EFFICACY OF ANTIMICROBIAL THERAPY IN THE COMPROMISED HOST

AN EXPERIMENTAL STUDY

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J. SPERNA WEILAND
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 15 OKTOBER 1980 DES NAMIDDAGS TE 3.45 UUR

DOOR

IRMINA ADRIANA JOHANNA MARIA BAKKER-WOUDENBERG
geboren te Utrecht

1980
DRUKKERIJ J.H. PASMANS B.V., 's-GRAVENHAGE

PROMOTOR : PROF. DR. M.F. MICHEL

CO-REFERENTEN: PROF. DR. J. ABELS

PROF. DR. O. VOS

voor Jaap

research *'Als alle gegevens bij elkander zijn*
zullen geleerden zich daarover buigen
en u bewijzen uit het ongerijmde'

Gerrit Achterberg

CONTENTS

GENERAL INTRODUCTION

For many years it has been recognized that patients with impaired host defense mechanisms due to hemoproliferative malignancy or its treatment are subject to episodes of fulminating, often lethal bacterial infection. The proper management of these infections is a problem, because antimicrobial agents which are known to be effective in the normal host, lose, at least partially, their efficacy in these patients. Therapeutic failures occur despite the organisms usual sensitivity. As a consequence, the use of combinations of antimicrobial drugs have been recommended for presumptive treatment of these life-threatening infections when the identity and susceptibility of the causative bacteria are unknown.

The high risk of infectious complications in these patients may be due to impairment of their defense mechanisms. This in turn is the consequence of the malignant process itself. The immune system and its functions are involved in many aspects of malignancies, especially in hematological malignancies. These each have a characteristic pattern of immunodeficiencies. For example, hematological malignancies may suppress humoral and cellular immunity and cause neutropenia or an impaired neutrophil function. Also the antineoplastic therapy by radiation and/or chemotherapeutic agents, interferes with specific and nonspecific host defense functions.

Several factors usually contribute simultaneously to the impairment of host defense in cancer patients. These include the underlying disease itself, invasive procedures and/or the antineoplastic therapy. As a result, a clear understanding of the relation between the efficacy of antimicrobial therapy and specific defects in host defense mechanisms is impossible. The critical role of individual host defense factors in the process of recovery during antimicrobial therapy can only be established in experimental infection. Two questions arise. To what extent does the susceptibility to infections and the efficacy of antimicrobial therapy depend on host defense mechanisms? Secondly,

can lack of host defense functions be partially compensated by intensification of the antibiotic treatment schedule. Animal studies may contribute to a deeper understanding of the relation between specific gaps in host defense and efficacy of antimicrobial therapy.

The purpose of our study, and the subject of this thesis is, to investigate the importance of intact host defense mechanisms for the success of antimicrobial treatment in experimental infections. Our studies have concentrated on deficiencies in host phagocytic activity, because leukocytes play a central role in the host's defense against infection. In rats and mice infections were produced by inoculation with *Streptococcus pneumoniae* and *Listeria monocytogenes*, respectively. These infectious agents were chosen because of the fact that the course of infection and the defense mechanisms of the host are completely different in both models:

- Intrabronchial inoculation of *S. pneumoniae*, an obligatory extracellular parasite, results in an acute inflammatory response and a rapidly-spreading pneumonia with extensive pulmonary lesions. Host defense against *S. pneumoniae* depends on phagocytosis by polymorphonuclear leukocytes (PMN). Without the presence of specific antibodies certain complement components act as opsonins. The spleen participates in clearing pneumococci from the blood.
- Intravenous inoculation of *L. monocytogenes*, a facultative intracellular parasite that can survive and multiply in macrophages particularly in the spleen and liver, results in a chronic infection. Host resistance to *L. monocytogenes* depends on T-cell mediated immunity resulting in activation of macrophages, the target cells of *Listeria*.

The host-bacteria relationship is relatively well-understood, but completely different in these experimental infection models. This enabled us to evaluate the change in efficacy of antimicrobial treatment as a function of selective elimination of several forms of host defense.

Chapters 1 through 3 describe the effect of impaired phagocytosis of *S. pneumoniae* by PMN upon the course of the infection and the efficacy of antimicrobial treatment of pneumococcal pneumonia in rats. Chapter 1 and chapter 2 deal with the activity of penicillin therapy in this model. In chapter 3 several aspects of clindamycin therapy were examined.

Another form of impaired host defense to pneumococcal infection we studied, was the removal of the spleen. In rats with pneumococcal bacteremia we studied the effect of splenectomy upon the course of the infection and the outcome of penicillin treatment. The results of these studies are reported in chapter 4.

Chapter 5 describes the effect of impaired T-cell mediated immune functions upon the course of infection and the efficacy of ampicillin treatment in mice with infection caused by *Listeria monocytogenes*. The results of rifampicin treatment in this model are reported in the appendix.

Finally a general discussion of the results of our experimental studies is presented in chapter 6.

Chapter 1

EFFICACY OF ANTIMICROBIAL THERAPY IN EXPERIMENTAL RAT PNEUMONIA: EFFECTS OF IMPAIRED PHAGOCYTOSIS*

Irma A.J.M. Bakker-Woudenberg, J.Y.T. de Jong-Hoenderop, and M.F. Michel

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, The Netherlands

The importance of intact host defense mechanisms for successful antimicrobial therapy was investigated in an animal model. Recovery from lobar pneumococcal pneumonia as a result of penicillin therapy was studied in normal rats and in rats treated with cobra venom factor. This factor was used to selectively suppress the phagocytosis of pneumococci as a result of complement depletion. Although complete recovery from the infection occurred in normal rats after appropriate penicillin therapy, this was not the case in cobra venom factor-treated rats. Within the limitations of this study, evidence is presented for loss of antibiotic activity as a consequence of impaired phagocytosis.

Introduction

Severe infections frequently occur in patients with hemoproliferative malignancies (5, 13, 15, 18, 19). They may be difficult to cure, as antimicrobial agents which are effective in normal hosts appear to have lost, at least in part, their efficacy in these patients. The high risk of infections in patients with malignancies may be due to an impairment of host defense mechanisms as a consequence of the malignant process itself or of the antineoplastic therapy (chemotherapy or radiotherapy), or both. It is known that both factors may suppress specific

*Infection and Immunity (1979) 25, 366-375.

and nonspecific host defense mechanisms, including cellular immunity (7, 8, 17, 22), antibody formation (7, 17, 22, 36), and the number or function of granulocytes (6, 7, 17, 26). Usually several factors contribute simultaneously to the impairment of host defense in cancer patients. The critical role of individual host defense factors in the process of recovery during antimicrobial therapy can only be established in experimental infection. In the present study several aspects of antimicrobial therapy of experimental pneumococcal pneumonia in normal rats and in rats with impaired phagocytosis were examined. Phagocytosis was impaired by complement depletion by using injections of cobra venom factor (CVF). This model permits the study of the role of the host defense factors in the process of recovery during antimicrobial therapy.

Materials and Methods

animals

Female R-strain albino rats (inbred strain specific pathogen free; 15 weeks old; weight, 180 to 210 g; bred in the Laboratory Animals Center of the Erasmus University Rotterdam) were used in all experiments.

bacteria

A *Streptococcus pneumoniae* type III strain isolated from a clinical case was used in all experiments. The minimal inhibitory concentration for penicillin was 0.024 μg/ml (tube dilution test). The virulence of the bacteria was maintained by passage in rats every 2 months and storage at $-70^{\circ}C$ in Todd-Hewitt broth (Oxoid) with 10% glycerol (Merck).

pneumonia

Experimental pneumonia was produced in the following manner. The rats were anesthetized with Hypnorm (Duphar) and pentobarbital. The left main stem bronchus was intubated, and the left lobe of the lung was inoculated with a suspension of 6×10^7 *S. pneumoniae* type III cells in a volume of 0.02 ml. To estimate the severity of the resulting pneumonia, five animals were observed during the entire course of

infection. In addition, dissections were made of groups of five animals at different intervals after inoculation, namely at 36, 84 and 108 h on rats who received no antibiotic therapy and at 36, 84 and 132 h on antibiotic-treated rats.

Parameters for the severity of the resulting pneumonia were as follows: roentgenogram of the chest, total body weight, rectal temperature, macroscopic examinations of the lesion in the left lobe of the lung, weight of the left lobe of the lung, quantitation of the number of viable bacteria in the left lobe and the four right lobes of the lung, and cultures of blood and pleural fluid.

histology

Lungs were fixed by injecting the trachea with 10% Formalin under a constant pressure to reexpand the lungs. The specimens were then dehydrated in ethanol and toluol, imbedded in paraffin, sectioned, and stained with hematoxylin-eosin or by the Gram stain technique.

antimicrobial treatment

The sodium salt of penicillin G in aqueous solution was injected intramuscularly into the thigh muscles of the rear legs. Every 12 h a dose of 2 mg/kg of body weight was administered. Nine injections were given, the first dose at 36 h after inoculation of the pneumococci.

treatment with CVF

The venom of the cobra (*Naja naja siamensis*) was purified and kindly supplied by I.J.C. Wilschut (Cell Tissue Kinet., in press). Rats were injected intraperitoneally 14 times with equal doses of CVF (0,7 units/kg of body weight). The first dose was administered at the time of pneumococcal inoculation, followed by five doses every 8 h and eight doses every 12 h. To determine complement depletion in vivo, rats were bled at several times during CVF treatment by orbital puncture. Sera were tested by an assay of complement hemolytic activity and an assay for complement component C3.

assay for complement hemolytic activity

The sera were tested in a mixture containing 0.2 ml of sheep erythrocytes (10^7 cells, sensitized with hemolysin), 0.2 ml of serially diluted serum,

and 0.8 ml of Veronal buffer (pH 7.2; Oxoid). After incubation for 60 min at 37°C, the mixture was centrifuged for 10 min at 1,300 x g, and the release of hemoglobin in the supernatant was recorded at 413 nm in a Zeiss spectrophotometer. Controls included 100 and 0% lysis (guinea pig complement and Veronal buffer, respectively, instead of rat serum). The data derived from this assay were presented as 50% complement hemolysis units per milliliter, corresponding to the reciprocal of the serum dilution that gave 50% hemolysis.

assay for complement component C3

Serum C3 concentration was measured by the rocket immunoelectrophoresis technique of Laurell, with some modifications (Wilschut, in press). Briefly, to avoid blunt precipitate peaks, the serum samples were carbamylated with 2 M potassium cyanate for 1 h at 37°C. To give a more precise measurement of the C3 concentration, the area enclosed by the precipitation peak was determined. Rabbit anti-rat C3 serum was purchased from Nordic Immunological Laboratories, Tilburg, The Netherlands.

in vitro phagocytosis

A mixture of 7×10^8 washed pneumococci and 1 ml of rat serum was tumbled gently at 14 rpm for 30 min at 37°C. After opsonization, the mixture was kept for 2 h at 4°C, which was followed by centrifugation of the pneumococci at 3,500 x g for 10 min at 4°C. A leukocyte suspension was obtained from rat peritoneal washings 21 h after intraperitoneal injection of 4.5 ml of thioglycolate (Difco Laboratories). The peritoneal cells were washed two times in Hanks bovine albumin-glucose solution (33) and pelleted at 300 x g for 10 min at 4°C. Eighty-six percent of the leukocytes were polymorphonuclear leukocytes (PMN). In the phagocytic test, 7×10^8 opsonized pneumococci and 7×10^7 leukocytes were tumbled gently at 14 rpm and 37°C in a total volume of 0.3 ml of buffer in polypropylene tubes (Falcon Plastics). (The buffer contained 0.14 M NaCl, 5 mM KCl, 0.4 mM $MgSO_4.7H_2O$, 0.5 mM $MgCl_2.6H_2O$, 1.3 mM $CaCl_2$, 0.4 mM $Na_2HPO_4.12H_2O$, 0.4 mM KH_2PO_4, 5.6 mM glucose, and 4 mM $NaHCO_3$). After 30 min

phagocytosis was stopped by dilution in cold buffer (see above). The cells were centrifuged at 25 x g for 10 min at $4^{\circ}C$ and washed to remove most of the extracellular bacteria. The leukocytes were then examined under a microscope in Gram-stained smears. A total of 400 leukocytes were observed, and the percentage of PMN containing pneumococci was calculated. Next, the average number of pneumococci per 100 PMN was estimated. The percent phagocytosis was calculated from the percentage of PMN containing pneumococci and the average number of pneumococci per cell. For instance, when 68% of the leukocytes contained pneumococci with an average of six pneumococci per cell, we calculated a total number of 1,632 pneumococci per 400 leukocytes. The estimation of the total number of pneumococci per 400 leukocytes, as seen after opsonization with normal rat serum, was called 100% phagocytosis.

Results

experimental pneumonia

After inoculation of the rats with 6×10^7 pneumococci, pneumonia developed within 24 h. Initially the infection was confined to the left lobe of the lung. By 108 h the pulmonary lesion involved the entire left lobe, and the infection was spreading to the right lobes. No spontaneous recovery occurred in untreated rats. During the course of the infection total body weight decreased constantly (Fig. 1A), and rectal temperature decreased below normal after an initial rise (Fig. 1B). The presence of an inflammatory response in the left lobe of the lung can be seen in a roentgenogram of the chest (Fig. 2B). Within the duration of our experiments no animals died spontaneously from infection. The animals were sacrificed at 36, 84, and 108 h after initiation of infection, and data are presented in Fig. 1C through F. In these figures each point represents one animal. The involvement of the lung tissue in the infectious process was reflected by a proportional increase in weight (up to sixfold) of the left lobe of the lung (Fig. 1C). At 108 h the pulmonary lesion was very extensive. During the course of infection, the number of pneumococci cultured

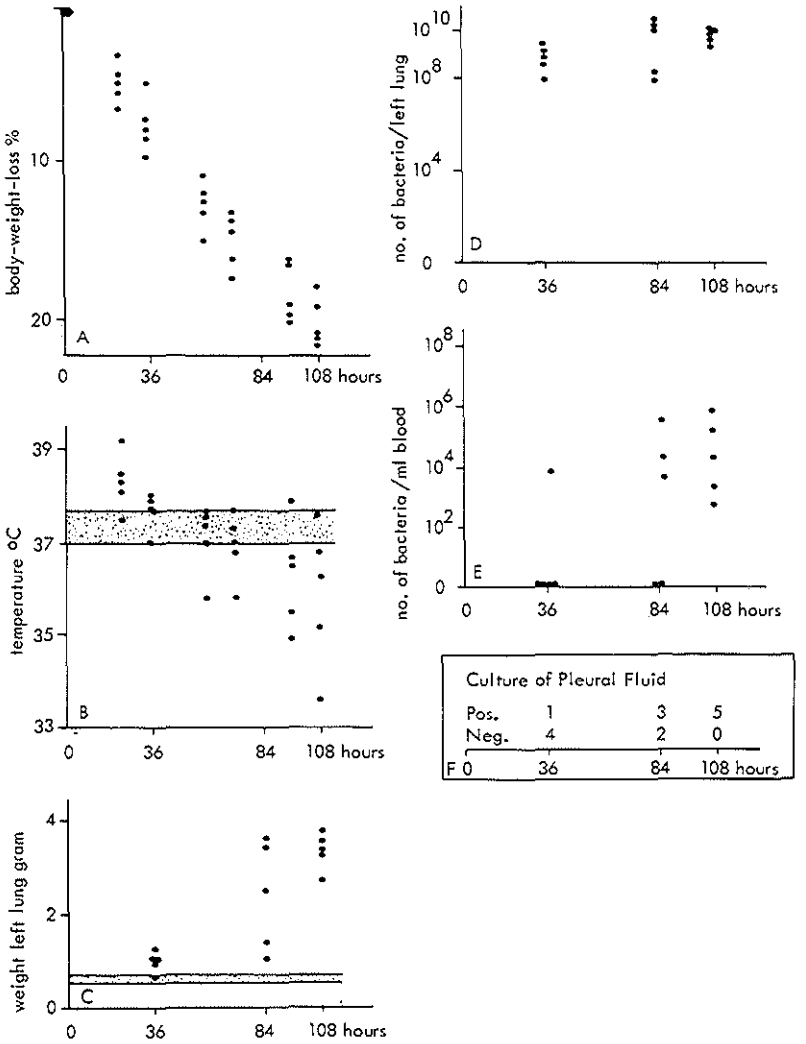


Fig. 1. *Course of pneumonia in normal rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

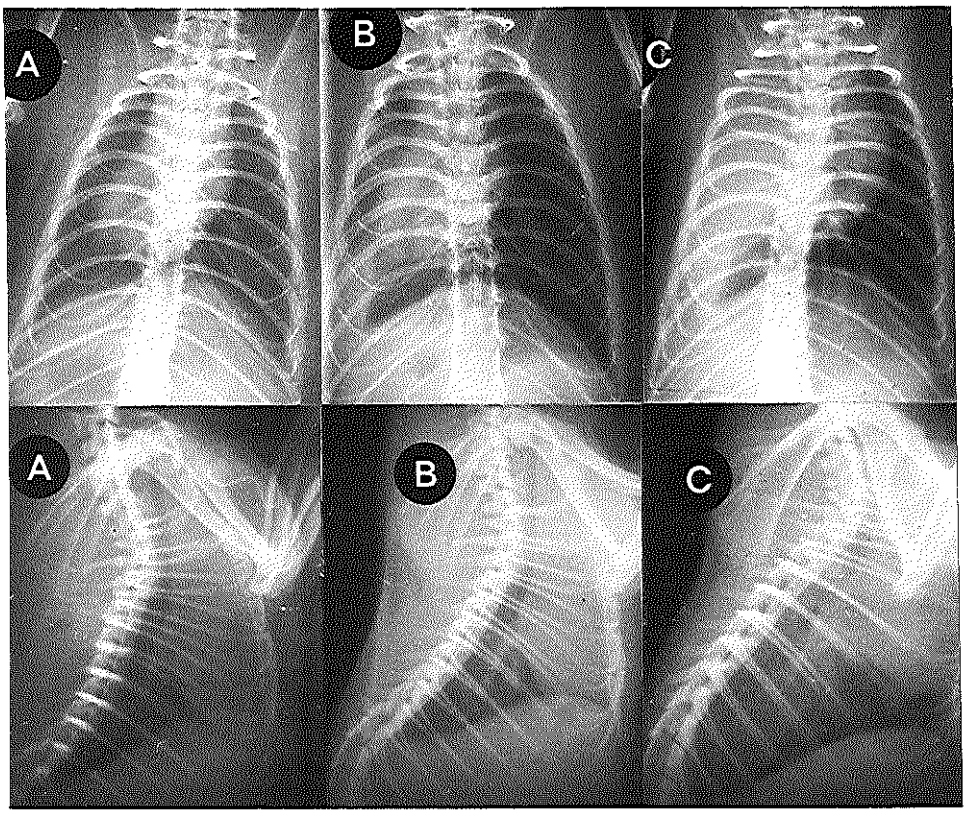
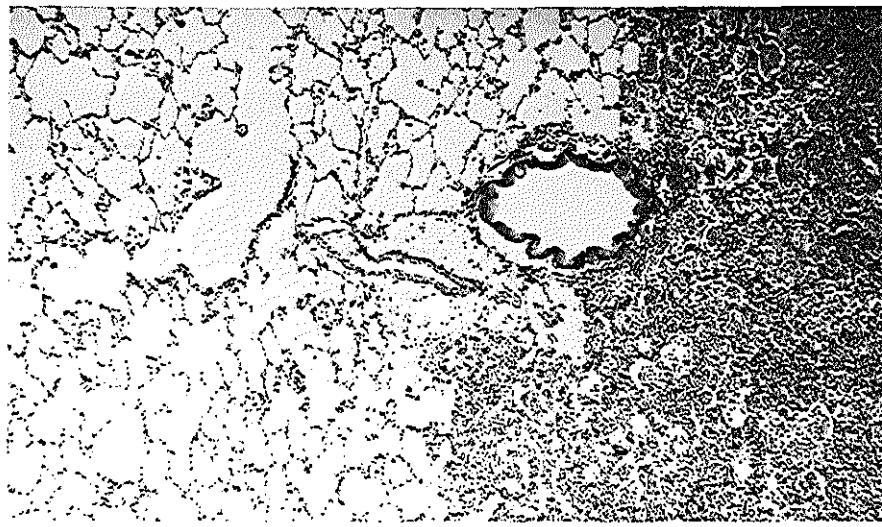


Fig. 2. *(A) Chest roentgenograms of normal rats. (B) and (C) Chest roentgenograms of rats who developed pneumonia 3 days after inoculation with 6 x 10^7 type III pneumococci. (B) Untreated rats. (C) CVF-treated rats.*

from the left lobe of the lung increased and reached an average number of 6 x 10^9 bacteria at 108 h (Fig. 1D). Sooner or later the pneumonia caused a pneumococcal invasion of the bloodstream and pleural cavity. At 108 h this was reflected by positive cultures of blood and pleural fluid in all animals (Fig. 1E and F). A control experiment revealed that the technique of intubation itself did not influence total body weights or rectal temperatures.

A



B

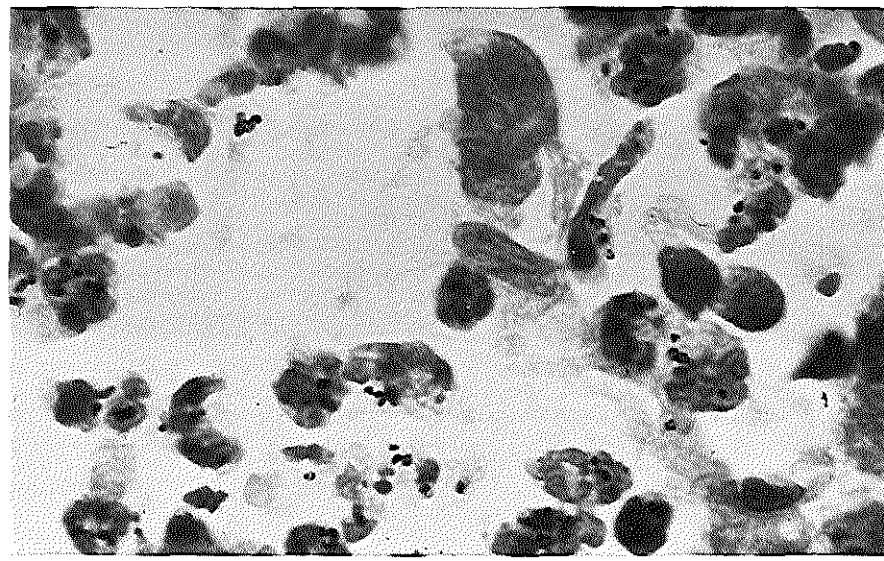


Fig. 3. *Sections of the left lobes of the lungs from rats inoculated with type III pneumococci. (A) Section stained with hematoxylin-eosin (x16). At the margin of the lesion in the edema zone there are few PMN in the alveoli. More to the center of the lesion are edema-filled alveoli containing many PMN. In the center of the lesion zone of advanced consolidation, alveoli are packed with PMN. (B) Section stained with Gram stain (x400). Alveoli contain PMN and pneumococci, many of which have already been phagocytized by PMN.*

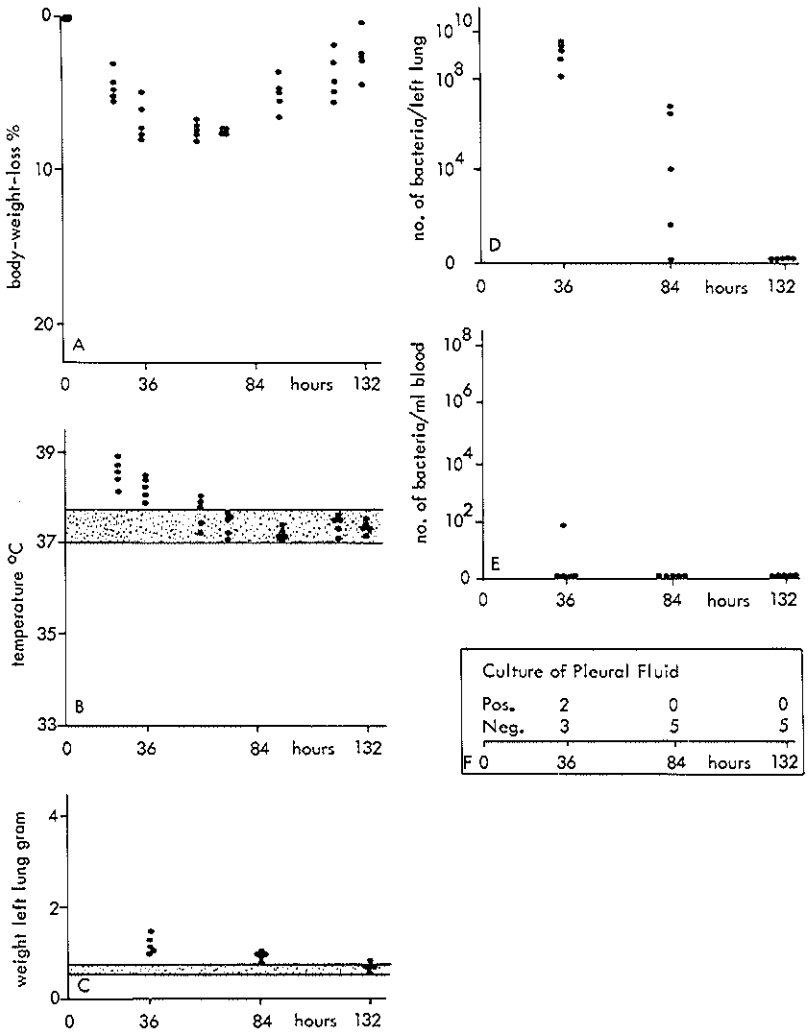


Fig. 4. *Action of penicillin (2 mg/kg per 12 h, starting at 36 h) upon the course of pneumonia in normal rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

histology

The histological studies of the pulmonary lesion revealed that no lung abscesses were present. Neutrophils predominated in the lesion, and phagocytosis of pneumococci by PMN was observed in the alveoli (Fig. 3).

antibiotic therapy

Penicillin therapy with repeated injections of 2 mg/kg per 12 h was begun 36 h after the initiation of infection. Figures 4A through F show that antibiotic therapy was successful in normal rats. After an initial decrease the total body weight increased again (Fig. 4A). Rectal temperature normalized after an initial increase (Fig. 4B). The spreading lesion was promptly arrested, as shown by the moderate increase in weight of the left lobe of the lung (Fig. 4C). At 132 h all cultures of lungs, blood, and pleural fluid were sterile (Fig. 4D through F).

Table I. *Opsonic activities of sera from normal rats and rats treated with CVF*[a]

Preincubation medium	% phagocytosis
Normal rat serum undiluted	100
HBG[b]	2
Heated normal rat serum (56°C for 30 min)	18
C3-deficient rat serum[c]	43

[a] Type III pneumococci were preincubated in several media and added to the phagocytic system (see text).

[b] HBG, Hanks bovine albumin-glucose solution (32)

[c] Obtained from CVF-treated rats (see text).

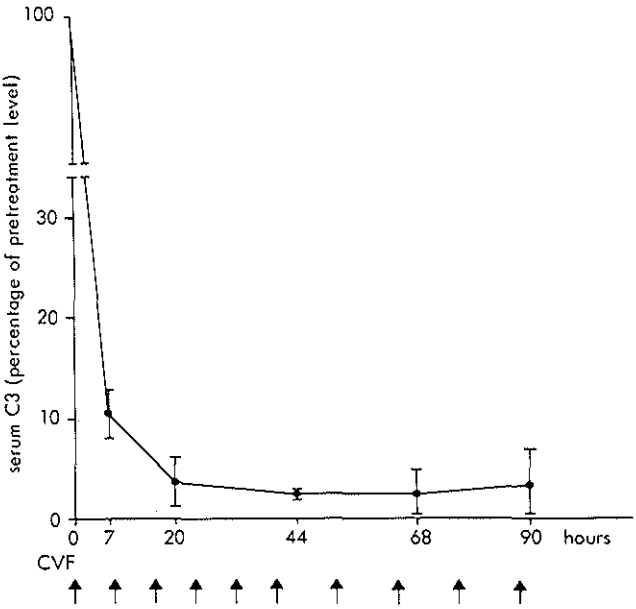


Fig. 5. *Effect of CVF (0.7 units/kg) on circulating C3 levels in serum. Each point represents the mean value of four rats (± standard deviation).*

in vivo effects of CVF

Whole complement hemolytic activity in serum decreased to less than 2% of normal values after multiple injections of CVF (0,7 units/kg). Figure 5 shows that the total levels of circulating C3 after the same CVF treatment fell to less than 3% of the pretreatment level.

influence of CVF on phagocytosis in vitro

Pneumococci were effectively phagocytized by PMN after opsonization with normal rat serum (100%). The opsonic activity of the serum decreased to 18% upon heating at 56°C for 30 min and was reduced to 43% when rats were treated with CVF, as evidenced by an impaired phagocytosis of pneumococci (Table 1).

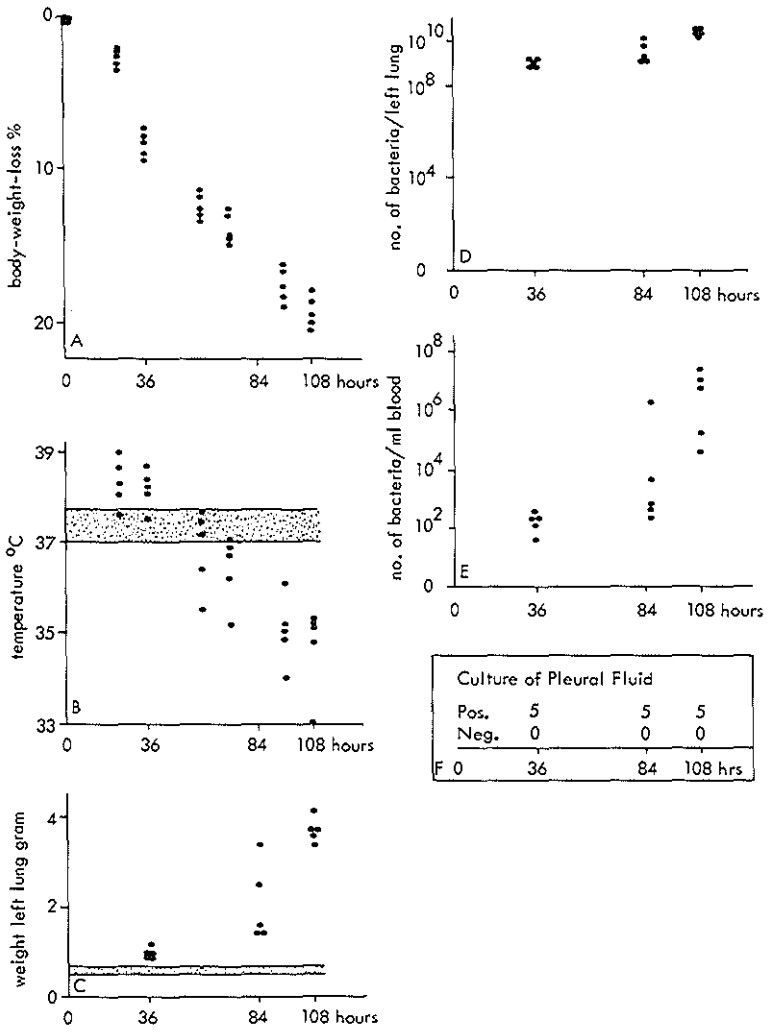


Fig. 6. *Course of pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

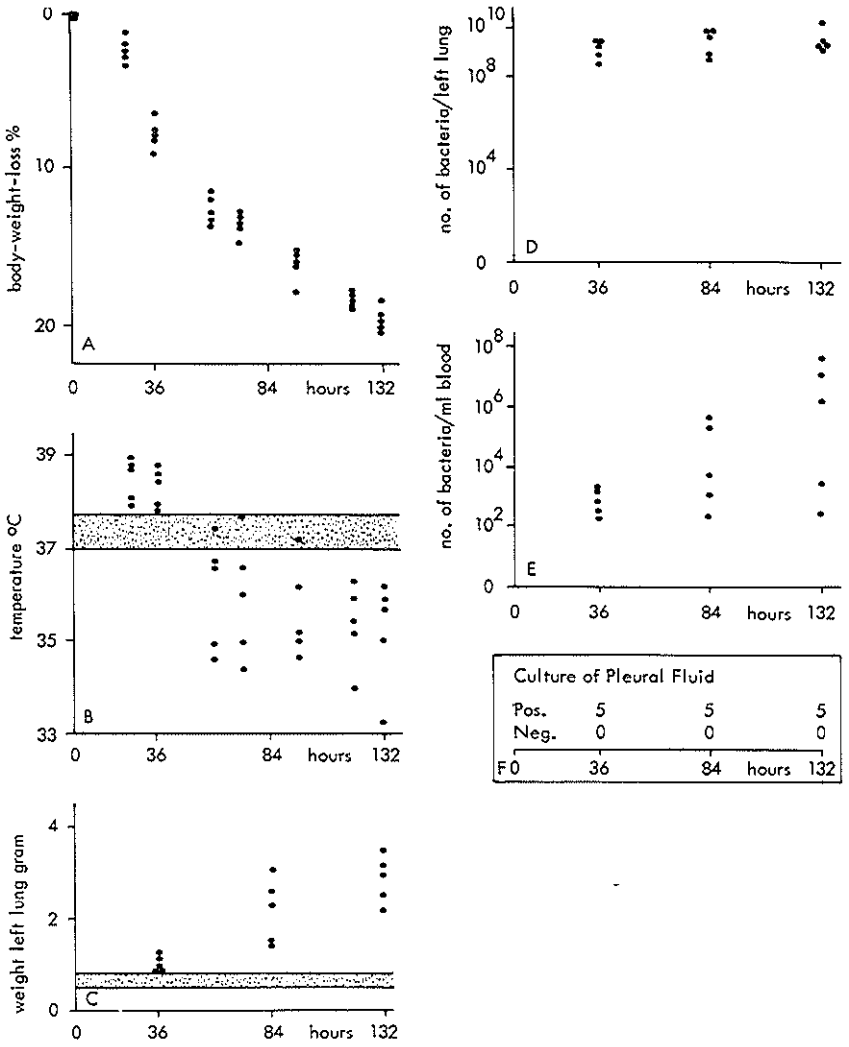


Fig. 7. *Action of penicillin (2 mg/kg per 12 h, starting at 36 h) upon the course of pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

pneumonia in CVF-treated rats

With the same number of pneumococci used in previous experiments pneumonia was produced in CVF-treated rats (Fig. 6A through F). The course of this infection was more severe in comparison with the infection in normal animals (Fig. 1A through F). As appeared from the roentgenograms, the pneumonia spread more rapidly in the CVF-treated rats (Fig. 2C) than in the rats not treated with CVF (Fig. 2B). No spontaneous recovery occurred in CVF-treated rats, as evidenced by a continuous decrease in total body weights (Fig. 6A) and rectal temperatures. The values of the latter finally fell far below normal (Fig. 6B). Lungs taken from animals sacrificed at 36, 84, and 108 h after inoculation also revealed more severe pneumonia as a result of CVF treatment. The pulmonary lesions were very extensive, as seen in the increase in weight (up to sevenfold) of the left lobe of the lung (Fig. 6C). By 108 h an average of 3×10^{10} pneumococci were cultured from the left lobe (Fig. 6D). In early stages of the infection pneumococci were seen in the blood and pleural fluid (Fig. 6E and F), and by 108 h the average number of bacteria per milliliter of blood had increased to 2×10^{6}.

A control experiment revealed that the CVF treatment itself did not influence total body weights or rectal temperatures.

antibiotic therapy of CVF-treated rats

Repeated injections of penicillin (2 mg/kg per 12 h starting 36 h after initiation of infection) was unsuccessful in eradicating the pneumococcal infection. There was also no increase in total body weights (Fig. 7A) and no normalization of rectal temperatures (Fig. 7B), and the cultures of lungs, blood, and pleural fluid remained positive (Fig. 7C through F). When the animals were sacrificed, the pneumonia was still actively spreading.

Discussion

The purpose of this study was to examine the efficacy of antimicrobial therapy in animals with selectively impaired host defense mechanisms.

Pneumonia and septicemia are the most common types of infections in compromised hosts (5, 14, 30) and major problems in patients with severe neutropenia (23). An experimental pneumococcal pneumonia in rats was chosen as an infection model, because the host-bacteria relationship in this model is relatively well understood (29, 33, 37, 39). The experimental pneumonia was characterized by a lobar consolidation as seen on the chest roentgenograms and at autopsy. Our histological studies of the pulmonary lesion revealed the same characteristics as described by Wood and Smith (42), except that there were no areas of abscess formation. In addition, bacteremia and extrapulmonary complications, such as pleurisy, were present as a rule. The pneumonia in the rats spread actively. Phagocytosis of pneumococci by PMN is the primary defense against this infection, and our histological results supported prior studies (42). However, phagocytosis alone was not sufficient for spontaneous recovery. To cure the infection, it was necessary to inject penicillin intramuscularly every 12 h at a dose of 2 mg/kg of body weight, starting 36 h after initiation of the infection. This dose was chosen because previous experiments with lower doses revealed that 2 mg/kg of body weight was the minimal effective dose required for the recovery of rats. Penicillin therapy was started 36 h after inoculation since at that time the pneumonia was developed. This permitted us to study the role of one host defense factor in the process of recovery during penicillin therapy. The influence of a selectively impaired host defense on the efficacy of antimicrobial therapy was examined by impairing phagocytosis in rats. Because phagocytosis of encapsulated pneumococci occurs after opsonization of the bacteria (28), impairment of phagocytosis was accomplished by removing the opsonins. In the early preantibody phase of infection, C3 is the principal component involved in the opsonization process (31, 38). C3 is directly activated by pneumococci (11, 34, 40), followed by fixation of C3b on bacterial surfaces. Through complement receptor sites on the surface of PMN (12, 21) binding of pneumococci to PMN occurs, resulting in phagocytosis.

These observations in animals explain why phagocytosis of the pneumococci was impaired by the depletion of plasma C3. This depletion was realized by repeated injections of the rats with CVF. It has been demonstrated that this factor may cause prolonged depletion of plasma C3 in several species (9, 20, 24). In the present study low levels of C3 were also observed in the sera of CVF-treated rats. The opsonic capacity of these sera was also grossly deficient, as measured by in vitro phagocytosis of pneumococci by PMN. As expected from these in vitro experiments, CVF-treated rats appeared to be incapable of producing adequate phagocytosis of pneumococci in the alveoli. This was confirmed by the fact that the spreading of the pulmonary infection in CVF-treated rats was more rapid and extensive than in untreated rats. These data are in accordance with the observation that patients with abnormalities of the complement system, especially C3, suffer from recurrent pneumococcal infections (1, 2, 25). Gross et al. found that hypocomplementemia predisposes mice to bacterial pneumonia (16). Despite therapy with penicillin, CVF-treated rats did not show signs of recovery. As the same therapy was successful in untreated animals, it must be concluded that penicillin shows a complete loss of efficacy during treatment of an infection caused by susceptible organisms in animals with impaired phagocytosis.

These data are in accordance with the findings of Smith and Wood, who reported that penicillin therapy of pneumococcal myositis lesions in irradiated mice was less effective than such therapy in normal mice (32). They concluded that the curative effect of penicillin was due to the combined effect of the drug and the cellular defenses of the host. The same conclusion also resulted from experiments of Dale et al. with irradiated dogs (10). However, the interpretation of these results is hindered by the fact that radiation not only induces granulocytopenia but also damages other systems like erythropoiesis and thrombopoiesis, and so may cause untimely death of the animals (41). Experimental studies of Biró and Iván (4), Trnka et al. (35), and Scott and Robson (27) revealed the effect of certain cancer chemotherapeutic agents

upon the efficacy of antimicrobial therapy. However, interpretation of these results is difficult because most cancer chemotherapeutic agents have multiple immunosuppressive actions both on B and T cells as well as on neutrophils. In this study, we suppressed host defense against the infectious organisms by an indirect and consequently selective way. Therefore, we may conclude that loss of activity of this penicillin treatment schedule results from selectively impaired phagocytosis of the infectious organisms. The total effect of antimicrobial therapy during an infection is the combined effect of the curative action of the antibiotic (penicillin in this case) in combination with the activity of the cellular defenses of the host (PMN in this case). If this hypothesis is correct, a lack of phagocytic functions of PMN might be compensated partially by an improved antibiotic treatment schedule, i.e. by increasing the dose or frequency of injections, or both. In a companion paper (3) observations related to the dose and administration schedule of penicillin in rats with impaired phagocytosis are reported.

Acknowledgements

We are grateful to R.C. Noble for his critical review of the manuscript, to M.E. Erkens-Versluis for performing the assay for complement component C3, and to A.L. Beukelman for secretarial help.
This study was supported in part by research grants from Beecham Farma B.V.

Literature cited

1. Alper, C.A., N. Abramson, R.B. Johnston, Jr., J.H. Jandl, F.S. Rosen, and L. Watson. 1970. Studies in vivo and in vitro on an abnormality in the metabolism of C3 in a patient with increased susceptibility to infection. J. Clin. Invest. 49: 1975-1985.
2. Alper, C.A., K.J. Bloch, and F.S. Rosen. 1973. Increased susceptibility to infection in a patient with type II essential hypercatabolism of C3. N. Engl. J. Med. 288: 601-606.
3. Bakker-Woudenberg, I.A.J.M., A.L.E.M. van Gerwen, and M.F. Michel.

1979. Efficacy of antimicrobial therapy in experimental rat pneumonia: antibiotic treatment schedules in rats with impaired phagocytosis. Infect. Immun. 25: 376-387.

4. Biró, L., and E. Iván. 1968. Study of bactericidal and bacteriostatic antibiotics in animals with normal and suppressed immunity. Chemotherapy 13: 100-107.
5. Bodey, G.P. 1975. Infections in cancer patients. Cancer Treat. Rev. 2: 89-128.
6. Bodey, G.P., M. Buckley, Y.S. Sathe, and E.J. Freireich. 1966. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann. Intern. Med. 64: 328-340.
7. Bodey, G.P., E.M. Hersh, M. Valdivieso, R. Feld, and V. Rodriguez. 1975. Effects of cytotoxic and immunosuppressive agents on the immune system. Postgrad. Med. 58: 67-74.
8. Brown, R.S., H.A. Haynes, H.T. Foley, H.A. Godwin, C.W. Berard, and P.P. Carbone. 1967. Hodgkin's disease. Immunologic, clinical, and histologic features of 50 untreated patients. Ann. Intern. Med. 67: 291-302.
9. Cochrane, C.G., H.J. Müller-Eberhard, and B.S. Aikin. 1970. Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. J. Immunol. 105: 55-69.
10. Dale, D.C., H.Y. Reynolds, J.E. Pennington, R.J. Elin, and G.P. Herzig. 1976. Experimental *Pseudomonas* pneumonia in leukopenic dogs: comparison of therapy with antibiotics and granulocyte transfusions. Blood 47: 869-875.
11. Edwards, M., and J.M. Stark. 1977. The ability of smooth and rough strains of *Streptococcus pneumoniae* to activate human complement by the alternative pathway. J. Med. Microbiol. 11: 7-14.
12. Ehlenberger, A.G., and V. Nussenzweig. 1977. The role of membrane receptors for C3b and C3d in phagocytosis. J. Exp. Med. 145: 357-371.
13. Feld, R., and G.P. Bodey. 1977. Infections in patients with

malignant lymphoma treated with combination chemotherapy. Cancer 39: 1018-1025.

14. Folland, D., D. Armstrong, S. Seides, and A. Blevins. 1974. Pneumococcal bacteremia in patients with neoplastic disease. Cancer: 33: 845-849.
15. Gaya, H., M.H.N. Tattersall, R.M. Hutchinson, and A.S.D. Spiers. 1973. Changing patterns of infection in cancer patients. Eur. J. Cancer 9: 401-406.
16. Gross, G.N., S.R. Rehm, and A.K. Pierce. 1978. The effect of complement depletion on lung clearance of bacteria. J. Clin. Invest. 62: 373-378.
17. Hersh, E.M., and E.J. Freireich. 1968. Host defense mechanisms and their modification by cancer chemotherapy. Methods Cancer Res. 4: 355-451.
18. Inagaki, J., V. Rodriguez, and G.P. Bodey. 1974. Causes of death in cancer patients. Cancer 33: 568-573.
19. Klastersky, J., and D. Weerts. 1973. Recent experience with bacteremia in patients presenting cancer. Eur. J. Cancer 9: 69-76.
20. Kourounakis, L., R.A. Nelson, Jr., and M.A. Kupusta. 1973. The effect of a cobra venom factor on complement and adjuvant-induced disease in rats. Arthritis Rheum. 16: 71-76.
21. Lay, W.H., and V. Nussenzweig. 1968. Receptors for complement on leukocytes. J. Exp. Med. 128: 991-1007.
22. Lehane, D.E., and M. Lane. 1974. Immunocompetence in advanced cancer patients prior to chemotherapy. Oncology 30: 458-466.
23. Middleman, E.L., A. Watanabe, H. Kaizer, and G.P. Bodey. 1972. Antibiotic combinations for infections in neutropenic patients. Cancer 30: 573-579.
24. Pepys, M.B. 1975. Studies in vivo of cobra factor and murine C3. Immunology 28: 369-377.
25. Reed, W.P., M.S. Davidson, and R.C. Williams, Jr. 1976. Complement system in pneumococcal infections. Infect. Immun. 13: 1120-1125.
26. Rosner, F., I. Valmont, P.J. Kozinn, and L. Caroline. 1970.

Leukocyte function in patients with leukemia. Cancer 25: 835-842.

27. Scott, R.E., and H.G. Robson. 1976. Synergistic activity of carbenicillin and gentamicin in experimental *Pseudomonas* bacteremia in neutropenic rats. Antimicrob. Agents Chemother. 10: 646-651.
28. Shin, H.S., M.R. Smith, J. Winkelstein, and A. Nicholson. 1977. Heat labile opsonin system to pneumococcus, p. 563-566. *In* D. Bergsma et al. (ed.), Immunodeficiency in man and animals. Birth Defects: Original Article Series, the National Foundation, vol. 11, no. 1. Robert E. Krieger Publishing Co., Inc., New York.
29. Shin, H.S., M.R. Smith, and W.B. Wood, Jr. 1969. Heat labile opsonins to pneumococcus. II. Involvement of C3 and C5. J. Exp. Med. 130: 1229-1241.
30. Sickles, E.A., V.M. Young, W.H. Green, and P.H. Wiernik. 1973. Pneumonia in acute leukemia. Ann. Intern. Med. 79: 528-534.
31. Smith, M.R., H.S. Shin, and W.B. Wood, Jr. 1969. Natural immunity to bacterial infections: the relation of complement to heat-labile opsonins. Proc. Natl. Acad. Sci. U.S.A. 63: 1151-1156.
32. Smith, M.R., and W.B. Wood, Jr. 1956. An experimental analysis of the curative action of penicillin in acute bacterial infections. II. The role of phagocytic cells in the process of recovery. J. Exp. Med. 103: 499-508.
33. Smith, M.R., and W.B. Wood, Jr. 1969. Heat labile opsonins to pneumococcus. I. Participation of complement. J. Exp. Med. 130: 1209-1227.
34. Stephens, C.G., R.C. Williams, Jr., and W.P. Reed. 1977. Classical and alternative complement pathway activation by pneumococci. Infect. Immun. 17: 296-302.
35. Trnka, L., M. Kvapilová, and P. Mison. 1976. Influence of immunosuppression on the action of antimycobacterial drugs in experimental tuberculosis. Chemotherapy 22: 353-361.
36. Ultmann, J.E., W. Fish, E. Osserman, and A. Gellhorn. 1959. The clinical implications of hypogammaglobulinemia in patients with

chronic lymphocytic leukemia and lymphocytic lymphosacroma. Ann. Intern. Med. 51: 501-516.

37. Winkelstein, J.A., H.S. Shin, and W.B. Wood, Jr. 1972. Heat labile opsonins to pneumococcus. III. The participation of immunoglobulin and of the alternate pathway of C3 activation. J. Immunol. 108: 1681-1689.
38. Winkelstein, J.A., M.R. Smith, and H.S. Shin. 1975. The role of C3 as an opsonin in the early stages of infection. Proc. Soc. Exp. Biol. Med. 149: 397-401.
39. Winkelstein, J.A., and A.J. Swift. 1975. Host defense against the pneumococcus in T-lymphocyte deficient, nude mice. Infect. Immun. 12: 1222-1223.
40. Winkelstein, J.A., and A. Tomasz. 1977. Activation of the alternative pathway by pneumococcal cell walls. J. Immunol. 118: 451-454.
41. Wood, W.B., Jr., and E.N. Irons. 1946. Studies on the mechanism of recovery in pneumococcal pneumonia. II. The effect of sulfonamide therapy upon the pulmonary lesion of experimental pneumonia. J. Exp. Med. 84: 365-376.
42. Wood, W.B., Jr., and M.R. Smith. 1950. Host parasite relationships on experimental pneumonia due to pneumococcus type III. J. Exp. Med. 92: 85-99.

Chapter 2

EFFICACY OF ANTIMICROBIAL THERAPY IN EXPERIMENTAL RAT PNEUMONIA: PENICILLIN TREATMENT SCHEDULES IN RATS WITH IMPAIRED PHAGOCYTOSIS*

Irma A.J.M. Bakker-Woudenberg, A.L.E.M. van Gerwen, and M.F. Michel

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, The Netherlands

Pneumococcal pneumonia in rats with intact host defense mechanisms could be successfully cured by penicillin. The efficacy of this antibiotic therapy was lost in cobra venom factor-treated rats which had selectively impaired phagocytic functions. In these animals the effect of penicillin therapy was improved by increasing the daily dose and the frequency of injections and by earlier initiation of the therapy. The efficacy of penicillin in the cobra venom factor-treated rats was restored either by markedly increasing the daily dose of penicillin or by increasing the daily dose in combination with a reduced interval of the penicillin injections.

Introduction

In another paper (1) it was shown that an experimental pneumococcal pneumonia in normal rats can be cured by penicillin. In rats with phagocytosis of the pneumococci by neutrophils selectively impaired, the infection could not be cured with the same amount of penicillin. These data are in accordance with the clinical findings that patients with impaired phagocytosis as a consequence of their malignancy or the administration of antitumor agents suffer from recurrent infections and often respond poorly to antimicrobial therapy (9). Because of the diminished efficacy of antimicrobial therapy, different antibiotic

*Infection and Immunity (1979) 25, 376-387.

dosage schedules have been examined in these patients (4). In this study we investigated to what extent the antimicrobial response in rats with selectively impaired phagocytosis can be improved by alterations in the antimicrobial treatment schedule.

Materials and Methods

general methods

The production of lung infection and treatment with cobra venom factor (CVF) were performed as described in another paper (1). Five animals were observed during the course of infection in order to measure body weights and rectal temperatures. Animals were sacrificed in groups of five at 36, 84, and 132 h after inoculation in order to measure the weight of the left lobe of the lung, count the number of viable bacteria present in the left lobe of the lung, and culture blood and pleural fluid.

antimicrobial treatment

The sodium salt of penicillin G in aqueous solution was used. Individual doses of penicillin amounted to 2, 4, 24, 34, 44, and 50 mg/kg of body weight. Penicillin injections were given either every 12 or every 8 h intramuscularly into the thigh muscles of the rear legs. The first penicillin injection was given either 36 or 24 h after inoculation of pneumococci. The last injection was given 132 h after inoculation.

antibiotic assay

Penicillin levels were estimated by a standard large-plate agar diffusion technique with Oxoid diagnostic sensitivity test agar and a *Staphylococcus aureus* strain as the indicator organism (2). The tests were performed with 200 µl of rat serum per test. Blood specimens for antibiotic assay were obtained by orbital puncture under light anesthesia. From each rat five blood samples were collected at different times after intramuscular injection of the antibiotic.

Results

The effect of different penicillin dosage schedules upon the course of pneumococcal pneumonia in CVF-treated rats (Fig. 1 through 9) may be compared with the efficacy of penicillin at a dosage rate of 2 mg/kg per 12 h started at 36 h after inoculation of pneumococci into normal and CVF-treated rats (1). In all different treatment schedules, total body weight decreased constantly in some animals, whereas in other animals body weight remained constant at a subnormal level during treatment (Fig. 1 through 9). All different treatment schedules eventually caused normalization of rectal temperatures. This was also the case in some of the rats who received penicillin at a rate of 4 mg/kg per 12 h starting at 36 h (Fig. 2). These animals suffered from subnormal temperatures for several days during infection. When penicillin injections were started as early as 24 h after inoculation of pneumococci (Fig. 1), bacteria were still cultured from the left lobe of the lung at 132 h. Similarly, when the dose of penicillin was doubled (Fig. 2), the lungs were not sterilized. Figures 5 through 7 show the effect of three successive dosage schedules upon pneumonia in the CVF-treated rats; an increase in dosage of at least 22 times (44 versus 2 mg) was needed to sterilize the lungs of most of the CVF-treated animals (Fig. 7). Other experiments (data not shown) revealed that a penicillin dosage of 50 mg/kg per 12 h starting at 36 h was successful in clearing the lungs of all CVF-treated animals at 132 h.

When the interval between the 2 mg/kg injections was shortened from 12 to 8 h (Fig. 3), the lungs were not sterilized. This increase in frequency of injections from 12 to 8 h in combination with a doubled penicillin dose (4 mg/kg per 8 h) sterilized the lungs of only one of five animals (Fig. 4). Figures 8 and 9 show that a 17-fold increase (34 versus 2 mg) in penicillin dose was sufficient to sterilize the lungs when the interval between the injections was shortened from 12 to 8 h.

Intramuscular injections of penicillin doses of 34 and 50 mg/kg gave

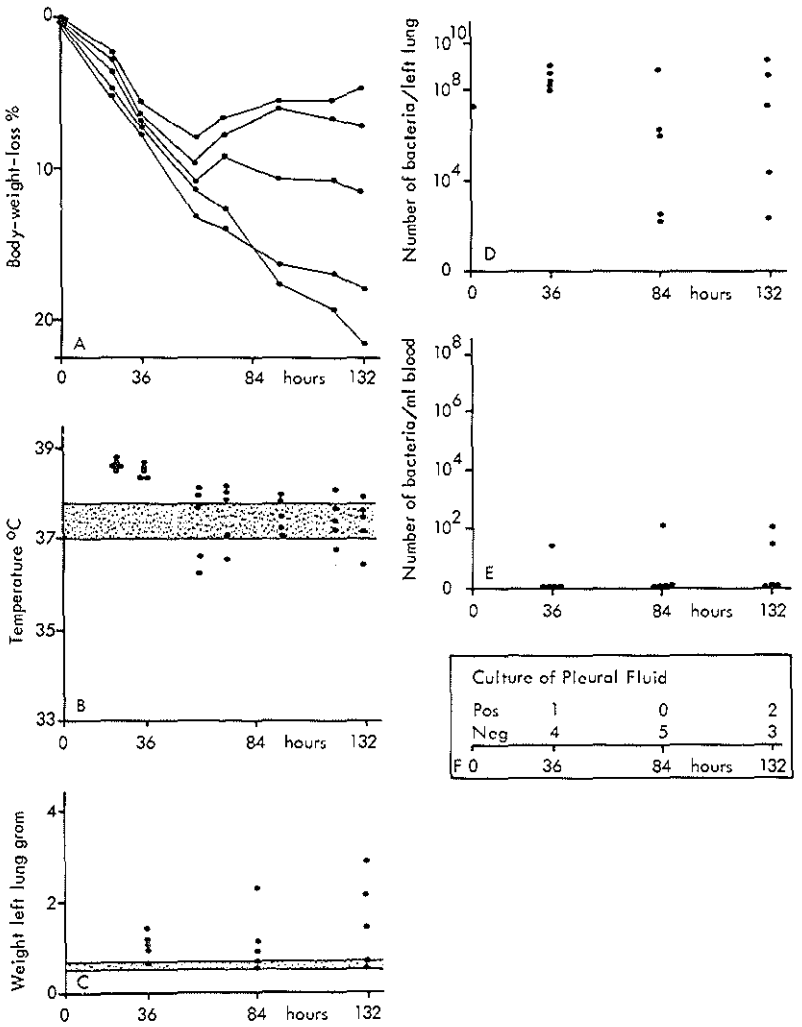


Fig. 1. *Action of penicillin at 2 mg/kg per 12 h starting at 24 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

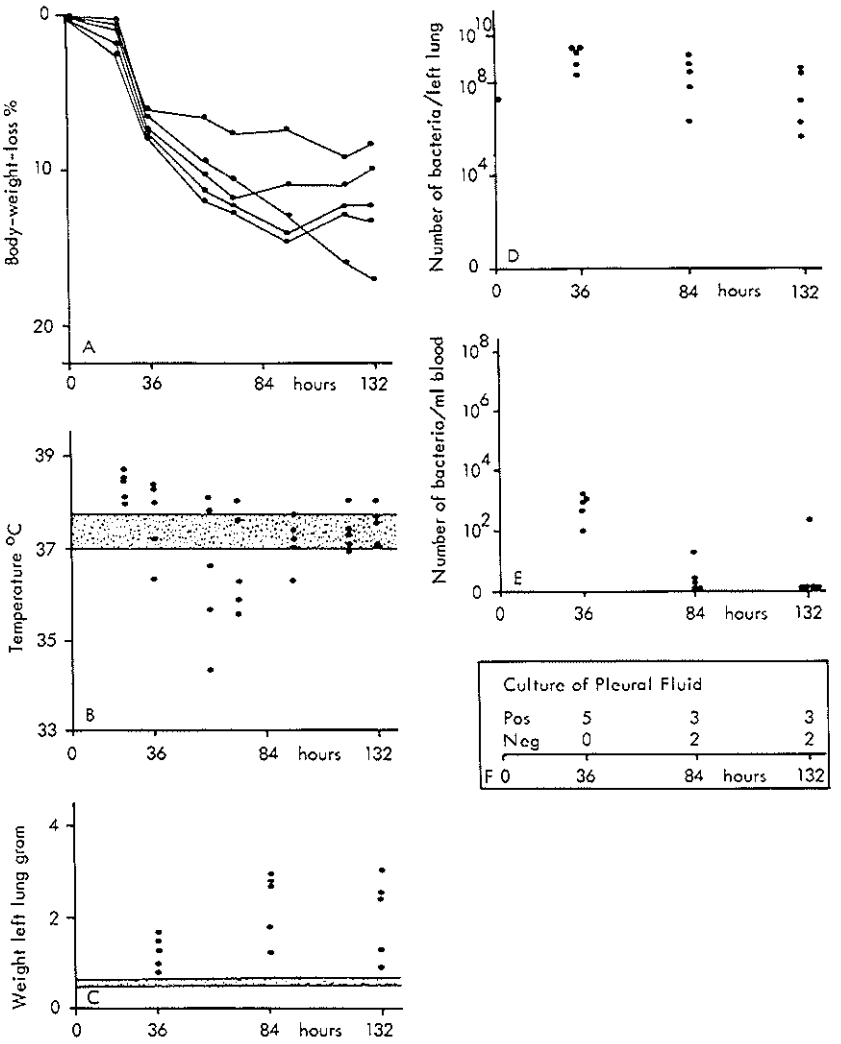


Fig. 2. *Action of penicillin at 4 mg/kg per 12 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

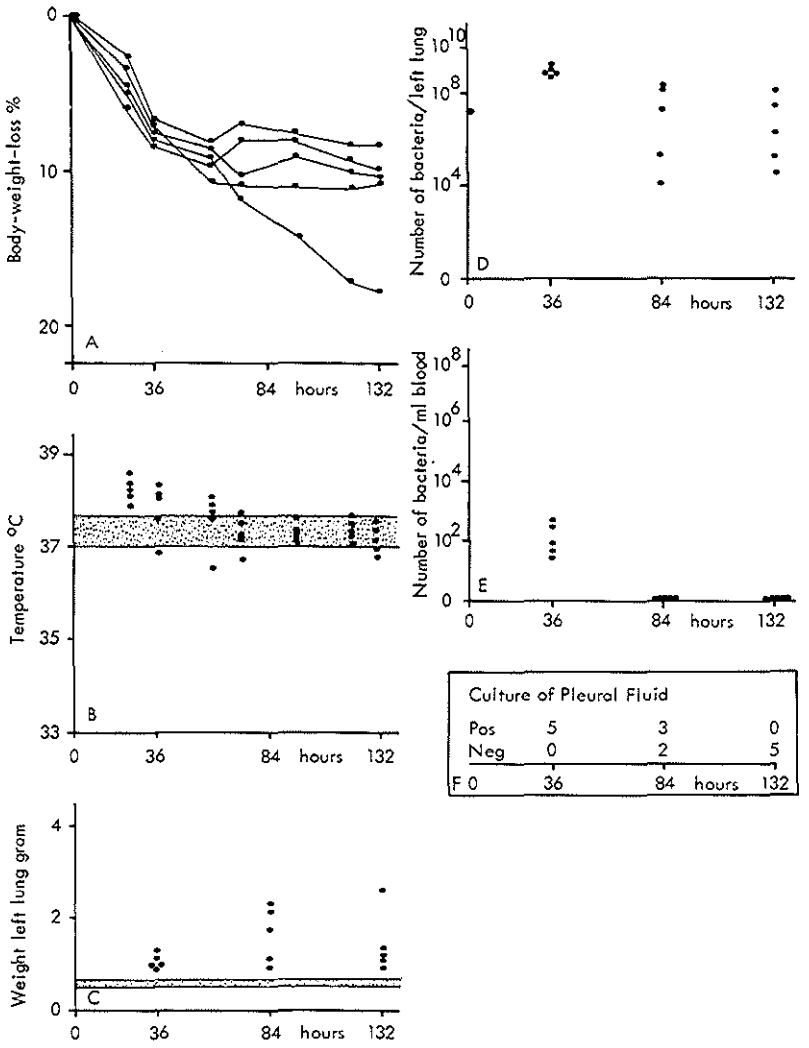


Fig. 3. *Action of penicillin at 2 mg/kg per 8 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

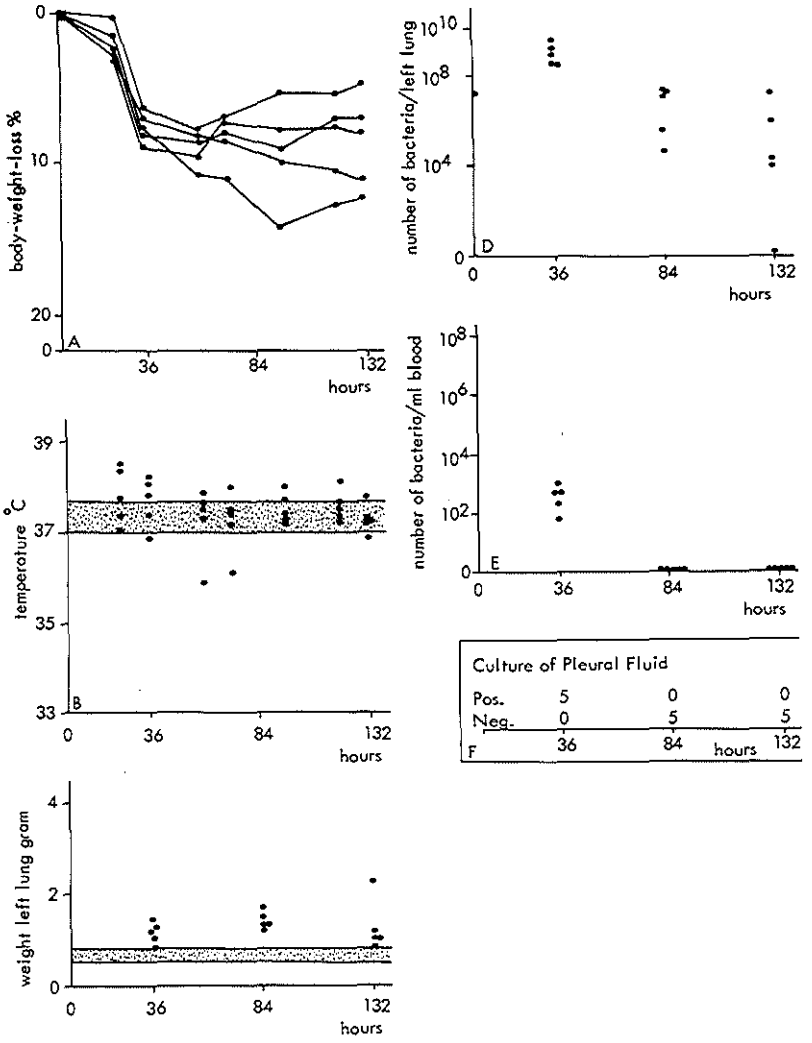


Fig. 4. *Action of penicillin at 4 mg/kg per 8 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

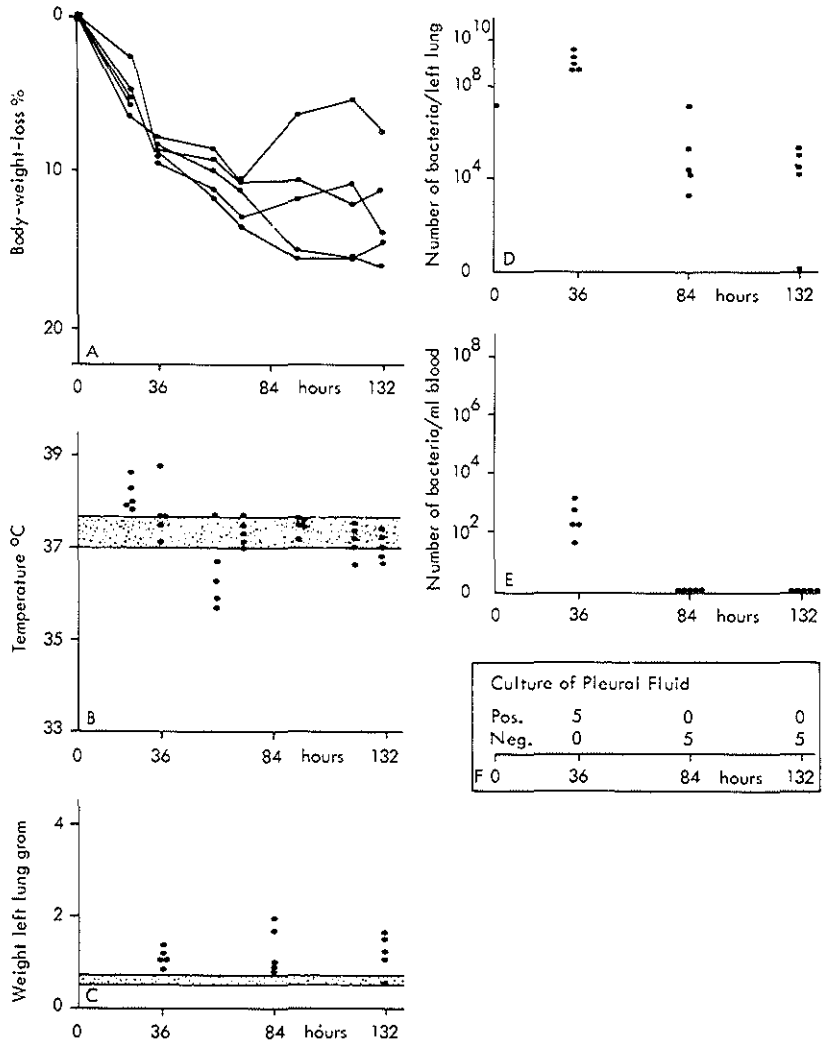


Fig. 5. *Action of penicillin at 24 mg/kg per 12 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

rise to penicillin levels which differed in peak heights and times during which the penicillin concentration exceeded the minimal inhibitory concentration of the pneumococcus (0.024 µg/ml); administration of 34 and 50 mg/kg gave peak concentrations of 49 and 76 µg/ml, respectively, and the durations of detectable concentrations of penicillin (0.125 µg/ml) were 119 and 212 min, respectively (Fig. 10). Estimated minimal effective plasma concentrations of 0.024 µg of penicillin per ml or greater were present for 134 and 250 min, respectively. When the frequency of penicillin injections was increased, blood and pleural fluid became sterile in all rats at 84 h after initiation of infection (Fig. 3 and 4). However, penicillin at 4 mg/kg per 12 h was only partially successful in clearing the infectious organisms from blood and pleural fluid (Fig. 2). On the other hand, a penicillin dose of 24 mg/kg per 12 h completely cleared blood and pleural fluid (Fig. 5). By advancing the penicillin treatment schedule of 2 mg/kg per 12 h by 12 h (starting at 24 instead of 36 h), the blood and pleural fluid of only some of the animals were cleared of pneumococci (Fig. 1).

Discussion

In another paper evidence was presented that penicillin therapy cured rats with pneumococcal pneumonia, but was ineffective in rats with selectively impaired phagocytosis (1). In this study we demonstrated that, in rats with impaired phagocytic function, the therapeutic effect of the penicillin therapy could be partially restored either by earlier initiation of the therapy or by increasing the dose or the frequency of the penicillin injections.

Injection schedules of 50 mg/kg per 12 h or 34 mg/kg per 8 h, both starting 36 h after initiation of the infection in CVF-treated rats, equaled the success of the 2 mg/kg per 12 h dosage schedule starting at 36 h in rats with intact host defense mechanisms. That the minimal effective penicillin dose can be reduced when the

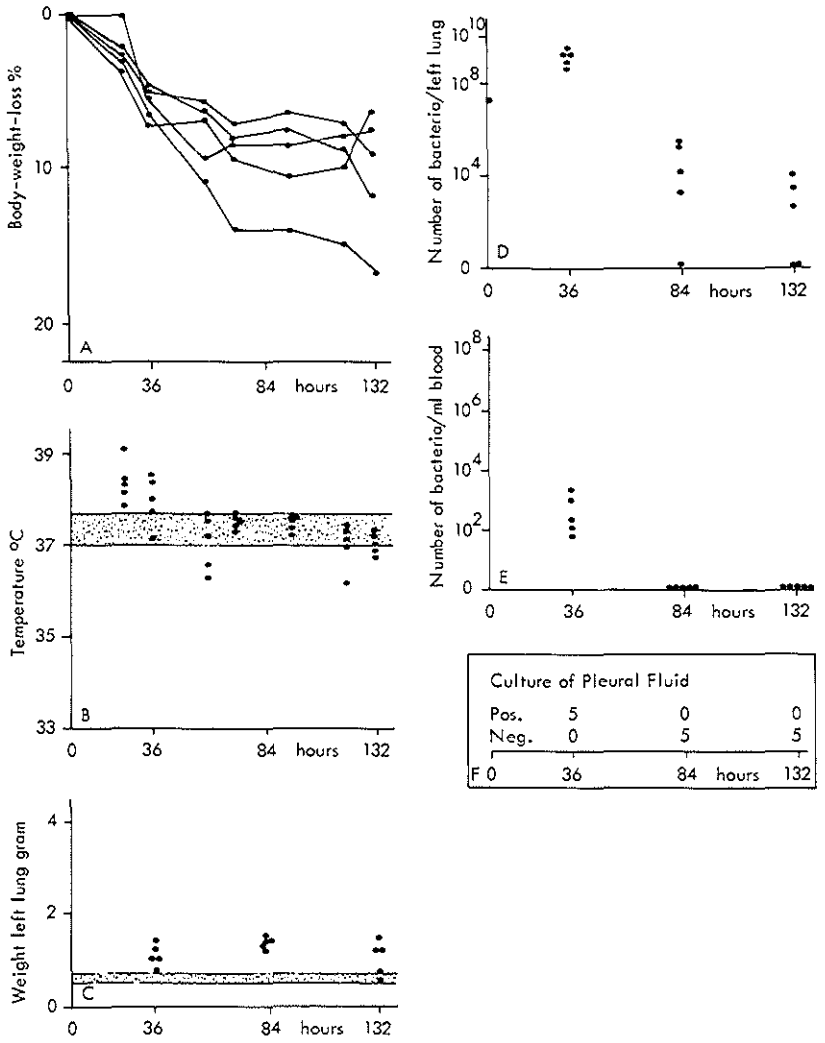


Fig. 6. *Action of penicillin at 34 mg/kg per 12 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

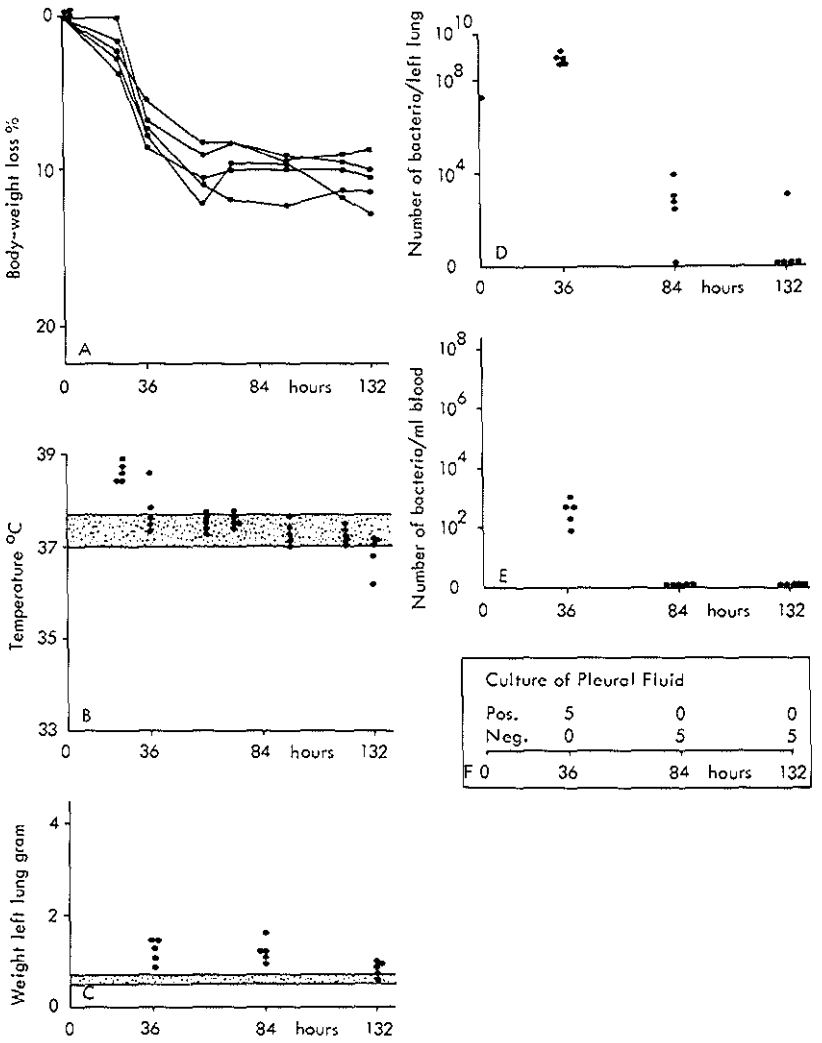


Fig. 7. *Action of penicillin at 44 mg/kg per 12 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

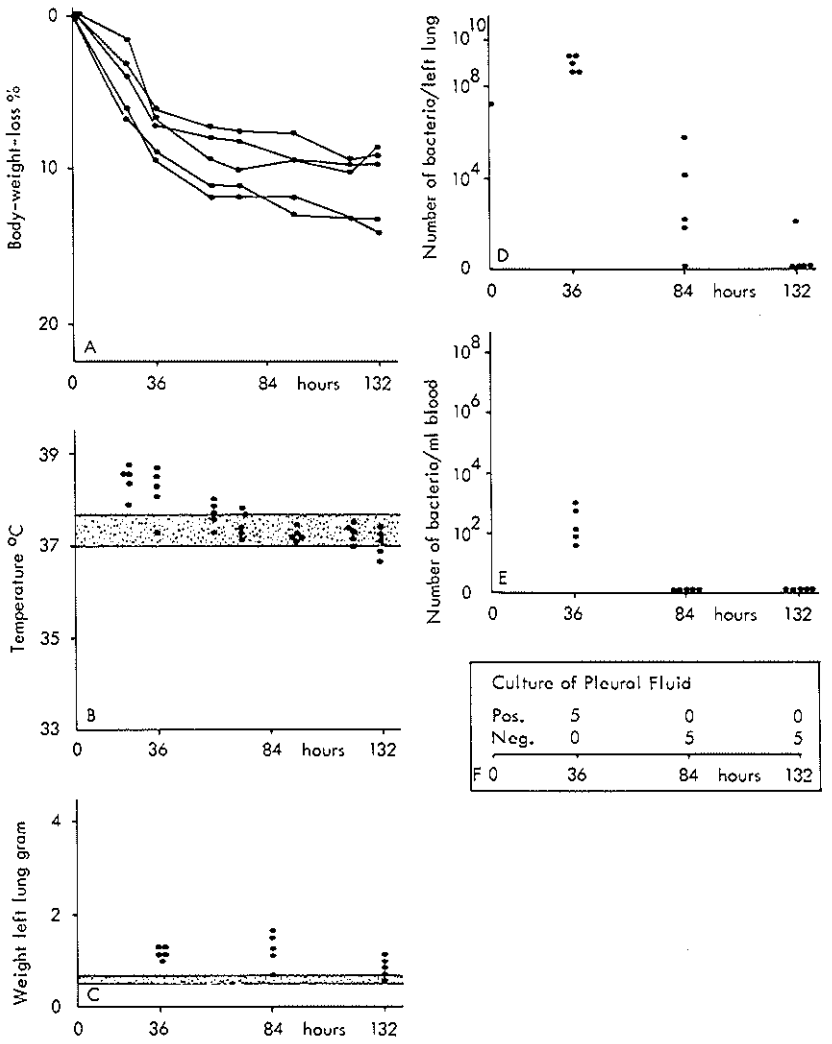


Fig. 8. *Action of penicillin at 24 mg/kg per 8 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

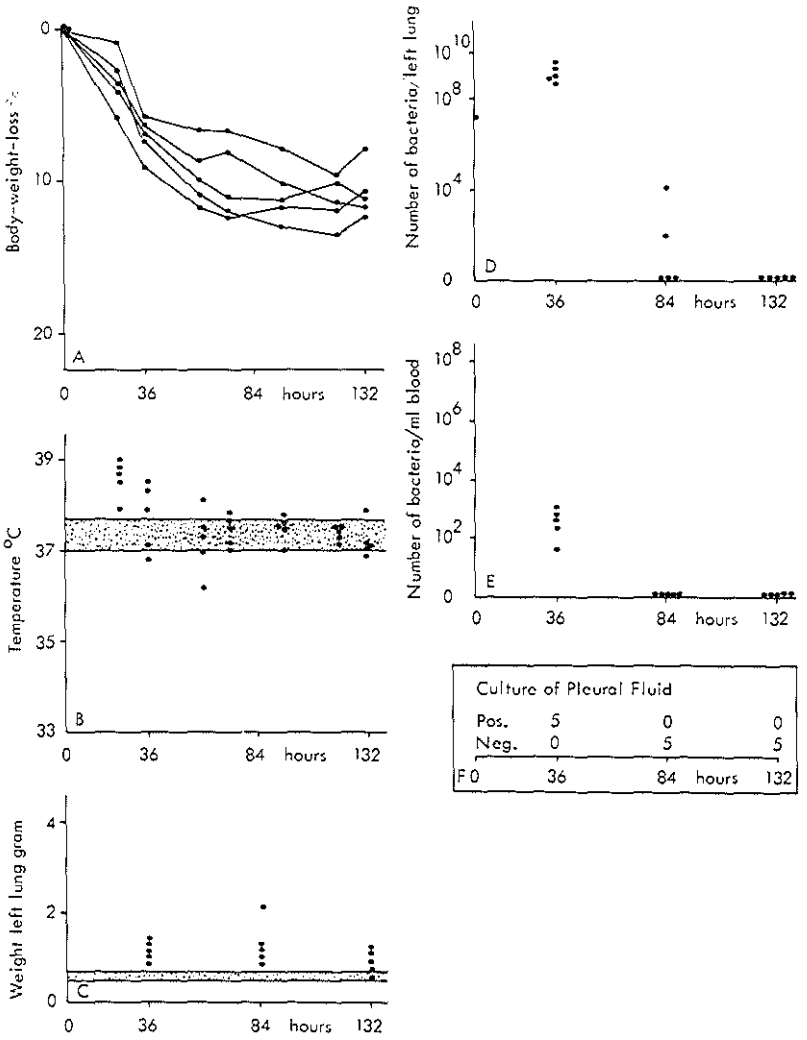


Fig. 9. *Action of penicillin at 34 mg/kg per 8 h starting at 36 h upon the course of the pneumonia in CVF-treated rats after inoculation with 6 x 10^7 type III pneumococci. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter of blood. (F) Culture of pleural fluid.*

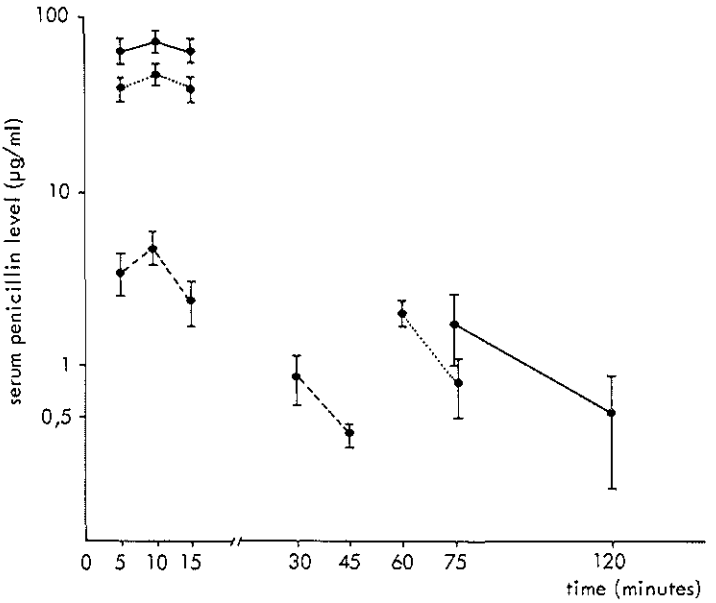


Fig. 10. *Serum concentrations of penicillin. Rats were injected intramuscularly with penicillin doses of*
2 mg/kg (- - -),
34 mg/kg (·····), or
50 mg/kg (——).
Each point represents the mean value of five rats (± standard deviation).

interval between the injections is shortened from 12 to 8 h may be explained from data on the plasma levels of the drug at varying times after intramuscular administration. Injections of 34 and 50 mg of penicillin per kg gave rise to drug levels which differed in height and time during which the antibiotic concentration exceeded the minimal inhibitory concentration of the pneumococcus. It might be concluded that improvement of the antibiotic treatment schedule obtained by increasing the penicillin dose or frequency of penicillin

injections or both can compensate at least in part for the impaired phagocytosis of pneumococci by polymorphonuclear leukocytes in CVF-treated rats. This is consistent with the hypothesis that the total effect of antimicrobial therapy during an infection is due to the combined effect of the antimicrobial drug and the cellular defenses of the host.

Brewin at al. (5) studied what beneficial effect varying doses of procaine penicillin G had on the treatment of hospitalized patients with pneumococcal pneumonia of varying severity. His conclusion was that there was no advantage in treating patients with massive dosages of penicillin. In this experimental study evidence was presented that animals with impaired host defense mechanisms benefit more from massive dosages of penicillin than from moderate dosages.

When the interval between penicillin injections was reduced from 12 to 8 h, pneumococci were cleared from blood and pleural fluid. This is in accordance with the findings of Schmidt and Walley, who tried various dosage regimens of penicillin against type I pneumococcal pneumonia in normal rats (11). They found that administration of the antibiotic was most effective when it was given every 8 h.

In the present study a considerable amount of penicillin was required to eradicate the pneumococci from the lungs of all CVF-treated animals. Experiments of Scott et al. revealed the loss of antimicrobial activity in cyclophosphamide-treated rats injected with several doses of gentamicin (12). Whether the loss of antibiotic activity resulted from impairment of a single host defense factor cannot be concluded from their study. Cyclophosphamide is not a selective immunosuppressive agent, but acts on T and B cells as well as on neutrophils. Also, the antibiotic therapy was instituted early after the onset of infection (2 h), when the role of host defense mechanisms in the infectious process is more difficult to evaluate. Our data in rats with selectively impaired phagocytosis are in accordance with the clinical findings concerning the effect of various dosage regimens of antibiotics against infections in cancer patients (4). In severe infections in these

patients, it is advisable to institute antimicrobial therapy immediately at the onset of infection (4, 8, 10). Based on clinical experience, Bodey and others have suggested that the results in antimicrobial therapy can be improved in neutropenic patients by administering combinations of antibiotics that have synergistic in vitro activity (3, 6, 7, 9, 10). In further studies several antibiotic combinations will be applied in this experimental infection model in rats with impaired host defense mechanisms. Infections with organisms other than the pneumococci will also be studied.

Acknowledgements

This study was supported in part by research grants from Beecham Farma B.V.

Literature cited

1. Bakker-Woudenberg, I.A.J.M., J.Y.T. de Jong-Hoenderop, and M.F. Michel. 1979. Efficacy of antimicrobial therapy in experimental rat pneumonia: effects of impaired phagocytosis. Infect. Immun. 25: 366-375.
2. Bennet, J.V., J.L. Brodie, E.J. Benner, and W.M.M. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. 14: 170-177.
3. Bodey, G.P. 1975. Infections in cancer patients. Cancer Treat. Rev. 2: 89-128.
4. Bodey, G.P., V. Rodriguez, M. Valdivieso, and M. Keating. 1977. Gram-negative bacillary infections in cancer patients. S. Afr. Med. J. 52: 1049-1055.
5. Brewin, A., L. Arango, W.K. Hadley, and J.F. Murray. 1974. High-dose penicillin therapy and pneumococcal pneumonia. J. Am. Med. Assoc. 230: 409-413.
6. Hahn, D.M., S.C. Schimpff, V.M. Young, C.L. Fortner, H.C. Standiford, and P.H. Wiernik. 1977. Amikacin and cephalothin: empiric regimen for granulocytopenic cancer patients. Antimicrob. Agents Chemother. 12: 618-624.

7. Klastersky, J., C. Hensgens, and L. Debusscher. 1975. Empiric therapy for cancer patients: comparative study of ticarcillin-tobramycin, ticarcillin-cephalothin, and cephalothin-tobramycin. Antimicrob. Agents Chemother. 7: 640-654.
8. Klastersky, J., and M. Staquet. 1973. Optimal antibiotic therapy for infections in patients with cancer: prospects for the future. Eur. J. Cancer 9: 397-399.
9. Middleman, E.L., A. Watanabe, H. Kaizer, and G.P. Bodey. 1972. Antibiotic combinations for infections in neutropenic patients. Cancer 30: 573-579.
10. Schimpff, S.C., S. Landesman, D.M. Hahn, H.C. Standiford, C.L. Fortner, V.M. Young, and P.H. Wiernik. 1976. Ticarcillin in combination with cephalothin or gentamicin as empiric antibiotic therapy in granulocytopenic cancer patients. Antimicrob. Agents Chemother. 10: 837-844.
11. Schmidt, L.H., and A. Walley. 1951. The influence of the dosage regimen on the therapeutic effectiveness of penicillin-G in experimental lobar pneumonia. J. Pharmacol. Exp. Ther. 103: 479-488.
12. Scott, R.E., and H.G. Robson. 1976. Synergistic activity of carbenicillin and gentamicin in experimental *Pseudomonas* bacteremia in neutropenic rats. Antimicrob. Agents Chemother. 10: 646-651.

Chapter 3

EFFICACY OF ANTIMICROBIAL THERAPY IN EXPERIMENTAL RAT PNEUMONIA: CLINDAMYCIN TREATMENT SCHEDULES IN RATS WITH IMPAIRED PHAGOCYTOSIS*

Irma A.J.M. Bakker-Woudenberg, J.C. van de Berg, W.B. van Leeuwen, and M.F. Michel

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, The Netherlands

Pneumococcal pneumonia in rats with intact host defense mechanisms was successfully cured by clindamycin. In cobra venom factor-treated rats with impaired phagocytosis of pneumococci, the efficacy of the same clindamycin treatment was lost. In these rats the therapeutic effect of the clindamycin treatment was restored by a four-fold increase in dosage. The efficacy of clindamycin - in terms of minimal dosage and treatment schedule required for recovery of normal rats and rats with impaired host defense - differed from the efficacy of penicillin (described earlier). In vitro experiments showed that penicillin was not inactivated by homogenates of pneumococcal-infected lung tissue of cobra venom factor-treated rats. The serum levels of clindamycin were determined at different intervals after its administration, and compared to penicillin levels (described earlier). The duration of the periods where the antibiotic serum levels exceeded the minimal inhibitory concentration (MIC) for the pneumococci appear to be much longer after injection of clindamycin than of penicillin. Within the limits of this study evidence is presented for the superiority of clindamycin over penicillin in the treatment of experimental pneumococcal pneumonia in rats with impaired host defense. In these animals it is important to maintain prolonged inhibitory serum concentration of antibiotic.

*In shortened version submitted for publication.

Introduction

In previous papers it has been shown that an experimental pneumococcal pneumonia in normal rats was successfully cured by penicillin (2, 3). The efficacy of this antibiotic therapy was lost in cobra venom factor-treated rats with impaired opsonization of pneumococci resulting in impaired phagocytosis. In these rats, the therapeutic effect of the penicillin therapy was restored by 25-fold increase in daily dosage, or by a combination of an increased daily dosage (17-fold) and a shortened interval between penicillin injections (every 8 h instead of every 12 h). In the present study, the efficacy of clindamycin therapy was studied in normal rats and in rats with impaired phagocytosis.

Materials and Methods

animals

Female R-strain albino rats (inbred strain; specific pathogen free; 14-16 weeks old; weight 180 to 210 g; bred in the Laboratory Animals Center of the Erasmus University Rotterdam) were used in all experiments.

bacteria

A *Streptococcus pneumoniae* type III strain isolated from a clinical case was used in all experiments. The minimal bactericidal concentration of clindamycin was 0.024 µg/ml (tube dilution test). The virulence of the bacteria was maintained by passage in rats every 4 months and by storage at -70°C in Todd-Hewitt broth (Oxoid) with 10% glycerol (Merck).

treatment with cobra venom factor

Rats were treated with cobra venom factor (CVF) as described earlier (2). Rats were injected intraperitoneally 14 times with equal doses of CVF (0.7 units/kg of body weight). One unit of CVF amounted to 12 µg of the purified material. The first dose was administered at the time of pneumococcal inoculation, followed by five doses at 8 hourly intervals and eight doses at 12 hourly intervals. Assays for complement component C3 in sera of CVF-treated rats revealed that total levels of

circulating C3 fell to less than 3% of the pretreatment level (2).

pneumonia

Experimental pneumonia was produced as described earlier (2). Under anesthesia the left main stem bronchus was intubated, and the left lobe of the lung was inoculated with a suspension of 6×10^7 *S. pneumoniae* type III cells in a volume of 0.02 ml. Parameters for the severity of the pneumonia were: total body weight, rectal temperature, weight of the left lobe of the lung, quantification of the number of viable bacteria in the left lobe of the lung, and cultures of blood and pleural fluid. After inoculation of the rats with 6×10^7 pneumococci, pneumonia developed initially in the left lobe of the lung. The course of pneumococcal pneumonia in CVF-treated rats was more severe than the infection in normal animals (2). The pneumonia spread more rapidly in the CVF-treated rats.

antimicrobial treatment

Aqueous solutions of clindamycin (Dalacin C phosphate, Upjohn) were injected intramuscularly into the thigh muscles of the rear legs. Individual doses of clindamycin amounted to 2, 4 or 8 mg/kg of body weight. Nine clindamycin injections were given every 12 h, the first dose 36 h after inoculation of the pneumococci, the last dose 120 h after inoculation.

penicillin inactivation assay

Inactivation experiments were performed by adding 0.1 ml of the appropriate concentration of penicillin to 4 ml uninfected or pneumococcal-infected lung homogenates. Final concentrations of penicillin were 1 and 10 µg/ml. The infected left lobes of the lungs of normal rats and of CVF-treated rats were removed and homogenized (each lobe in 20 ml of saline) three days after pneumococcal inoculation. The mixtures were tumbled gently at 14 rpm at 37°C. Immediately after mixing (0 h), and after 1½ and 6 h incubation at 37°C, the mixtures were assayed against penicillin standards in normal lung homogenates (see antibiotic assay).

serum antibiotic levels

Serum antibiotic levels were determined after intramuscular injections of clindamycin. Individual doses of clindamycin amounted to 2 or 8 mg/kg of body weight. Blood specimens for antibiotic assay were obtained by orbital puncture under light ether anesthesia. From each rat blood was collected at five different times after intramuscular injection of the antibiotic.

antibiotic assay

Clindamycin levels were estimated by a standard agar diffusion method (7) with some modifications: sensitivity test agar* and a *Bacillus stearothermophilus* strain as the indicator organism were used, and incubations were performed at 56°C. (Sensitivity of the assay system was 0.06 µg/ml for clindamycin). Clindamycin hydrochloride (Upjohn) was used for preparation of standard solutions. Tests were performed with 200 µl of rat serum per test.

Results

clindamycin therapy of normal rats with pneumococcal pneumonia

After pneumococcal inoculation of the rats, pneumonia developed as described earlier (2), in short: during the course of the infection total body weight decreased constantly and rectal temperature decreased below normal after initial rise. There was an increase in weight (up to sixfold) of the left lobe of the lung. Cultures of lungs, blood and pleural fluid were positive; by 108 h an average of 6×10^9 pneumococci were cultured from the left lobe of the lung, and the number of pneumococci cultured from the blood reached an average number of 5×10^4 per milliliter.

Repeated injections with 2 mg clindamycin per kg every 12 h starting 36 h after the initiation of the infection was the minimal effective treatment required for the recovery of normal rats within 132 h (Fig. 1). Total body weights increased again, and rectal temperature

*0.5% tryptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar (Difco)

normalized (Fig. 1A and 1B). There was a moderate increase in weight of the left lobe of the lung (Fig. 1C). This clindamycin treatment schedule could sterilize the lungs, blood and pleural fluid of all animals (Fig. 1D trough 1F).

clindamycin therapy of CVF-treated rats with pneumococcal pneumonia

The course of pneumococcal pneumonia in CVF-treated rats was more severe than the infection in normal animals as described earlier (2), in short: the total body weight and rectal temperature decreased, the value of the latter to far below normal. The increase in weight of the left lobe of the lung was sevenfold. By 108 h the number of pneumococci cultured from the left lobe of the lung reached an average number of 3×10^{10} pneumococci. Soon after initiation of the infection cultures of blood and pleural fluid were positive; by 108 h an average of 2×10^{6} bacteria per milliliter of blood were cultured. In CVF-treated rats the clindamycin therapy of 2 mg/kg every 12 h was unsuccessful in eradicating the pneumococcal infection. After an initial decrease, total body weight remained constant at a subnormal level (Fig. 2A). Rectal temperature normalized (Fig. 2B). The weight of the left lobe of the lung doubled (Fig. 2C). By 132 h, an average of 6×10^{7} pneumococci were cultured from the left lobe of the lung (Fig. 2D). All cultures of blood and pleural fluid were sterile at 132 h (Fig. 2E and 2F). After injections of double doses of clindamycin, i.e. 4 mg/kg every 12 h starting at 36 h, the lungs were still not sterilized; an average of 8×10^{5} pneumococci was still cultured from the left lobe of the lung at 132 h (Fig. 3D). An increase in clindamycin dosage of 4 times was required for complete recovery of the CVF-treated rats (Fig. 4). The clindamycin dosage of 8 mg/kg every 12 h starting at 36 h, was successful in sterilizing the lungs of all CVF-treated rats at 132 h (Fig. 4D).

penicillin inactivation in vitro

Three days after pneumococcal inoculation homogenates of infected lungs from normal and CVF-treated rats were tested for their capacity to inactivate penicillin. When penicillin was added to the lung

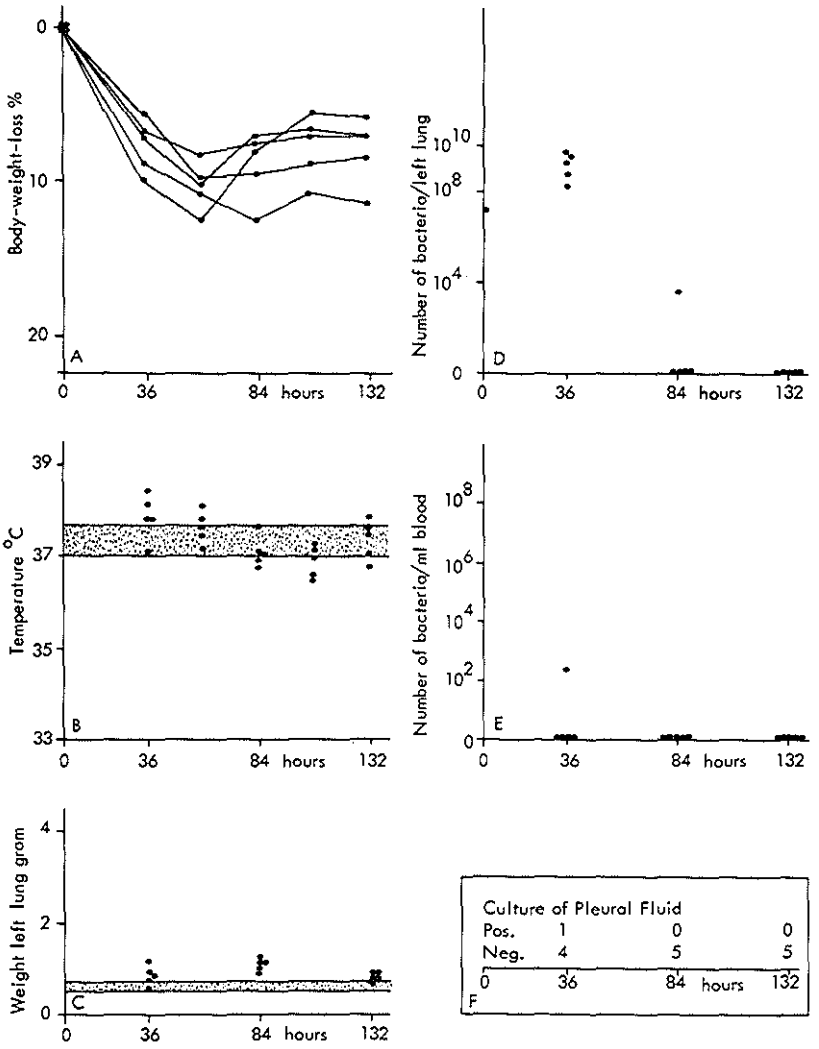


Fig. 1. *Action of clindamycin at 2 mg/kg body weight every 12 h starting 36 h after inoculation with 6 x 10^7 type III pneumococci upon the course of the pneumonia in normal rats. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter blood. (F) Culture of pleural fluid.*

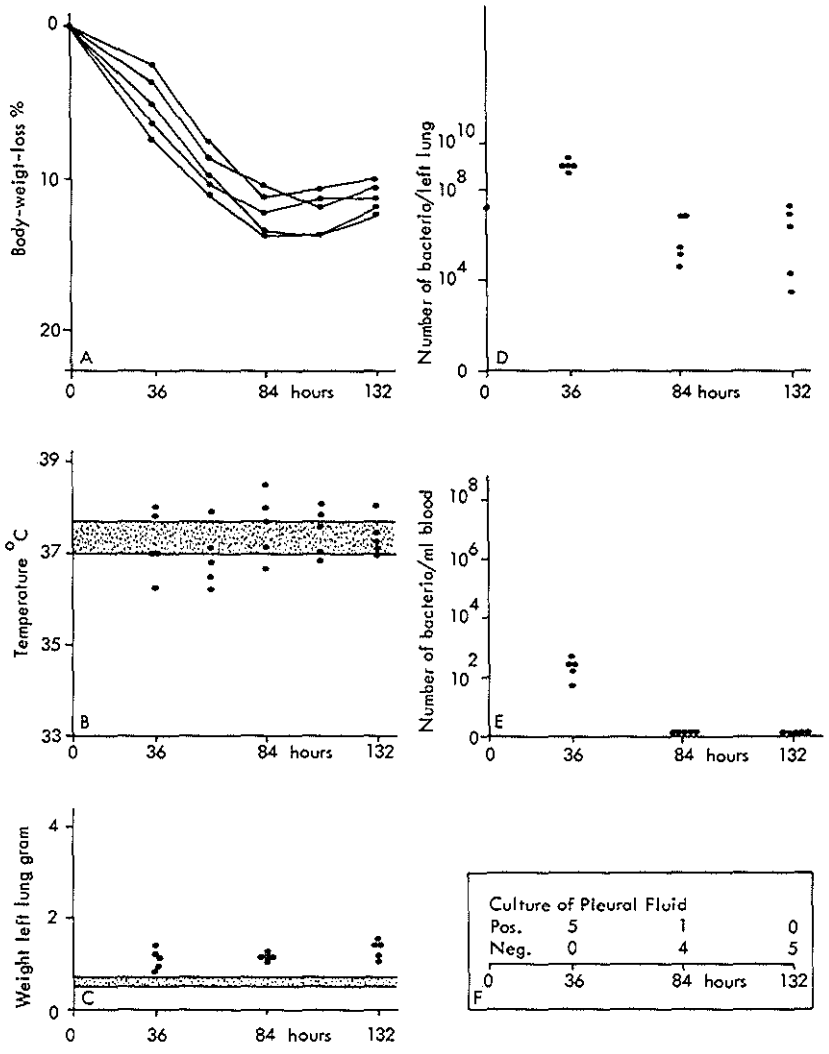


Fig. 2. *Action of clindamycin at 2 mg/kg body weight every 12 h starting 36 h after inoculation with 6 x 10^7 type III pneumococci upon the course of the pneumonia in CVF-treated rats. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter blood. (F) Culture of pleural fluid.*

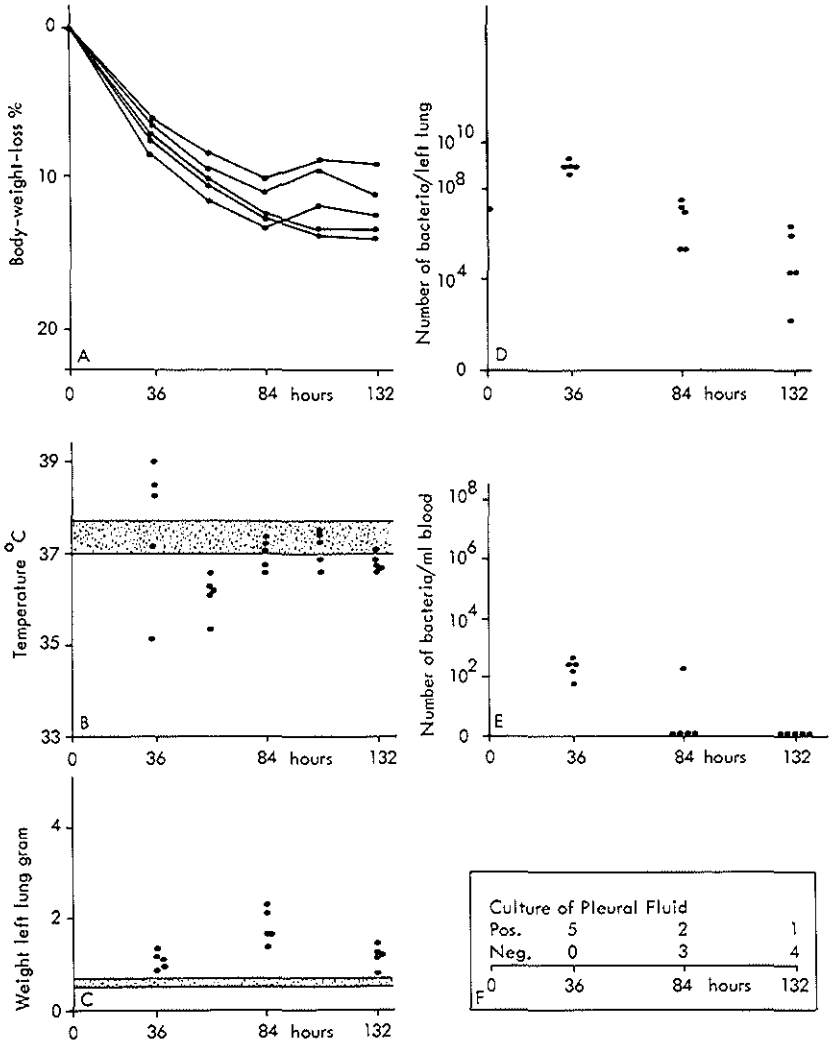


Fig. 3. *Action of clindamycin at 4 mg/kg body weight every 12 h starting 36 h after inoculation with 6 x 10^7 type III pneumococci upon the course of the pneumonia in CVF-treated rats. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter blood. (F) Culture of pleural fluid.*

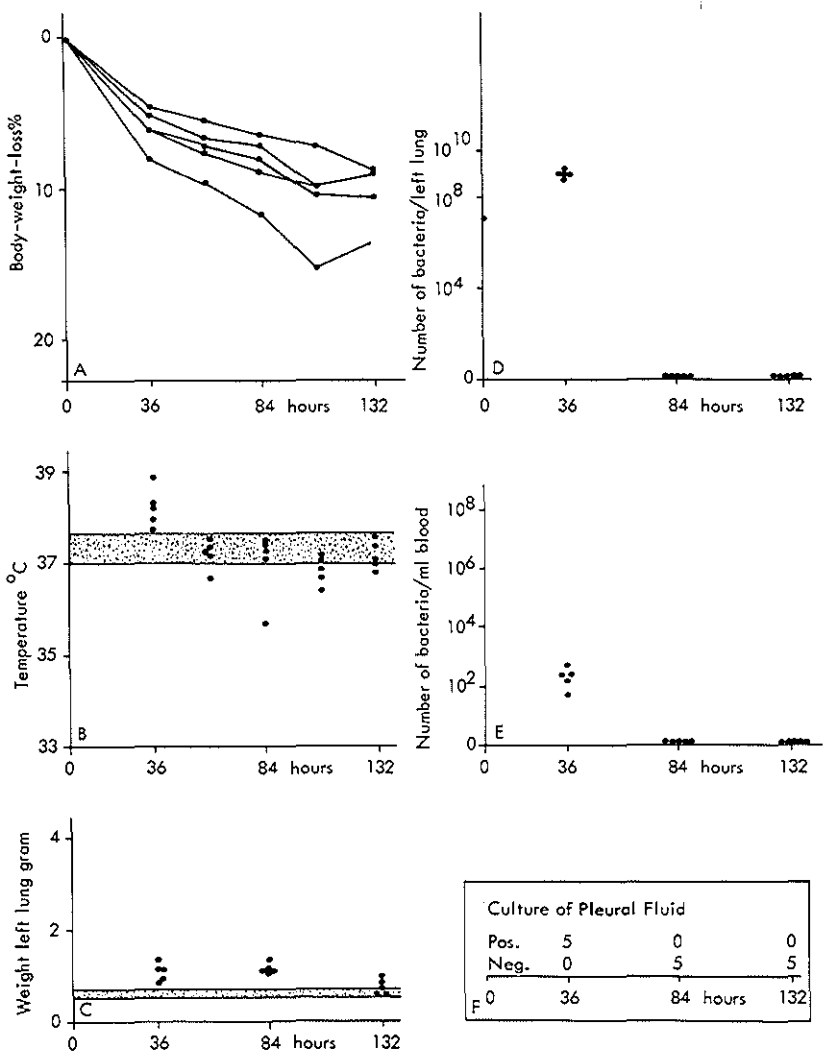


Fig. 4. *Action of clindamycin at 8 mg/kg body weight every 12 h starting 36 h after inoculation with 6 x 10^7 type III pneumococci upon the course of the pneumonia in CVF-treated rats. (A) Total body weight loss (percentage) of five rats during infection. (B) Rectal temperature of five rats during infection (normal temperature, 37.4 ± 0.34°C). The following determinations were made after sacrificing 15 rats in groups of 5. (C) Weight of the left lobe of the lung (normal weight, 0.6 ± 0.15 g). (D) Number of viable pneumococci in the left lobe of the lung. (E) Number of viable pneumococci per milliliter blood. (F) Culture of pleural fluid.*

homogenates to a final concentration of 1 µg/ml, 60-70% was found immediately after mixing (0 h), and after 1½ and 6 hours incubation the concentration of penicillin was unchanged. When penicillin was added to the lung homogenates to a final concentration of 10 µg/ml, 80-90% was found immediately after mixing (0 h); samples tested after 1½ and 6 hours incubation, yielded the expected concentration of penicillin. These experiments revealed that the amount of penicillin added to the homogenates of infected lungs of normal and of CVF-treated rats did not decrease substantially within 6 hours of incubation at 37°C. It must be concluded that the slight decrease in penicillin concentration immediately after mixing, was due not to inactivation of the penicillin but probably to an initial binding of penicillin to the tissue homogenate. As a control, homogenates of healthy lungs were mixed with penicillin (final concentration 1 and

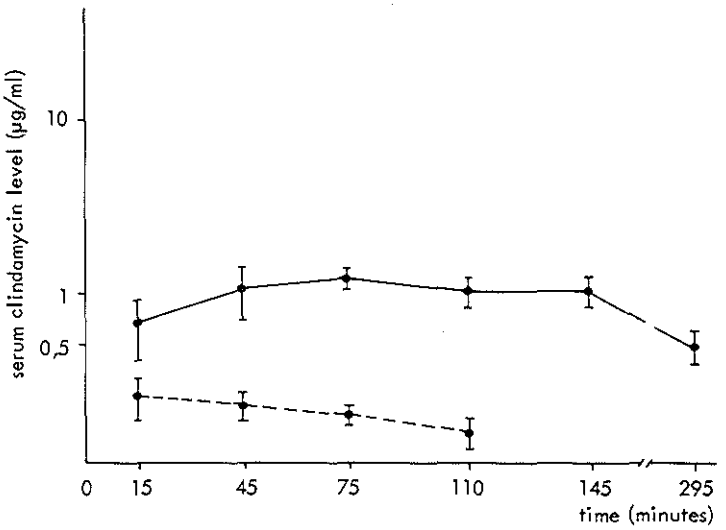


Fig. 5. *Serum concentrations of clindamycin. Rats were injected intramuscularly with clindamycin doses of 2 mg/kg (---) and 8 mg/kg (——). Each point represents the mean value of five rats (± standard deviation).*

10 µg/ml) and assayed against penicillin standards in phosphate buffered saline, pH= 7.3 that were stored at 4°C. In these experiments 40-50% of the penicillin added to the lung homogenates was found immediately after mixing (0 h) and the same after 1½ and 6 hours of incubation. Obviously penicillin had lost ± 50% of its activity probably as a result of binding to the healthy lung tissue. Six hours of incubation at 37°C did not alter the amount of free penicillin in healthy lung homogenate.

serum antibiotic levels

Intramuscular injections of 2 and of 8 mg/kg clindamycin gave peak concentrations in the serum of 0.25 µg/ml and 1.2 µg/ml respectively. Levels exceeding the MIC of pneumococci for clindamycin i.e. 0.024 µg/ml were present for about 6 and 12 h respectively (Fig. 5).

Discussion

Clinical experience has established the efficacy of clindamycin for treatment of mixed aërobic and anaërobic pulmonary infections. Several investigators studied the therapeutic effect of penicillin and clindamycin on lungabcesses generally caused by anaërobic or mixed aërobic and anaërobic bacteria (1, 6, 8, 9, 12). Most of these studies revealed that there was no difference between the efficacy of these two antibiotics. Clinical experience with penicillin as well as with clindamycin in the management of these infections has been good. Balachandar demonstrated the efficacy of clindamycin in children with pneumonia, whose throat cultures revealed *Streptococcus pneumoniae*; the clinical response of these children to intramuscular clindamycin phosphate was excellent (4).

In general it can be concluded from this and from other studies that clindamycin can be an effective drug in the treatment of severe infections, particularly in patients who are hypersensitive to penicillin, and in those whose infections are caused by organisms resistant to penicillin (4).

In this study we investigated the efficacy of clindamycin treatment to

cure pneumococcal infection in normal rats and in rats with impaired phagocytosis of pneumococci. We compared these results to results found earlier describing the efficacy of penicillin (2, 3). Impairment of phagocytosis was accomplished by removing complement component C3 which is the principal component involved in the opsonization of pneumococci before specific antibodies develop. C3 depletion was accomplished by repeated injections of the rats with cobra venom factor (2).

Although the antimicrobial sensitivity in vitro to both antibiotics was the same for the used pneumococcal strain, and although the antimicrobial activity in vivo of both drugs was the same in normal rats, the minimal effective dosage schedules of each antibiotic appears to be different in rats with impaired host defense mechanisms. The minimal effective dosage of clindamycin to cure experimental pneumonia in normal rats was 2 mg/kg every 12 h started 36 h after the initiation of the infection; this was similar to the treatment schedule with penicillin (2). It was demonstrated that for both antibiotics, penicillin and clindamycin, this treatment schedule was not successful in CVF-treated rats which had impaired host defense to pneumococcal infection (2, present study). The therapeutic effect of the antimicrobial therapy could be restored by a 25-fold increase in the daily dose for penicillin (3) and by a 4-fold increase for clindamycin (present study).

That the increase in dosage required for treatment of the pneumococcal infection in CVF-treated rats was considerably greater for penicillin than for clindamycin, might be explained by inactivation of the penicillin by inflammatory exudate at the site of infection. Recent literature reported probable enzymic inactivation of penicillin in inflammatory exudates; this agent which destroyes penicillin, was not derived from bacteria but apparently from host cells (5, 10, 11). Barnes et al. demonstrated that pus from a patient with an empyema due to a fully penicillin-sensitive hemolytic group B *Streptococcus* inactivated the penicillin added in vitro. Various antibiotics were tested. No important inactivation of clindamycin in the presence of pus was seen (5). De Louvois et al. reported that four out of 22 specimens of human pus were able to inactivate penicillin (11).

The results of our penicillin inactivation experiments make it improbable that the loss of activity of penicillin in the CVF-treated rats was due to inactivation of the antibiotic by inflammatory exudate. Although the pulmonary lesions in the CVF-treated rats were very extensive compared to the lesions in normal rats, the homogenates of 3 days'-infected lung tissue of these CVF-treated rats and normal rats did not inactivate added penicillin. Therefore, it is improbable that failure of penicillin treatment of pneumococcal pneumonia in CVF-treated rats is the result of inactivation of the penicillin at the site of the infection.

In an attempt to explain the difference in efficacy of the two antimicrobial drugs, the clindamycin serum level was determined at different times after its administration and compared to penicillin levels (described earlier). These estimations showed that intramuscular injections of the same doses of penicillin and of clindamycin gave rise to completely different serum levels in the rat, both with regard to peak values and as to duration of adequate levels. Serum levels of antibiotics above the MIC for pneumococci were maintained for a much shorter period with penicillin than with clindamycin. After injection of the drugs in a dosage rate of 2 mg/kg the duration of the periods where the levels exceeded the MIC for pneumococci were about 1½ h and 6 h for penicillin and clindamycin respectively (3, present study). Actually, serum levels constantly above or equal to the MIC of the pneumococcus to clindamycin were only found if a treatment schedule of 8 mg clindamycin per kg every 12 h was applied. At the site of infection clindamycin might therefore be more effective than penicillin. The course of pneumococcal infection in CVF-treated rats was more severe than the infection in normal rats; the pneumonia spread more rapidly in the CVF-treated rats, and the pulmonary lesions were more extensive (2). Evidently a continuous high level of the antimicrobial drug in the tissue is an essential condition for curing the infection in the CVF-treated rats. From the present experimental study and from other studies described earlier (2, 3) two conclusions can be drawn: first, the loss of

activity of antibiotic treatment (penicillin or clindamycin) of pneumococcal infection results from selectively impaired phagocytosis in the infected host, and secondly the loss of phagocytic functions of granulocytes can be compensated by an improved antibiotic treatment schedule. In the latter case the choice of antimicrobial agent depends not only on the antimicrobial susceptibility of the infecting organism, but also on the pharmacological properties of the antimicrobial drug. In pneumococcal pneumonia with extensive pulmonary lesions due to impaired phagocytosis, it is absolutely essential to apply antibiotic treatment schedules that give rise to prolonged inhibitory antibiotic serum levels in the interval between two successive injections.

Acknowledgements

We are grateful to M.E. Erkens-Versluis for supplying cobra venom factor, to B.K.B. Reimann-Knuth Winterfeldt for critical review of the manuscript, and to A.L. Beukelman for secretarial help. This study was supported in part by research grants from Beecham Farma B.V., The Netherlands.

Literature cited

1. Abernathy, R.S. 1968. Antibiotic therapy of lung abscess: effectiveness of penicillin. Dis. Chest 53: 592-598.
2. Bakker-Woudenberg, I.A.J.M., J.Y.T. de Jong-Hoenderop, and M.F. Michel. 1979. Efficacy of antimicrobial therapy in experimental rat pneumonia: effects of impaired phagocytosis. Infect. Immun. 25: 366-375.
3. Bakker-Woudenberg, I.A.J.M., A.L.E.M. van Gerwen, and M.F. Michel. 1979. Efficacy of antimicrobial therapy in experimental rat pneumonia: antibiotic treatment schedules in rats with impaired phagocytosis. Infect. Immun. 25: 376-387.
4. Balachandar, V., P.J. Collipp and B.J. Rising. 1973. Intramuscular clindamycin phosphate in children. Clin. Med. 80: 24-30.

5. Barnes, P., and P.M. Waterworth. 1977. New cause of penicillin treatment failure. Br. Med. J. I: 991-993.
6. Bartlett, J.G., and S.L. Gorbach. 1975. Treatment of aspiration pneumonia and primary lung abscess. J. Am. Med. Assoc. 234: 935-937.
7. Bennet, J.V., J.L. Brodie, E.J. Benner, and W.M.M. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. 14: 170-177.
8. Brook, I. 1979. Clindamycin in treatment of aspiration pneumonia in children. Antimicrob. Agents Chemother. 15: 342-345.
9. Finegold, S.M. 1977. Current status of therapy of anaerobic infections in humans. U.S.A.-Metronidazole proc. int. metronidazole conf. 438: 273-278.
10. Lacey, R.W. 1977. Inactivation of penicillin and cephalosporins by enzymes. Br. Med. J. I: 1285.
11. Louvois, J. de., and R. Hurley. 1977. Inactivation of penicillin by purulent exudates. Br. Med. J. I: 998-1000.
12. Weiss, W., and N.S. Cherniack. 1974. Acute non-specific lung abscess: a controlled study comparing orally and parenterally administered penicillin G. Chest 66: 348-351.

Chapter 4

EFFECT OF SPLENECTOMY UPON THE COURSE OF EXPERIMENTAL PNEUMOCOCCAL BACTEREMIA IN RATS AND THE EFFICACY OF PENICILLIN THERAPY*

Irma A.J.M. Bakker-Woudenberg, P. de Bos, A.L.E.M. van Gerwen, W.B. van Leeuwen, and M.F. Michel

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, The Netherlands

Evidence is presented about increased susceptibility to pneumococcal infection after splenectomy in young rats and after splenectomy in adult rats with low serum opsonic levels. The penicillin therapy which prevented mortality in normal intravenously infected rats, had lost its activity in splenectomized young rats and splenectomized adult rats with low serum opsonic levels. By markedly increasing the dose of penicillin the efficacy of the antibiotic was restored in those animals.

Introduction

For many years it has been recognized that patients with anatomic or functional asplenia are subject to episodes of fulminating, often lethal bacterial infections, frequently caused by the pneumococcus. The occurrence of such infections in patients with hyposplenism (9, 16) is documented by clinical studies in patients functionally asplenic by Sickle cell disease (19, 20) and in patients splenectomized for trauma (2, 13, 18) or Hodgkin's disease (6, 8, 10). It has become clear that the risk of developing postsplenectomy sepsis is related to a number of variables. One factor is the age of the patient at the time of splenectomy. Adults seem to be at less risk of postsplenectomy sepsis

* will be published in J. Infection.

than children. The reason for performing splenectomy is also an important factor. Some authors suggest that splenectomy per se carries some increased risk of acquiring overwhelming infections, even in otherwise normal individuals (2, 13, 18). Other authors have reported that the relative risk of postsplenectomy infection appears to be a function of the underlying disease. The risk is high in patients who received chemotherapy for haematologic malignancies such as Hodgkin's disease (6, 8, 10, 26, 27). The primary disease itself is associated with increased susceptibility to infection which in turn predisposes the patient to overwhelming and frequently fatal infection when the spleen is removed.

In this animal study we investigated the effect of splenectomy upon the course of pneumococcal bacteremia, and whether removal of the spleen influenced the amount of penicillin required for surviving the infection. Experimental conditions similar to clinical situations were chosen. Variables of age and opsonic capacity of the serum were introduced by using young and adult rats and by treating adult rats with cobra venom factor.

Materials and Methods

animals

Female R-strain albino rats (random bred; specific pathogen free; bred in the Laboratory Animals Center of the Erasmus University Rotterdam) were used in all experiments. Adults rats 14-18 weeks old, weight 180 to 210 g, young rats 3-4 weeks old, weight 50 to 80 g.

bacteria

A *Streptococcus pneumoniae* type III strain (isolated from a clinical case) was used in all experiments. The minimal inhibitory concentration (MIC) for penicillin was 0.024 µg/ml (tube dilution test).

The virulence of the bacteria was maintained by passage in rats every 4 months and storage at -70°C in Todd-Hewitt broth (Oxoid) with 10% glycerol (Merck).

splenectomy

Adult rats or young rats were splenectomized or sham operated. Splenectomies and sham operations were carried out under anesthesia with Hypnorm (Duphar) and pentobarbital (adult animals) or ether anesthesia (young animals). Splenectomy was performed through a small lateral left flank incision. The abdominal wall was closed with silk sutures; the skin was closed with skin clips (adult animals) or silk (young animals).

treatment with cobra venom factor

The venom of the Cobra (Naja naja siamensis) was purified and kindly supplied by I.J.C. Wilschut (29). One unit of cobra venom factor (CVF) amounted to 10 µg of the purified material. Rats were injected intraperitoneally 14 times with CVF (two doses 0.14 units/kg body weight, twelve doses 0.07 units/kg body weight). The first dose was administered 12 hours before pneumococcal inoculation, followed by five doses every 8 h and eight doses every 12 h. To determine complement depletion in vivo rats were bled at several times during CVF treatment by orbital puncture. Sera were tested by an assay of complement hemolytic activity as described earlier (1). The data derived from this assay were presented as 50% complement hemolysis units per milliliter, corresponding to the reciprocal value of the serum dilution that gave 50% hemolysis. Earlier experiments (1 and unpublished) revealed a correlation between the level of circulating C3 and the whole complement hemolytic activity in serum after CVF treatment.

in vitro phagocytosis

Experiments of in vitro phagocytosis of pneumococci by PMN were performed as described earlier (1). In short: pneumococci were preincubated in rat serum and added to a suspension of leukocytes obtained from rat peritoneal washings. After 30 min phagocytosis was stopped, the cells were washed and were examined under a microscope in Gram-stained smears.

intravenous inoculation

Adult rats and young rats were challenged by inoculation with 0.5 ml suspensions of 2 x 10^8 and 0.7 x 10^8 *S. pneumoniae* type III cells, respectively, into the tail vein. Mortality determinations were terminated one week after inoculation. For clearance studies blood samples (0.2 ml) were obtained by orbital puncture at 5, 15, 50, 100, 150 min after challenge and these were quantitated for viable bacteria.

antimicrobial treatment after intravenous pneumococcal inoculation

The sodium salt of penicillin G in aqueous solution was used. Individual doses of penicillin amounted to 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 45, 55 mg/kg body weight. Penicillin injections were given every 12 h intramuscularly into the thigh muscles of the rear legs. Fourteen injections were given, the first 2 h after intravenous inoculation of pneumococci.

serum antibiotic levels

Serum antibiotic levels were determined after intramuscular injections of penicillin amounting to 0.5, 2, 10 or 55 mg/kg body weight. Blood specimens for antibiotic assay were obtained by orbital puncture under light ether anesthesia. From each rat blood was collected at four different times after intramuscular injection of the antibiotic.

antibiotic assay

Penicillin levels were estimated by a standard agar diffusion method (3) with some modifications. For determinations of serum levels below 0.5 µg/ml penicillin, sensitivity test agar* and a *Bacillus stearothermophilus* strain as the indicator organism were used, and incubations were performed at 56°C (sensitivity of this assay system was 0.06 µg/ml penicillin). For determination of serum levels above 0.5 µg/ml penicillin, Oxoid diagnostic sensitivity test agar and a *Staphylococcus aureus* strain (ATCC 9144, strain Oxford) as the

*0.5% tryptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar (Difco)

indicator organism were used, and incubations were performed at 37°C (sensitivity of this assay system was 0.25 µg/ml penicillin). Tests were performed with 50 µl of rats serum per test.

Results

The effect of splenectomy upon the course of infection and the efficacy of antimicrobial therapy was studied in a model of pneumococcal bacteremia. Several groups of rats were used. Variables such as the age at the time of splenectomy, interval since splenectomy and increased susceptibility to pneumococcal infection due to impaired phagocytosis, were introduced in this study (Table I). In all experiments sham-operated rats were compared with age-matched normal control rats. We found no difference in the course of pneumococcal bacteremia in sham-operated rats and unoperated rats, not even when the animals were only 3 weeks old. Sham-operated rats will therefore not be mentioned further.

Table I.

Animals		Age at time of Splenectomy	Age at time of Pneumococcal infection
A	normal adult rats	-	18 weeks
B	normal adult rats	17 weeks	18 weeks
C	normal adult rats	14 weeks	18 weeks
D	normal adult rats	3 weeks	18 weeks
E	CVF-treated adult rats	-	18 weeks
F	CVF-treated adult rats	17 weeks	18 weeks
G	CVF-treated adult rats	14 weeks	18 weeks
H	CVF-treated adult rats	3 weeks	18 weeks
I	young rats	-	4 weeks
J	young rats	3 weeks	4 weeks

effects of cobra venom factor

By means of multiple injections of CVF (two injections 0.14 units/kg, twelve injections 0.07 units/kg) whole complement hemolytic activity in serum was kept at ± 25% of the normal values starting from 12 h after the first CVF injection. Compared to normal rat serum (100%) the opsonic activity of the serum of CVF-treated rats was reduced to 62%, as evidenced by an impaired phagocytosis of pneumococci in vitro by PMN.

	deaths/ no rats(1)	deaths/ no rats(2)	deaths/ no rats(3)
A	0/10	0/10	3/10
B	1/10	1/10	2/10
C	2/10	3/10	3/10
D	0/10	0/10	2/10
E	1/10	5/10	6/10
F	7/10	10/10	10/10
G	9/10	10/10	10/10
H	10/10	10/10	10/10
I	0/10	1/10	2/10
J	1/10	7/10	8/10

1) 12 hours after inoculation
2) 24 hours after inoculation
3) 1 week after inoculation

Table II. *Mortality in rats after i.v. inoculation of adult rats and young rats with respectively 2 x 10^8 and 0.7 x 10^8* Streptococcus pneumoniae. *type III. (A) Normal adult rats, (B) normal adult rats inoculated 1 week postsplenectomy, (C) normal adult rats inoculated 4 weeks postsplenectomy, (D) normal adult rats splenectomized at young age and inoculated 15 weeks postsplenectomy, (E) CVF-treated adult rats, (F) CVF-treated adult rats inoculated 1 week post-splenectomy, (G) CVF-treated adult rats inoculated 4 weeks postsplenectomy, (H) CVF-treated adult rats splenectomized at young age and inoculated 15 weeks postsplenectomy, (I) young rats, (J) young rats inoculated 1 week postsplenectomy.*

experimental pneumococcal bacteremia in splenectomized rats

Groups of 10 rats were inoculated intravenously. Parameters for the severity of pneumococcal bacteremia were: mortality after 12 h, 24 h and 1 week (Table II), and clearance of pneumococci from the blood stream (Fig. 1 through 3).

After intravenous inoculation of 2×10^8 pneumococci none of the adult rats died within the first 24 h; after 1 week the mortality was 30%. The mortality rate in rats splenectomized 1 week, 4 weeks or 15 weeks prior to infection, was slightly higher but not significantly (Fisher test $P > 0.05$). CVF-treated normal adult rats had an increased mortality within the first 24 h (Fisher test $P= 0.03$). All splenectomized CVF-treated rats died within the first 24 h; compared with the mortality rate of normal CVF-treated rats, mortality rates were significantly higher in these rats (Fisher test $P= 0.03$). The mortality rate in splenectomized young rats inoculated with 0.7×10^8 pneumococci was significantly higher than the mortality rate in normal young rats (Fisher test $P= 0.02$).

Clearance of pneumococci was determined at 5, 15, 50, 100, 150 minutes after intravenous injection of adult rats and young rats with 2×10^8 and 0.7×10^8 pneumococci, respectively. The clearance curves are shown in Fig. 1, 2, and 3. There was a rapid clearance of viable pneumococci in normal adult animals (Fig. 1). In splenectomized adult animals the clearance rate was slightly different compared to controls (Fig. 1). Within the first 100 minutes after the inoculation 85% of the pneumococci were cleared. CVF-treated animals cleared the pneumococci at a somewhat slower rate (Fig. 2). In contrast splenectomized CVF-treated animals were not able to clear pneumococci from the blood stream (Fig. 2). After a decrease during the first hour the number of bacteria increased again, and 150 minutes after the inoculation the number of viable bacteria in the blood was significantly higher than the number found in the controls (Wilcoxon rank-sum test $P < 0.01$). The clearance curve of the young animals (Fig. 3) compared well to the clearance curve in adult animals (Fig. 1) although within the first hour clearance seemed to be less in young animals. The clearance curve

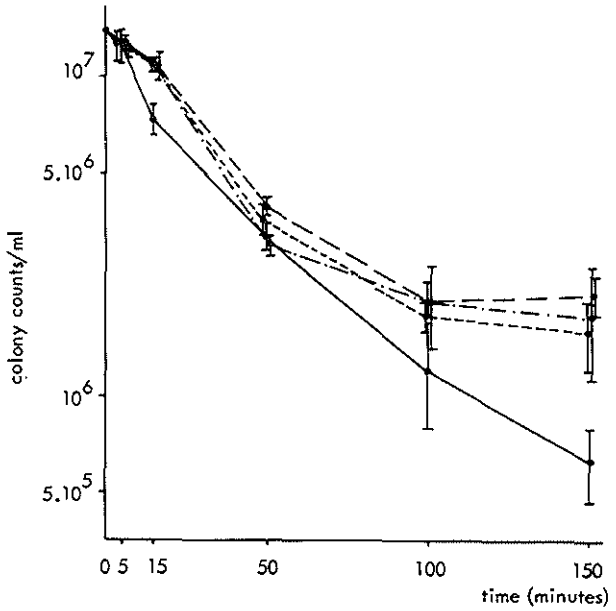

10^7
5.10^6
10^6
5.10^5
colony counts/ml
0 5 15 50 100 150
time (minutes)

Fig. 1.

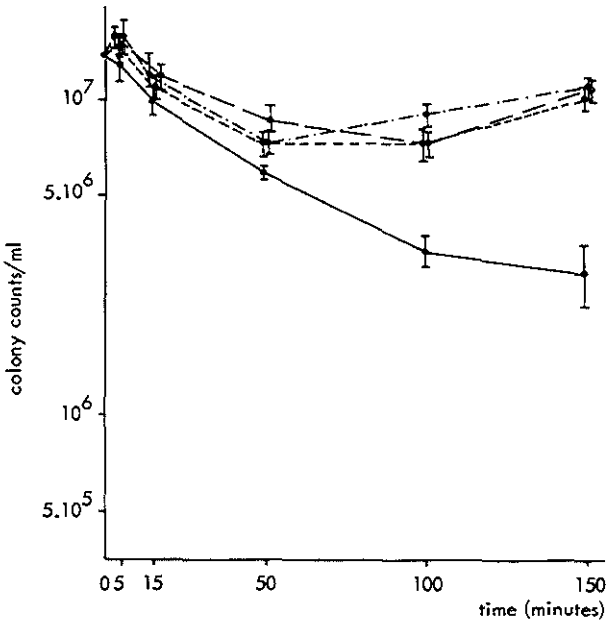

10^7
5.10^6
10^6
5.10^5
colony counts/ml
0 5 15 50 100 150
time (minutes)

Fig. 2.

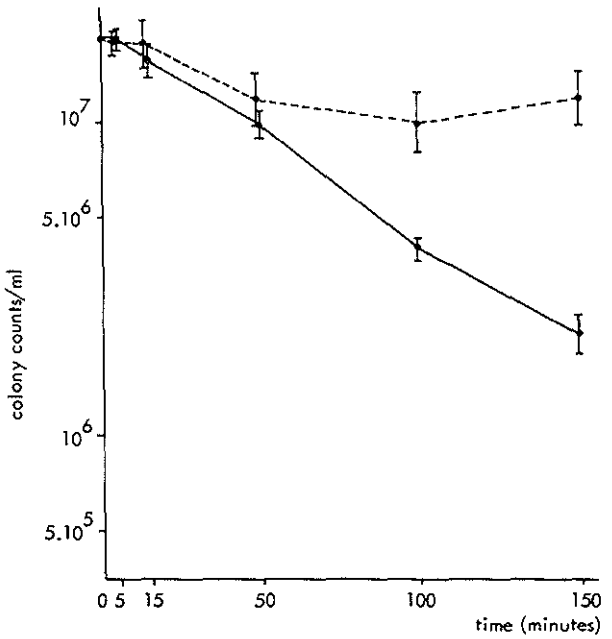


Fig. 3.

Fig. 1. *Viable bacterial counts in blood after i.v. inoculation of* 2 x 10^8 Streptococcus pneumoniae *type III. Each point represents the mean value of four rats (± standard deviation). (A) Normal adult rats (——), (B) normal adult rats inoculated 1 week postsplenectomy (- - -), (C) normal adult rats inoculated 4 weeks postsplenectomy (—·—), (D) normal adult rats splenectomized at young age and inoculated 15 weeks postsplenectomy (— —).*

Fig. 2. *Viable bacterial counts in blood after i.v. inoculation of* 2 x 10^8 Streptococcus pneumoniae *type III. Each point represents the mean value of four rats (± standard deviation). (E) CVF-treated adult rats (——), (F) (CVF-treated adult rats inoculated 1 week postsplenectomy (- - -), (G) CVF-treated adult rats inoculated 4 weeks postsplenectomy (—·—), (H) CVF-treated adult rats splenectomized at young age and inoculated 15 weeks postsplenectomy (— —).*

Fig. 3. *Viable bacterial counts in blood after i.v. inoculation of* 0.7 x 10^8 Streptococcus pneumoniae *type III. Each point represents the mean value of four rats (± standard deviation). (I) Young rats (——), (J) young rats 1 week postsplenectomy (- - -).*

of splenectomized young animals showed an initial decrease of pneumococci followed by bacterial multiplication (Fig. 3).

antimicrobial treatment of experimental pneumococcal bacteremia in splenectomized rats

Groups of ten rats (adult rats or young rats) were inoculated intravenously with pneumococci (2×10^8 or 0.7×10^8 respectively). After penicillin treatment at a dosage rate of 0.5 mg/kg/12 h starting 2 h after inoculation, normal adult rats did not die within the first week after inoculation. In CVF-treated rats a 20-fold increase in dosage (10 mg/kg/12 h) was required for survival, and in splenectomized

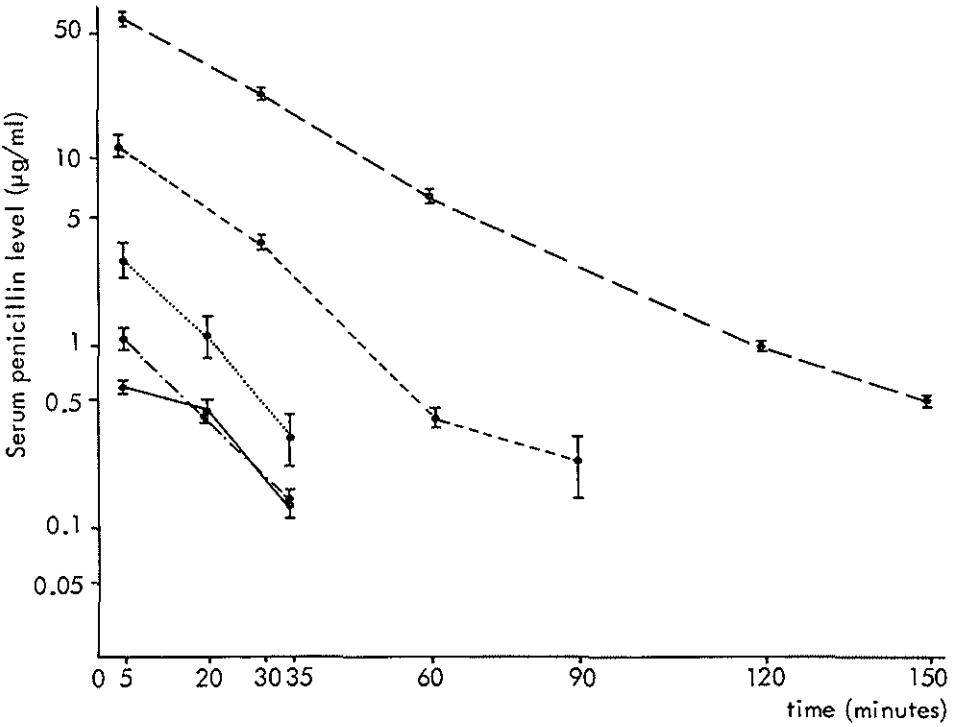


Fig. 4. *Serum concentrations of penicillin. Adult rats were injected intramuscularly with penicillin doses of 0.5 mg/kg (——), 10 mg/kg (- - - -) and 55 mg/kg (— —). Young rats were injected intramuscularly with penicillin doses of 0.5 mg/kg (— · — · —) and 2 mg/kg (· · · · · · · ·). Each point represents the mean value of five rats (± standard deviation).*

CVF-treated animals the minimal effective penicillin dose had to be increased even further (55 mg/kg/12 h). Young animals were cured by the same dosage of penicillin (0.5 mg/kg/12 h) as was used for adult animals. After splenectomy of the young rats the minimal effective penicillin dosage had to be increased to 2 mg/kg/12 h.

serum antibiotic levels

Intramuscular injections of adult rats with penicillin doses of 0.5, 10, and 55 mg/kg and of young rats with penicillin doses of 0.5 and 2 mg/kg, gave rise to penicillin levels which differed in peak heights and duration of penicillin concentrations equal to or above the minimal inhibitory concentration (MIC) for the pneumococcus (0.024 µg/ml) (Fig. 4). Administration of 0.5, 10 and 55 mg/kg to adult rats gave peak concentrations of 0.6, 12 and 62 µg/ml, respectively; estimated minimal effective serum concentrations of 0,024 µg of penicillin per ml or more were present for about 60, 130 and 250 min, respectively. Administration of 0.5 and 2 mg/kg to young animals gave peak concentrations of 1,1 and 2,9 µg/ml, respectively; estimated minimal effective serum concentrations of 0.024 µg of penicillin per ml or more were present for about 60 and 70 min, respectively.

Discussion

The effects of splenectomy upon the course of infection and the efficacy of antimicrobial therapy were studied in a rat model of pneumococcal bacteremia. Variables such as the age at the time of splenectomy, length of time since splenectomy and increased susceptibility to pneumococcal infection due to impaired phagocytosis, were introduced in this study. Phagocytosis of encapsulated pneumococci occur after opsonization of the bacteria (24), and impairment of phagocytosis was accomplished by removing the opsonins. Complement component C3 is the principal component involved in the opsonization process (30), and phagocytosis of pneumococci was therefore impaired by reducing the amount of C3. Reduction of C3 was accomplished by

repeated injections of the rats with cobra venom factor (CVF). It is known that this factor causes depletion of plasma C3 in several species (1, 15, 21). The present study showed that the CVF-treated rats had decreased levels of plasma C3 in their sera. The opsonic capacity of these sera was impaired, as measured by in vitro phagocytosis of pneumococci by PMN.

Our experiments showed that the course of infection after intravenous inoculation of pneumococci was influenced by removal of the spleen in young rats and in CVF-treated adult rats. In these two groups of animals asplenia gave rise to increased mortality, which appeared to be correlated with inability to remove pneumococci circulating in the blood. Splenic phagocytosis was of critical importance in the face of a quantitative deficiency in opsonins.

Other experimental studies also showed an increased mortality in splenectomized rats and mice after intravenous pneumococcal challenge (17, 25, 28). Coil demonstrated an increase in mortality after splenectomy in mice after pneumococcal infection via an aerosolized atmosphere (7). Our experimental data differ from the findings by Biggar who demonstrated a delay in clearance of ^{59}Fe-labelled pneumococci in adult rats (13 weeks old) if splenectomy preceded infection by five days; however sera from these rats, tested five days after splenectomy, had no demonstrable deficiency in capacity to promote in vitro phagocytosis of pneumcocci by PMN (4, 5). The discrepancies between these results may be due to the use of organisms with varying virulence, the use of different species and the use of animal strains with different susceptibilities. Selection of host and parasite is critical.

In our experiments inability to clear pneumococci after intravenous infection as a result of splenectomy was only demonstrated in young rats and in CVF-treated adult rats. In normal adult animals the opsonic capacity of the serum is sufficient for adequate phagocytosis of pneumococci. When the C3 level was reduced by treatment with CVF, pneumococcal opsonic capacity of the serum was diminished as evidenced by impaired

phagocytosis of the pneumococci in vitro by PMN. This resulted in a delay in pneumococcal clearance and increased mortality. Pneumococcal clearance studies in guinea pigs treated with CVF also demonstrated that although a clearance was initiated, bacteria started to multiply again, a condition that led to the animals' death (12).
Some clinical investigations showed that increased susceptibility for pneumococcal infections was found in splenectomized individuals with an abnormality of the alternate complement pathway and in patients with Sickle cell disease (14, 23). The absence of the spleen permits the organisms to circulate and therefore multiply for a longer time, and, as seen in the clearance curves, this may lead in a short time to a dangerously high blood stream concentration of bacteria.
In splenectomized animals with increased mortality to intravenous pneumococcal infection, the efficacy of penicillin therapy was tested. Penicillin in a dosage rate of 0.5 mg/kg/12 h starting 2 h after inoculation of pneumococci prevented mortality of normal adult rats and young rats. Inhibitory serum concentrations of 0.024 µg/ml or more of penicillin were present for 65-70 min in both the adult and the young rats. To cure CVF-treated rats, splenectomized four weeks before infection, or young rats, splenectomized one week before infection, the penicillin dosage had to be increased (0.5 to 55 mg/kg/12 h or 0.5 to 2 mg/kg/12 h respectively). These observations suggest a correlation between a need for an increased penicillin dosage and an increased bacteria count in the blood. This supports previous studies of Perry a.o. suggesting that the lethal outcome of pneumococcal infection was related to the total mass of multiplying pneumococci (22). Our findings correspond to some clinical reports that hyposplenic patients did not respond well to penicillin therapy (11, 13). Prompt penicillin treatment in high doses of suspected postsplenectomy pneumococcal infection is desirable.

Acknowledgements

We are grateful to M.E. Erkens-Versluis for supplying Cobra Venom

Factor, to B.K.B. Reimann-Knuth Winterfeldt for critical review of the manuscript and to A.L. Beukelman for secretarial help. This study was supported in part by research grants from Beecham Farma B.V., The Netherlands.

Literature cited

1. Bakker-Woudenberg, I.A.J.M., J.Y.T. de Jong-Hoenderop, and M.F. Michel. 1979. Efficacy of antimicrobial therapy in experimental rat pneumonia: effects of impaired phagocytosis. Infect. Immun. 25: 366-375.
2. Balfanz, J.R., M.E. Nesbit, Jr., C. Jarvis, and W. Krivit. 1976. Overwhelming sepsis following splenectomy for trauma. J, Pediatr. 88: 458-460.
3. Bennet, J.V., J.L. Brodie, E.J. Benner, and W.M.M. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. 14: 170-177.
4. Biggar, W.D., B. Holmes, and R.A. Good. 1972. Impaired phagocytosis of Pneumococcus Type 3 in splenectomized rats. Proc. Soc. Exp. Biol. Med. 139: 903-908.
5. Bogart, D., W.D. Biggar, and R.A. Good. 1972. Impaired intravascular clearance of Pneumococcus Type 3 following splenectomy. J. Reticuloendothel. Soc. 11: 77-87.
6. Chilcote, R.R., R.L. Baehner, and D. Hammond. 1976. Septicemia and meningitis in children splenectomized for Hodgkin's disease. N. Engl. J. Med. 295: 798-800.
7. Coil, J.A., J.D. Dickerman, and E. Boulton. 1978. Increased susceptibility of splenectomized mice to infection after exposure to an aerosolized suspension of type III Streptococcus pneumonia. Infect. Immun. 21: 412-416.
8. Desser, R.K., and J.E. Ultman. 1972. Risk of severe infection in patients with Hodgkin's disease or Lymphoma after diagnostic laparotomy and splenectomy. Ann. Intern. Med. 77: 143-146.
9. Dickerman, J.D. 1976. Bacterial infection and the asplenic host: a Review. J. Trauma 16: 662-668.

10. Donaldson, S.S., E. Glatstein, and K.L. Vosti. 1978. Bacterial infections in pediatric Hodgkin's disease. Relationship to radiotherapy, chemotherapy and splenectomy. Cancer 41: 1949-1958.
11. Ertel, I.J., E.T. Boles, Jr., and W.A. Newton, Jr. 1977. Infection after splenectomy. N. Engl. J. Med. 296: 1174.
12. Frank, M.M. 1979. The complement system in host defense and inflammation. Rev. Infect. Dis. 1: 483-501.
13. Gopal, V., and A.L. Bisno. 1977. Fulminant pneumococcal infections in 'normal' asplenic hosts. Ann. Intern. Med. 137: 1526-1530.
14. Johnston, R.B., Jr., S.L. Newman, and A.G. Struth. 1973. An abnormality of the alternate pathway of complement activation in Sickle-cell disease. N. Engl. J. Med. 288: 803-808.
15. Kourounakis, L., R.A. Nelson, Jr., and M.A. Kupusta. 1973. The effect of a cobra venom factor on complement and adjuvant-induced disease in rats. Arthritis Rheum. 16: 71-76.
16. Krivit, W. 1977. Overwhelming postsplenectomy infection. Am. J. Hematol. 2: 193-201.
17. Leung, L.E., G.J. Szal, and R.H. Drachman. 1972. Increased susceptibility of splenectomized rats to infection with Diplococcus pneumoniae. J. Infect. Dis. 126: 507-513.
18. Maron, B.J., and J.R. Maloney. 1972. Septicemia following traumatic or incidental splenectomy. Johns Hopkins Med. J. 130: 266-268.
19. Overturf, G.D., D. Powars, and L.J. Baraff. 1977. Bacterial meningitis and septicemia in Sickle cell disease. Am. J. Dis. Child. 131: 784-787.
20. Pearson, H.A. 1977. Sickle cell anemia and severe infections due to encapsulated bacteria. J. Infect. Dis. 136 S, S25-S30.
21. Pepys, M.B. 1975. Studies in vivo of cobra venom factor and murine C3. Immunology 28: 369-377.
22. Perry, J.E., and L.E. Cluff. 1963. Manifestations of fatal pneumococcal infection in rabbits. J. Lab. Clin. Med. 62: 549-558.
23. Polhill, R.B., Jr., and R.B. Johnston. 1975. Diminished alternative complement pathway (ACP) activity after splenectomy. Pediatr. Res. 9: 333.

24. Shin, H.S., M.R. Smith, J. Winkelstein, and A. Nicholson. 1977. Heat labile opsonin system to pneumococcus, p. 563-566. *In* D. Bergsma et al. (ed.), Immunodeficiency in man and animals. Birth Defects: Original Article Series, the National Foundation, vol. 11, no. 1. Robert E. Krieger Publishing Co., Inc., New York.
25. Shinefield, H.R., C.R. Steinberg, and D. Kaye. 1966. Effect of splenectomy on the susceptibility of mice inoculated with Diplococcus pneumoniae. J. Exp. Med. 123: 777-794.
26. Sullivan, J.L., H.D. Ochs, G. Schiffman, M.R. Hammerschlag, J. Miser, E. Vichinsky, and R.J. Wedgwood. 1978. Immune response after splenectomy. Lancet I: 178-181.
27. Weitzman, S., and A.C. Aisenberg. 1977. Fulminant sepsis after the successful treatment of Hodgkin's disease. Am. J. Med. 62: 47-50.
28. Whitaker, A.N. 1968. The effect of previous splenectomy on the course of pneumococcal bacteraemia in mice. J. Pathol. 95: 357-376.
29. Wilschut, I.J.C., M.E. Erkens-Versluis, R.E. Ploemacher, R. Benner, and O. Vos. 1979. Studies on the mechanism of haemopoietic stem cell (CFU's) mobilization. A role of the complement system. Cell Tissue Kinet. 12: 299-311.
30. Winkelstein, J.A., M.R. Smith, and H.S. Shin. 1975. The role of C3 as an opsonin in the early stages of infection. Proc. Soc. Exp. Biol. Med. 149: 397-401.

Chapter 5

EFFICACY OF ANTIMICROBIAL THERAPY IN EXPERIMENTAL LISTERIOSIS IN MICE: AMPICILLIN TREATMENT SCHEDULES IN MICE WITH IMPAIRED T-CELL-MEDIATED IMMUNE RESPONSE*

Irma A.J.M. Bakker-Woudenberg, P. de Bos, W.B. van Leeuwen, and M.F. Michel

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, The Netherlands

The importance of intact host defense mechanisms for successful antimicrobial therapy was investigated in an animal model. Recovery from experimental infections caused by *Listeria monocytogenes* as a result of ampicillin therapy was studied in normal mice and in congenitally athymic (nude) mice. Nude mice were used for these experiments because antimicrobial resistance to *Listeria monocytogenes* depends on the development of cellular immunity, induced specifically by a T-cell-mediated reaction. These experimental studies revealed that the ampicillin therapy that cured normal mice infected with *Listeria monocytogenes*, had lost its activity in mice with impaired cell-mediated immunity. Lack of cellular immunity can not be compensated by intensification of the ampicillin treatment schedule, in terms of higher dose and/or shorter dosage interval and/or prolonged treatment.

Introduction

Listeria monocytogenes is an uncommon cause of septicemia and meningitis in healthy adults but can produce severe infections in neonates and in patients with compromised defense mechanisms.

*A shortened version will be published in Antimicrob. Agents Chemother.

Patients with underlying malignancies such as lymphoma's and leukemia's, and immunosuppressed patients such as renal transplant recipients receiving azathioprine and corticosteroids have shown considerable susceptibility to infections due to opportunistic pathogens such as *Listeria monocytogenes* (1, 10, 13, 19, 22, 25, 29). The susceptibility of patients with malignant diseases of lymphatic cells to these infections may be ascribed to defects in their cell-mediated immune mechanisms (6, 11). Such defects may be inherent to the underlying disease, but the inhibition of cell-mediated immune response by immunosuppressive drugs must also be weighed.
Data from several studies suggest that ampicillin is the drug of choice for the treatment of infections due to *Listeria monocytogenes* (12, 17, 27). However, despite the organism's usual sensitivity, therapeutic failures have occurred in patients with impaired host defense mechanisms (14, 25, 26, 32). Reasons for the failure of apparently appropriate antibiotic therapy are discussed by Watson et al. (32) and the synergistic effect of certain antibiotic combinations in vitro against *Listeria* was demonstrated by Moellering (23) and Espąze (9).
In the present study we investigated the role of T-cell-mediated immunity in relation to ampicillin therapy of listeric infection in normal mice and mice with impaired T-cell-mediated immunity.

Materials and Methods

animals

Female B10LP nude mice homozygote for the mutation 'nude' (nu/nu) and their age-matched immunologically normal controls heterozygote for the nude gene (nu/+) (specific pathogen free; 10-12 weeks old; bred in the Laboratory Animals Center TNO Zeist, the Netherlands) were used in all experiments.

bacteria

A *Listeria monocytogenes* type 4b strain (isolated from a clinical case) was used in all experiments. The minimal inhibitory concentration

for ampicillin was 0.16 µg/ml (tube dilution test). The virulence of the bacteria was maintained by passage into the yolk sac of 10-day old chicken embryos. After death of the embryo (after 3-5 days) the yolk sac material was inoculated on blood agar, and incubated for 16 h at 37°C. Beef broth was inoculated with these *Listeria* and after incubation, for 16 h at 37°C, this stock culture was stored at -70°C in small aliquots. A stock culture was thawed and used to seed a fresh culture for each animal inoculation. The bacterial culture was incubated for 16 h at 37°C. After incubation the stationary phase culture was kept at 4°C. The culture contained 2×10^9 viable organisms per ml (ranges $1.8-2.2 \times 10^9$). To prepare the inocula, the 16 h culture was appropriately diluted with physiological saline. To ensure identical inocula, for each experiment the number of viable organisms was determined before and just after animal inoculation.

experimental infection

Mice (10-12 weeks old) were infected by injection of 0.5 ml of a suspension of *Listeria monocytogenes* cells into the tail vein. Inocula amounted to 5×10^4 (ranges $4.5-5.5 \times 10^4$), 10^5 (ranges $0.9-1.1 \times 10^5$) and 5×10^5 (ranges $4.5-5.5. \times 10^5$) bacteria. The severity of the infection was indicated by the number of viable *Listeria* present in the spleen, liver and blood. At different intervals following the intravenous inoculation and at least 5 h after injection of ampicillin, the mice were sacrificed and the spleen and liver were removed. Individual organs were homogenized separately in 20 ml physiological saline (VirTis homogenizer, 30 sec. 10.000 rpm). The tissue homogenates were diluted serially 10-fold with saline, and 0.2 ml of each dilution was spread on blood agar base; finally the total homogenate was tested for viable bacteria by the pour-plate method. After dissection and macroscopic examination no thymus could be found in nude mice.

antimicrobial treatment

The sodium salt of ampicillin (Beecham Farma B.V., The Netherlands) was administered in aqueous solutions. Individual doses of

ampicillin amounted to 5, 10, 25 and 50 mg/mouse. Ampicillin injections were given either every 12 or every 8 h subcutaneously. Eight, twelve, sixteen or thirty-two injections were given, the first dose 36 h after the intravenous bacterial inoculation.

antibiotic assay

Bloodspecimens for antibiotic assay were obtained by orbital puncture under light anesthesia with ether. From each mouse blood was collected at three different times after ampicillin injection. Ampicillin levels were estimated by a standard large-plate agar diffusion technique with Oxoid diagnostic sensitivity test agar and a *Staphylococcus aureus* strain (ATCC 9144 strain Oxford) as the indicator organism (2). Sensitivity of the assay system was 0.25 µg/ml ampicillin. The coefficient of variation (CV) of the antibiotic determination was estimated for solutions containing concentrations within the range 0.5-16 µg/ml ampicillin. The CV of 12 determinations was 1-3%. Antibiotic assay were performed with 50 µl of mouse serum per test.

Results

The course of infection is indicated by the number of viable *Listeria* in the spleen and liver, presented in figures.

listeric infection in untreated mice

Figure 1 summarizes the results of *Listeria* spleen and liver counts in normal (nu/+) mice after intravenous inoculation of 5×10^4, 10^5 and 5×10^5 *Listeria monocytogenes* cells respectively. The course of infection depended on the number of bacteria injected. After inoculation of 5×10^5 *Listeria* all mice died within 48-72 h. Blood cultures were positive. After 36 h the average number of bacteria per milliliter blood was 380. *Listeria* multiplied rapidly in the tissues. At 36 h average numbers of 3×10^7 and 10^7 bacteria were cultured from spleens and livers respectively. Inoculation of normal mice with 10^5 *Listeria* resulted in a progressive increase in bacterial populations of the spleen and liver followed by a decrease in number of organisms between the third and the fifth day. The blood was sterile. Two mice out of

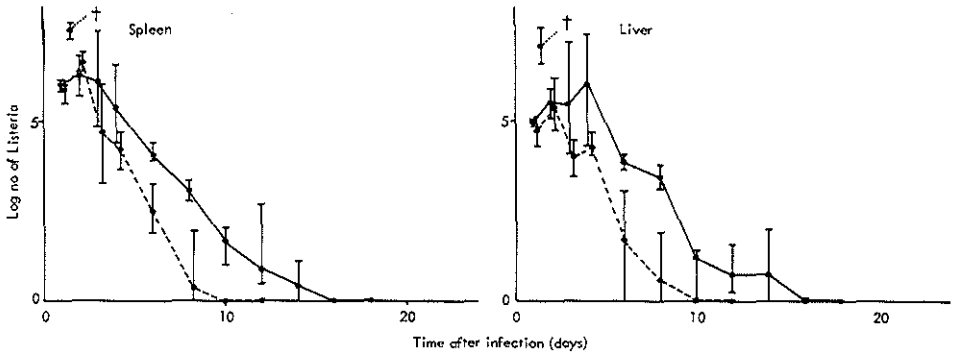


Fig. 1. *Growth curves of* Listeria monocytogenes *in spleens and livers of normal (nu/+) mice after intravenous inoculation of* 5 x 10^5 *bacteria (...),* 10^5 *bacteria (——) and* 5 x 10^4 *bacteria (---). Each point represents the mean and ranges of five mice (linear scale).*
† *mice died during the experiment.*

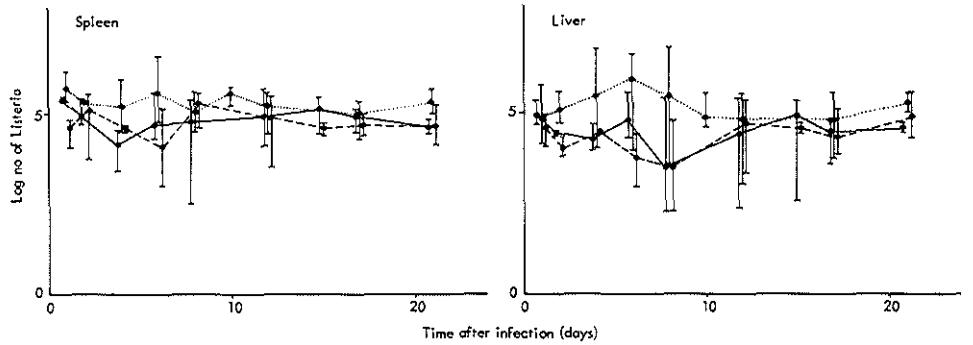


Fig. 3. *Growth curves of* Listeria monocytogenes *in spleens and livers of nude (nu/nu) mice after intravenous inoculation of* 5 x 10^5 *bacteria (...),* 10^5 *bacteria (——) and* 5 x 10^4 *bacteria (---). Each point represents the mean and ranges of five mice (linear scale).*

55 mice died on the fourth and the fifth day respectively. The remaining animals recovered, and by day 16 spleens and livers were sterile. The spleens were now enlarged and there were signs of splenic necrosis. After inoculation of 5×10^4 *Listeria* all normal mice recovered quickly. The blood was sterile all the time. After 10 days no bacteria could be cultured from the spleen and the liver. The course of listeric infection in nude mice did not depend on the size of the inocula investigated (Fig. 3). The nude mice inoculated with 5×10^5, 10^5 or 5×10^4 *Listeria monocytogenes* did not recover from the infection, but they did not die. From the start of the infection the spleens were enlarged, no lesions or signs of splenic necrosis were seen. Independantly of the number of injected bacteria, average numbers of 9.10^4 and 4.10^4 bacteria were cultured from spleens and livers respectively. Of the 45 nude mice infected with 5×10^5 *Listeria* only one mouse had bacteria in the blood on day six after inoculation: 819 bacteria per milliliter blood was counted. The blood of all nude mice infected with 10^5 or 5×10^4 *Listeria* was sterile.

The efficacy of ampicillin therapy was studied in normal mice and nude mice infected with 5×10^5 or 10^5 *Listeria*.

ampicillin therapy of normal (nu/+) mice and nude (nu/nu) mice after inoculation of 5×10^5 *L. monocytogenes*

Eight injections of 5 mg ampicillin per mouse every 12 h, starting 36 h after initiation of the infection, could sterilize spleen, liver and blood of all normal mice within 14 days after bacterial inoculation (Fig. 2). This ampicillin treatment was unable to cure nude mice infected with the same number of *Listeria* (Fig. 4). Spleens and livers of ampicillin-treated mice contained the same numbers of bacteria as untreated mice. In an attempt to cure the nude mice with listeric infection, the ampicillin treatment was intensified by markedly increasing the daily dose of ampicillin (10, 25, 50 mg versus 5 mg) and/or the frequency of injections (from 12 h to 8 h) and/or the number of injections (32 versus 8 injections). Figures 5 through 7 show the results of these experiments. None of these intensified

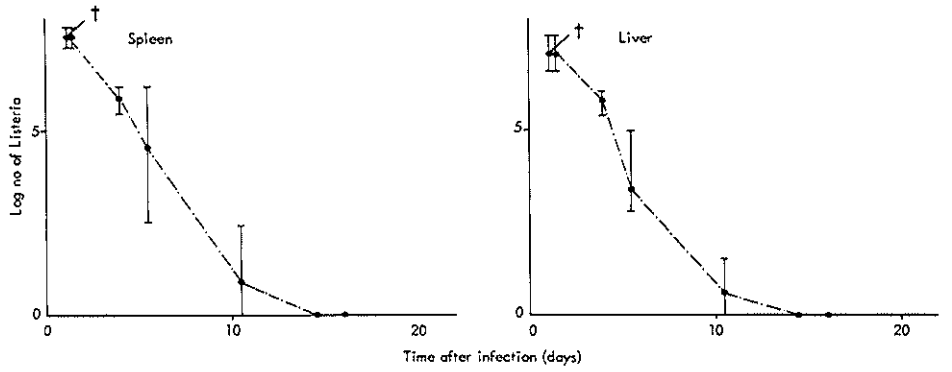


Fig. 2. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 5 x 10^5 *bacteria into normal (nu/+) mice which received ampicillin therapy 8 injections of* 5 *mg/mouse every* 12 *h starting at* 36 *h (-·-) and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).* † *mice died during the experiment.*

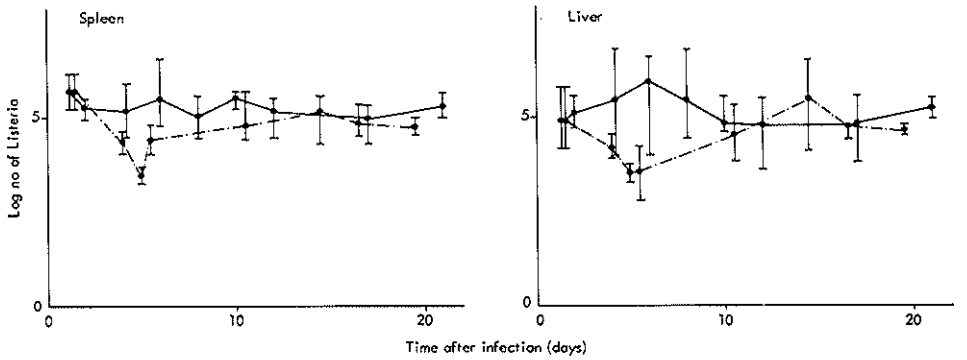


Fig. 4. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 5 x 10^5 *bacteria into nude (nu/nu) mice which received ampicillin therapy 8 injections of* 5 *mg/mouse every* 12 *h starting at* 36 *h (-·-) and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

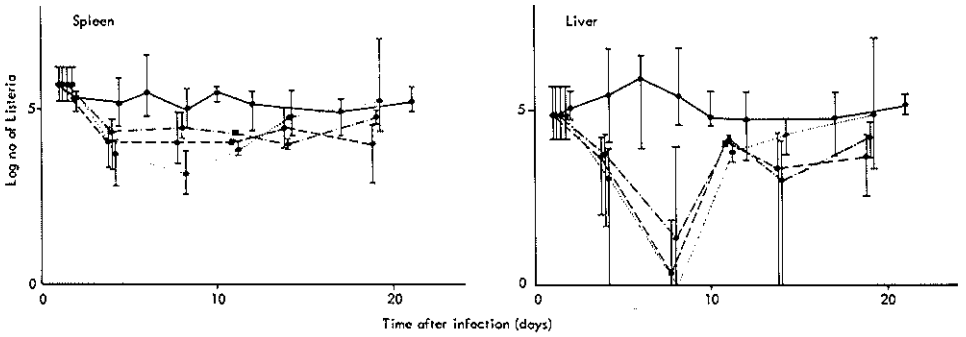


Fig. 5. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of 5 x 10^5 bacteria into nude (nu/nu) mice which received ampicillin therapy*
8 injections of 10 mg/mouse every 12 h starting at 36 h (-•-),
8 injections of 25 mg/mouse every 12 h starting at 36 h (---),
8 injections of 50 mg/mouse every 12 h starting at 36 h (...),
and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).

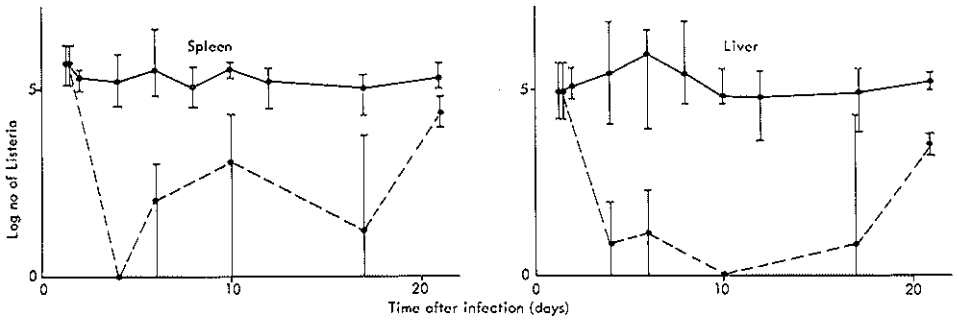


Fig. 6. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of 5 x 10^5 bacteria into nude (nu/nu) mice which received ampicillin therapy 12 injections of 25 mg/mouse every 8 h starting at 36 h (---) and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

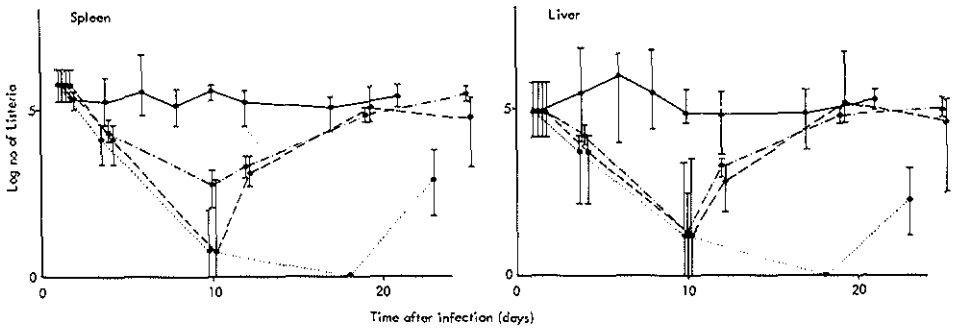


Fig. 7. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 5 x 10^5 *bacteria into nude (nu/nu) mice which received ampicillin therapy*
16 injections of 5 mg/mouse every 12 h starting at 36 h (-·-),
16 injections of 25 mg/mouse every 12 h starting at 36 h (---),
32 injections of 25 mg/mouse every 12 h starting at 36 h (...),
and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).

treatment schedules were successful. After ampicillin treatment with increased dosages (Fig. 5) the number of bacteria especially those cultured from the liver decreased initially, but increased again as soon as ampicillin therapy was terminated. Six mice out of 105 treated with 8 injections of respectively 10 mg, 25 mg and 50 mg ampicillin every 12 h died within 14 days, probably as a result of the high ampicillin dosages. Ampicillin treatment with an increased frequency and number of injections in combination with an increased daily dose (12 injections of 25 mg/mouse every 8 h), apparently sterilized spleens and livers of most animals (Fig. 6). At day ten the numbers of bacteria cultured from spleen and liver of ampicillin treated mice was significantly lower than in untreated controls (Wilcoxon rank-sum test, P= 0.008). However, recovery was not complete. Three mice out of 30 died within 20 days after inoculation. The numbers of bacteria cultured from spleen and liver at day 21 increased again to

pretreatment levels. After ampicillin treatment with an increased number of injections (16 versus 8 injections) in combination with an increased dose (25 versus 5 mg), the numbers of bacteria cultured from spleens and livers initially decreased, but returned to the pretreatment level ten days after the termination of the therapy (Fig. 7). Treatment with 32 injections of 25 mg ampicillin per mouse every 12 h seemed to be successful (Fig. 7). One day after the last ampicillin injection no bacteria could be cultured from spleens and livers of nude mice. However, although the numbers of bacteria cultured from spleen and liver were significantly lower compared to untreated controls (Wilcoxon rank-sum test, P= 0.008) five days after the last ampicillin injection, *Listeria* could again be found in spleens and livers of all mice. The bacteria isolated from these organs were found to retain their ampicillin sensitivity.

ampicillin therapy of normal (nu/+) mice and nude (nu/nu) mice after inoculation of 10^5 *L. monocytogenes*

Eight injections of 5 mg ampicillin per mouse every 12 h starting 36 h after bacterial inoculation, could sterilize spleen and liver of all normal mice within six days after initiation of the infection (Fig. 8). This ampicillin treatment was unable to cure nude mice infected with 10^5 *Listeria* (Fig. 9). After an initial decrease, the number of bacteria present in spleens and livers increased again to pretreatment levels. In an attempt to cure the nude mice with listeric infection the ampicillin treatment was intensified by markedly increasing the daily dose of ampicillin (25 versus 5 mg) and/or the frequency of injections (from 12 h to 8 h) and/or the number of injections (32 versus 8 injections). The results of these experiments are summarized in figures 10 and 11. Ampicillin treatment with an increased frequency and number of injections in combination with an increased daily dose, (12 injections of 25 mg/mouse every 8 h), apparently sterilized spleens and livers of most nude mice (Fig. 10). However, although the numbers of bacteria cultured from spleen and liver were significantly lower compared to untreated controls (Wilcoxon rank-sum test, P= 0.008) fifteen days after the termination of the

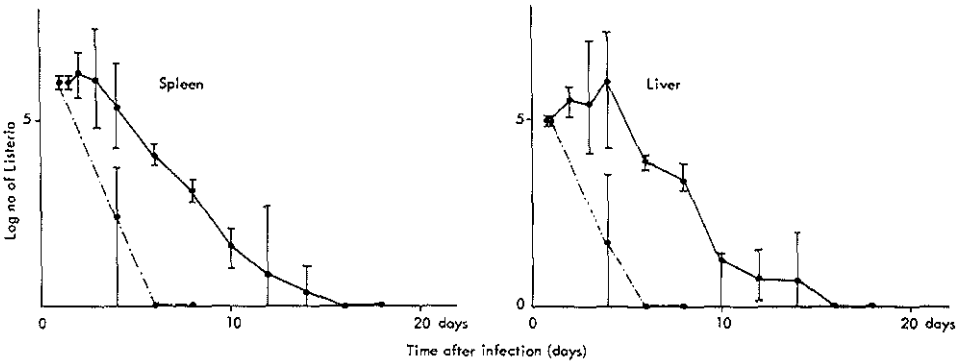


Fig. 8. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into normal (nu/+) mice which received ampicillin therapy 8 injections of 5 mg/mouse every 12 h starting at 36 h (-•-) and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

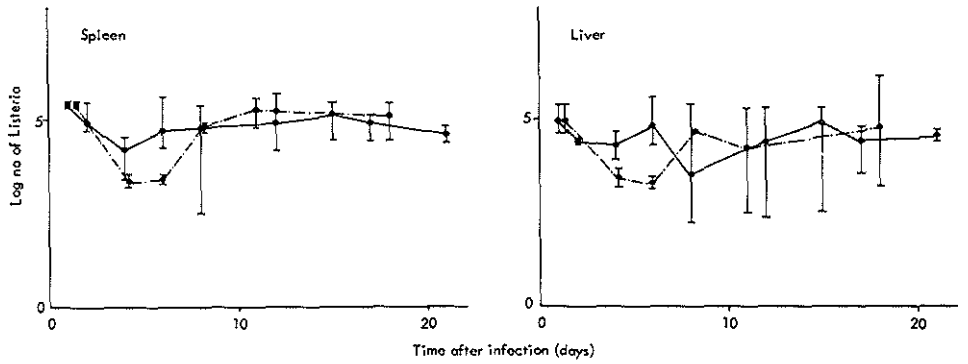


Fig. 9. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into nude (nu/nu) mice which received ampicillin therapy 8 injections of 5 mg/mouse every 12 h starting at 36 h (-•-), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

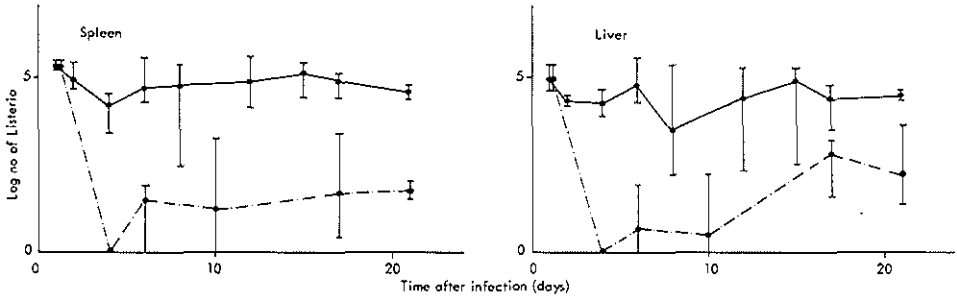


Fig. 10. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into nude (nu/nu) mice which received ampicillin therapy 12 injections of 25 mg/mouse every 8 h starting at 36 h (-·-), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

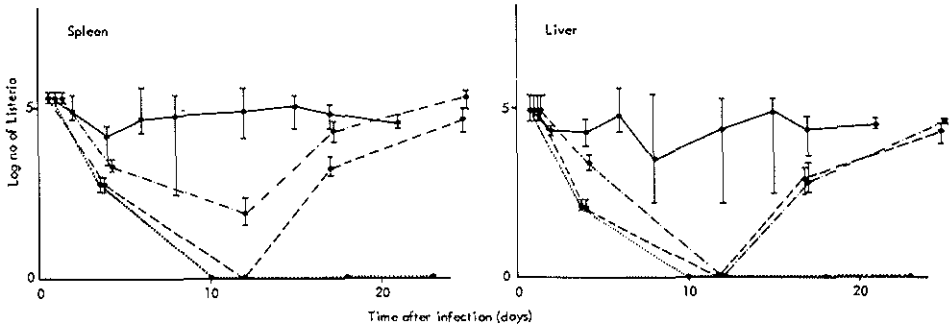


Fig. 11. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into nude (nu/nu) mice which received ampicillin therapy*
16 injections of 5 mg/mouse every 12 h starting at 36 h (-·-),
16 injections of 25 mg/mouse every 12 h starting at 36 h (---),
32 injections of 25 mg/mouse every 12 h starting at 36 h (...),
and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).

therapy, *Listeria* were still cultured from spleens and livers of all mice. The ampicillin sensitivity of the isolated bacteria was unchanged. One mouse out of 35 died within 19 days after inoculation. The excessive therapy of 32 injections of 25 mg ampicillin per mouse every 12 h was needed for complete recovery of nude mice (Fig. 11). With this therapy sterility of cultures of spleens and livers was maintained.
Figure 12 shows the ampicillin serum levels after subcutaneous injections of ampicillin doses of 5 mg/mouse and 25 mg/mouse, determined in normal mice and nude mice. There were no substantial differences between the curves of serum ampicillin levels in nude mice and their littermates. Subcutaneous injections of 5 mg and 25 mg ampicillin gave

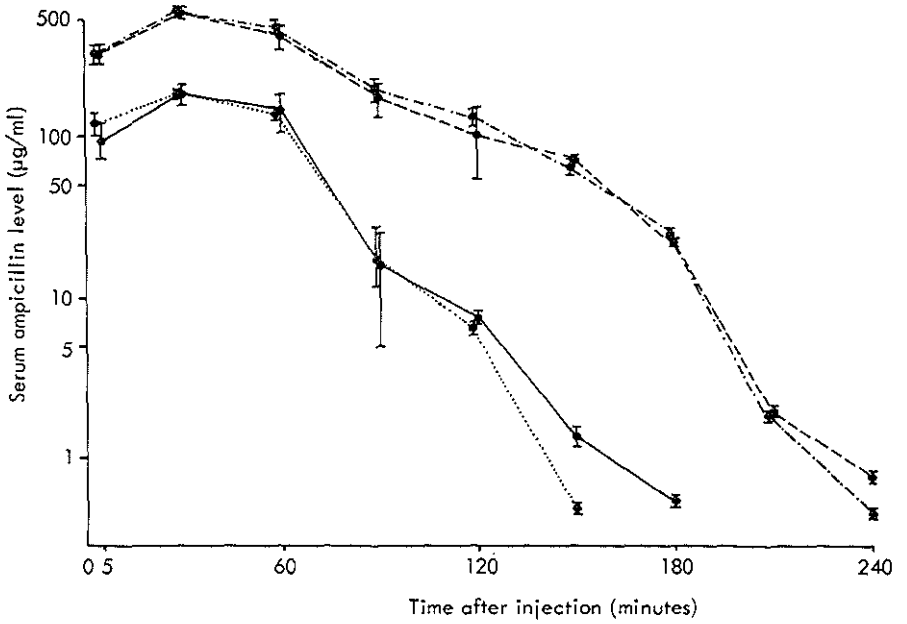


Fig. 12. *Serum concentrations of ampicillin after subcutaneous injections of 5 mg/mouse into normal (nu/+) mice (——) and nude (nu/nu) mice (...), and injections of 25 mg/mouse into normal (nu/+) mice (---) and nude (nu/nu) mice (—•—). Each point represents the mean of five mice (± standard deviation).*

rise to peak concentrations of 180 µg/ml and 550 µg/ml respectively; concentrations exceeding the MIC of *Listeria* for ampicillin i.e. 0.16 µg/ml, were present for about 3½ h and 5 h respectively. The serum concentrations of ampicillin after subcutaneous injections of 5 mg and 25 mg ampicillin during the course of listeric infection, were the same for uninfected mice and infected mice.

Discussion

Listeria monocytogenes is a facultative intracellular bacterial parasite that can survive and multiply in macrophages (20) particularly in the spleen and liver. Resistance to infection caused by *Listeria monocytogenes* has been studied extensively by Mackaness and coworkers. It is clear that host resistance to listeric infection correlates with the capacity to develop cellular immunity, induced specifically by a T-cell mediated reaction. These sensitized T-lymphocytes are able to activate macrophages. Activated macrophages possess a number of remarkable properties including a greatly enhanced capacity for phagocytosis and destruction of *Listeria monocytogenes* (3, 16, 20, 21, 34). Based upon these studies thymus-less animals are expected to have impaired host defense mechanisms against infection caused by *Listeria monocytogenes*. In our experiments we used mice homozygote for the mutation "nude" (nu/nu) which are described as deficient in thymus and thymus-derived (T) lymphocytes (15). Congenitally athymic nude (nu/nu) mice and their littermates, heterozygote for the mutation nude (nu/+) and immunologically normal, were challenged with different numbers of *Listeria monocytogenes* cells. The course of the infection was investigated by determining the number of *Listeria* in their spleens, livers and blood. The curves of bacterial multiplication in the spleen and liver and the outcome of infection (mortality or recovery) in nu/+ mice, differ with different numbers of inoculated bacteria. Normal mice developed a short-term listeriosis. Nude mice developed a chronic infection independent of the inoculum, with no signs of illness. Nude mice were able to restrain bacterial population - in fact their resistance was superior to that of normal mice - but were unable to

eradicate the infection. The course of listeric infection of nude mice and their littermates, as seen in our experiments, confirm the findings of other investigators (5, 7, 24, 30). Emmerling showed that in nude mice no significant changes in *Listeria* spleen counts occurred during 35 days after bacterial challenge. During the initial phase of infection nude mice were capable of resisting the number of *Listeria* which killed normal mice. This enhanced resistance to infection of nude mice in the time period before a specific immune response developed, was evidently nonspecific. Large numbers of macrophages with enhanced bactericidal properties are supposed to exist in these mice lacking functional T-cells before the introduction of *Listeria* organisms (7).

Ampicillin in large doses effectively cured normal mice from listeric infection. Studies by other investigators whose experiments differ in route of infection and bacterial inocula, also revealed that high doses of ampicillin are required to cure experimental listeric infections (8, 28, 31, 33). Ampicillin therapy that was adequate to cure normal mice, showed a complete loss of efficacy in the nude mice. Intensification of the ampicillin treatment in terms of an increase in dose (5-10 times) and/or frequency (8 h instead of 12 h) and/or number of injections (4 times) was not able to cure nude mice infected with 5×10^5 *Listeria*. When the inoculum was lowered to 10^5 *Listeria* it remained extremely difficult to cure nude mice. A treatment schedule of not less than 32 injections of 25 mg ampicillin per mouse every 12 h was required for recovery. Ampicillin serum levels exceeding 500 µg/ml were observed with this therapy.

We may conclude from these experiments that loss of efficacy of antibiotic therapy resulted from impaired host defense mechanisms against the infectious organisms. Obviously killing of these intracellular bacteria in normal mice is due to the combined effect of the curative action of the antibiotic and the activity of the cellular defenses of the host. Lack of cellular immunity can not be compensated by intensification of the ampicillin treatment schedule. Ampicillin treatment failure because of the development of antimicrobial

resistance is not likely, because of the fact that bacteria isolated from nude mice after extensive ampicillin treatment retained their ampicillin sensitivity. Therefore two possibilities may account for the survival of phagocytized *Listeria* when serum ampicillin levels are high. First, the antimicrobial drug does not penetrate phagocytic cells and thus does not reach the bacteria in their intracellular location. Secondly, the metabolic activity of the intraleukocytic bacteria in nude mice is low so that they are not susceptible to drug action. In vitro studies suggest that ampicillin penetrates poorly into cells (4, 18). Our experiments also suggest a poor penetration of phagocytes by ampicillin as high doses of ampicillin were needed for complete recovery of normal mice. However, it must be realized that inhibitory ampicillin serum levels were present only during part of the dosage interval due to the short serum half-life of ampicillin in mice. Nevertheless, an intensified treatment (increased frequency of injections in combination with an increased ampicillin dose and number of injections) was only partly successful in nude mice. That ampicillin treatment was successful in normal mice might suggest that loss of ampicillin activity in nude mice is not only due to lack of penetration of the phagocyte by the antibiotic. Some evidence to support the hypothesis of low metabolic activity of the intraleukocytic bacteria might be inferred from the *Listeria* counts in spleen and liver from nude mice, these counts suggest no bacterial multiplication in spleen and liver. It must be realised, however, that from total viable counts of spleen and liver, it can not be concluded whether these curves reflect a chronic infection or are a composite of extracellular multiplication and intracellular killing of the organisms.

With the object to investigate the reason for the failure of ampicillin therapy to cure listeric infections in the compromised host, these studies will be continued. Other antibiotics will be applied in this experimental infection model, especially antibiotics which differ from ampicillin in cell-permeability and serum half-life.

Acknowledgements

We are grateful to A.L. Beukelman for secretarial help, and to B.K.B. Reimann-Knuth Winterfeldt for critical review of the manuscript. This study was supported in part by research grants from Beecham Farma B.V., The Netherlands.

Literature cited

1. Ascher, N.L., R.L. Simmons, S. Marker, and J.S. Najarian. 1978. Listeria infection in transplant patients. Five cases and a review of the literature. Arch. Surg. 113: 90-94.
2. Bennet, J.V., J.L. Brodie, E.J. Benner, and W.M.N. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. 14: 170-177.
3. Blanden, R.V. and R.E. Langman. 1972. Cell-mediated immunity to bacterial infection in the mouse. Thymus-derived cells as effectors of acquired resistance to Listeria monocytogenes. Scand. J. Immunol. 1: 379-391.
4. Brown, K.N., and A. Percival. 1978. Penetration of antimicrobials into tissue culture cells and leukocytes. Scand. J. Infect. Dis., Suppl. 14: 251-260.
5. Cheers, C., and R. Waller. 1975. Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice. J. Immunol. 115: 844-847.
6. Cline, M.J. 1973. Defective monunuclear phagocyte function in patients with myelomonocytic leukemia and in some patients with lymphoma. J. Clin. Invest. 52: 2185-2190.
7. Emmerling, P., H. Finger, and H. Hof. 1977. Cell-mediated resistance to infection with Listeria monocytogenes in nude mice. Infect. Immun. 15: 382-385.
8. Emmerling, P., H. Hof, and H. Finger. 1978. Resistance to infection with Listeria monocytogenes in normal and thymusless mice treated with ampicillin. Zbl. Bakt. Hyg., I. Abt. Orig. A. 240: 339-346.
9. Espaze, E.P., Y.G. Roubeix, J.Y. le Berre, and A.L. Courtieu. 1978. In vitro susceptibility of Listeria monocytogenes to some antibiotics and their combinations. Zbl. Bakt. Hyg., I. Abt. Orig. A. 240: 76-85.

10. Gantz, N.M., R.L. Myerowitz, A.A. Medeiros, G.F. Carrera, R.E. Wilson, and T.F. O'Brien. 1975. Listeriosis in immunosuppressed patients. A cluster of eight cases. Am. J. Med. 58: 637-643.
11. Han, T., J. Minowada. 1978. Impairment of cell-mediated immunity in untreated Hodgkin's disease. N.Y. State J. Med. 78: 216-221.
12. Hudgins, L.B., and S.R. Acchiardo. 1978. Diagnosis and treatment of Listeria monocytogenes infection in renal transplant patients. Dialysis Transplant. 7: 1023-1027.
13. Isiadinso, O.A. 1975. Listeria sepsis and meningitis. A complication of renal transplantation. J. Am. Med. Assoc. 234: 842-843.
14. Johnson, M.L., and E.W. Colley. 1969. Listeria monocytogenes encephalitis associated with corticosteroid therapy. J. Clin. Pathol. 22: 465-469.
15. Kindred, B. 1979. Nude mice in immunology. Prog. Allergy 26: 137-238.
16. Lane, F.C., and E.R. Unanue. 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. 135: 1104-1112.
17 Lavetter, A., J.M. Leedom, A.W. Mathies, D. Ivler, and P.F. Wehrle. 1971. Meningitis due to Listeria monocytogenes.A review of 25 cases. N. Engl. J. Med. 285: 598-603.
18. Lobo, M., and G.L. Mandell. 1973. The effect of antibiotics on Escherichia coli ingested by macrophages. Proc. Soc. Exp. Biol. Med. 142: 1048-1050.
19. Louria, D.B., T. Hensle, D. Armstrong, H.S. Collins, A. Blevins, D. Krugman, and M. Buse. 1967. Listeriosis complicating malignant disease. A new association. Arch. Intern. Med. 67: 261-281.
20. Mackaness, G.B. 1962. Cellular resistance to infection. J. Exp. Med. 116: 381-406.
21. Mackaness. G.B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. 129: 973-992.
22. Medoff, G., L.J. Kunz, A.N. Weinberg. 1971. Listeriosis in humans: an evaluation. J. Infect. Dis. 123: 247-250.

23. Moellering, R.C., G. Medoff, I. Leech, C. Wennersten, and L.J. Kunz. 1972. Antibiotic synergism against Listeria monocytogenes. Antimicrob. Agents Chemother. 1: 30-34.
24. Nickol, A.D., and P.F. Bonventre. 1977. Anomalous high native resistance of athymic mice to bacterial pathogens. Infect. Immun. 18: 636-645.
25. Nieman, R.E., and B. Lorber. 1980. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968-1978. Rev. Infect. Dis. 2: 207-227.
26. Nirmul, G., S. Glabman, M. Haimov, E. Leiter, and L. Burrows. 1971. Listeria monocytogenes meningitis during immunosuppression. N. Engl. J. Med. 285: 1323.
27. Schröter, G.P.J., and R. Weil. 1977. Listeria monocytogenes infection after renal transplantation. Arch. Intern. Med. 137: 1395-1399.
28. Seeliger, H.P.R., U. Laymann, and H. Finger. 1967. Untersuchungen zur Frage der Wirksamkeit von Ampicillin auf Listeria monocytogenes. Dtsch. Med. Wochenschr. 92: 1095-1097.
29. Simpson, J.F. 1971. Listeria monocytogenes meningitis: an opportunistic infection. J. Neurol. Neurosurg. Psychiatry 34: 657-663.
30. Takeya, K., S. Shimotori, T. Taniguchi, and K. Nomoto. 1977. Cellular mechanisms in the protection against infection by Listeria monocytogenes in mice. J. Gen. Microbiol. 100: 373-379.
31. Vischer, W.A., and C. Rominger. 1978. Rifampicin against experimental listeriosis in the mouse. Chemotherapy 24: 104-111.
32. Watson, G.W., T.J. Fuller, J. Elms, and R.M. Kluge. 1978. Listeria cerebritis. Relapse of infection in renal transplant patients. Arch. Intern. Med. 138: 83-87.
33. Weingärtner, L., and S. Ortel. 1967. Zur Behandlung der Listeriose mit Ampicillin. Dtsch. Med. Wochenschr. 92: 1098-1104.
34. Zinkernagel, R.M., R.V. Blanden, and R.E. Langman. 1974. Early appearance of sensitized lymphocytes in mice infected with Listeria monocytogenes. J. Immunol. 112: 496-501.

Chapter 6

GENERAL DISCUSSION

The relation between host defense mechanisms and the efficacy of antibiotic therapy of bacterial infections has been studied in this thesis. Because the critical role of individual host defense factors in the process of recovery during antimicrobial therapy can not be established easily in clinical situations, we resorted to an experimental approach. Animal models of experimental infections with *S. pneumoniae* and *L. monocytogenes* in rats and mice were used. The host-bacteria relationship with these two microorganisms is relatively well-understood but completely different. This enabled us to evaluate the change in efficacy of antimicrobial therapy as a function of selective elimination of several forms of host defense. The results were compared with the response to antimicrobial therapy of infections in normal animals.

Phagocytic cells such as circulating polymorphonuclear leukocytes (PMN) or fixed macrophages of the spleen, are primarily important for host defense against bacterial infection. Phagocytosis of *Streptococcus pneumoniae* involves interaction between bacteria and PMN's, where components of the complement system promote attachment of bacteria to phagocytic cell membranes before opsonizing antibodies develop (10, 11). The spleen also participates in clearing *Streptococcus pneumoniae* from the blood stream. Host resistance to *Listeria monocytogenes*, however, depends on a T-cell mediated reaction resulting in activation of macrophages, the target cells of *Listeria* (6, 12). Defects in phagocytic activity as a consequence of low serum opsonic levels correlate with the occurrence of infections by bacteria with polysaccharide mucoid capsules, such as *Streptococcus pneumoniae*. In that case pneumococcal clearance from the blood stream is dependent on optimal splenic function (2). Defects in T-cell mediated functions are associated with an increased incidence of infections due to bacteria such as

Listeria monocytogenes, which are located intracellularly in macrophages (8).

Chapters 1 through 3 show the importance of intact host defense mechanisms for successful antimicrobial therapy of pneumococcal pneumonia. This was studied in normal rats and in rats treated with cobra venom factor. This factor was used to suppress the phagocytosis by PMN of *Streptococcus pneumoniae* as a result of complement depletion. From the studies described in Chapter 1 it appeared that, compared to normal rats, the spreading of the pneumococcal infection was more rapid and extensive in rats with inadequate phagocytosis of the pneumococci. In man abnormalities in the alternate complement pathway, are also known to be associated with increased susceptibility to pneumococcal infections (9). Pneumococcal pneumonia in rats with intact host defense mechanisms could be successfully cured by low doses of penicillin or clindamycin (Chapters 1 and 3). The efficacy of both antibiotics - in terms of minimal dosage and treatment schedule required for complete recovery - was equal. The antimicrobial treatment schedule with penicillin or clindamycin which cured normal rats, was not successful in eradicating the pneumococcal infection in rats with impaired phagocytosis (Chapters 1 and 3). Therefore we investigated to what extent the therapeutic effect of the antibiotic therapy in these rats could be restored by alterations in the treatment schedule. Observations related to the dose and administration schedule of penicillin and clindamycin are reported in respectively Chapter 2 and Chapter 3. The increase in dosage required for the successful treatment of pneumococcal infection in rats with impaired phagocytosis was considerably greater for penicillin (25-fold, or 17-fold in combination with a shortened interval between injections) than for clindamycin (4-fold). This observation could not be explained by a difference in in vitro sensitivity of the pneumococcal strain for both antibiotics, nor by inactivation of penicillin by inflammatory exudate at the site of infection (Chapter 3). The results might, however, be explained on the basis of a distinct pharmacological behavior of both antibiotics as expressed by substantial

differences in peak values and serum half-life values of the drugs. It was therefore concluded that in normal rats, antimicrobial treatment schedules with periodically subinhibitory serum levels were apparently sufficient to eradicate pneumococcal infection in the lung. On the other hand in rats with extensive pulmonary lesions due to impaired phagocytosis of pneumococci, it was absolutely essential to maintain prolonged effective serum levels during the whole treatment period. Presumably such a treatment schedule is required to achieve effective antibacterial levels at the site of infection.

Another form of impaired host defense to pneumococcal infection i.e. removal of the spleen, was studied in chapter 4. As is known from clinical studies, the risk of postsplenectomy pneumococcal sepsis is increased in children under 3 years of age, in patients with hematologic disorders and in patients who received chemotherapy for malignancies such as Hodgkin's disease (2, 4). In these patients the underlying disease itself is associated with increased susceptibility to infection. This in turn predisposes the patient to overwhelming and frequently fatal infection with pneumococci or other pathogens when the spleen is removed. Increased susceptibility to pneumococcal infection appears to be correlated with a reduced ability to remove pneumococci circulating in the blood. This may be the consequence of low serum opsonic levels, as found in young individuals and in adults with complement deficiences or an impaired antibody response. In the animal studies of Chapter 4, experimental conditions similar to clinical situations were chosen. The age at the time of splenectomy and the opsonic capacity of the serum were introduced as variables. Increased susceptibility to intravenous pneumococcal infection after splenectomy was demonstrated in rats at young age and in adult rats with deficient serum opsonic capacity. Splenic phagocytosis of pneumococci appeared to be critically important in the face of a quantitative deficiency in opsonins. In these specific groups of rats penicillin therapy which could cure pneumococcal infection in non-splenectomized rats, had lost its effectiveness. The effect of

the penicillin therapy could be restored by an increase in daily penicillin dose of four times in splenectomized young rats, of 20 times in adult rats with low serum opsonic levels, and of 100 times in splenectomized adult rats with low serum opsonic levels. The observations suggested the existence of a relationship between a need for an increased penicillin dose and an increased bacteria count in the blood.

In Chapter 5 we examined the importance of intact host defense mechanisms for successful antimicrobial therapy of infections caused by *Listeria monocytogenes*. Cell-mediated immune responses play a major role in host defense against these facultative intracellular bacteria, induced specifically by a T-cell mediated reaction. These sensitized thymus-dependent lymphocytes activate macrophages harboring the *Listeria* resulting in destruction of the bacteria. Thymus-less (nude) mice have impaired host defense mechanisms against infections caused by *Listeria monocytogenes*. The effect of impaired cell-mediated immunity upon the course of the infection and the efficacy of ampicillin therapy, was therefore studied in thymus-less nude mice and their littermates. Experimental infections were produced with different numbers of *Listeria*. Normal mice developed short-term listeriosis the course of which depended upon numbers of inoculated bacteria. Nude mice developed a chronic infection independent of the inoculum. Although the animals demonstrated no signs of illness, they were not able to eradicate the *Listeria* infection. This corresponds with the clinical observation that infections due to opportunistic pathogens such as *Listeria monocytogenes* result from depression of cellular immune responses, and therefore may complicate lymphatic malignancy such as Hodgkin's disease, especially during intensive chemotherapy (8). Experiments described in Chapter 5 revealed that ampicillin in large doses effectively cured normal mice from listeric infection. The same ampicillin treatment was completely ineffective in mice with impaired host defense mechanisms. Intensification of ampicillin treatment schedules could not compensate for lack of cellular immunity. Antibiotic

treatment failure because of the development of antimicrobial resistance is not a likely explanation, because of the fact that bacteria isolated from mice after extensive ampicillin treatment retained their ampicillin sensitivity. Therefore two possibilities may account for the survival of phagocytized *Listeria* in animals with impaired host defense. First, ampicillin does not penetrate phagocytic cells and thus does not reach the bacteria in their intracellular location. Secondly, the metabolic activity of the intracellular bacteria is at such a low level that they are not susceptible to drug action. To see whether loss of ampicillin activity in nude mice is due to lack of penetration the effect of rifampicin to cure listeric infection in normal mice and nude mice was investigated in recent experiments (reported in the appendix). It is known that rifampicin is able to penetrate phagocytic cells (3, 5, 7) and has a longer serum half-life in mice than does ampicillin (serum half-life values of rifampicin and ampicillin in mice are 6 h and ½ h respectively). Rifampicin in low dosage cured listeric infection in normal mice. If the treatment schedule was intensified in terms if a 40-fold increased dosage in combination with an increased number of injections (4 to 16 injections) this drug was also able to eradicate *Listeria* from nude mice. Thus the loss of cell-mediated immune functions could be compensated only by a treatment schedule with long-term effective serum levels of an antimicrobial drug which is capable of penetrating into host cells. These results favor the second hypothesis of low metabolic activity of the intraleukocytic bacteria in the nude mice. Bacteria located within cells whose intracellular killing mechanisms fail to function, probably persist in a stationary phase.

From our experimental studies in the animal host outlined in Chapters 1 through 5, some basic conclusions on the relation between impaired host defense mechanisms and the efficacy of antimicrobial therapy can be drawn:

Loss of activity of antimicrobial therapy results from selectively impaired host defense against the infectious organisms. This seems to

be true irrespective of whether the microorganism is a classice pathogen such a *Streptococcus pneumoniae* which produces disease by growing outside the polymorphonuclear leukocytes, or a microorganism such as *Listeria monocytogenes* that belongs to the endogenous flora, multiplies within macrophages and causes serious infection in hosts lacking normal defense mechanisms. Obviously the total effect of antimicrobial therapy is the combined effect of the curative action of the antimicrobial drug and the activity of the cellular defenses of the host.

Whether lack of host defense functions can be compensated by intensification of the antibiotic treatment schedule, i.e. by manipulating the dose and/or frequency of administration, seems to depend on the infectious agent (obligatory extracellular parasite or facultative intracellular parasite) and on the experimental infection (acute inflammatory response or chronic infection). In the case of pneumococcal infections, lack of PMN phagocytic activity either with or without lack of splenic functions can be compensated by considerable increase in antibiotic dosage. In pneumococcal pneumonia with extensive pulmonary lesions due to impaired phagocytosis, antimicrobial treatment schedules that give rise to prolonged inhibitory antibiotic serum levels in the interval between two successive injections, are of prime importance. In the case of listeric infections, lack of cell-mediated immunity can be compensated only by a treatment schedule with long-term inhibitory serum levels of an antimicrobial drug which is capable of penetrating into host cells. Low metabolic activity of the intraleukocytic bacteria in the immunodeficient host may possibly account for failure of conventional antimicrobial therapy.

In these experimental studies in animals with compromised defense systems, the critical role of host defenses in antimicrobial treatment has been demonstrated. Evidence is presented that selectively impaired host defense mechanisms may result in a subtantial loss of efficacy of antimicrobial therapy. Successful treatment of bacterial infection in the compromised host must not only be based upon characterization of the infecting organism in terms of species and in vitro sensitivity to antibiotics, but also the judicious use of

antibacterial agents is essential. In this respect it is of prime importance to maintain effective antibiotic concentrations of the drug at the site of infection.

These studies support clinical data suggesting that the schedule of drug administration may play an important role in the outcome of antibiotic therapy in patients with impaired host defense mechanisms (1).

Literature cited

1. Bodey, G.P., M. Valdivieso, and B.S. Yap. 1980. The role of schedule in antibiotic therapy of the neutropenic patient. Infection 8 Suppl. 1: S75-S81.
2. Dickerman, J.D. 1976. Bacterial infection in the asplenic host: a review. J. Trauma 16: 662-668.
3. Ezer, G., and J.F. Soothill. 1974. Intracellular bactericidal effects of rifampicin in both normal and chronic granulomatous disease polymorphs. Arch. Dis. Child. 49: 463-466.
4. Krivit, W. 1977. Overwhelming postsplenectomy infection. Am. J. Hematol. 2: 193-201.
5. Lobo, M.C., and G.L. Mandell. 1973. The effect of antibiotics on Escherichia coli ingested by macrophages. Proc. Soc. Exp. Biol. Med. 142: 1048-1050.
6. Mackaness, G.B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. 129: 973-992.
7. Mandell, G.L., and T.K. Vest. 1972. Killing of intraleukocytic Staphylococcus aureus by Rifampicin: in-vitro and in-vivo studies. J. Infect. Dis. 125: 486-490.
8. Nieman, R.E., and B. Lorber. 1980. Listeriosis in adults: a changing pattern. Report of eight cases and a review of the literature, 1968-1978. Rev. Infect. Dis. 2: 207-227.
9. Reed, W.P., M.S. Davidson, and R.C. Williams, Jr. 1976. Complement system in pneumococcal infections, Infect. Immun. 13: 1120-1125.

10. Shin, H.S., M.R. Smith, J. Winkelstein, and A. Nicholson. 1977. Heat labile opsonin system to pneumococcus, p. 563-566. *In* D. Bergsma et al. (ed.), Immunodeficiency in man and animals. Birth Defects: Original Article Series, the National Foundation, vol. 11, no. 1. Robert E. Krieger Publishing Co., Inc., New York.
11. Winkelstein, J.A., and A. Tomasz. 1977. Activation of the alternative pathway by pneumococcal cell walls. J. Immunol. 118: 451-454.
12. Zinkernagel, R.M., R.V. Blanden, and R.E. Langman. 1974. Early appearance of sensitized lymphocytes in mice infected with Listeria monocytogenes. J. Immunol. 112: 496-501.

APPENDIX

Rifampicin therapy of normal (nu/+) mice and nude (nu/nu) mice after inoculation of 5 x 10^5 *Listeria monocytogenes*

Four injections of 0.024 mg rifampicin per mouse every 24 h, starting 36 h after initiation of the infection, could sterilize spleen, liver and blood of all normal mice within 14 days after bacterial inoculation (Fig. 1). This rifampicin treatment was unable to cure nude mice infected with the same number of *Listeria* (Fig. 2). To cure the nude mice the rifampicin treatment was intensified by two-fold increases of the daily dose of rifampicin (0.024 mg to 1.92 mg/mouse) or by increasing the daily dose in combination with an increased number of injections (4 to 16 injections). By increasing the number of injections (to 16) as well as the daily dose (to 0.96 mg) rifampicin could cure the nude mice (Fig. 3).

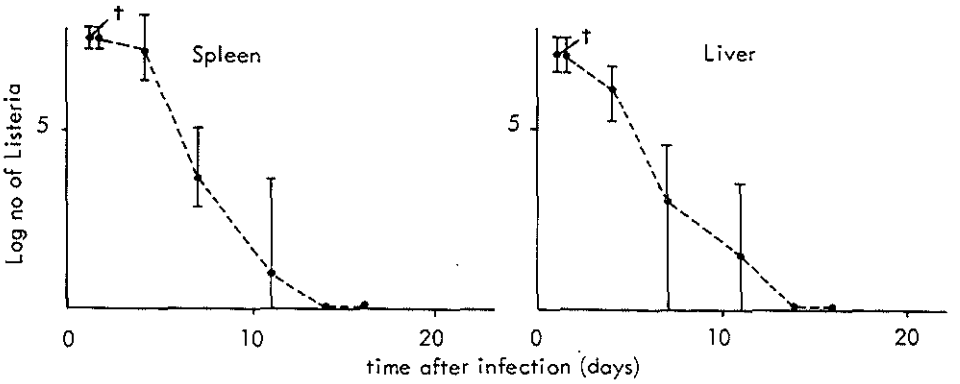


Fig. 1. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of 5 x 10^5 bacteria into normal (nu/+) mice which received 4 injections of 0.024 mg rifampicin per mouse every 24 h starting at 36 h (---), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

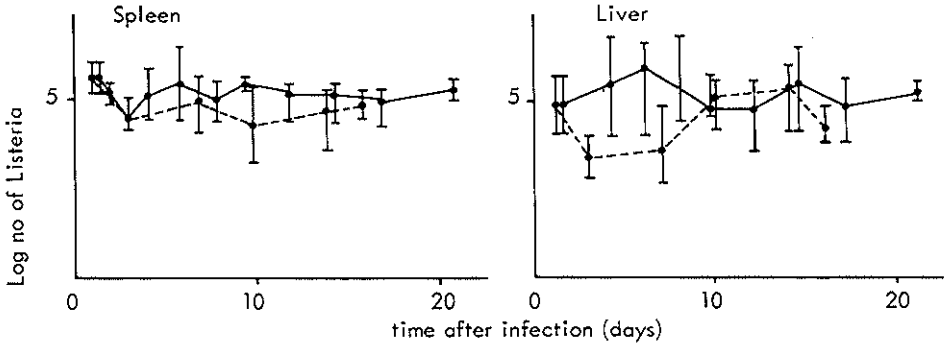


Fig. 2. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of 5 x 10^5 bacteria into nude (nu/nu) mice which received 4 injections of 0.024 mg rifampicin per mouse every 24 h starting at 36 h (---), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

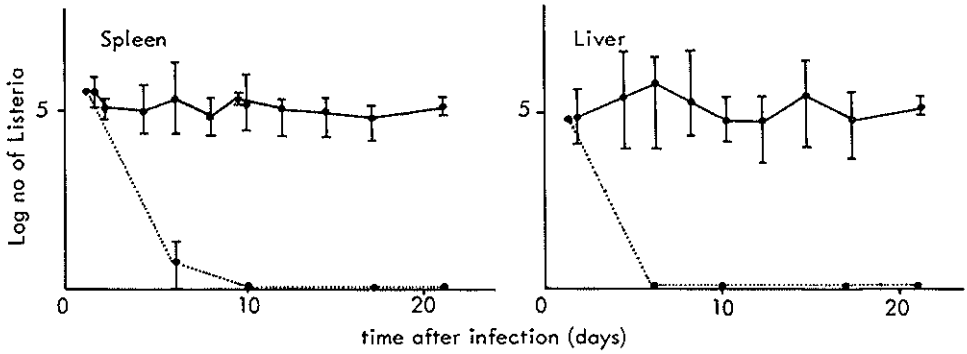


Fig. 3. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of 5 x 10^5 bacteria into nude (nu/nu) mice which received 16 injections of 0.96 mg rifampicin per mouse every 24 h starting at 36 h (...), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

Rifampicin therapy of normal (nu/+) mice and nude (nu/nu) mice after inoculation of 10^5 *Listeria monocytogenes*

Four injections of 0.012 mg rifampicin per mouse every 24 h, starting 36 h after initiation of the infection, could sterilize spleen, liver and blood of all normal mice within six days after bacterial inoculation (Fig. 4). This rifampicin treatment was unable to cure nude mice infected with the same number of *Listeria* (Fig. 5). To cure the nude mice the rifampicin treatment was intensified by two-fold increases of the daily dose of rifampicin (0.012 to 1.92 mg/mouse) or by increasing the daily dose in combination with an increased number of injections (4 to 16 injections). By increasing the number of injections (to 16) as well as the daily dose (to 0.96 mg) rifampicin could cure the nude mice (Fig. 6).

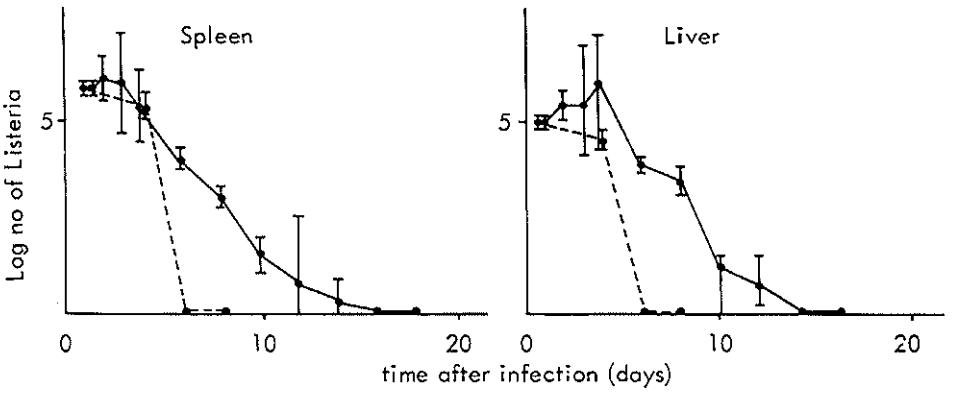


Fig. 4. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into normal (nu/+) mice which received 4 injections of 0.012 mg rifampicin per mouse every 24 h starting at 36 h (---), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

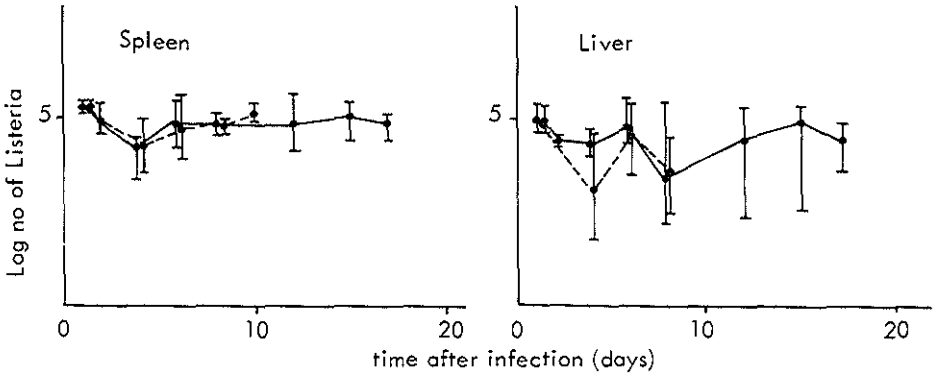


Fig. 5. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into nude (nu/nu) mice which received 4 injections of 0.012 mg rifampicin per mouse every 24 h starting at 36 h (----), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

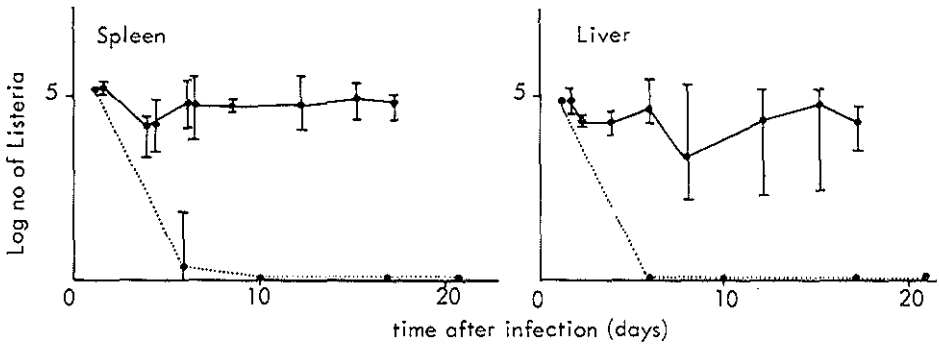


Fig. 6. *Growth curves of* Listeria monocytogenes *in spleens and livers after intravenous inoculation of* 10^5 *bacteria into nude (nu/nu) mice which received 16 injections of 0.96 mg rifampicin per mouse every 24 h starting at 36 h (·····), and untreated controls (——). Each point represents the mean and ranges of five mice (linear scale).*

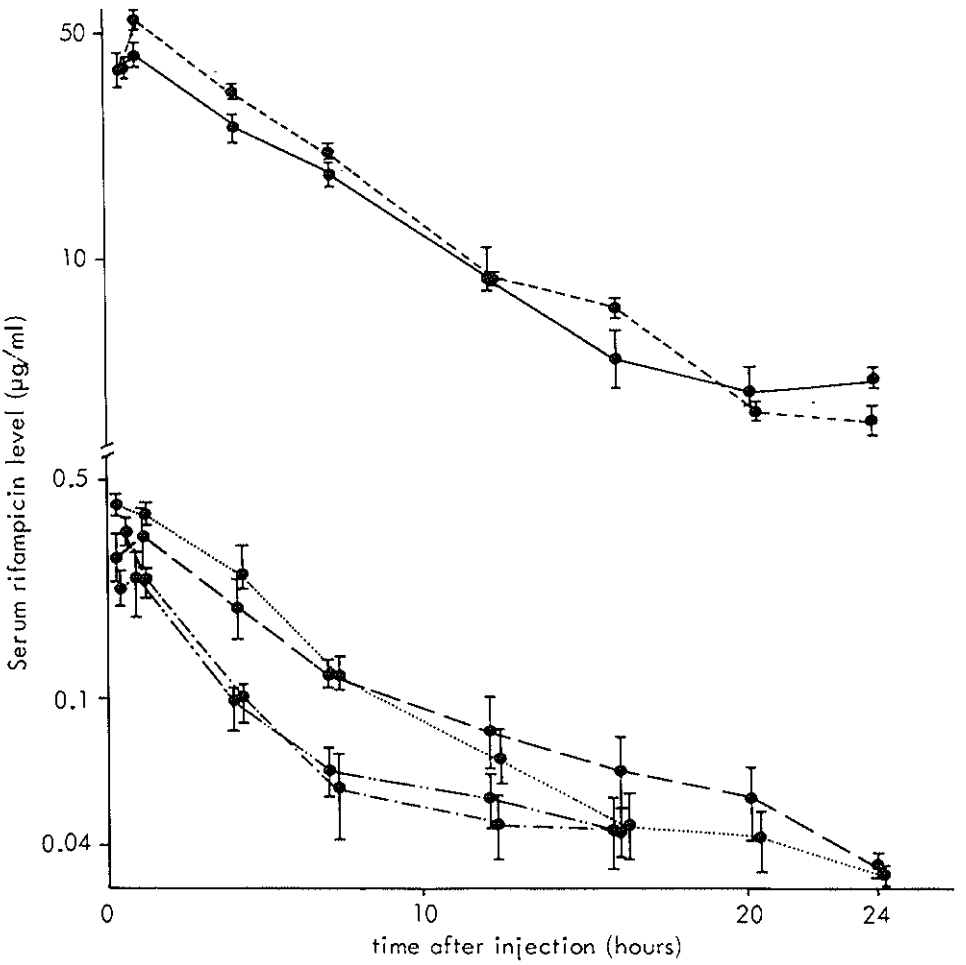
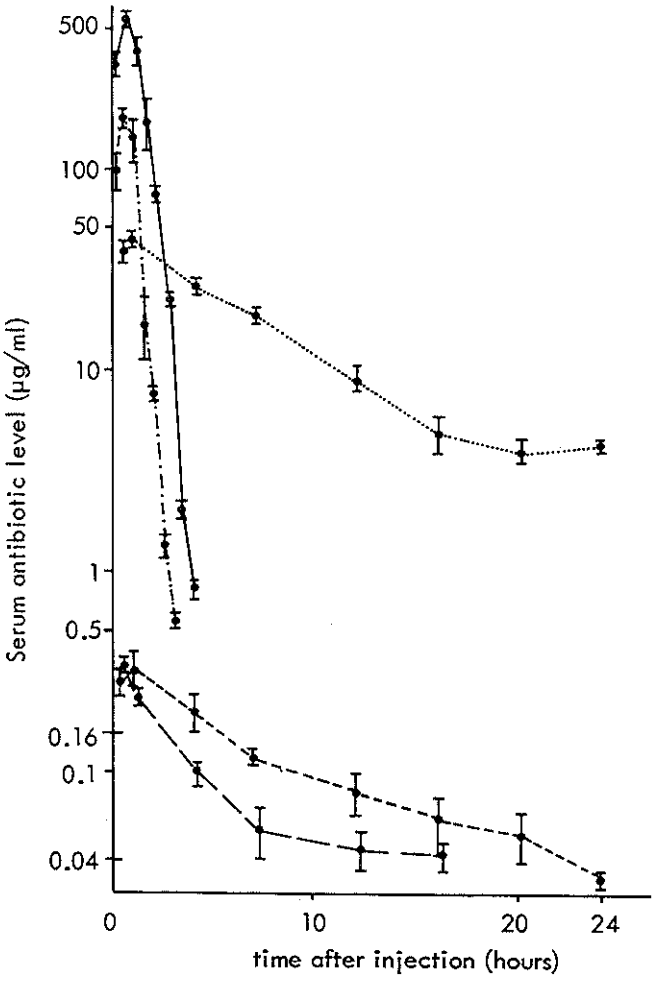


Fig. 7. *Serum concentrations of rifampicin after subcutaneous injections of 0.024 mg/mouse into normal (nu/+) mice (— —) and nude (nu/nu) mice (·····), 0.012 mg/mouse into normal mice (—·—) and nude mice (—··—) and 0.96 mg/mouse into normal mice (——) and nude mice (- - - -). Each point represents the mean of five mice (± standard deviation) (linear scale).*

Fig. 8. *Serum concentrations of rifampicin and ampicillin. Normal (nu/+) mice were injected subcutaneously with rifampicin doses of 0.024 mg/mouse (---), 0.012 mg/mouse (— —) and 0.96 mg/mouse (·····) or with ampicillin doses of 5 mg/mouse (—·—) and 25 mg/mouse (——). Each point represents the mean of five mice (± standard deviation). These data show that subcutaneous injections of rifampicin and ampicillin gave rise to completely different serum levels in the mice with regard to duration of adequate levels. Serum antibiotic levels above the minimal inhibitory concentration (MIC) for* Listeria monocytogenes *were maintained for a much longer period with rifampicin than with ampicillin. Actually, during the interval between two injections of 0.024 mg rifampicin per mouse, serum levels were 23-24 h above or equal to the MIC of* L. monocytogenes *to rifampicin (0.04 µg/ml); serum concentrations exceeding the MIC for* L. monocytogenes *to ampicillin (0.16 µg/ml) were present for about 3½ and 5 h if treatment with ampicillin doses of respectively 5 mg and 25 mg per mouse was applied.*

Fig. 8.


500
100
50
10
1
0.5
0.16
0.1
0.04
Serum antibiotic level (µg/ml)
0
10
20
24
time after injection (hours)

SUMMARY

This thesis deals with the evaluation of the change in efficacy of antimicrobial agents in the treatment of experimental infection in rats and mice with selectively impaired host defense mechanisms.
As outlined in the General Introduction patients with impaired host defense mechanisms due to malignant disease or its treatment (radiotherapy, chemotherapy) have a high degree of infectious complications. These may be difficult to cure as antimicrobial therapy that is usually effective in the normal host, may be inadequate in patients with seriously compromised host defenses. Therapeutic failures occur despite the organisms usual sensitivity. In these patients the initial loss of host resistance is related to the underlying disease or brought about by the antineoplastic therapy, resulting in a marked reduction in number or function of polymorphonuclear leukocytes (PMN), decreased antibody formation or suppressed cellular immune reactions. Two questions arise. To what extent does the susceptibility to infections and the efficacy of antimicrobial therapy depend on host defense mechanisms? And secondly, can lack of host defense functions be partially compensated by intensification of the antibiotic treatment schedule. As it is difficult to delineate the change in efficacy of antimicrobial therapy as a function of specific defects in host defense in clinical studies, these problems were investigated by an experimental approach. The animal studies described in chapters 1 through 5 provide some relevant information with regard to these questions. Models of experimental infections with *S. pneumoniae* and *L. monocytogenes* in rats and mice were used. Because the host-bacteria relationship with these two microorganisms is relatively well-understood but completely different, we were able to study the effect of elimination of several forms of host defense upon the activity of antimicrobial therapy.
Chapters 1 through 3 deal with the critical role of phagocytosis by PMN's in relation to antimicrobial therapy of pneumococcal pneumonia

in rats. From these studies it can be concluded that impaired phagocytosis of the pneumococci by PMN's results in loss of efficacy of antimicrobial treatment by penicillin or clindamycin. Loss of PMN phagocytic activity can be compensated by an intensified antibiotic treatment schedule in terms of a considerable increase in dosage and an increased injection frequency. For treatment of pneumococcal pneumonia in rats with extensive pulmonary lesions due to impaired phagocytosis of pneumococci, the choice of an antimicrobial agent is not only determined by the in vitro sensitivity of the infecting organism for the antimicrobial drug, also the pharmacological properties of the drug appear to be critical. Treatment schedules that give rise to prolonged inhibitory antibiotic serum levels in the interval between two successive injections are of prime importance.

Chapter 4 reports on the effects of splenectomy upon the course of pneumococcal bacteremia in rats, and the efficacy of penicillin therapy. From these experiments it can be concluded that increased susceptibility to intravenous pneumococcal infection and loss of efficacy of penicillin treatment results from splenectomy in young rats and in adult rats with minimal serum opsonic levels. In these specific groups of rats, the capacity to remove circulating pneumococci from the blood is reduced. Penicillin treatment which can prevent mortality of rats with intact spleens, has lost its activity in these groups of rats. A considerable increased amount of antibiotic can compensate for lack of splenic functions.

In chapter 5 the critical role of T-cell mediated immunity in relation to antimicrobial therapy of intracellular listeric infection in mice is studied. These experiments reveal the occurrence of chronic listeric infections in animals with impaired T-cell mediated immunity. Ampicillin treatment which can cure normal mice, has lost its activity. Even excessive ampicillin treatment schedules are not able to cure the listeric infection in the mice with impaired T-cell functions. In recent experiments (described in the appendix) infected nude mice were treated with rifampicin which has a better cell penetrating capacity and a

longer serum half-life in mice than does ampicillin. From these studies it appears that prolonged rifampicin treatment in high doses is able to eradicate listeric infection in nude mice. Thus, lack of T-cell mediated immunity can only be compensated by a treatment schedule with long-term effective serum levels of an antimicrobial drug which is capable of penetrating into host cells.

A general discussion of the results of our experimental studies is presented in chapter 6.

In these experimental studies in animals whose basic defense systems have been compromised in various ways, evidence is presented that selectively impaired host defense mechanisms may result in a substantial loss of efficacy of antimicrobial therapy. Successful treatment of infection in the compromised host not only depends on the isolation of the causing organism and the estimation of its susceptibility to antibiotics, but also on the judicious use of antibacterial agents. In this respect the maintenance of effective levels of the drug at the site of infection appears to be of prime importance.

SAMENVATTING

Met het dierexperimenteel onderzoek, beschreven in dit proefschrift, wordt getracht meer inzicht te geven in de relatie tussen afweerfactoren en de werkzaamheid van antibiotica bij het bestrijden van bacteriële infecties.
In de klinische geneeskunde wordt men in toenemende mate geconfronteerd met patienten die lijden aan aanwijsbare vormen van weerstandsvermindering, bijvoorbeeld als gevolg van maligniteiten en de behandelingen daarvan (bestraling of chemotherapie). Ernstige infekties komen als gevolg hiervan frekwent voor en hebben niet zelden een fataal verloop omdat de antimicrobiële therapie die werkzaam is bij de normale gastheer, in deze patienten vaak geheel of gedeeltelijk faalt. Verschillende factoren dragen gelijktijdig bij aan de vermindering van de weerstand. Maligniteiten en chemotherapeutische behandelingen kunnen aanleiding geven tot onderdrukking van humorale of cellulaire immuniteit, alsook vermindering van het aantal of de funktie van polymorphonucleaire leukocyten (PMN). Een tweetal vragen komen naar voren. Ten eerste, in welke mate is de werkzaamheid van een antibioticum therapie afhankelijk van weerstandsfactoren van de gastheer? Ten tweede, kan een verzwaring van het antibioticum therapieschema het gemis aan eigen afweerfactoren geheel of gedeeltelijk compenseren? Omdat de klinische situatie weinig ruimte biedt voor de bestudering van deze vragen, is gekozen voor een dierexperimentele benadering waarbij het mogelijk is weerstandsfactoren selectief weg te nemen. Door middel van de studies beschreven in de hoofdstukken 1-5 proberen wij een bijdrage te leveren aan de beantwoording van deze vragen. Door de keuze van de experimentele infecties, veroorzaakt door *Streptococcus pneumoniae* of *Listeria monocytogenes*, was het mogelijk de relatieve betekenis van afweerfactoren afzonderlijk voor de werkzaamheid van antibiotica te bestuderen.
De hoofdstukken 1-3 beschrijven een experimentele pneumococcen pneumonie bij de rat. In dit model is het belang van intacte afweerfactoren voor het welslagen van antimicrobiële therapie bestudeerd. Aangetoond is

dat de antimicrobiële behandeling door penicilline of clindamycine, die wel normale dieren kan genezen, faalt als gevolg van een verminderde phagocytose door PMN van pneumococcen. Het verlies van pneumococcen-phagocyterend vermogen door PMN kan grotendeels gecompenseerd worden door een verzwaring van het antibioticum behandelingsschema in de vorm van een dosisverhoging, al dan niet in combinatie met een verhoogde injectiefrekwentie. Omdat het hierbij gaat om de behandeling van een zich snel-verspreidende longinfectie als gevolg van inadekwate phagocytose, dient bij de keuze van een antibioticum niet alleen gelet te worden op het antibioticum-gevoeligheidspatroon van de verwekker, maar ook op de pharmacologische eigenschappen van het middel. Behandelingsschema's die langdurig-werkzame antibioticum serumspiegels geven tussen twee opeenvolgende injecties zijn van het grootste belang.
Hoofdstuk 4 bespreekt de gevolgen van splenectomie voor het verloop van een pneumococcen bacteriaemie in ratten enerzijds, anderszijds de werkzaamheid van een penicilline therapie. Deze experimenten laten zien dat het wegnemen van de milt resulteert in een verhoogde mortaliteit als gevolg van de intraveneuze pneumococcen infectie in ratten op jonge leeftijd, en in volwassen ratten die al een verhoogde pneumococcen-infectiegevoeligheid hadden als gevolg van een verminderd pneumococcen-opsonizerend vermogen van het serum. De verhoogde gevoeligheid voor infectie blijkt gecorreleerd te zijn met een gereduceerd vermogen om circulerende pneumococcen uit het bloed te verwijderen. In deze twee groepen van dieren heeft de behandeling met penicilline die nodig is om sterfte van niet-gesplenectomeerde ratten te vóórkomen, zijn werkzaamheid verloren. Een aanzienlijke verhoging in penicilline doses kan het gemis aan miltfunkties compenseren.
In hoofdstuk 5 wordt het belang van cellulaire immuniteit in relatie tot de antimicrobiële therapie van intracellulaire *Listeria* infecties bij muizen bestudeerd. Deze experimenten tonen aan dat de infectie met *L. monocytogenes* in dieren met verminderde T-cell funkties een chronisch verloop heeft; in deze muizen heeft de behandeling met ampicilline die muizen met een intacte afweer kan genezen, zijn werkzaamheid verloren.

Zelfs behandelingsschema's met frekwente injecties van hoge doses ampicilline zijn niet in staat de *Listeria* infectie in de muizen met verminderde cellulaire immuniteit te genezen. In recente experimenten (beschreven in de appendix) zijn de geïnfecteerde muizen behandeld met rifampicine, een antibioticum dat een beter cel-penetrerend vermogen en een langere serum half-waarde tijd heeft dan ampicilline. Uit deze studies blijkt dat een langdurige behandeling met rifampicine in hoge doses in staat is de *Listeria* infectie in de muizen met verminderde afweer te beëindigen. Blijkbaar kan gemis aan cellulaire immuniteit slechts gecompenseerd worden door een behandelingsschema met langdurig-werkzame serumspiegels van een antibioticum dat het vermogen heeft in gastheercellen binnen te dringen.

In hoofdstuk 6 worden de resultaten van deze experimentele studies besproken in een algemene discussie. Geconcludeerd wordt dat voor een succesvolle behandeling van infecties een samenspel van twee componenten vereist is, te weten intacte afweerfactoren en gerichte, adekwate toediening van antibiotica. Selectieve vermindering van afweermechanismen resulteert in een substantieel verlies van werkzaamheid van antimicrobiële therapie. Wanneer de afweermechanismen van de gastheer niet meer intact zijn, is succesvolle behandeling van infecties niet alleen afhankelijk van de isolatie van de verwekker en het bepalen van het antibioticumgevoeligheidspatroon daarvan, maar ook van het zorgvuldig gebruik van antimicrobiële middelen. In dit opzicht is het van het grootste belang dat werkzame concentraties op de plaats van de infectie gehandhaafd worden.

DANKWOORD

Mijn dank gaat uit naar een ieder die bij het tot stand komen van dit proefschrift betrokken is geweest, in het bijzonder:
mijn ouders, die mijn academische opleiding mogelijk maakten,
Prof. Dr. M.F. Michel, mijn promotor, die het initiatief nam voor het onderzoek, mij alle mogelijkheden bood het onderzoek uit te voeren, en dit met een diepgaande interesse en kritische opmerkingen begeleidde,
de co-referenten Prof. Dr. J. Abels en Prof. Dr. O. Vos, voor de beoordeling van het proefschrift en hun waardevolle suggesties,
Yvonne de Jong-Hoenderop, Toon van Gerwen en Willem van Leeuwen, voor hun zorg, bereidvaardigheid en enthousiasme bij het uitvoeren van de experimenten, ook op ongemakkelijke tijden. Hun kritische werkwijze stond borg voor optimale resultaten,
Ada Beukelman, voor haar steun en toewijding, en voor de accurate en vakkundige verwerking van het manuscript,
Bob Noble en Karin Reimann-Knuth Winterfeldt, voor de bereidwilligheid tot corrigeren van het manuscript,
Pieter de Bos,
Joke van de Berg en Cor Schipper,
Corina Looten,
Yvonne Elgersma-Huizer en Wil Schneider,
de heer M. van Vliet,
de heer H. van de Velden,
en andere medewerkers van de afdeling Klinische Microbiologie en Antimicrobiële Therapie voor hun samenwerking en behulpzaamheid,
Ina Wilschut en Mieke Erkens-Versluis van de afdeling Celbiologie II,
Kees van Doorn van het Proefdierenbedrijf,
Trix Fassotte-van Leeuwen en John Fassotte van het Laboratorium voor Chirurgie,
mevr. E.C. van Reede van de afdeling Dermatologie, en
de heer H. Kneefel en andere medewerkers van het Audio Visueel Centrum.

curriculum vitae

De schrijver van dit proefschrift werd in 1949 geboren te Utrecht.

1968 Eindexamen Gymnasium β aan het R.K.Lyceum O.L.Vrouw ter Eem te Amersfoort.

1968–1975 Studie in de biologie aan de Rijks Universiteit Utrecht

1971 Kandidaatsexamen richting B4

1975 Doctoraalexamen met als hoofdrichting microbiologie en als bijvakken immunologie en haematologie.

1975–heden als wetenschappelijk medewerker werkzaam op de afdeling Klinische Microbiologie en Antimicrobiële Therapie van de Erasmus Universiteit Rotterdam, alwaar het in dit proefschrift beschreven onderzoek werd verricht.