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Effects of Immunization and Anticoagulation on the Development of Experimental *Escherichia coli* Endocarditis

LOUIS THÖRIG,* JAN THOMPSON, AND RALPH VAN FURTH

Department of Infectious Diseases, University Hospital, Leiden, The Netherlands

The effects of immunization and anticoagulation in experimental *Escherichia coli* endocarditis were studied. Immunization of rabbits with *E. coli* resulted in the development of specific agglutinating and opsonic activity of the serum, but not in bactericidal activity. These antibody activities also developed in nonimmunized rabbits during the course of bacterial endocarditis. Immune serum promoted phagocytosis in vitro but did not enhance intracellular killing of *E. coli* by elicited rabbit peritoneal macrophages. The presence of specific antibodies in rabbits after immunization had no effect on the induction or course of *E. coli* infection of endocardial vegetations. Anticoagulation was found to affect the induction of the infection. In anticoagulated rabbits, larger bacterial inocula were needed to induce an infection, but in animals with bacterial endocarditis the number of bacteria in the vegetations did not differ significantly from that of the control animals.

Gram-negative bacteria represent only a minority in the spectrum of bacteria causing endocarditis on natural heart valves, although they are more frequently involved in the endocarditis of patients with prosthetic heart valves, intravenous narcotic users (28), and immunocompromised hosts (10). This probably reflects the difficulty these bacteria encounter in forming septic metastatic foci (22), in all likelihood caused by the bactericidal activity of specific antibodies and an intact complement system. This mechanism might prevent the colonization of endocardial vegetations by blood-borne bacteria in patients with gram-negative bacteremia (4, 14). This seems to be confirmed by three recent studies showing that experimental endocarditis could only be induced with serum-insensitive strains of gram-negative bacteria (2, 7, 12). However, in these studies the role of non-bactericidal antibodies in the induction of bacterial endocarditis (BE) was not explored.

Although preexisting type-specific antibodies were formerly thought to be a predisposing factor for bacterial endocarditis, recent studies on preformed antibody in the serum of rabbits immunized with *Streptococcus sanguis, Streptococcus mutans* (8, 20), *Staphylococcus epidermidis* (25), or *Pseudomonas aeruginosa* (3) have shown that the presence of such antibodies does not play a significant role in the genesis of endocarditis.

After attachment, bacteria on the surface of the vegetation are covered by fibrin. Fibrin formation could be initiated by the generation of tissue thromboplastin by monocytes on the vegetational surface, activated by the phagocytosis of bacteria (27) or by bacterial endotoxin (18). However, endotoxin could also trigger local blood coagulation via other pathways (1, 16, 29). The contribution of fibrin formation to the development of bacterial endocarditis is probably not uniform, since anticoagulant treatment with warfarin has an inhibitory effect on the development of *S. epidermidis* endocarditis (23) but does not affect the development of *S. sanguis* endocarditis (11, 23).

The aim of the present study was to investigate the effect on the development of an experimental *Escherichia coli* endocarditis of increased levels of specific serum antibodies after immunization and of reduced blood coagulability after warfarin treatment.

MATERIALS AND METHODS

Experimental design. The study was done with male Chinchilla rabbits weighing 2.0 to 2.5 kg that were raised in the Central Institute for the Breeding of Laboratory Animals, Bilthoven, The Netherlands.

For immunization, rabbits were given a daily intravenous injection of bacterial vaccine on 12 consecutive days. The dose injected was 0.5 ml on the first 3 days and 1.0 ml on the following 9 days. Concomitantly, a group of control rabbits was given daily intravenous injections of equal volumes of saline according to the same scheme.

For anticoagulation, a group of normal rabbits was given warfarin daily as in our previous studies (23, 24). Anticoagulant treatment was started on day 1 after nonbacterial thrombotic endocarditis (NBTE) induction.

NBTE was induced by the method of Durack and Beeson (6) by inserting a plastic catheter into the left

ventricle via the left carotid artery. Three days after the catheterization for NBTE induction, control, immunized, and anticoagulated rabbits were inoculated with various numbers of live *E. coli* to induce bacterial endocarditis.

Microorganisms. *E. coli* 054 was cultured overnight in nutrient broth no. 2 (Oxoid Ltd., London), harvested by centrifugation at 1,500 × *g* for 10 min, and washed twice with phosphate-buffered saline (PBS) (pH 7.2). This strain is known to be serum resistant (no bacterial death occurs when 10^6 *E. coli* are incubated in 90% normal rabbit serum for 24 h). Bacterial vaccine containing 10^9 heat-killed bacteria per ml was prepared by heating the overnight cultures at 60°C for 2 h.

Anticoagulant treatment. Warfarin sodium (kindly donated by ENDO Laboratories Inc., New York, N.Y.) was used as anticoagulant. A daily dose of 8 mg was given intramuscularly except for the first dose, which was given intraperitoneally. Within about 48 h (i.e., just before the *E. coli* injection) this treatment resulted in a reduction of the factor II, VII, and X activity levels from control values of 86, 30, and 62% to 25.3, 1.3 and 5.2%, respectively. Furthermore, there was a corresponding prolongation of prothrombin and partial thromboplastin times from control values of 11 and 26.2 to 82.7 and 40.7 s, respectively. Within about 72 h (i.e., day 1 of manifest infection) the factor II, VII, and X activity levels were reduced to 17, 1.2, and 2.3%, and the prothrombin and partial thromboplastin times were prolonged to more than 180 and 48.8 s, respectively.

Sera. For serological studies, serum samples were taken on the day of catheterization for NBTE induction and at various time points after that. To prepare the sera, we allowed the blood to clot for 4 h at room temperature and then centrifuged it for 20 min at 1,500 × *g*, after which the supernatant serum was filtered through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). The sera were stored at −20°C until use.

Mononuclear phagocyte suspensions. For the measurement of phagocytosis and intracellular killing of bacteria, we elicited exudates in the peritoneal cavity of normal rabbits as indicated elsewhere (5). Of the harvested cells, about 90% were macrophages and about 5% were lymphocytes; granulocytes accounted for less than 5% of the cells. Washouts gave an average yield of 5.5×10^7 macrophages per rabbit. After the cells had been washed twice with PBS (pH 7.2), a suspension of 10^7 cells/ml in Hanks balanced salt solution (HBSS) (pH 7.2) containing 0.1% (wt/vol) gelatin (Difco) (gelatin-HBSS) was prepared. Siliconized glass tubes were used throughout.

Agglutination assay. Agglutinating activity of serum was investigated by incubation of equal volumes of serial twofold dilutions of serum in PBS with 10^8 heat-killed bacteria per ml in microtiter plates at room temperature for 24 h. The agglutinating activity of serum is expressed as the reciprocal value of the highest dilution showing flake-like agglutination, read macroscopically.

Opsonization of bacteria. For the investigation of opsonic activity, we incubated 5×10^6 bacteria per ml with various concentrations of immune or normal serum for 30 min at 37°C under slow rotation (4 rpm), followed by centrifugation at 1,500 × *g* for 10 min and two washings in ice-cold gelatin-HBBS to remove excess serum. The bacteria were then suspended in gelatin-HBBS to a concentration of about 10^7/ml for the phagocytosis assay (26).

Phagocytosis assay. Phagocytosis was measured as described elsewhere (26) and expressed as the percent decrease of the initial number of viable bacteria calculated according to the formula $F(60) = (1 - N_{60}/N_0) \times 100$, in which $F(60)$ is the phagocytic index at 60 min, N_0 is the number of viable extracellular bacteria at 0 min, and N_{60} is the number of viable extracellular bacteria at 60 min.

Intracellular killing assay. Intracellular killing of bacteria was measured independently of phagocytosis as described elsewhere (15). At 0 and 60 min, the number of viable intracellular bacteria was determined and expressed as the percent decrease of the initial number of viable intracellular bacteria calculated according to the formula $K(60) = (1 - N_{60}/N_0) \times 100$, in which $K(60)$ is the intracellular killing index at 60 min, N_0 is the number of viable intracellular bacteria at 0 min, and N_{60} is the number of viable intracellular bacteria at 60 min.

Evaluation of bactericidal activity of serum. For this assay, overnight cultures of *E. coli* in nutrient broth no. 2 were diluted to a concentration of about 10^6 bacteria per ml. This suspension was incubated for 1 h at 37°C in a shaking water bath. Next, 0.5-ml samples of the culture were added to 4.5 ml of various kinds of rabbit serum and reincubated at 37°C for 24 h, and then 0.1-ml samples were taken at given time points and the number of viable bacteria was determined as described elsewhere (13).

Quantitative bacteriology for blood and endocardial vegetations. The methods used for quantitation of bacteria in blood and infected vegetations have been described elsewhere (23, 24). The number of bacteria in the blood was determined in samples from an ear vein at various time points between the onset of an infection and at the time at which the animal was killed, and expressed as the number of bacteria per milliliter. The degree of infection of the endocardial vegetations was expressed as the number of bacteria per gram of vegetation (23, 24).

Statistical analysis. The results obtained in the various groups were compared by a two-tailed Student's *t* test.

RESULTS

Agglutinating activity of serum. Normal serum had low agglutinating activity for *E. coli*. Immunization of rabbits resulted in a high agglutinating activity of the sera against *E. coli* (Table 1); agglutinating antibodies also appeared during *E. coli* endocarditis (Table 1).

Opsonic activity of serum. During immunization, opsonic activity for *E. coli* developed in the serum. With normal serum, maximal phagocytosis of *E. coli* by rabbit peritoneal macrophages was obtained after pre-opsonization with 10% (vol/vol) serum, and virtually no

TABLE 1. *Levels of serum agglutinating activity*[a]

Microorganism	Normal rabbit serum[b]	Immune rabbit serum[b]	BE rabbit serum[d]
E. coli	16 (2-64)	40,960 (10,240-163,840)	2,560 (1,024-5,120)

[a] Each value is the median of at least four sera (range given in parentheses); serum dilutions are expressed reciprocally.

[b] Collected from control rabbits on the day of catheterization.

[c] Collected from immunized rabbits on the day of catheterization.

[d] Collected from nonimmunized rabbits with BE taken at the time of sacrifice.

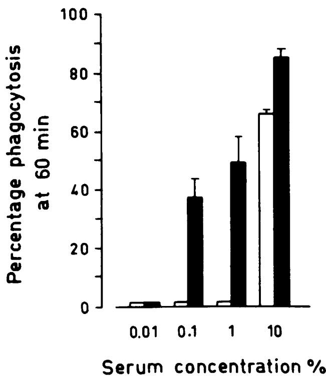

FIG. 1. *Effect of immunization on the opsonic activity of serum, as measured with a phagocytosis assay (26). For this assay, bacteria were preopsonized with various percentages of normal serum (□) or immune serum (■). Phagocytosis occurred at a bacteria-to-macrophage ratio of 1:1. The values are means ± standard deviation of three experiments. At all concentrations the difference between phagocytosis of bacteria preopsonized with immune and normal serum is significant (P < 0.01).*

phagocytosis occurred when 1% (vol/vol) serum was used (Fig. 1). With immune serum, a significantly higher level of ingestion was obtained after pre-opsonization of the bacteria with 0.1% (vol/vol) or more (Fig. 1). During *E. coli* endocarditis opsonic antibodies developed, and phagocytosis was obtained with 1% (vol/vol) BE serum (taken just before death from nonimmunized rabbits with BE) (phagocytic index, 42%).

Bactericidal activity of serum. There is a slight difference between the growth of *E. coli* in normal serum, serum from immunized rabbits, and serum from rabbits with BE (Fig. 2), but this difference is not statistically significant ($P > 0.2$).

Effect of serum on intracellular killing of bacteria. For the measurement of intracellular killing, *E. coli* was preopsonized with 10% (vol/vol) normal or immune serum and incubated with peritoneal macrophages for 1 min under rotation. When normal serum was used, 5×10^6 macrophages contained 8×10^5 to 1.9×10^6 viable intracellular bacteria after phagocytosis and 5.9×10^6 to 7.4×10^6 when preopsonized with serum of immunized rabbits. The difference between these values is significant ($P < 0.01$). Next, these macrophages with ingested bacteria were reincubated in the presence of 10% (vol/vol) serum, and the survival of the intracellular bacteria was assessed. The killing index obtained in the presence of normal serum did not differ from that in the presence of immune serum ($P > 0.2$) (Table 2).

Effects of immunization and of anticoagulation on the induction of bacterial endocarditis. To find out whether immunization or anticoagulation influences the induction of infection in sterile heart vegetations, control, immunized, and anticoagulated rabbits were infected with microorganisms after the establishment of NBTE. Immunization had no effect on the induction of *E. coli* endocarditis, as judged from the number of rabbits with an infected vegetation 1 day after the injection of various numbers of bacteria (Table 3). Anticoagulation, however, seems to have an influence on the induction of

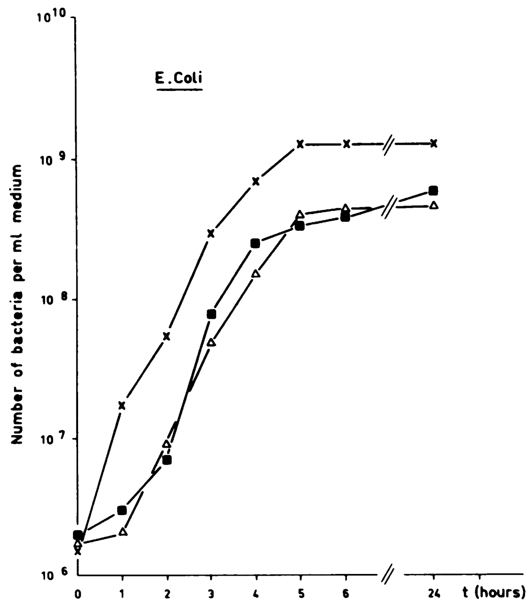

FIG. 2. *Effect of immunization on bactericidal activity of serum. Growth of E. coli in various rabbit sera was measured. Normal (90%, ×) and immune (90%, ■) were sampled on the day of catheterization. BE serum (90%, △) was collected from nonimmunized rabbits just before the animals were killed. Each curve represents the results of three experiments.*

infection of the sterile vegetations, since the anticoagulated rabbits required larger inocula for infection. In this group the injection of 10^8 *E. coli* resulted in infected vegetations in all of the animals, whereas 10^5 microorganisms failed to infect any of them (Table 3). The 50% infective dose, calculated according to Spearman-Kärber (9), was 1.0×10^6 for the control and 1.3×10^7 for the anticoagulated rabbits. The difference between these values is significant ($P < 0.01$) (Table 3).

Effects of immunization and of anticoagulation on survival. Control and immunized rabbits with *E. coli* endocarditis survived at least 14 days. However, anticoagulated rabbits with *E. coli* endocarditis were already moribund on day 3 of infection. At autopsy, extensive pulmonary edema and pulmonary hemorrhages were found.

Effects of immunization and of anticoagulation on the degree of infection of vegetations. All rabbits had infected vegetations at autopsy (Table 4). The number of bacteria increased significantly from day 1 to day 14 in both control and immunized animals, but this increase was absent from day 1 to day 3 in both control and anticoagulated animals. All of the latter died or were moribund on day 3. The number of bacteria in the vegetations did not differ significantly between the groups at any of the three time points.

Effects of immunization and of anticoagulation on bacteremia during bacterial endocarditis. After induction of BE with 10^7 *E. coli,* the control, immunized, and anticoagulated rabbits all showed a low-grade bacteremia (Table 5). The difference between the control and immunized groups is only significant for day 1 of infection. For later time points the difference between the three groups is not significant.

DISCUSSION

The results of the present study show that when live *E. coli* are injected into rabbits with NBTE, a preexisting high level of humoral immunity induced by immunization has no effect on the induction and course of an *E. coli* endocarditis. However, decreased coagulability has an inhibitory effect on the induction of infection of the vegetations and shortened the survival time of rabbits with *E. coli* endocarditis.

The rarity of *E. coli* endocarditis in humans is thought to be related to the bactericidal activity of serum against many *E. coli* strains (4, 7, 14). To exclude such an effect, which would hamper the induction of BE in rabbits with an NBTE, we used a serum-resistant *E. coli* strain in the present study.

TABLE 2. *Effect of serum on intracellular killing of E. coli by peritoneal macrophages*[a]

Phagocytosis[b] of *E. coli* preopsonized with	Intracellular killing index after 60 min of incubation with	
	10% Normal serum	10% Immune serum
10% Normal serum	96 ± 2	95 ± 5
10% Immune serum	91 ± 8	95 ± 4

[a] Mean values ± standard deviation of three experiments.

[b] For 3 min at a bacteria-to-macrophage ratio of 1:1.

TABLE 3. *Effects of immunization and of anticoagulation on induction of bacterial endocarditis*[a]

Experimental conditions	No. of *E. coli* injected intravenously				
	10^8	10^7	10^6	10^5	10^4
Controls	ND	12/12	5/10	1/4	0/5
Immunized	ND	4/4	2/5	1/5	0/4
Anticoagulated	5/5	5/8	1/6	0/4	ND

[a] Expressed as the number of rabbits with infected vegetations per total number of rabbits killed. The animals were killed 1 day after the injection of *E. coli.* ND, Not determined.

TABLE 4. *Effects of immunization and of anticoagulation on the degree of infection of vegetations*

Days after infection[a]	Bacteria/g of vegetation[b]		
	Control	Immunized	Anticoagulated
1	2.5×10^8 (12)[c]	1.4×10^8 (4)[d]	8.1×10^7 (5)[e]
	(5.0×10^6–7.2×10^8)	(1.0×10^8–2.2×10^8)	(6.9×10^5–3.4×10^9)
3	4.4×10^8 (5)	ND[h]	2.0×10^8 (5)
	(4.3×10^7–1.7×10^9)		(8.6×10^4–2.8×10^{10})
14	1.5×10^9 (5)[f]	3.4×10^9 (4)[g]	—[i]
	(1.0×10^9–4.6×10^9)	(1.4×10^9–7.4×10^9)	

[a] *E. coli* (10^7) were administered intravenously.

[b] Expressed as geometric means; range and number are given in parentheses.

[b, c, d, e, f, g] *c* versus *f*, $P < 0.001$; *d* versus *g*, $P < 0.001$; *c* versus *e*, $P > 0.2$.

[h] ND, Not determined.

[i] Anticoagulated rabbits were already moribund on day 3 of infection.

TABLE 5. *Effects of immunization and anticoagulation on E. coli bacteremia in rabbits*

Days after infection[a]	Control rabbits[b]	N[c]	Immunized rabbits[b]	N[c]	Anticoagulated rabbits[b]	N[c]
1	58[d] (5–420)	16/22	10[e] (4–46)	5/8	71 (3–953)	9/13
3	19 (4–801)	4/5	ND[f]		89 (5–542)	4/5
4	56 (2–768)	3/5	27 (2–368)	2/4	—[g]	
11	34	1/5	9 (2–20)	4/4	—[g]	
14	46 (3–320)	3/5	15 (3–106)	3/4	—[g]	

[a] *E. coli* (10^7) administered intravenously.
[b] Number of *E. coli* per milliliter of blood is expressed as geometric means (ranges given in parentheses).
[c] Number of rabbits with bacteremia per total number of rabbits infected.
[d, e] *d* versus *e*, $P < 0.05$.
[f] ND, Not determined.
[g] Anticoagulated rabbits were already moribund on day 3 of infection.

Immunization of rabbits with *E. coli* of this strain resulted in high levels of agglutinating and opsonic activity of the serum, but no bactericidal activity was induced. In vitro, pre-opsonization with the immune serum promoted phagocytosis of *E. coli* by macrophages as compared with normal serum, whereas this immune serum had no effect on the intracellular killing of this microorganism by these cells. Despite the in vitro findings, we found that in vivo an enhanced immunity did not influence the colonization of endocardial vegetations by *E. coli.*

It should be kept in mind here that the effect of immunization on the induction and course of endocarditis may depend on the classes of antibody induced during immunization. However, it is noteworthy that nonimmunized rabbits develop during *E. coli* endocarditis serum agglutinating and opsonic activity similar to that induced by immunization. The term activity is used here instead of antibody because the functional tests were done with whole serum and not with immunoglobulin fractions, but opsonic and agglutinating antibodies of both immunoglobulin classes are present in the serum of patients with BE (21).

In view of the importance of fibrin formation for the genesis of *E. coli* endocarditis, the question is raised of how during the colonization local activation of the clotting mechanism occurs on the surface of the vegetations. One factor contributing to this process could be the local generation of tissue thromboplastin by monocytes activated by the phagocytosis of bacteria on the surface of the vegetations (27) or by bacterial endotoxin (18). However, other pathways could also lead to fibrin formation, for instance, endothelial damage caused by *E. coli* endotoxin, resulting in the exposure of collagen at the site of NBTE, which in turn might induce platelet aggregation and release of platelet factor 3 as well as activation of the Hageman factor (1, 16, 28). Impaired fibrin formation due to anticoagulant treatment could hamper both the attachment to and outgrowth of the bacteria in the vegetations. This would be reflected in the need for larger inocula to induce an infection of the NBTE, or in the numbers of bacteria in the vegetations. We found that in anticoagulated rabbits significantly higher inocula were needed to induce an infection with *E. coli,* but the bacterial numbers in the infected vegetations were unaffected. Thus, anticoagulation hampers only the attachment of *E. coli* to the vegetations, but not their outgrowth once they have settled. This could mean that once *E. coli* have attached to the vegetations, even in intensively anticoagulated rabbits, sufficient procoagulant activity is generated in the microenvironment via the abovementioned mechanisms to make it possible for the bacteria after the fibrin network has formed to multiply unhindered by host factors such as antibodies and phagocytic cells. The effects of warfarin treatment on the induction and course of *E. coli* endocarditis are intermediate between those on *S. sanguis,* in which neither attachment nor outgrowth is affected, and on *S. epidermidis,* in which both these processes are hampered. These differences make it likely that these microorganisms adhere to the vegetations and maintain themselves there in different ways.

The survival of warfarin-treated rabbits with *E. coli* endocarditis was significantly shortened by this treatment. Like the animals in our previous studies with *S. sanguis* and *S. epidermidis* (23, 24), at autopsy the lungs of these animals showed extensive hemorrhages and pulmonary congestion, which presumably precipitated their early death.

Since immunization had no effect on bacterial

numbers in the blood, it is evident that the clearance of bacteria from the blood of rabbits with *E. coli* endocarditis is not promoted by increased levels of opsonic antibodies. Bacterial numbers in the blood of these rabbits were higher than those found in rabbits with *S. epidermidis* endocarditis (24) but lower than in rabbits with *S. sanguis* endocarditis (23). Furthermore, the 50% infective dose (1.0×10^6) in nonimmunized rabbits was significantly higher for *E. coli* ($P < 0.01$) than for *S. sanguis* (1.3×10^4; unpublished data) and *S. epidermidis* (6.1×10^4) (24). This indicates a lower propensity of *E. coli* to attach to endocardial vegetations than that of the two gram-positive bacteria, probably as a result of a lack of specific bacterial properties (such as dextran production by *S. sanguis*) that promote their attachment to the vegetations (17, 19). Thus, not only serum bactericidal activity but also specific bacterial properties might contribute to the lower incidence of gram-negative endocarditis.

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LITERATURE CITED

1. **Angrist, A., M. Oka, and K. Nakao.** 1967. Vegetative endocarditis. Pathol. Ann. **2**:155-212.
2. **Archer, G., and F. R. Fekety.** 1976. Experimental endocarditis due to *Pseudomonas aeruginosa*. I. Description of a model. J. Infect. Dis. **134**:1-7.
3. **Archer, G. L., and J. L. Johnston.** 1979. Effect of type-specific active immunization on the development and progression of experimental *Pseudomonas aeruginosa* endocarditis. Infect. Immun. **24**:167-173.
4. **Cohen, P. S., T. F. O'Brien, S. C. Schoenbaum, and A. A. Medeiros.** 1978. The risk of endothelial infection in adults with Salmonella bacteremia. Ann. Intern. Med. **89**:931-932.
5. **Conrad, R. E., L. C. Yang, and H. B. Herscowitz.** 1977. Mononuclear phagocytic cells in peritoneal exudates of rabbits: a comparison of inducing agents. RES J. Reticuloendothel. Soc. **21**:103-107.
6. **Durack, D. T., and P. B. Beeson.** 1972. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. Br. J. Exp. Pathol. **53**:44-49.
7. **Durack, D. T., and P. Beeson.** 1977. Protective role of complement in experimental *Escherichia coli* endocarditis. Infect. Immun. **16**:213-217.
8. **Durack, D. T., B. C. Gilliland, and R. G. Petersdorf.** 1978. Effect of immunization on susceptibility to experimental *Streptococcus mutans* and *Streptococcus sanguis* endocarditis. Infect. Immun. **22**:52-56.
9. **Finney, D. J.** 1962. Probit analysis, p. 39. Cambridge University Press, Cambridge.
10. **Garvey, G. J., and H. C. Neu.** 1978. Infective endocarditis—an evolving disease. Medicine **57**:105-127.
11. **Hook, E. W., and M. A. Sande.** 1974. Role of the vegetation in experimental *Streptococcus viridans* endocarditis. Infect. Immun. **10**:1433-1438.
12. **Kaspar, R. L., and D. J. Drutz.** 1977. Perihepatitis and hepatitis as complications of experimental endocarditis due to *Neisseria gonorrhoeae* in the rabbit. J. Infect. Dis. **136**:37-42.
13. **Kunst, M. W., and H. Mattie.** 1978. Cefazolin and Cephradine: relationship between antibacterial activity in vitro and in mice experimentally infected with *Escherichia coli*. J. Infect. Dis. **137**:391-402.
14. **Leddy, J. P., and R. T. Steigbigel.** 1979. Complement, serum bactericidal activity, and disseminated Gram-negative infection. Ann. Intern. Med. **90**:984-985.
15. **Leijh, P. C. J., M. Th. van den Barselaar, T. L. van Zwet, M. R. Daha, and R. van Furth.** 1979. Requirement of extracellular complement and immunoglobulin for intracellular killing of micro-organisms by human monocytes. J. Clin. Invest. **63**:772-784.
16. **Morrison, D. C., and C. G. Cochrane.** 1974. Direct evidence for Hageman factor (factor XII) activation by bacterial lipopolysaccharides (endotoxins). J. Exp. Med. **140**:797-811.
17. **Ramirez-Ronda, C. H.** 1978. Adherence of glucan-positive and glucan-negative streptococcal strains to normal and damaged heart valves. J. Clin. Invest. **62**:805-814.
18. **Rivers, R. P. A., W. E. Hathaway, and W. L. Weston.** 1975. The endotoxin coagulant activity of human monocytes. Br. J. Haematol. **30**:311-316.
19. **Scheld, W. M., J. A. Valone, and M. A. Sande.** 1978. Bacterial adherence in the pathogenesis of endocarditis. Interaction of bacterial dextran, platelets, and fibrin. J. Clin. Invest. **61**:1394-1404.
20. **Scheld, W. M., J. H. Thomas, and M. A. Sande.** 1979. Influence of preformed antibody on experimental *Streptococcus sanguis* endocarditis. Infect. Immun. **25**:781-785.
21. **Shanson, D. C., and C. Hince.** 1978. An immunofluorescent method for detecting antibodies against viridans streptococci in Streptococcus viridans endocarditis. J. Clin. Pathol. **31**:292-293.
22. **Siroky, D. B., R. A. Moylan, G. Austen, and Carl A. Olsson.** 1976. Metastatic infection secondary to genitourinary tract sepsis. Am. J. Med. **61**:351-360.
23. **Thompson, J., F. Eulderink, H. Lemkes, and R. van Furth.** 1976. Effect of warfarin on the induction and course of experimental endocarditis. Infect. Immun. **14**:1284-1289.
24. **Thörig, L., J. Thompson, and F. Eulderink.** 1977. Effect of warfarin on the induction and course of experimental *Staphylococcus epidermidis* endocarditis. Infect. Immun. **17**:504-509.
25. **Thörig, L., J. Thompson, F. Eulderink, and R. van Furth.** 1979. The role of host factors in the pathogenesis of bacterial endocarditis. Neth. J. Med. **22**:174-175.
26. **van Furth, R., T. L. van Zwet, and P. C. J. Leijh.** 1978. In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes, chapter 32, p. 1. *In* D. M. Weir (ed.), Handbook of experimental immunology, 3rd ed. Blackwell Scientific Publications, Oxford, England.
27. **van Ginkel, C. J. W., L. Thörig, J. Thompson, J. I. H. Oh, and W. G. van Aken.** 1979. Enhancement of generation of monocyte tissue thromboplastin by bacterial phagocytosis: possible pathway for fibrin formation on infected vegetations in bacterial endocarditis. Infect. Immun. **25**:388-395.
28. **Weinstein, L., and R. H. Rubin.** 1973. Infective endocarditis. Prog. Cardiovasc. Dis. **16**:239-255.
29. **Zuker, M. B.** 1974. Platelets, p. 511. *In* B. W. Zweifach, L. Grant, and R. T. McCluskey (ed.), The inflammatory process, vol. I. Academic Press Inc., New York.