Chemotherapy of Experimental Streptococcal Endocarditis

I. COMPARISON OF COMMONLY RECOMMENDED PROPHYLACTIC REGIMENS

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ABSTRACT The effectiveness of various antibiotics commonly recommended for the prophylaxis of bacterial endocarditis has been evaluated in experimental streptococcal endocarditis in rabbits. High doses of penicillin G did not prevent the development of this infection. The only consistently successful prophylactic regimens using penicillin alone were those which provided for both an early high serum level and more than 9 h of effective antimicrobial action. Vancomycin was the only other drug which proved uniformly successful when given alone, even though the duration of its antimicrobial action in the blood was only 3 h. However, combined therapy using penicillin G or ampicillin with streptomycin was always effective in prophylaxis. Treatment with single injections of ampicillin, cephaloridine, cephalexin, clindamycin, cotrimoxazole, rifampicin, streptomycin, erythromycin, and tetracycline failed to prevent infection.

The findings provide information on the effect of antimicrobials in vivo and may be applicable to the chemoprophylaxis of infective endocarditis in clinical practice.

INTRODUCTION

Patients with valvular heart disease or intracardiac prostheses are presumed to be at risk of bacterial endocarditis after dental work, urinary tract instrumentation, and other surgical procedures which are commonly attended by bacteremia. It has therefore become routine practice, under these circumstances, to administer antimicrobial agents in order to protect the patient from this disease. However, it has been impossible to determine the efficacy of prophylaxis because the chance that organisms will localize on a damaged valve during any single bacteremic episode is very small (1). Occasional reports of endocarditis occurring despite the use of antibiotics have appeared (2, 3), and have cast some doubt upon the success of attempted prophylaxis. Because controlled clinical trials of the efficacy of prophylaxis are virtually impossible, recommendations have had to be based largely on the in vitro sensitivities of probable infecting organisms. The current situation has been summarized as follows: "There is no proof that prophylaxis with antibiotics is effective in persons with valvular or congential heart disease undergoing procedures associated with transient bacteremia. However, the use of prophylactic antibiotics appears to be a reasonable approach to the problem and the consensus of opinion strongly supports the use of antibiotics in this situation" (4).

Garrison and Freedman (5) in 1970 described a comparatively simple model for producing bacterial endocarditis in rabbits; we have modified their procedure so that the time of onset of the infection can be accurately defined, and have studied bacterial growth in the vegetations (6, 7). We report here a series of experiments on prophylaxis of streptococcal endocarditis in this experimental model.

METHODS

Production of bacterial endocarditis. Young New Zealand White rabbits of either sex weighing 1–2 kg were anesthetized with 40–60 mg of pentobarbitone intravenously. To induce right-sided lesions the jugular vein was exposed and opened between ligatures. The lower ligature was loosened and a polyethylene catheter of external diameter 0.8 mm and internal diameter 0.4 mm containing sterile saline was passed toward the heart until resistance and pulsation indicated that it had entered the right atrium or ventricle. The catheter was then secured in place by tightening the ligatures; any excess was cut off and the upper end sealed with a heated spatula. The skin was then closed over the catheter with silk sutures. For left-sided lesions a catheter was passed down the carotid artery toward the

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heart until pulsation, resistance, and reflux of arterial blood indicated that it had reached the aortic valve or passed beyond into the left ventricle; it was then secured as described. The rabbits were left undisturbed for 1–3 days, during which time small sterile vegetations developed on the valve in contact with the catheter. Approximately 10^8 colony-forming units of the test organism were then given by intravenous injection. If the catheter is correctly placed this procedure produces bacterial endocarditis in every animal. All the experiments reported here refer to right-sided lesions with the exception of the six rabbits which received rifampicin (Table III); these had left-sided catheters.

Test organism. A strain of *Streptococcus viridans* isolated from a patient with bacterial endocarditis, and used in previous studies, was employed throughout. This organism has been identified as *Streptococcus sanguis* serotype 2 by Mr. J. M. Hardie of the London Hospital Medical College, England. Minimal inhibitory concentrations (MIC)[1] of antibiotics for this strain are as follows:

Ampicillin	0.03 μg/ml
Cephaloridine	0.16 μg/ml
Clindamycin	0.02 μg/ml
Erythromycin	0.13 μg/ml
Penicillin G	0.01 μg/ml
Rifampicin	0.02 μg/ml
Streptomycin	8.0 μg/ml
Tetracycline	0.20 μg/ml
Vancomycin	0.62 μg/ml.

Administration of antibiotics. Standard commercial preparations were used, except for rifampicin, which was supplied in an injectable form by Ciba Laboratories. Penicillin G, ampicillin, cephaloridine, and streptomycin were given intramuscularly; erythromycin, vancomycin, tetracycline, clindamycin, and rifampicin were given intravenously. Cotrimoxazole was administered by stomach tube. All drugs were given 30 min before injection of streptococci except where stated otherwise, in the dosages to be described.

Serum levels. To measure the level of antimicrobial activity in serum after administration of antibiotics, blood was drawn from the ear vein and allowed to clot. Serial twofold dilutions of serum were made in glucose broth, and approximately 10^4 colony-forming units of the test strain of *Streptococcus viridans* were added to 1 ml of each dilution. The highest dilution failing to show visible growth after 18 h at 37°C was taken as the end point.

Evaluation of infection. 24 h after injection of streptococci the rabbits were killed by intravenous injection of pentobarbitone. The hearts were removed with antiseptic precautions and dipped briefly into boiling water to eliminate contaminants. The chambers of the hearts were opened, the vegetations were excised, weighed, homogenized in glass tissue grinders, and suspended in glucose broth. The number of colony-forming units of streptococci per gram of vegetation was determined after incorporating serial dilutions of the homogenate into blood agar pour plates. The volume of agar used was such that the final dilution of the homogenized vegetation was never less than 500:1, thus diluting any antibiotic contained in the vegetation below an effective concentration. Penicillinase was added to each plate when penicillin had been used.

[1] *Abbreviation used in this paper:* MIC, minimal inhibitory concentrations.

TABLE I

Results of Attempted Prophylaxis with Different Preparations and Doses of Penicillin

Penicillin preparation	Dose*	no. infected/no. animals
	mg/kg i.m.	
Penicillin G	6	6/6
	30	6/6
	150	19/21
Procaine penicillin	10	8/8
	50	1/8‡
	250	0/9‡
Benzathine penicillin	7.5	5/5
Benzathine penicillin plus penicillin G	7.5 150	0/4

* The low, intermediate, and high doses of each of these forms of penicillin are equal in terms of penicillin activity (U).
‡ Both these doses of procaine penicillin are more effective than the highest dose of penicillin G ($P < 0.001$ by Fisher's exact test).

RESULTS

Penicillins. The results of attempted prophylaxis with various forms of penicillin in 67 rabbits are summarized in Table I. It will be seen that a single intramuscular injection of penicillin G was rarely effective. Even with the high dose of 150 mg/kg (250,000 U/kg), which on a weight basis would be equivalent to 20,000,000 U in man, only 2 of 21 vegetations were sterilized. The number of streptococci recovered from the infected vegetations did not differ significantly from that which was found in untreated animals.

To test the effect of repeated administration of penicillin G, four rabbits were given 30 mg/kg every 4 h for six doses, starting 30 min before bacteria were injected; all four remained infected. When the dose was raised to 150 mg/kg every 4 h for six doses, three of four had sterile vegetations and a low count of streptococci was found in the other. Although this dose is too high for anything but experimental use, the result suggested that prolonged administration of penicillin might be effective.

In order to achieve more prolonged penicillin effect after a single treatment, 25 rabbits were given injections of procaine penicillin. A dose of 10 mg/kg was ineffective; however, by increasing the dose to 50 mg/kg the infection was prevented in seven of eight, and when 250 mg/kg was given all of nine rabbits were protected. Both 50 and 250 mg/kg of procaine penicillin were more effective than the highest dose of penicillin G ($P < 0.001$ by Fisher's exact test). Four rabbits from the group given 250 mg procaine penicillin received the drug 30 min after the bacteria; because the result was

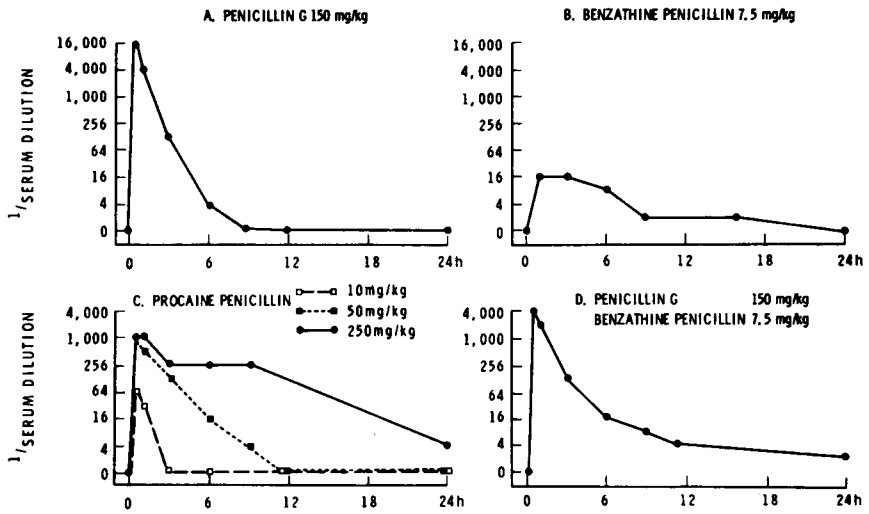

FIGURE 1 Reciprocal of highest inhibitory dilution of serum for the test strain of *Streptococcus viridans* after intramuscular administration of different penicillin preparations to rabbits.

the same as when penicillin was given 30 min before, the results have been reported together.

In order to separate the influence of serum concentration and duration of action, prophylaxis was attempted with 7.5 mg/kg of benzathine penicillin. All animals so treated developed bacterial endocarditis. On the other hand, a combination of 150 mg/kg of penicillin G plus 7.5 mg/kg of benzathine penicillin prevented infection in the four animals tested.

Six rabbits were given 30 mg/kg of penicillin V-K, a soluble form of penicillin V, intramuscularly. Three were treated 30 min before bacteremia, and all became infected. In three the drug was given at six 1-h intervals for four doses, beginning 30 min before inoculation of streptococci; bacterial endocarditis developed in two of the three rabbits.

Serum levels in rabbits. The inhibitory activity of rabbit serum for this organism after intramuscular injections of penicillin is shown in Fig. 1. High levels were achieved after penicillin G, but the drug was excreted rapidly (Fig. 1 *A*), while a lower peak level and longer duration were found after benzathine penicillin (Fig. 1 B). Neither of these regimens was capable of preventing bacterial endocarditis.

TABLE II

Results of Attempted Prophylaxis with Bacteriostatic Drugs

	Dose	no. infected/no. animals
	mg/kg	
Tetracycline	15	5/5
Erythromycin	15	5/5
Clindamycin	5	5/5

Levels many times greater than the MIC were found after the administration of procaine penicillin but inhibitory activity could not be demonstrated for longer than 6 h unless the dose was 50 mg/kg or more (Fig. 1 C). These results indicate that the curve of serum activity after administration of procaine penicillin to rabbits differs from that observed in man, being characterized by a higher early peak and shorter duration.

When penicillin G and benzathine penicillin were given in combination (Fig. 1 D), the peak and duration of penicillin activity were similar to that achieved with large doses of procaine penicillin (Fig. 1 C). Both these regimens were successful in preventing streptococcal endocarditis.

Serum levels in man. An attempt was made in man to approximate the pattern of serum activity which prevented endocarditis in rabbits. After several regimens had been tried in a healthy 80 kg male volunteer, it was found that 2,00,000 U of penicillin G plus 600,000 U of procaine penicillin given intramuscularly produced a curve of serum activity very similar to that which had proved effective in rabbits.

Alternatives to penicillin. Although some form of penicillin is most often employed in prophylaxis, situations in which an acceptable alternative is desired arise frequently in clinical practice. Patients may be allergic to penicillin, or organisms resistant to penicillin may be anticipated. Several antibiotics have been recommended in such situations. These include erythromycin, tetracycline, and cephaloridine (8–11). Vancomycin has been advocated for patients already receiving penicillin (12), while newer agents such as clindamycin and rifampicin have also been suggested (13). These drugs

were therefore tested in an attempt to evaluate relative efficacy in vivo.

The results of attempted prophylaxis with the bacteriostatic drugs erythromycin, tetracycline, and clindamycin are summarized in Table II. Vegetations from all rabbits which received these drugs were found to be infected at the end of 24 h.

Attempted prevention of streptococcal endocarditis with bactericidal drugs is reported in Table III. Penicillin, ampicillin, cephaloridine, cephalexin, rifampicin, cotrimoxazole, and streptomycin failed to prevent infection in most rabbits. In contrast, all of 11 rabbits given vancomycin had sterile vegetations ($P < 0.005$).

There was no significant reduction in the number of streptococci per gram of vegetation 24 h after injection of *Streptococcus viridans* compared with untreated animals studied previously (6) after any drug except vancomycin.

The inhibitory activity of rabbit serum after administration of some of these antibiotics in shown in Figs. 2 and 3. Antimicrobial activity was substantially in excess of the MIC for the test organism with every drug except streptomycin which only exceeded the MIC at 30 min; moreover, the highest levels found were 30 min after administration, at the time streptococci were injected. Except after clindamycin, the duration of detectable serum activity was 6 h or less.

Attempted prophylaxis with two drugs. Combinations of penicillin or ampicillin with streptomycin were tested in the same system. None of the three agents used singly

TABLE III

Results of Attempted Prophylaxis with Bactericidal Drugs

	Dose	no. infected/no. animals
	mg/kg	
Penicillin G	150	19/21
Ampicillin	30	4/5
Cephalexin	30	3/3
Cephaloridine	30	5/6
Cotrimoxazole		
Sulphamethoxazole	17	
Trimethoprim	3.4	4/4
Rifampicin	20	6/6
Streptomycin	15	6/9
Vancomycin	30	0/11*
Penicillin G plus Streptomycin	150 15	0/6‡
Ampicillin plus Streptomycin	30 15	0/4‡

* Vancomycin is more effective than any other single drug ($P < 0.005$ by Fisher's exact test).

‡ Each of the combinations using streptomycin is more effective than penicillin G or streptomycin alone ($P < 0.05$ by Fisher's exact test).

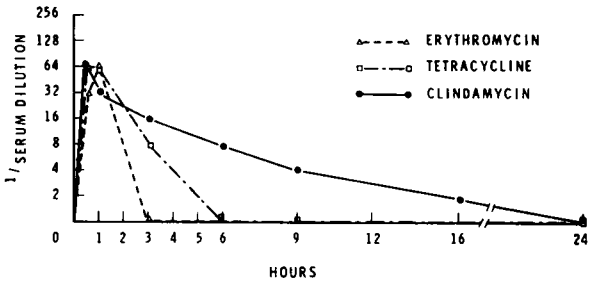

FIGURE 2 Reciprocal of highest inhibitory dilution of serum for the test strain of *Streptococcus viridans* after administration of bacteriostatic antibiotics to rabbits.

was uniformly effective, although some cures were obtained with streptomycin. On the other hand, a combination of streptomycin with either of the penicillins was uniformly effective (Table III); each of these two combinations was significantly better than penicillin G or streptomycin alone ($P < 0.05$ by Fisher's exact test).

Bactericidal action in vitro. In an attempt to shed further light on the in vivo findings, bacterial killing was studied in vitro. The counts per milliliter of this strain of *Streptococcus viridans* in broth after incubation at 37°C with penicillin (6 μg/ml) or penicillin (6 μg/ml) plus streptomycin (20 μg/ml) are shown in Fig. 4. A low count of "persisters" was present after incubation with penicillin for 24 h. However, when streptomycin was included the culture became sterile between the 6th and 24th h.

DISCUSSION

No definitive evidence that antimicrobial prophylaxis of bacterial endocarditis is effective has been presented previously. Clinical studies are difficult to perform because of the very low incidence of infection after any

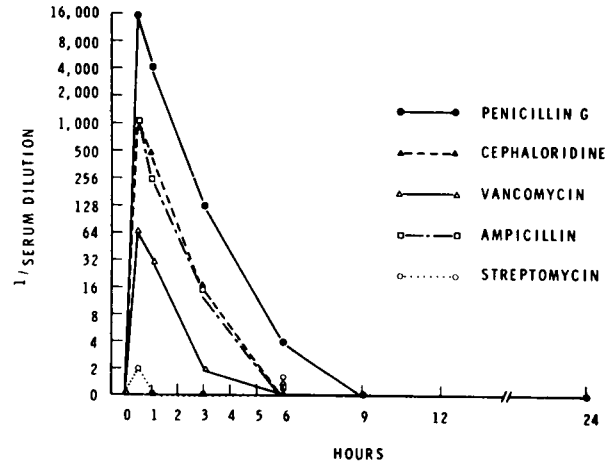

FIGURE 3 Reciprocal of highest inhibitory dilution of serum for the test strain of *Streptococcus viridans* after administration of bactericidal antibiotics to rabbits.

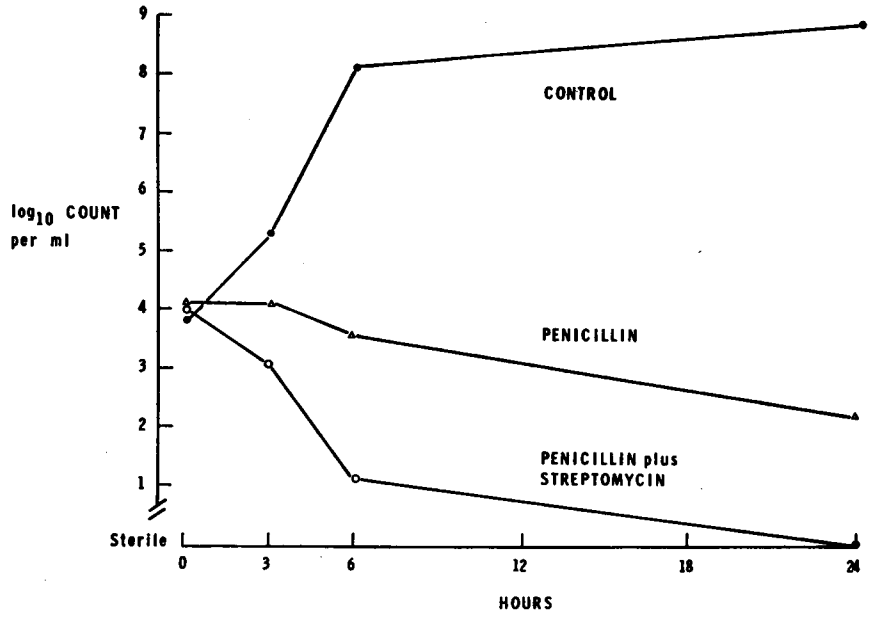

FIGURE 4 Comparison of the bactericidal action of penicillin alone with penicillin plus streptomycin, against penicillin-sensitive *Streptococcus viridans* in vitro.

single bacteraemic episode in a patient at risk; a properly controlled clinical trial would require far too many patients to be practicable. This situation has led to acceptance of indirect criteria to indicate the best choice of antibiotic. The ability of a drug to reduce the count of organisms in the blood after dental procedures has often been equated with effectiveness in preventing endocarditis. This assumption may not be correct; for example, it has led to the recommendation of a tetracycline as an "effective" prophylactic (9) even though the drug is bacteriostatic. Evidence provided by in vitro tests may be misleading when the drug acts against the organism outside the intact animal.

Experimental evaluation of prophylaxis of bacterial endocarditis has previously been impracticable due to the lack of a simple and dependable model. Elaborate procedures were required to produce infection reliably (14), whereas simpler methods often resulted in a lower proportion of infected valves (15, 16). For these reasons there have been few studies on treatment of experimental endocarditis and apparently only one on its prevention (17).

The technique used in these experiments has been shown previously to induce experimental endocarditis reliably and reproducibly (5, 6). It has unique advantages for study of antimicrobial prophylaxis and treatment. The sterile endocardial lesions can be converted to bacterial endocarditis by a single intravenous injection of streptococci; the onset of infection can therefore be timed precisely. The bacteria on the vegetations multiply in a predictable pattern, and the histological picture closely resembles that found in human endocarditis.

The studies reported here demonstrated unexpected difficulty in preventing circulating bacteria from lodging on the sterile lesion. Although this streptococcus was highly sensitive to penicillin in vitro, and was rapidly cleared from the blood in normal rabbits (6), it proved remarkably difficult to eradicate in an endocardial collection of fibrin and platelets. Despite the presence of very high penicillin concentrations in the blood, infection could not be prevented with a single injection of penicillin G; it appears therefore that this system poses an extremely severe test for any drug.

Comparison of the serum concentrations resulting from various treatment programs suggests that a certain relationship between level and duration of penicillin is required for prophylaxis. The failure of penicillin G indicates that high serum levels alone are inadequate to prevent circulating bacteria from infecting the valve. Likewise, failure of benzathine penicillin indicates that a prolonged penicillin effect at a lower level is also inadequate. However, the combination of penicillin G and benzathine penicillin provided both a high initial level and prolonged action, and was uniformly successful. Equally successful were high doses of procaine penicillin, which in the rabbit provided both an early peak and prolonged duration. In fact, the curves of serum penicillin concentration after the two successful regimens were strikingly similar.

These findings in experimental animals suggest that current recommendations for penicillin prophylaxis in

man should be reconsidered. Several regimens, including penicillin G (18), procaine penicillin (8, 19), and oral penicillin V (8), are in wide clinical use today. It is possible that the presumed success of prophylactic therapy may be due more to the inherently low infection rate than to the antibiotics. According to our studies an optimum regimen for prophylaxis with penicillin alone should provide both an early peak of serum activity and a duration of at least 24 h.

Several other antibiotics that have been advocated for man on empirical grounds proved incapable of preventing bacterial endocarditis in this model. It seems unlikely that the presence of any antimicrobial agent would prevent the actual lodgement of circulating bacteria on a suitable nidus, because seeding of the vegetation occurs less than 30 min after bacteria enter the circulation (6) before any drug present could be expected to have completed its antibacterial action. Therefore, all effective chemoprophylactics must be capable of eliminating bacteria after they have lodged on the valve. The finding that high doses of procaine penicillin were equally effective in preventing bacterial endocarditis when given 30 min before or after streptococci were injected intravenously is consistent with this concept. Therefore bacteriostatic drugs, which do not kill organisms *in situ* on the valve, cannot be expected to work as prophylactic agents when given in short courses because organisms lodged on the lesion could remain quiescent until the agent disappeared from the blood, and then multiply. However, this does not exclude the possibility that a bacteriostatic drug present over a long enough period could be an effective preventive agent.

The failure of several bactericidal agents requires another explanation. It is probable that although a majority of the streptococci were killed by these drugs, a small residual population of persisters remained. The in vitro study showing that a low count of streptococci persisted after incubation with penicillin for 24 h, parallels the findings with penicillin-sensitive staphylococci reported in a previous study of the phenomenon of persistence (20). In contrast, penicillin plus streptomycin completely sterilized the culture in less than 24 h. These findings may explain both the failure of penicillin, and the success of penicillin plus streptomycin, in the prevention of this infection. In other locations persisting bacteria would probably be disposed of by phagocytes, but in the vegetation where phagocytes are scanty organisms could survive until the penicillin was excreted and then multiply to cause bacterial endocarditis. The short courses of antimicrobial agents used for chemoprophylaxis must therefore be aimed at achieving "total kill," since the death of even 99.9% of the organisms may not suffice to prevent development of bacterial endocarditis.

The synergism between penicillin and streptomycin against an organism fully sensitive to penicillin alone may be of clinical importance. Previous authors generally have not recommended this combination for dental procedures, on the grounds that in vitro tests showed most oral streptococci to be highly sensitive to penicillin alone. However, in vitro tests on combinations sometimes correlate poorly with clinical results (21). On the basis of these studies the routine use of streptomycin with penicillin for prophylaxis during dental procedures should be considered, not on the grounds that a wider spectrum of organisms will be sensitive, but because the bactericidal action of the combination is more rapid and complete.

It may be argued that the experimental conditions used here are prejudicial to the optimal action of the antibiotic, because the drug acts for a short time against a large inoculum. It is impossible to be sure how many bacteria gain access to the circulation during dental procedures, because a proportion must be cleared before the remainder reach the peripheral venous blood. Since counts of 20 or more per milliliter venous blood have been reported (22) it can be calculated that a total of more than 10^5 bacteria were in the circulation of those patients at the moment blood was drawn. Nevertheless the number of streptococci injected here was probably larger than that introduced into the bloodstream during dental work. Although infection could be initiated with irregular frequency using injections of as few as 10^3 bacteria,[3] it was necessary, in order to compare different prophylactic regimens, to inject enough bacteria to ensure that some would lodge on the small sterile vegetation in all animals. In any case, only 10^3–10^4 reach the vegetation after injection of 10^8 bacteria; the vast majority is promptly cleared by the reticuloendothelial system (6). The number of organisms reaching the vegetation is therefore in the range usually chosen for routine in vitro sensitivity tests. Thus it seems unlikely that inoculum size alone can explain why penicillin and several other drugs were ineffective.

These experiments deal almost exclusively with right-sided endocarditis, and it may be objected that in human endocarditis the vegetations are most often left-sided. In further experiments, which are to be reported,[3] we treated established right- and left-sided endocarditis and found that both lesions responded similarly during the first 4 days. We feel, therefore, that the present findings are pertinent to prophylaxis of infection on either side.

[3] Durack, D. T., and R. G. Petersdorf. Chemotherapy of experimental streptococcal endocarditis II. Treatment of established infection, with special reference to synergism between penicillin and streptomycin. Submitted for publication.

It may also be argued that by leaving the plastic catheter *in situ,* a condition was created that is not relevant to the problem of the patient with valvular heart disease. However, we have found that the sterile vegetation can be infected even after the catheter has been withdrawn.[3] It is, of course, possible that regimens which were ineffective in the presence of a catheter might have been successful if the foreign body had been withdrawn before bacteria were introduced. Nevertheless, it must be recognized that bacterial endocarditis now often affects patients who have prosthetic valves, or intracardiac catheters (23–26). The point to be emphasized is that these experiments have defined the conditions under which a short course of antimicrobial therapy can prevent bacterial endocarditis even in the presence of a foreign body.

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