Chemotherapy of Experimental Streptococcal Endocarditis

IV. FURTHER OBSERVATIONS ON PROPHYLAXIS

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ABSTRACT The ability of antibiotics to prevent *Streptococcus sanguis* endocarditis was tested in rabbits. Only vancomycin or a combination of penicillin G plus streptomycin always prevented infection when administered as a single dose. A loading dose of 30 mg/kg of phenoxymethyl penicillin (penicillin V) followed by additional 7.5 mg/kg doses for 48 h proved to be the only successful prophylactic program that could be given orally to man. Cefazolin alone or with streptomycin in multiple doses was also an effective alternative to penicillin or penicillin derivatives. Erythromycin uniformly failed to protect animals from bacterial endocarditis but showed greater prophylactic efficacy when a low inoculum of streptococci was used.

INTRODUCTION

Bacteremia with viridans streptococci following tooth extraction (1–3), dental manipulation (4, 5), and oral surgery (6, 7) is occasionally associated with the development of bacterial endocarditis (8–10). This observation has led the American Heart Association (11) to recommend prophylactic administration of antibiotics to patients with known heart disease undergoing dental procedures. However, due to the low incidence of endocarditis after dental bacteremia, it has not been possible to determine whether antibiotics can successfully abort the development of endocarditis or to compare the relative efficacy of different prophylactic programs in man (12). Some other means of evaluating antibiotic efficacy is necessary that would avoid the necessity for a long-term, multicenter study and that also would determine directly the presence of endocardial infection without relying on indirect observations such as blood cultures.

In previous reports (13–15) Durack, Petersdorf, and colleagues showed that antibiotics prevented the development of experimental endocarditis in rabbits and that certain regimens were more effective than others. Using a modification of the experimental model first described by Garrison and Freedman (16), they demonstrated that sterile endocardial platelet and fibrin vegetations could be induced in rabbits by the placement of a polyethylene catheter into the heart (17). These sterile vegetations were then invariably infected by the intravenous injection of approximately 10^8 colony-forming units (cfu)[1] of a strain of *Streptococcus sanguis*, isolated from a patient with bacterial endocarditis (18). The success or failure of antibiotics administered 30 min before the injection of bacteria in preventing the colonization of endocardial vegetations was determined by the excision and quantitative culture of the endocardial vegetations 24 h after the administration of antibiotics.

These studies showed that intramuscular penicillin G 150 mg/kg combined with streptomycin 15 mg/kg, large doses of procaine penicillin 250 mg/kg, penicillin G 150 mg/kg combined with benzathine penicillin 7.5 mg/kg, and intravenous vancomycin 30 mg/kg were the only regimens that always prevented endocarditis after a single administration of the drugs. Bacteriostatic agents were uniformly ineffective in preventing endocarditis.

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[1] *Abbreviations used in this paper:* cfu, colony-forming unit; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

Penicillin required both an early high peak level as well as serum bactericidal activity for at least 9 h in order to prevent endocardial colonization. Of the drugs tested, none that could be administered orally in man was effective, and vancomycin was the only effective alternative to penicillin.

These results generated a series of questions that are the subject of this report:

(*a*) Were agents other than those tested previously effective in preventing endocarditis? (*b*) Would multiple doses of drugs that had failed to protect animals after a single dose be successful when given over a 48 h period, as in clinical practice? (*c*) Were there any effective drugs in the rabbit that could be administered orally to man? (*d*) Were there reliable alternatives to the penicillins that would be easier to administer than vancomycin? (*e*) Was left-sided endocarditis more difficult to prevent than right-sided endocarditis? (*f*) Were antibiotics more effective when the inoculum of streptococci was reduced to levels closer to those estimated to be present during bacteremia in man?

METHODS

Experimental model. New Zealand white rabbits of either sex weighing 1.5–2.0 kg were anesthetized with 25–30 mg/kg of pentobarbital intravenously. To induce left-sided lesions a polyethylene catheter of external diameter 0.8 mm and internal diameter 0.4 mm containing sterile saline was passed in retrograde fashion down the right common carotid artery toward the aortic valve until pulsation and resistance indicated that the catheter had reached the aortic valve or had passed into the left ventricle. The catheter was then tied in place with silk, and any excess catheter was cut off and sealed with a heated spatula. The skin was closed over the catheter with a continuous silk suture. 24 h later, after which a formation of small sterile fibrin and platelet vegetations on the aortic valve and endocardium has been shown to occur (17), antibiotics were administered, and 30 min later approximately 10^8 cfu of *S. sanguis* was given intravenously. Groups of untreated control animals received the same inoculum of streptococci and were sacrificed at the same time intervals as antibiotic-treated animals.

Test organism. A single strain of *S. sanguis,* serotype II (NCTC 7864) used in previous experiments (13–15) and originally isolated from a patient with subacute bacterial endocarditis, was utilized throughout these studies. In vitro growth characteristics and virulence were maintained by periodic passage through rabbits in the endocarditis model. Rabbits were inoculated intravenously through a marginal ear vein with 1.0-ml portions of 18-h trypticase soy yeast extract (Difco Laboratories, Detroit, Mich.) broth cultures of *S. sanguis* diluted in broth to a standard inoculum of approximately 10^8 cfu/ml by using absorbance at 550 nm determined on a Coleman Junior spectrophotometer (Coleman Instruments Div., Perkin Elmer Corp., Oak Brook, Ill.) as a guide. All inocula were quantitated by serial 10-fold dilution in saline and culture of portions in blood agar. pour plates to determine the number of cfu injected.

In vitro susceptibility studies. The minimum inhibitory concentrations (MIC) of various antibiotics were determined by incubating 10^4 cfu/ml of *S. sanguis* with serial twofold tube dilutions of antibiotic in Mueller Hinton broth (Difco Laboratories). The highest dilutions failing to show visible growth after 18 h at 35°C were taken as the MIC. The minimum bactericidal concentration (MBC) was read as the highest dilution showing no growth on blood agar after subculture of 0.01 ml from each clear tube. The MIC and MBC of various antibiotics for this strain were:

	MIC	MBC
	μg/ml	
Pencillin G	0.02	0.02
Penicillin V	0.04	0.04
Ampicillin	0.04	0.04
Cephalexin	6.25	6.25
Cefazolin	0.08	0.08
Cephaloridine	0.08	0.08
Cephalothin	0.31	0.31
Tetracycline	0.20	0.20
Streptomycin	32.0	32.0
Erythromycin	0.08	0.15
Clindamycin	0.02	0.02
Vancomycin	0.62	0.62

Antibiotics. Commercially available antibiotics authorized for use in man were used except for penicillin V standard powder and parenteral sodium cephalexin supplied by Eli Lilly Laboratories. All antibiotics were prepared for intravenous or intramuscular administration according to the manufacturer's directions and were used within 24 h of reconstitution, except for the penicillin V standard powder, which was solubilized in pH 7.2 phosphate buffer and was used within 12 h of reconstitution.[2]

Serum levels. 3-ml blood samples were drawn from ear veins of rabbits at time intervals after administration of antibiotic and were allowed to clot. Serum antibiotic concentrations were measured by the modified agar-well diffusion assay method of Bennett, Brodie, Benner, and Kirby (19). Serum inhibitory activity against a concentration of 10^4 cfu/ml of the test organism was determined by standard serial twofold dilution of serum in trypticase soy broth for some antibiotics but are not reported because of close correlation with the agar-well diffusion assay method. Each reported serum level was the average of determinations in three different rabbits.

Evaluation of infection. Rabbits were killed by intravenous injection of 150 mg of pentobarbital 24 h after the last administration of antibiotic, and the hearts were removed by an antiseptic technique. After brief immersion in boiling water to destroy surface contaminants the hearts were opened, and the endocardial vegetations excised, weighed, and homogenized in glass and Teflon tissue grinders in 1.0 ml of trypticase soy yeast broth. The number of cfu per milligram of vegetation was determined by counting colonies after incorporating serial 10-fold dilutions of the homogenate into blood agar pour plates and incubating 48 h at 37°C. Penicillinase was added to culture plates when penicillin was used. The final dilution of homogenized vegetations was never less than 500:1, so that any residual antibiotic contained in the vegetation was diluted below an

[2] We acknowledge the valuable advice and assistance of Dr. H. R. Black, Clinical Research Division, Lilly Laboratory for Clinical Research.

TABLE I

Results of Single-Dose Antibiotic Prophylaxis Programs in Rabbits with Right or Left Heart Catheters Using an Inoculum of $\log_{10}$ 8.0 cfu S. sanguis

Antibiotic	Route	Dose	Results		Right vs. left response	
			Right	Left	Fisher test	Wilcoxon test
		mg/kg				
Vancomycin	IV	30	0/6*	0/6	NS‡	NS
Penicillin G	IM	150	0/10	0/9	NS	NS
+ streptomycin	IM	15				
Benzathine penicillin	IM	7.5	0/12	3/10	NS	NS
+ penicillin G	IM	150				
Procaine penicillin	IM	250	11/24	22/24	$P \leqq 0.005$	$P \leqq 0.01$
Erythromycin	IV	15	4/6	11/12	NS	NS
Untreated controls	—	—	7/7	30/30	NS	NS

* Number of animals infected/total number of animals.
‡ $P > 0.05$.

effective concentration. Vegetations were considered sterile when no growth was seen on pour plates containing 0.5 ml of undiluted tissue homogenate incubated at 37°C for 48 h.

Statistical analysis. Experimental results were expressed in two forms: the proportion of infected animals in each group (presented in tables) and the number of streptococci cultured per milligram of vegetation (depicted in graphic form). Significance tables calculated by Finney, Latscha, Bennett, and Hsu (20) from the one-sided Fisher exact test were used to determine the probability value of whether the observed difference in the proportion of infected animals in different groups was due to chance alone. The two-sided Wilcoxon rank sum test (21) was used to determine significance of observed differences in the number of streptococci per milligram of vegetation. Differences in the proportion of infected animals and vegetation colony counts were recorded as significant if the probability value was 0.05 or less.

Scattergrams (Figs. 1, 3, 6, 7, 9, 10) of the number of streptococci cultured from endocardial vegetations provided additional data not previously reported (13, 14) and were analyzed by the Wilcoxon rank sum test due to the non-normal distribution of the culture results. The quantitative culture results were plotted as the $\log_{10}$ of the number of cfu per milligram of vegetation with vegetation size ranging from 2 to 150 mg. Since the largest vegetation weighed 150 mg, the minimum number of streptococci detectable was 1 cfu/150 mg; converted to $\log_{10}$ this value was graphed as -2.2 $\log_{10}$ cfu/mg.

Probit analysis. In order to establish the relationship between the inoculum size and the percentage of animals infected, groups of rabbits with sterile left heart vegetations were injected with $\log_{10}$ 3.0, 4.3, 5.0, and 8.0 cfu of streptococci, and the proportion of animals infected in each group was determined. A linear dose-response curve was observed when the probit transformation of the proportion of infected animals in each group was plotted against the $\log_{10}$ of inoculum size. A best-line fit for the observed points was determined by the working probit method of Finney (22).

RESULTS

Response of right- vs. left-sided endocarditis. Single-dose antibiotic programs that had been totally effective in preventing endocarditis in rabbits with right-sided endocardial vegetations (13) were administered to groups of animals with either right or left heart catheters. The results of these experiments are summarized in Table I and Fig. 1. Intravenous vancomycin 30 mg/kg or intramuscular penicillin G 150 mg/kg combined with streptomycin 15 mg/kg were the only single-dose antibiotic regimens that uniformly protected all animals with left- or right-sided endocardial vegetations. Benzathine penicillin 7.5 mg/kg plus penicillin G 150 mg/kg effectively prevented infection in animals with right heart vegetations (0/12 animals infected), but 3 of 10 rabbits with left heart vegetations developed endocardial infections. This difference was not significant ($P \leqq 0.05$, Fisher exact test). Treatment with high-dose procaine penicillin 250 mg/kg failed to prevent infection in

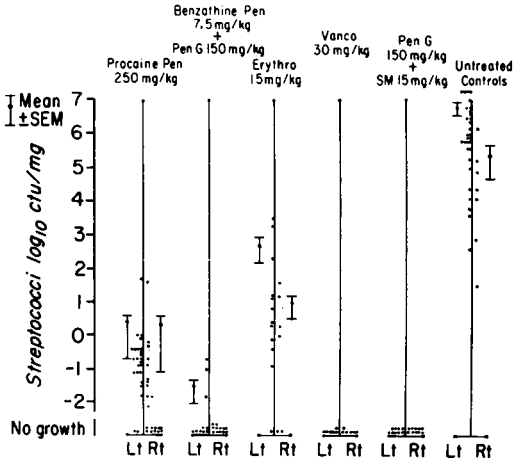

FIGURE 1 Results of quantitative cultures of endocardial vegetations from rabbits with left or right heart vegetations after single-dose antibiotic prophylaxis using an inoculum of $\log_{10}$ 8.0 cfu *S. sanguis*.

TABLE II

Comparison of the Results of Single-Dose Antibiotic Prophylaxis Programs in Rabbits with Left Heart Vegetations Using an Inoculum of $\log_{10}$ 8.0 cfu S. sanguis

Antibiotic	Route	Dose	Results	Statistical comparison of antibiotic vs.: Controls (30/30) P*	Controls (30/30) P‡	Penicillin V (4/22) P*	Penicillin V (4/22) P‡	Penicillin G + streptomycin (0/9) P*	Penicillin G + streptomycin (0/9) P‡
		mg/kg							
Penicillin V	IM	30	4/22§	0.005	0.01	—	—	NS¶	NS
Ampicillin	IM	30	6/7	NS	0.01	0.025	0.02	0.005	0.01
Ampicillin	IV	30	4/4	NS	NS	0.05	0.01	0.01	0.01
Cefazolin	IM	30	8/8	NS	0.01	0.01	0.01	0.005	0.01
Cefazolin	IM	30	6/10	0.01	0.01	NS	NS	0.025	0.05
+ streptomycin	IM	15							
Clindamycin	IM	10	6/6	NS	0.01	0.01	0.01	0.005	0.01
Clindamycin	IV	10	4/5	NS	0.01	0.01	0.05	0.025	0.025
Cephalexin	IV	30	6/6	NS	NS	0.01	0.01	0.005	0.01
Tetracycline	IV	15	6/7	NS	0.01	0.025	0.02	0.005	0.01

* Fisher exact test.

‡ Wilcoxon rank sum test.

§ Number of infected animals/total number of animals.

¶ $P > 0.05$.

11 of 21 animals with right heart vegetations and 22 of 24 rabbits with left heart vegetations. The differences in response between rabbits with left- or right-sided vegetations was significant ($P \leqq 0.005$, Fisher exact test). However, with two exceptions a very small number of streptococci (less than 1 cfu/mg of vegetation) were cultured from the vegetations of the infected animals after prophylaxis with procaine penicillin 250 mg/kg. Erythromycin 15 mg/kg intravenously failed to prevent infection on either side of the heart but significantly decreased the number of streptococci cultured from the endocardial vegetations compared to untreated controls ($P \leqq 0.01$, Wilcoxon rank sum test). No significant difference in response between animals with right- or left-sided lesions was observed after erythromycin.

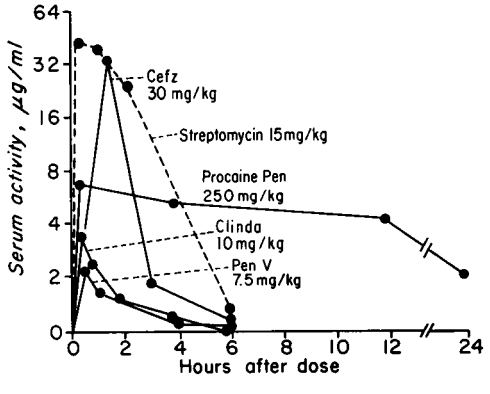

FIGURE 2 Antibiotic serum levels determined by the cup plate assay method after *intramuscular* administration with each point representing the average of levels in three rabbits.

These experiments demonstrated that vancomycin or the combination of penicillin G with streptomycin were the only totally effective single-dose antibiotic prophylaxis regimens. The response to antibiotic prophylaxis in animals with either right- or left-sided vegetations was the same except after high-dose procaine penicillin therapy.

Persistence of organisms after procaine penicillin. The persistence of a very small number of organisms (< 1 cfu/mg) in 11 of 21 right-sided endocardial vegetations had not been previously observed after procaine penicillin 250 mg/kg (13). Since procaine penicillin (Fig. 2) and the combination of benzathine penicillin plus penicillin G (13) were the only single-dose antibiotic regimens that provided serum levels beyond the 24 h sacrifice point, a further experiment was performed to determine whether these organisms would be eradicated by sustained penicillin levels 24 h after procaine penicillin. Groups of 11 rabbits with right heart vegetations and 10 rabbits with left heart vegetations were sacrificed 6 days after the administration of a single dose of procaine penicillin 250 mg/kg 30 min before the injection of an ID_{100} of streptococci. Positive cultures were obtained in only two animals in each group. One animal in each group had quantitative colony counts compatible with endocarditis ($\log_{10}$ 4.8 and 3.2 cfu/mg) while the other two infected animals had persistently low counts below 1 cfu/mg.

A single intramuscular dose of procaine penicillin reduced the number of streptococci in the vegetations to very low numbers within 24 h after administration and continued to sterilize vegetations beyond that point so that the significant difference in response between right- and left-sided endocardial vegetations in animals sacrificed at 24 h was eliminated in animals sacrificed 6 days after infection. The number of animals with right-sided infections was reduced to 2 of 11 animals, which is not significantly different from a previously reported 0/9 infection rate (13). Moreover, procaine penicillin was the only drug with which there was any difference in the response between right- and left-sided lesions, and no differences were observed with any other antimicrobial that was examined (Table I).

Prevention of endocarditis after administration of a single dose of antibiotic. The response of left-sided endocarditis to additional single-dose antibiotic regimens was studied. The results are summarized in Table II and Fig. 3. Intramuscular penicillin V 30 mg/kg was the most effective of the additional antibiotics tested with only 4 of 22 rabbits remaining infected. Penicillin V in preliminary studies (13) failed to prevent infection when administered in the same dosage. The difference in response between the two studies may be explained by earlier failure to dissolve the relatively insoluble parenteral preparation in phosphate buffer at pH 7.2 to permit improved absorption from intramuscular sites of administration.

The significant advantage of penicillin V over intravenous or intramuscular ampicillin in the same dosage ($P \leqq 0.025$, Fisher exact test) was unexepcted because the *S. sanguis* strain used in these experiments was susceptible to the same MIC of 0.04 μg/ml for both drugs. Determination of the antibiotic serum levels following intramuscular administration to rabbits showed that ampicillin peaked at a higher level (28.3 μg/ml) 30 min after a dose and then declined more rapidly than penicillin V, so that ampicillin activity lasted only 4 h (Fig. 4). Although the peak level of penicillin V 30 min after a dose (6.4 μg/ml) was lower than ampicillin, penicillin V was still present in a concentration (0.81 μg/ml) that exceeded the MIC of this organism by 20-fold, 6 h after administration. This sustained serum level probably explains why penicillin V was more effective in preventing infection than ampicillin.

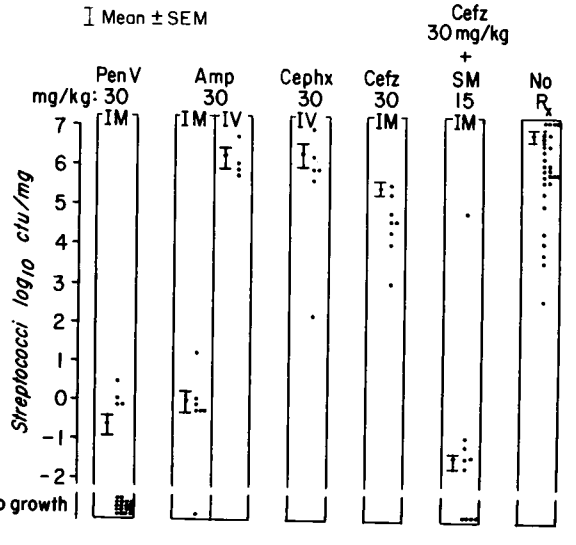

FIGURE 3 Results of quantitative cultures of endocardial vegetations from rabbits with left heart vegetations after single-dose antibiotic prophylaxis using an inoculum of $\log_{10}$ 8.0 cfu *S. sanguis*.

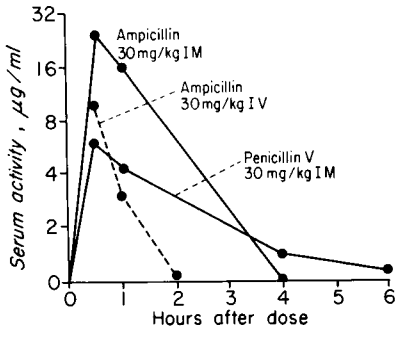

FIGURE 4 Ampicillin and penicillin V serum levels determined by the cup plate assay method with each point representing the average of levels in three rabbits.

Further evidence of the importance of sustained serum bactericidal activity in the prevention of endocarditis was provided by the difference in response between intravenously and intramuscularly administered ampicillin. While neither intramuscular nor intravenous ampicillin prevented endocarditis in the rabbits, intramuscular administration resulted in a significantly lower number of streptococci cultured from endocardial vegetations ($P \leqq 0.02$, Wilcoxon rank sum test). Rabbits treated with a single dose of ampicillin intravenously had the same number of streptococci in endocardial vegetations as untreated control animals (Fig. 3). After intravenous administration ampicillin serum levels declined more rapidly than after intramuscular administration and were almost undetectable 2 h after a dose (Fig. 4).

A single intramuscular administration of cefazolin 30 mg/kg failed to prevent infection in rabbits with left heart vegetations (8/8 animals infected) despite a peak serum level of 33.2 μg/ml (Fig. 2). When the same dose of cefazolin was combined with 15 mg/kg of streptomycin some of the animals were protected (6/10 infected), and there was a significant reduction in the number of streptococci in endocardial vegetations ($P \leqq$

TABLE III

Results of Multiple-Dose 12-Hourly Prophylaxis Programs in Rabbits with Left Heart Vegetations Using an Inoculum of $\log_{10}$ 8.0 cfu S. sanguis

Antibiotic	Route	Dose	Frequency	Results	Statistical comparison to controls	
					Fisher test	Wilcoxon test
		mg/kg				
Tetracycline	IV	15	×4	10/11*	NS‡	0.01
Clindamycin	IV	10	×4	4/5	NS	0.01
Erythromycin	IV	15	×4	3/6	NS	0.01
Ampicillin	IV	30	×4	6/6	NS	NS
Cephalexin	IV	30	×4	6/6	NS	NS
Untreated controls	—	—	—	8/8	—	—

* Number of infected animals/total number of animals.

‡ $P > 0.05$.

0.01, Wilcoxon rank sum test) compared to cefazolin alone (Fig. 3). The greater efficacy of cefazolin and streptomycin was probably due to the more rapid rate of bacterial killing of the combination observed in standard bacterial killing curves in vitro.

Bacteriostatic agents such as tetracycline 15 mg/kg intravenously and clindamycin 10 mg/kg intramuscularly or intravenously significantly reduced the number of streptococci cultured from endocardial vegetations when treated animals were compared to untreated controls but failed to protect animals from infection (Table II). Intravenous sodium cepalexin 30 mg/kg was rapidly eliminated from the serum so that 1 h after a dose serum assay (Fig. 5) showed that the serum concentration of cephalexin was below the relatively high MIC of cephalexin (6.2 μg/ml) for this organism.

Attempts to prevent infection with multiple doses of antibiotics. In order to establish whether antibiotics that failed to protect rabbits with left heart vegetations when given as a single dose might succeed when given in multiple doses, groups of rabbits were treated at 6–12-h intervals for a total of 48 h commencing 30 min before injection of streptococci.

The results of treatment programs with several antibiotics administered at 12-h intervals are summarized in Table III and Fig. 6. None of the antibiotics consistently prevented endocardial infections. Compared to untreated controls, multiple doses of tetracycline, clindamycin, and erythromycin significantly reduced the number of streptococci cultured from vegetations but did not eliminate them. Ampicillin and cephalexin failed to reduce significantly the number of organisms cultured from vegetations. The failure of these bactericidal antibiotics to prevent infection my be explained by the

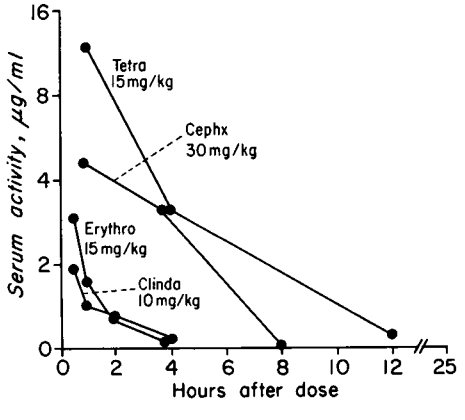

FIGURE 5 Antibiotic serum levels determined by the cup plate assay method after *intravenous* administration with each point representing the average of levels in three rabbits.

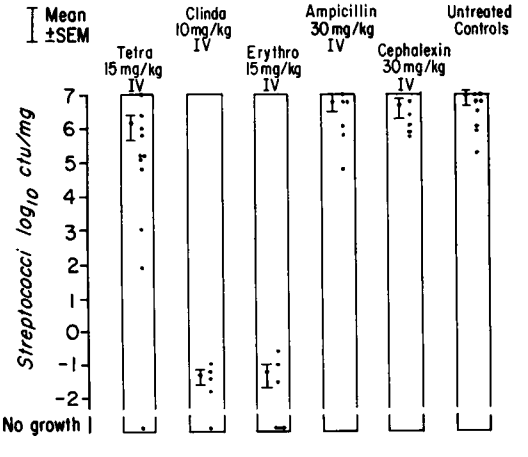

FIGURE 6 Results of quantitative cultures of endocardial vegetations from rabbits with left heart vegetations after multiple-dose 12-hourly antibiotic prophylaxis using an inoculum of $\log_{10}$ 8.0 cfu *S. sanguis*.

TABLE IV

Results of Multiple-Dose 6-Hourly Prophylaxis Programs in Rabbits with Left Heart Vegetations Using an Inoculum of $\log_{10}$ 8.0 cfu S. sanguis

Antibiotic	Route	Dose	Frequency	Results	Comparison of antibiotic vs.: Controls (8/8) Fisher test	Controls (8/8) Wilcoxon test	Cefazolin (1/12) Fisher test	Cefazolin (1/12) Wilcoxon test
		mg/kg						
Cefazolin	IM	30	Q. 6 h × 8	1/12*	0.005	0.01	—	—
Penicillin V	IM	7.5	Q. 6 h × 8	8/10	NS‡	0.01	0.005	0.01
Penicillin V	IM	30	×1, then	0/8	0.005	0.01	NS	NS
	IM	7.5	Q. 6 h × 4					
Clindamycin	IM	10	Q. 6 h × 8	6/11	0.05	0.01	0.025	0.01
Erythromycin	IV	15	Q. 6 h × 8	7/11	NS	0.01	0.01	0.02
Untreated controls	—		—	8/8	—	—	—	—

* Number of infected animals/total number of animals.
‡ $P > 0.05$.

lack of sustained antibiotic serum levels, and for this reason, these drugs were not examined further. Determination of serum antibiotic activity showed that no antibiotic exhibited activity for more than 6 h after administration except for tetracycline, where a serum level of 0.29 μg/ml existed 12 h after one intravenous dose.

Because the duration of antibiotic activity was inadequate with 12-h dosage intervals, additional groups of animals were treated every 6 h for a total of 48 h. The first dose was given 30 min before the injection of bacteria. The results of these experiments are summarized in Fig. 7 and Table IV. Penicillin V and cefazolin were the only antibiotics tested that proved effective in preventing the colonization of endocardial vegetations.

More importantly, only when penicillin V was given in a loading dose of 30 mg/kg followed by 7.5 mg/kg at 6-h intervals was infection prevented in all animals. The loading dose appears to be important because without it intramuscular penicillin V in 7.5 mg/kg multiple doses at 6-h intervals left 8 of 10 rabbits with left heart vegetations infected, although the number of organisms cultured from the endocardial vegetations was significantly lower than untreated controls ($P \leqq 0.01$, Wilcoxon rank sum test).

Antibiotic serum levels resulting from these 6-h multiple-dose regimens were more comparable to those resulting from treatment schedules used in man. Under these conditions penicillin V and cefazolin were significantly more effective in preventing streptococcal endocarditis than clindamycin or erythromycin.

The effect of different inoculum sizes on the prevention of infection. In all the experiments described to this point rabbits with endocardial vegetations were challenged with an intravenous inoculum of 2–5 × 10^8 cfu of *S. sanguis* 30 min after the administration of prophylactic antibiotics. This inoculum infects all untreated rabbits with left or right heart catheters, but the number of streptococci per milliliter blood probably exceeds that found in clinical bacteremias by several-fold. A series of experiments was therefore performed in order to determine if various prophylactic antibiotic programs that failed at the high inoculum size might be more effective at a lower inoculum size of 2–3 × 10^5 cfu of *S. sanguis*. From the linear dose-response relationship (Fig. 8) between the proportion of infected animals after the administration of different inoculum sizes plotted against the $\log_{10}$ of the inoculum it was predicted that approximately 60% of untreated animals

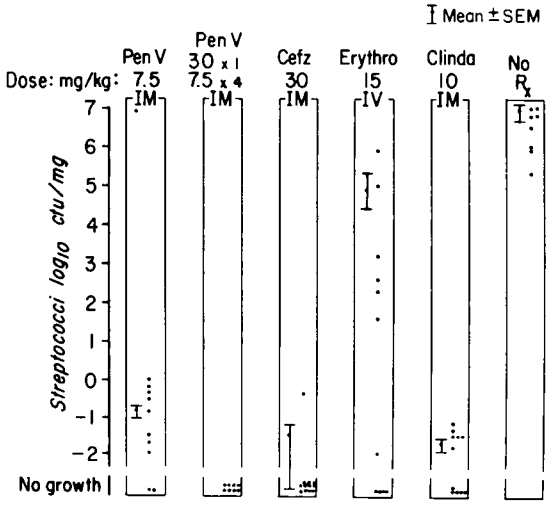

FIGURE 7 Results of quantitative cultures of endocardial vegetations from rabbits with left heart vegetations after multiple-dose 6-hourly antibiotic prophylaxis using an inoculum of $\log_{10}$ 8.0 cfu *S. sanguis*.

TABLE V

Results of Antibiotic Prophylaxis Programs in Rabbits with Left Heart Vegetations Using an Inoculum of $\log_{10}$ 5.4 cfu S. sanguis

Antibiotic	Route	Dose	Frequency	Results	Comparison of antibiotic vs.: Controls (18/30) Fisher test	Controls (18/30) Wilcoxon test	Erythromycin (2/20) Fisher test	Erythromycin (2/20) Wilcoxon test
		mg/kg						
Erythromycin	IV	15	×1	2/20*	0.005	0.01	—	—
Clindamycin	IM	10	×1	7/10	NS‡	NS	0.005	0.01
Cefazolin	IM	30	×1	7/10	NS	NS	0.005	0.01
Penicillin V	IM	7.5	Q. 6 h × 8	4/14	NS	0.02	NS	NS
Untreated controls	—	—	—	18/30	—	—	—	—

* Number of infected/total number of animals.

‡ $P > 0.05$.

would be infected at an inoculum of $\log_{10}$ 5.4 cfu. 18 of 30 control animals with left heart vegetations (60%) receiving the same low inoculum as antibiotic-treated animals were observed to be infected, as predicted from the dose-response curve.

The results of antibiotic prophylaxis experiments in rabbits with left-sided vegetations given an inoculum of $\log_{10}$ 5.4 cfu of *S. sanguis* are summarized and compared to previous comparable $\log_{10}$ 8.0 cfu inoculum experiments in Table V and Fig. 9. Single intramuscular doses of clindamycin 10 mg/kg and cefazolin 30 mg/kg failed to prevent infection or significantly reduce the number of streptococci cultured from endocardial vegetations. In contrast, a single intravenous dose of erythromycin 15 mg/kg prevented infection in 18 of 20 rabbits, compared to 18 infected among 30 untreated controls ($P \leqq 0.005$, Fisher exact test). Thus, erythromycin was more effective against an inoculum of $\log_{10}$ 5.4 cfu than against $\log_{10}$ 8.0 cfu inoculum. In contrast, penicillin V 7.5 mg/kg in 6-hourly doses for 48 h, comparable to doses used clinically, did not show reliable protection against endocarditis at either inoculum size.

To determine if an inoculum greater than $\log_{10}$ 8.0 cfu of streptococci would decrease the efficacy of prophylaxis programs, antibiotics were tested in rabbits with left heart vegetations challenged with an inoculum of $\log_{10}$ 9.2 cfu. The results are summarized in Fig. 10 and Table VI. A single administration of penicillin V 30 mg/kg intramuscularly was significantly less effective in preventing infection with 9/9 animals infected compared to the number of animals infected after a $\log_{10}$ 8.0 cfu inoculum ($P \leqq 0.005$, Fisher exact test). Although ampicillin 30 mg/kg intramuscularly failed to prevent infection at either inoculum size, the number of streptococci cultured from endocardial vegetations was significantly higher after the $\log_{10}$ 9.2 cfu inoculum

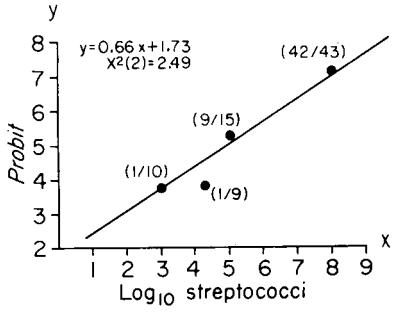

FIGURE 8 Relationship between the probit transformation of the proportion of animals infected and the $\log_{10}$ inoculum size of *S. sanguis* injected into rabbits with left heart vegetations.

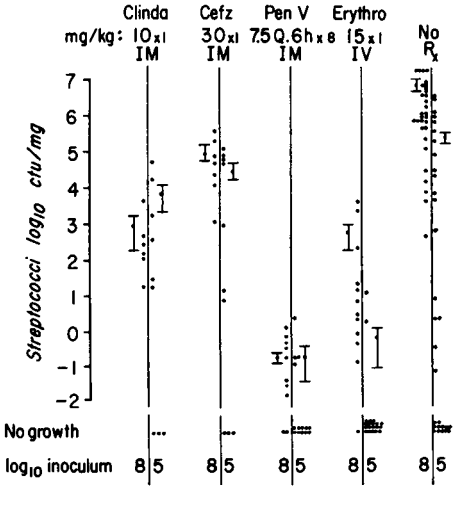

FIGURE 9 Comparison of the quantitative cultures of endocardial vegetations from rabbits with left heart vegetations after antibiotic prophylaxis using an inoculum of $\log_{10}$ 5.4 or $\log_{10}$ 8.0 cfu *S. sanguis*.

TABLE VI

Results of Antibiotic Prophylaxis Programs in Rabbits with Left Heart Vegetations Using an Inoculum of $\log_{10}$ 9.2 cfu S. sanguis

Antibiotic	Route	Dose	Frequency	Results	Comparison of response ($\log_{10}$ 8.0 vs. 9.2 cfu inoculum)	
					Fisher test	Wilcoxon test
		mg/kg				
Penicillin V	IM	30	×1	9/9*	0.005	0.01
Penicillin V	IM	30	×1, then			
	IM	7.5	Q. 6 h × 4	2/9	NS‡	NS
Ampicillin	IM	30	×1	9/9	NS	0.01

* Number of infected/total number of animals.
‡ $P > 0.05$.

($P \leqq 0.01$, Wilcoxon rank sum test). A loading dose of penicillin V 30 mg/kg followed by four 7.5 mg/kg doses at 6-h intervals remained effective at the high inoculum (2/9 animals infected).

DISCUSSION

The present studies expand upon previous observations by Durack, Petersdorf, and Southwick (13, 15) on the antimicrobial prophylaxis of *S. sanguis* endocarditis. For the first time certain parameters affecting the response of endocardial infections to antibiotic prophylaxis were examined in controlled experiments comparing: the response of right-sided vs. left-sided endocarditis, the effectiveness of multiple-dose vs. single-dose endocarditis regimens, the importance of peak vs. sustained antibiotic serum levels, the results of new antibiotics vs. previously tested antibiotics, and the influence of inoculum size on outcome.

In general, there was remarkable reproducibility with this experimental preparation. With one exception which will be commented upon in detail below, all the experiments carried out in Oxford (13) were repeated in Seattle, with identical results.

Initial studies comparing the response of left-sided *S. sanguis* endocarditis to right-sided endocarditis were prompted by the observations of Perlman and Freedman (23) that right-sided staphylococcal endocarditis in the rabbit model spontaneously healed more often than left-sided infection after removal of intracardiac catheters from infected animals. Durack, Beeson, and Petersdorf (18), using the same experimental preparation described in this paper, also showed that 25% of rabbits with right-sided endocarditis and retained catheters spontaneously sterilized their vegetations. Since these studies suggested a difference between right- and left-sided lesions and since the previous prophylaxis studies (13, 14) were conducted almost entirely in rabbits with right-sided endocarditis, previously successful single-dose prophylactic regimens were administered to rabbits with either right-sided or left-sided vegetations, and the animals were sacrificed 24 h after infection to see if there were significant differences in the incidence of infection or the number of streptococci cultured from the endocardial vegetations.

As previously observed (13) either vancomycin 30 mg/kg or the combination of penicillin G 150 mg/kg with streptomycin 15 mg/kg regularly prevented infec-

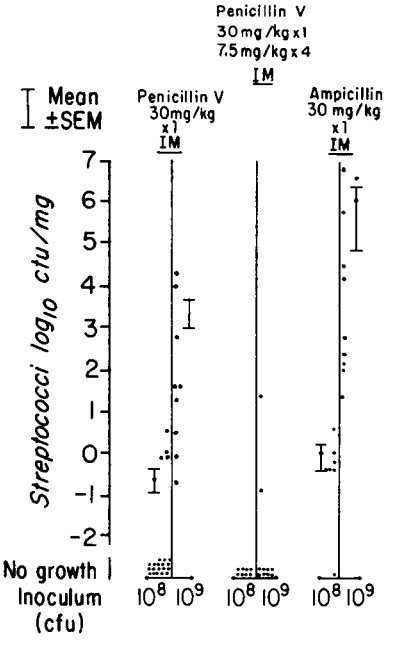

FIGURE 10 Comparison of the quantitative cultures of endocardial vegetations from rabbits with left heart vegetations after antibiotic prophylaxis using an inoculum of $\log_{10}$ 8.0 or $\log_{10}$ 9.2.

tion in rabbits with right-sided vegetations when administered as a single dose 30 min before infection with an intravenous bolus of 10^8 cfu of *S. sanguis*. Both vancomycin and the combination of penicillin G plus streptomycin also were 100% effective in preventing left-sided endocarditis. Benzathine penicillin 7.5 mg/kg combined with penicillin G 150 mg/kg was again shown to be highly effective leaving only 3 of 22 animals colonized with fewer than 1 cfu/mg vegetation. As reported previously, erythromycin 15 mg/kg did not prevent colonization of the right side of the heart, and no difference in the proportion of animals infected or the number of organisms cultured from the vegetations was observed after erythromycin prophylaxis in animals with left-sided vegetations.

In contrast to earlier studies large doses of procaine penicillin seemed less effective because streptococci persisted in 11 of 21 animals with right-sided lesions. This result is not inconsistent with the bactericidal activity of penicillin against viridans streptococci. Hunter (24) and we (25) have shown that penicillin in vitro fails to kill *S. sanguis* completely within 24 h. These results were expanded by Wolfe and Johnson (26), who showed that 48 of 49 penicillin-sensitive strains of viridans streptococci were not completely killed after exposure to the drug for 24 h, although the number of organisms was greatly reduced. A similar phenomenon was observed in vivo in this experimental model because the number of bacteria persisting in the vegetations was very low, usually less than 1 cfu/mg of vegetation. In fact, at 24 h we are observing the tail-end of an in vivo killing curve, which is approaching total eradication of bacteria. Therefore with larger numbers of animals it would not be unexpected that a few viable streptococci were occasionally isolated from the vegetations of penicillin-treated animals. This observation reinforces our previous conclusion that penicillin combined with streptomycin kills viridans streptococci more effectively than penicillin alone both in vitro and in vivo (13, 25).

It also follows from the above that culturing the vegetation 24 h after infection, may not be a suitable end point in animals treated with procaine penicillin. At that time the serum still has bactericidal activity, and killing of persistent bacteria will continue. (The average serum level of penicillin after administration of procaine penicillin 250 mg/kg in two rabbits was 2.84 μg/ml at 24 h, 1.64 μg/ml at 48 h, and 0.21 μg/ml at 72 h). This was substantiated by the results obtained when the animals were sacrificed 6 days after a single dose of procaine penicillin, when the number of animals infected was significantly less than at 24 h.

In contrast, all the other drugs tested except tetracycline, which lasted 12 h, were excreted in 6 h or less after a single administration. This means that for all other drugs, vegetations were cultured in the absence of measurable antibiotic, providing a reproducible index of infection or cure.

Further prophylactic regimens using single and multiple doses were tested in rabbits with left heart vegetations to find practical and reliable programs that might be used in man. Bactericidal drugs were the only effective agents. Multiple administration of bacteriostatic agents including tetracycline (15), clindamycin, and erythromycin consistently failed to prevent infection under the same experimental conditions in which bactericidal agents such as penicillin V and cefazolin were effective.

Study of bactericidal activity in serum showed that both an early peak as well as sustained levels for 6–8 h or longer were required to prevent infection. For example, penicillin V administered at 6-h intervals for 2 days in 7.5 mg/kg doses failed to prevent infection because the initial peak was not achieved. However, when a loading dose of 30 mg/kg provided such a high initial peak, subsequent 7.5 mg/kg doses at 6-h intervals successfully protected all the animals. In other experiments sustained serum levels of cefazolin after 6-h administration for 2 days were protective, when single doses, which produced high but not sustained serum levels, were ineffective. Comparison of the serum levels after intramuscular ampicillin and penicillin V after administration in the same dosage showed that ampicillin produced a higher initial peak but had a shorter duration. After injection of *S. sanguis,* which had the same MIC for both drugs, infection occurred significantly less frequently in animals treated with penicillin V, presumably because serum levels were more prolonged in this group. Only with vancomycin and the combination of penicillin G and streptomycin did duration of serum activity seem less important; with these drugs more rapid rates of in vitro killing were observed.

The inoculum size of streptococci injected into the rabbits was found to influence the effectiveness of several antibiotics. At a low inoculum of $\log_{10}$ 5.4 cfu, which infected 60% of the rabbits, only erythromycin was more effective in preventing infection. Clindamycin, cefazolin, and multiple 7.5 mg/kg doses of penicillin V still failed to prevent infection reliably. This may be of clinical importance, since bacteremia with viridans streptococci in man is probably associated with fewer organisms than were used in these experiments and the drug's increased activity at lower inoculum sizes may explain its apparent effectiveness. While it would be desirable to compare the effectiveness of different antibiotics at even lower inoculum sizes, such experiments would require hundreds of rabbits in order to compare just two antibiotics at a statistical level of significance. For example, the inoculum-infectivity relationship (Fig. 8) for the *S. sanguis* used in these studies shows

that if the inoculum were reduced to log 3.0 cfu only 15 of 100 untreated animals would be infected. To attain statistical significance, antibiotic treatment groups would have to consist of at least 100 animals. Moreover, the relative efficacy of several antibiotics in these studies was not altered by reducing the inoculum size, confirming the relative value of different antibiotics established with high inoculum experiments. Further studies with low inoculum sizes would offer little additional information.

These experiments with rabbit streptococcal endocarditis, together with previous work (13–15), constitute the only firm experimental evidence that the development of bacterial endocarditis is averted by administration of antibiotics and that certain regimens are more effective than others. Successful prophylaxis resulted from the administration of bactericidal agents in doses providing an initial high serum level followed by sustained serum bactericidal activity. The duration of therapy required to prevent infection appeared to depend on the rapidity of bacterial killing by the antibiotic and varied from 8 h with penicillin G and streptomycin to over 24 h with penicillin alone.

To translate the results of experiments in rabbits into recommendations for prophylaxis of streptococcal endocarditis in man certain dissimilar elements between these models must be considered. The use of relatively high inocula and the presence of the cardiac catheter probably make infections more difficult to prevent in rabbits than in man. Therefore, antibiotics that were successful in preventing infection under these conditions probably have a wide margin of safety. Moreover, the pharmacokinetics of antibiotics in small animals are different from man and do not permit direct translation of doses from animals to man.

Although a number of prophylaxis failures have been reported (27–31) no systematic evaluation of currently recommended prophylactic regimens has been performed. Since the chance of developing bacterial endocarditis after procedures known to produce bacteremia with the viridans streptococci is very low, clinical studies of the relative efficacy of different antibiotics in the prevention of streptococcal endocarditis yielding statistically significant results would require large numbers of patients. They are not likely to be done. In the absence of such studies, data from these experiments on the relative efficacy of antibiotics in the prophylaxis of bacterial endocarditis should be considered in developing clinical recommendations.

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