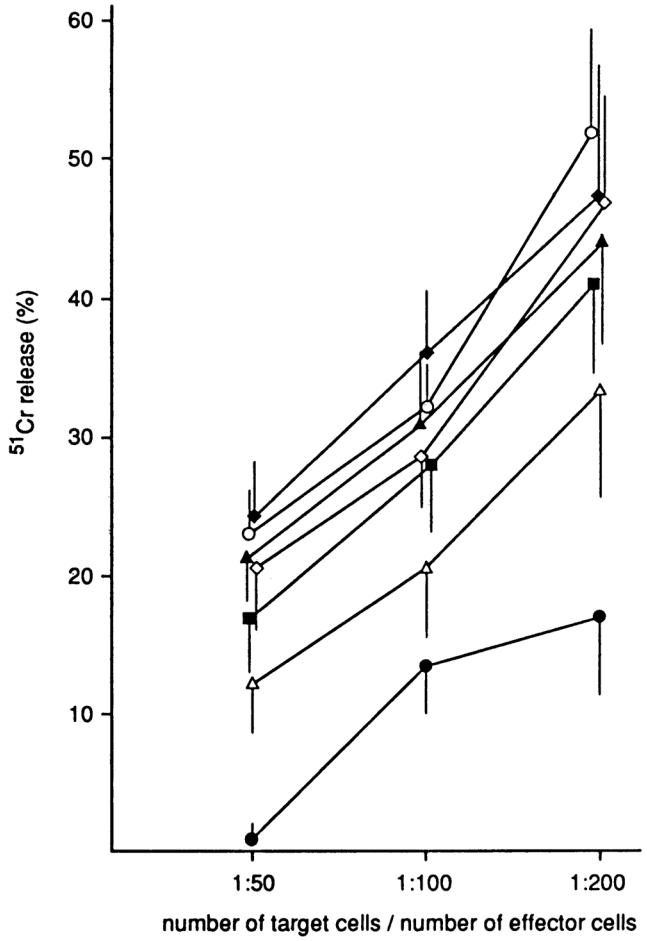

Figure 6: Influence of antibiotic treatment on spleen lymphocytes from C3H mice against YAC-1 cells.
(○: control, ✦: penicillin G, △: piperacillin, ■: cefotaxime, ✧: cephalotin, ▲: cefamandole, ●: mezlocillin)

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]$p<0.01$

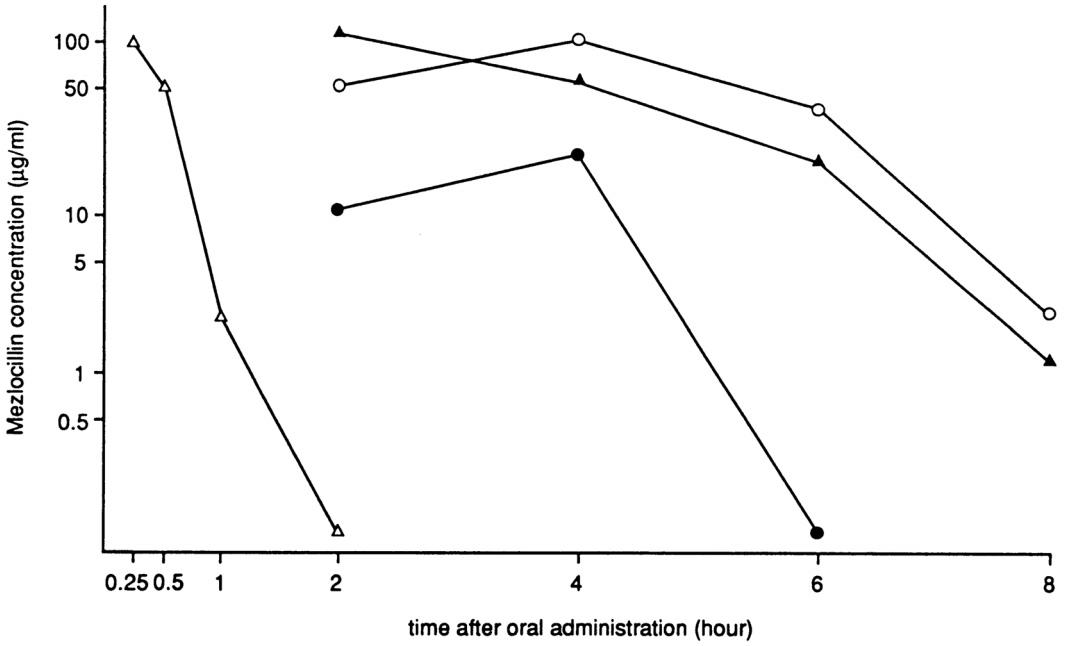

Figure 7: Concentration of mexlocillin in mouse serum and coecum contents after subcutaneous and peroral administration of the drug.
△: serum concentration (dose: 150 mg/kg body weight s.c.)
▲: coecum concentration (dose: 150 mg/kg body weight s.c.)
❍: coecum concentration (dose: 75 mg/kg body weight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

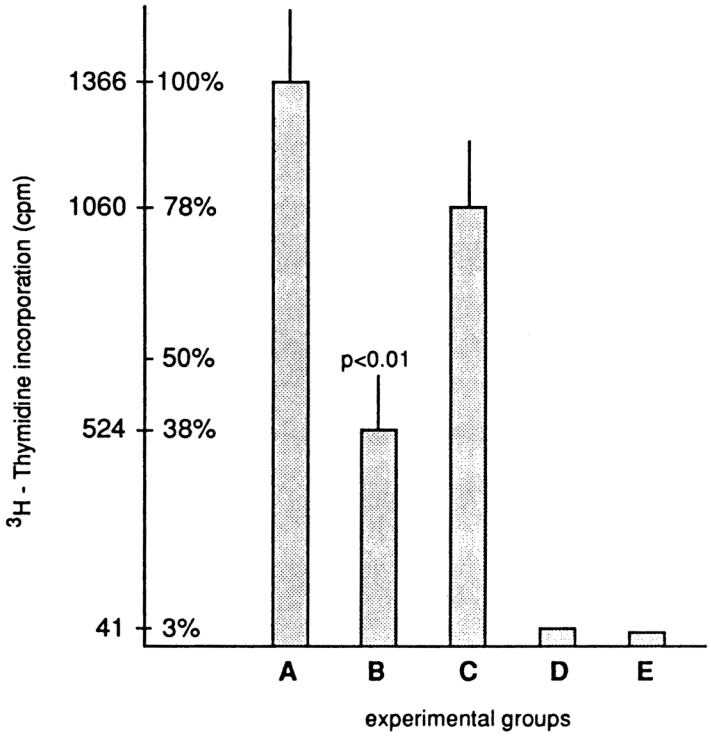

Figure 8: ^{3}H-thymidine incorporation in sarcoma L-1 cells (**A**), in sarcoma L-1 cells and control macrophages (**B**), in sarcoma L-1 cells and macrophages from decontaminated mice (**C**), in control macrophages (**D**), and in macrophages from decontaminated mice (**E**).

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.

LITERATURE

Abrams, G.D., Bauer, H., and Sprinz, H.: Influence of normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germfree and conventional mice. Lab. Invest. 12, 335-364 (1963).

Bassi, L., Di Bernardino, I., Arioli, V., Silvestrini, L.G., and Liegniere, E.L.: Conditions for immunosuppression by rifampicin. J. Inf. Dis. 128, 736-744 (1973).

Crabbe, P.A., Bazin, H., Eysen, H., and Heremans, J.F.: The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. In. Arch. Allergy 34, 362-375 (1968).

Forsgren, A., and Banck, G.: Influence of antibiotics on lymphocyte function *in vitro*. Infection Suppl.I, 91-97 (1978).

Gillissen, G.J.: Influence of Cephalosporins on humoral immune response. In: The Influence of Antibiotics on the Host-Parasite Relationship (Eds.: Eicenberg, H.U., Hahn, H., and Opferkuch, W.). Springer Verlag, Berlin, Heidelberg, New York, 5-11 (1982).

Hoeprich, P.D., and Martin, C.H.: Effects of tetracycline, polymycin B and rifampicin on phagocytosis. Clin .Pharmacol. Ther. 11, 418-422 (1970).

Janik, P.: Kinetyka i mechaniszmy zmian w populacji komorkowej nowotworow o zatrzymanym wzroscie lub zanikajacych w nastepstwie odczynow immunologicznych organiszmu. Post. Biol. Komorki 4, 405-412 (1977).

Janik, P., and Roszkowski, W.: The *in vivo* cytostatic effect induced by *Propionibacterium granulosum* on murine tumor cells. J. Cancer Res. Clin. Oncol. 98, 51 (1980).

Kabir, S., Rosenstreich, D.L., and Mergenhagen, S.E.: Bacterial endotoxins and cell membranes. In: Bacterial Toxins and Cell membranes (Eds.: Jeljaszewicz, J., and Wadstrom, T.). Academic Press, London, New York, San Francisco, 59-87 (1978).

Kasamaki, A., Furuya, K., and Kanamitsu, H.: Immune response in rabbits treated with tetracycline. J. Toxicol. Sci. 389-404 (1979).

Mandel L.A.: The effects of antibacterial, antiviral and antifungal drugs on the phagocyte, microbicidal functions of the human polymorphonuclear leukocyte. In: The Influence of Antibiotics on the Host-Parasite Relationship (Eds.: Eicenberg, H.U., Hahn, H., and Opferkuch, W.). Springer Verlag, Berlin, Heidelberg, New York, 40-55 (1982).

Nessi, R., Palanza, R., and Fows, G.: Rifampicin and immunosuppression. Arzneim. Forsch. 94, 832-836 (1974)

Rook, G.A.: Is the macrophage the site of immunosuppression of rifampicin? Tubercle 54, 291-295 (1973).

Serrou, B., Solassol, A., Joyeux, H., Pujol, H., and Romieu, C.: Immunosuppressive effect of rifampicin. Transplantation 14, 654 (1972).

Thong, Y.H., and Ferrante, A.. Effect of tetracycline treatment on immunological response in mice. Clin. Exp. Immunol. 39, 728-732 (1980).

Wiegersma, N., Jansen, G., van der Waaij, D.: Effect of twelve antimicrobial drugs on the colonization resistance of the digestive tract of mice and on endogenous potentially pathogenic bacteria. J. Hyg. Camb. 88, 221-230 (1982).