The 1976 Paul B. Beeson Lecture
Some Observations on Experimental Endocarditis

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INTRODUCTION

Holding forth in this amphitheater is a true deja vu experience for me because years ago I conducted Grand Rounds many times from the very same podium. I am delighted to see that in the 18 years since my departure, the Fitkin amphitheater has retained the same threadbare gentility that characterized it during my tenure here as a student and house officer.

It is now almost 24 years to the day since I first met Paul Beeson. He had just arrived from Emory, where he had been Chairman of the Department of Medicine a few short days before. In order to get acquainted with the students and housestaff, he used to go to the housestaff dining room for his meals. He had heard of the industry and dedication of the Yale housestaff and since he believed that getting to know them was very important, on his first morning in New Haven he went to the dining room at 7:15 a.m. to join them for breakfast. To his surprise, none of the house officers was there. That impressed him a great deal because he figured that they were already on the wards, so the next morning he came in at 7:00 o’clock and again failed to find the housestaff. On the third day, he came in at 6:45 a.m. only to find the dining room closed. He eventually learned that the usual breakfast time for Yale house officers was closer to 8:15 than 7:15 [1].

I first met him at dinner. It was a month or two before I was to graduate and I was subinterning on ENT, eating the usual diet of kale in which the dining room of the New Haven Hospital specialized at that time, when Beeson sat down with me. He had learned that I was going to be an intern on the medical service and asked what I was planning to do with my future. I allowed that I was interested in clinical medicine and teaching, and that perhaps that combination would be good enough for an academic career. He pointed out that the only true road to success was clinical investigation. He was certainly right about that at the time—in fact, he may still be right about it. Although my beginning with Beeson may have been less than auspicious, we soon became fast friends. He took great interest in his housestaff, and came to know them professionally and personally. Those of us who had the privilege of being chief residents formed particularly warm relationships with him.

Beeson settled in quickly at Yale; he had brought with him two bright young men—Bennett and Bondy—the first to help him set up his fever laboratory and the
second to play a vital role in the metabolic unit. Soon he recruited other faculty, many of whom like Lish Atkins, Aaron Lerner, Gil Glaser and Howard Spiro, to mention just some, are still here. By 1954, he had the service firmly under control and he had put his stamp indelibly upon it. His stamp was that of a doctor and a teacher. He was never too busy to see a patient—day or night—and he took exquisite pains to show younger physicians the importance of a sensitive patient-doctor relationship. For example, he always chose a patient with a fatal disease as the subject of his first lecture to the freshman medical students. Once such a patient was selected by the resident, he would spend several hours with him each day so he could grasp the nuances that characterize the fragile bond between a dying patient and his physician. He always went to great lengths to point out how poorly the housestaff succeeded in ministering to this type of patient’s complaints; not only his pain, but his insomnia, his diet, and even his constipation. Then there was the time that the chief resident took him around to see three or four patients with complex febrile illnesses. Each of these patients was receiving multiple antimicrobials and none was getting better. His advice in each instance was the same—stop the drugs. At the conclusion of the rounds the resident commented that they really had not done much that day. The enigmatic Mona Lisa smile that some of us came to know so well crossed Beeson’s face and he agreed that they probably had not, but we all knew differently. It was by this kind of teaching that he left lasting impressions on his students and housestaff.

While at Yale, Beeson rejuvenated research in the field of urinary tract infections. Later he took a sabbatical—probably the first chairman in the history of the school to do so—and went to the Wright-Fleming Institute where he and Rowley demonstrated clearly the effect of ammonia on the susceptibility of the kidney to infection [2]. When he left on sabbatical, his department had grown in size and stature and when he returned all felt that he would never leave again. With the major recruiting and building behind him, he had a chance to plan for the future and it was largely his efforts that culminated in the grant from the Commonwealth Fund for the Laboratory of Clinical Investigation. It came as a great surprise, therefore, when he announced in 1965, after 13 years at the helm, that he was leaving Yale to accept the Nuffield Professorship of Medicine at Oxford. As at Yale, he wrought a number of important changes at that institution. In addition, he served as host for a variety of visiting professors—many from Yale such as Tom Ferris, Lish Atkins and Bill Hollingsworth, as well as from elsewhere. In 1971–72, there were four American professors in Beeson’s firm in Oxford, three of whom (Louis Welt, Franklin Epstein and myself) had strong ties with Yale.

During my year at Oxford, Beeson rejuvenated my investigative career. Although I had had an active laboratory dealing with fever, experimental meningitis, antibiotic pharmacology and urinary tract infections, I was working more and more through surrogates and was getting farther and farther away from the bench. When I came to Oxford, I had no specific plans but it soon became apparent that the way to get the best from Beeson was to take advantage of his remarkable gift for asking the right clinical questions and then taking them to the bench for rigorous examination in the laboratory. My contact with endocarditis began in Oxford in 1971 and has continued ever since. It seems appropriate that my lecture today should be on a subject on which Beeson and I worked together under such pleasant circumstances.

As most of you know, my personal relationship with Beeson has continued and since October 1974, he has been a member of the Department of Medicine in Seattle and Distinguished Professor at the Seattle Veterans Administration Hospital. Although he is no longer active in the laboratory, he is doing all of the other things as
well as ever—rounding, teaching and doctoring, and I am delighted that the students and housestaff at Washington now have the same privilege of being exposed to Paul Beeson that we had when we were students and house officers at Yale (Fig. 1).

FIG. 1. Paul B. Beeson, M.D.

THE EXPERIMENTAL MODEL

When I arrived at the Radcliffe Infirmary in Oxford, Beeson and a young doctoral candidate in his laboratory, David Durack, had just modified the experimental model for endocarditis previously produced by Garrison and Freedman\(^3\) [3]. The Garrison-Freedman rabbit model consisted of a plastic polyethylene catheter that was inserted into the right heart through the femoral vein, filled with a culture of staphylococci, and subsequently sealed at the distal end. Several days after insertion of the catheter, the rabbits developed staphylococcal endocarditis in the area of the tricuspid valve. If the catheter was filled with sterile saline, small sterile vegetations developed at the site where the catheter was in contact with the valve in the endocardium. The Beeson-Durack modification was as follows: Reasoning that bacterial endocarditis often developed when a pre-existing sterile vegetation was seeded by circulating organisms, they inserted a catheter into the right heart through the jugular vein and left it in place for 24–48 hours. By that time, a sterile vegetation had invariably formed. If a culture of virulent organisms was then injected intravenously, the bacteria lodged on the sterile vegetation, converting it to an infected vegetation. This model lent itself to ready quantification because the vegetation could be excised in toto, homogenized, and the number of organisms enumerated [4].

A further modification of this model, which has been used by most investigators, consists of the production of left-sided endocarditis by the insertion of a catheter into the carotid artery and its placement in the aorta at the site of the aortic valve [5]. This invariably results in the formation of a sterile vegetation in the area of the aortic valve. The sterile vegetation provides a favorable nidus for colonization by circulating organisms. When streptococci were injected into the bloodstream, they lodged on

\(^{3}\)Dr. Lawrence R. Freedman was Editor-in-Chief of The Yale Journal of Biology and Medicine from 1965 to 1973.
the vegetation and entered a logarithmic phase of growth, while in organs such as the liver or spleen, they were gradually killed [4].

Pathologically, the vegetations are remarkably like those found in man. They consist of a relatively amorphous mass of fibrin, platelets and platelet debris, and bacteria. Leukocytes are scanty. Organisms may be found deep within the fibrin matrix or, early in the infection, on the surface. When right-sided infection is produced with *Streptococcus sanguis*, about one-third of animals recover spontaneously, while the other two-thirds die with continuing infection and heart failure. On the left side of the heart, infection is progressive and death is almost invariable.

PREVENTION OF EXPERIMENTAL ENDOCARDITIS WITH ANTIBIOTICS

It is well known that cases of endocarditis may develop following dental manipulation, or surgery of the genital, urinary and gastrointestinal tracts. Chemoprophylaxis of bacterial endocarditis has therefore become accepted practice, but its efficacy has not been put to clinical trial. As a matter of fact, a clinical trial would be virtually impossible because of the relatively low incidence of bacterial endocarditis following bacteremia. Studies in an animal model therefore seemed to be the best alternative available. In our prophylaxis experiments [6,7] we gave antibiotics 30 minutes before intravenous injection of bacteria into catheterized rabbits.

The experiments were performed with a strain of *Streptococcus sanguis* which was sensitive to 0.02 micrograms per ml of penicillin G. We thought, therefore, that the administration of penicillin to rabbits within 30 minutes of inducing bacteremia should prevent colonization of the vegetation. To our surprise, doses of aqueous penicillin that were comparable on a weight basis to those deemed to be effective in preventing infection in man were not effective in these rabbits (Table 1) [5]. In fact, not until very large doses of penicillin were administered repeatedly was prevention achieved. We thought that the surprising failure of aqueous penicillin might be related to its rapid excretion, and for this reason procaine penicillin was tested. Again, however, this longer-acting form was ineffective until large doses—150,000 units per kilo, which is equivalent on a weight basis to about 20 million units in man—were used.

In view of the failure of procaine penicillin in ordinary doses, we wondered whether it might not be necessary to provide penicillin in the blood for a more prolonged period. For this reason, we gave the rabbits benzathine penicillin, a formulation which is excreted very slowly. However, it also was ineffective.

These data suggested that with penicillin an initial high peak followed by a demonstrable serum level for a relatively prolonged period of time was necessary for the drug to be effective. We tested this hypothesis by treating the animals with a combination of aqueous crystalline penicillin, which was excreted very rapidly, along with a modest dose of benzathine penicillin to provide relatively long exposure (Table 1). This regimen worked in every instance. Measurements of serum levels in the animals showed that regimens that provided a high initial peak, followed by moderate levels for 12–24 hours were effective. These included large doses of procaine penicillin, and the combination of aqueous penicillin and benzathine penicillin. On the other hand, regimens that failed to achieve the high initial peak or to maintain an adequate level of drug in the animal's serum were not effective.

Attempts to simulate these serum levels in man showed that a single administration of procaine penicillin and repeated administration of penicillin V would not provide the initial peak. Only a combination of crystalline aqueous penicillin plus aqueous procaine penicillin provided the initial high peak and the relatively prolonged levels that we sought.
TABLE I
Comparison of Various Antibiotic Regimens
in Prevention of Experimental Strep. Sanguis Endocarditis

| Dose                               |
|------------------------------------|
| IN EFFECTIVE                       |
| Ampicillin                         | 30 mg/kg |
| Aqueous penicillin G               | 6–150 mg/kg |
| Penicillin V                       | 7.5 mg/kg |
| Cefazolin, cephalaxin, cephaloridine | 30 mg/kg |
| Clindamycin                        | 5 mg/kg |
| Erythromycin                       | 15 mg/kg |
| Rifampin                           | 20 mg/kg |
| Tetracycline                       | 15 mg/kg |
| Trimethoprim                       | (17 mg/kg) |
| —sulfamethoxazole                  | (3.4 mg/kg) |
| PARTIALLY EFFECTIVE                |
| Procaine penicillin G              | 50–250 mg/kg |
| Erythromycin (repeated 6 hourly x 8)| 15 mg/kg |
| Streptomycin                       | 15 mg/kg |
| Cefazolin plus streptomycin (both) | 15 mg/kg |
| Penicillin V                       | 30 mg/kg |
| EFFECTIVE                          |
| Aqueous penicillin G               | (150 mg/kg) |
| plus benzathine penicillin         | (7.5 mg/kg) |
| Penicillin V—loading dose,         | 30 mg/kg |
| then 4 further doses, 6 hourly     | 7.5 mg/kg |
| Aqueous penicillin G (or ampicillin| 150 mg/kg |
| 30 mg/kg)                          | 15 mg/kg |
| plus streptomycin                  | 15 mg/kg |
| Vancomycin                         | 15–30 mg/kg |

Interesting as these data were, they did not provide us an ideal regimen for use in man because many dentists would not be willing to administer parenteral penicillin to their patients prior to dental manipulation or surgery. We therefore sought alternatives.

Among the several alternative bactericidal drugs tested, only vancomycin was uniformly effective. The reason for vancomycin’s effectiveness is not solely the high level which could be achieved in serum. Other drug regimens that were not successful resulted in serum bactericidal dilutions in excess of those resulting from vancomycin. Vancomycin’s effectiveness is probably related to its dual mechanism of action which we believe is both on the cell wall and on other components of the microorganism. Of the bacteriostatic drugs tested, none was effective despite the fact that the levels achieved with those drugs exceeded the minimum inhibitory concentrations several-fold.

In looking for an effective regimen, we harkened back to some experiments done by Hunter almost 30 years ago demonstrating that in vitro the combination of penicillin and streptomycin was more effective against sensitive viridans streptococci than penicillin alone [8]. Penicillin kills off the vast majority of a culture of sensitive organisms but leaves a number of persisters. These bacteria are probably in the stationary phase, not synthesizing cell wall material and hence not susceptible to penicillin. Complete killing can be achieved by use of a second drug such as streptomycin, which acts at a different site in the bacterial cell. Synergism between penicillin and streptomycin cannot be demonstrated in ordinary tube-dilution or serum inhibi-
tion tests because these streptococci are overwhelmingly sensitive to penicillin alone. If a bacterial killing curve is performed, it shows that a handful of organisms persist after exposure to penicillin for 24 hours. In contrast, there are no persisting organisms when streptomycin is added to penicillin, even though the concentration of streptomycin is not inhibitory [6,9].

When these observations were transferred to the experimental model, we showed that in every instance the combination of penicillin G and streptomycin sterilized the vegetations when neither drug alone was able to do so.

To this point, then, the results may be summarized as follows:
1. The most effective regimen in preventing experimental Strep. viridans endocarditis in rabbits was a single dose of penicillin and streptomycin. Neither of these drugs was effective when used alone.
2. Of the various penicillins, only procaine penicillin in large doses or multiple large doses of aqueous penicillin were effective.
3. Bacteriostatic drugs were ineffective.
4. Vancomycin was the only alternative drug that was capable of preventing infection.

Repeated administration of a number of agents was then tested. These included penicillin V, ampicillin, erythromycin, tetracycline, clindamycin and cefazolin [7]. Although some of these regimens reduced the number of bacteria in vegetations, none was effective in preventing infection entirely.

Although penicillin V was not effective when given at 6-hourly intervals, we found one characteristic of this drug that made it attractive. While ampicillin was excreted rapidly, penicillin V was excreted more slowly and we worked on this observation to attempt to find an oral regimen that could be recommended for use in man. This program consists of the administration of a loading dose of penicillin V followed by four maintenance doses at 6-hourly intervals. Unfortunately, an effective oral alternative to penicillin is not yet at hand.

Of course, this model represents a severe test of the efficacy of any antibiotic regimen because we used an inoculum which would render virtually every animal infected—in other words, an ID100. Hence, the number of organisms injected in these experiments is greater than would be expected under natural circumstances in man. In a lengthy experiment we calculated the ID50 for this organism and determined that it was approximately $10^{5.5}$ bacteria. We then infected groups of animals with the lower inoculum and found that the lower inoculum made little significant difference in the relative response to the various drugs, with the exception that erythromycin, which had been ineffective at the higher inoculum size, was more effective at the lower one.

In summary, we extended our observations on the prophylaxis of streptococcal endocarditis to include the following:
1. Pen V is effective with a 2 gram loading dose and repeated administration.
2. Cefazolin is partially effective when used in combination with streptomycin.
3. Erythromycin may be a good second-line drug.

**PREVENTION OF ENTEROCOCCAL ENDOCARDITIS**

Next, let me turn briefly to a series of experiments that we have just completed on the prophylaxis of enterococcal endocarditis [10]. This is the organism that is most likely to cause endocarditis following operative procedures involving the urinary, genital and gastrointestinal tracts. A total of 11 strains of enterococci were studied in the rabbit model. Ninety-six percent of untreated animals were infected. Ampicillin
alone left 67% infected and even when the drug was given repeatedly over 48 hours, 50% of animals remained infected. Gentamicin alone was not effective, but the combination of ampicillin and gentamicin or ampicillin and streptomycin was effective in preventing infection in approximately 80% of animals. High dose vancomycin was the best regimen tested, but a lower dose was less successful. Streptomycin plus the lower dose of vancomycin was very effective against strains that were not highly streptomycin resistant. Cefazolin was ineffective against two strains, even when combined with gentamicin.

There were notable variations between individual strains, and results in vivo could not always be predicted from in vitro sensitivity data. For example, cefazolin was ineffective at doses which gave serum levels that were well above the MIC's for the strains tested, while streptomycin alone prevented infection in about half the animals tested even though serum levels of streptomycin were well below the MIC's for these enterococci. These results indicate that the efficacy of prophylactic antibiotic regimens cannot be predicted reliably from in vitro MIC's.

There are a number of significant differences between this animal model and the clinical situation; it is therefore important that our findings on prophylaxis of bacterial endocarditis should not be extrapolated too literally to man. We have emphasized that the correct use of these experimental findings is to compare antibiotic regimens in vivo and rank them in order of efficacy. In other words, use of the rabbit model cannot define precisely which regimens will be successful in patients and which will fail, but it can indicate which regimens should provide the widest margin of safety [11].

TREATMENT OF EXPERIMENTAL ENDOCARDITIS

I would next like to illustrate how this model can be used to evaluate therapeutic regimens [12,13]. In the initial prophylaxis experiments we included a group of animals that did not receive antibiotics until 6 hours after injection of bacteria. Prophylactic regimens that were successful when given within 30 minutes of injecting bacteria failed after a 6-hour delay. Cure could only be achieved by giving repeated doses of penicillin. Synergism was demonstrated between penicillin and streptomycin in this short-term model for therapy of endocarditis [12], and soon afterward was independently confirmed by Sande [14]. In order to simulate the situation in man more closely, we then interposed a longer delay between infection and treatment, allowing the vegetation to evolve for 48 hours before administering antibiotics. When these animals were treated with penicillin alone, it took approximately 10 days to sterilize the vegetations in 50% of the animals, and even after two weeks not all were cured. Penicillin and streptomycin cured half the animals in approximately 6 days, and all within two weeks. Animals from which the catheters were removed responded slightly better to therapy but in both instances penicillin and streptomycin was better than penicillin alone [12].

STUDIES IN PROGRESS

Finally, I want to summarize briefly some of the studies that are presently in progress. We and others are trying to define the factors in pathogenesis affecting infectivity or susceptibility to bacterial endocarditis.

It has been postulated that certain structural components of oral streptococci, namely dextrans, render them more likely to cause endocarditis by increasing their "stickiness" [15]. However, in some recent experiments we have shown this not to be the case. Taking a dextran-producing organism and removing dextran by selecting
out a dextran-negative mutant made no difference to that organism's infectivity [16].

We are looking at the role of humoral antibody in the pathogenesis of this infection. Bacterial endocarditis is an infection that progresses despite rising titers of antibody. It is presumed that antigen-antibody complexes are formed between the bacterial antigen and its antibody, and it may be these complexes that cause tissue damage, including nephritis [17]. They are also suspect in causing persistent infection on the heart valves. This subject has assumed particular importance recently because of the suggestion that immunization against oral streptococci may lead to the prevention of dental caries. The question then arises whether such immunization could alter susceptibility to endocarditis; this question can be examined conveniently in our rabbit model. We are in the process of doing some experiments to test the effect of prior immunization on the susceptibility of rabbits to bacterial endocarditis. If immunization renders animals more susceptible to infection, it would probably affect the eventual manufacture of these vaccines.

We have recently shown that serum-resistant strains of *E. coli*, that is, strains that are not susceptible to the bactericidal effect of serum, are much more prone to produce endocarditis than serum-sensitive strains of *E. coli*. It appears that this phenomenon is mediated by complement, because when rabbits genetically deficient in C6 were tested, a serum-sensitive strain that did not infect normal rabbits caused *E. coli* endocarditis in the C6 deficient rabbits. The bactericidal action of serum may be a major factor determining the relative rarity of endocarditis due to *E. coli* and other bacteria that are killed by serum [18].

Sanford and his colleagues have devised a technique whereby they incubate excised dog heart valves with various bacteria. Gram-positive organisms, which are much more prone to cause endocarditis, stuck to the valve in greater numbers than gram-negative organisms. This is, of course, a somewhat artificial *in vitro* system, but the correlation with what happens in patients is noteworthy [19].

The role of leukocyte and reticuloendothelial function in the development of infection has not been evaluated. Equally important is the question whether the infection can be caused or perpetuated by incomplete bacterial forms.

CONCLUDING REMARKS

I am sure that by now you have thought of any number of experiments that you yourself may want to do. This model is so simple to work with that it has in a sense become the "Volkswagen" of experimental infectious disease.

There are now at least a dozen groups in this country working on the problem, where in 1972 there were only two. The availability of this easily reproducible and quantifiable experimental model may make bacterial endocarditis one of the best-studied infections. Moreover, there is every likelihood that some of the results obtained will be applicable to man. In fact, the American Heart Association is currently reviewing their recommendations for SBE prophylaxis in the light of our findings.

Experimental endocarditis in rabbits is a good example of Beeson's great gift for identifying an important clinical problem and finding the right way to study it in the laboratory. He has scored again.

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