EXPERIMENTAL ENDOCARDITIS. II: STAPHYLOCOCCAL INFECTION OF THE AORTIC VALVE FOLLOWING PLACEMENT OF A POLYETHYLENE CATHETER IN THE LEFT SIDE OF THE HEART

INTRODUCTION
Proceeding from the clinical observation that intravascular polyethylene catheters easily become infected in man, the introduction of a polyethylene catheter into the right side of the heart has recently been shown to induce the formation of sterile endocarditis in rabbits. It was further noted that inoculation of as few as $10^2$ staphylococci through the catheter would regularly result in staphylococcal endocarditis. In these experiments the catheter remained in the right heart until the animal was sacrificed; it thus served as a possible source of mechanical trauma and as a nidus for the seeding of microorganisms.

The present study was undertaken to test whether it was possible to establish sterile and infective endocarditis in the left side of the heart utilizing techniques that previously had been shown to be effective on the right side.

MATERIALS AND METHODS
The experimental animals were five-pound, white New Zealand rabbits. The animals received 50-60 mg. of sodium pentobarbital intravenously and inhaled ethyl ether as anaesthesia. The cannula utilized was a sterile polyethylene catheter, "Intramedic" (Clay-Adams), PE 90, radiopaque, I.D. 0.034, O.D. 0.050.

The right carotid artery was exposed through a two-inch longitudinal incision in the neck, ligated and incised, and the catheter inserted a distance of about $3\frac{3}{4}$ inches until resistance was met. It was then withdrawn slightly, perhaps 1/16 to 1/8 of an inch and tied in place. The tip thus remained in a position just above the semilunar cusps of the aortic valve. The catheter was then filled with the appropriate fluid, the free end bent over and tied upon itself, and the skin incision closed with silk over the free end of the catheter. The catheter remained in situ until the death or sacrifice of the animal. Although rigorous sterile techniques were not followed, local wound infection was not encountered.

The microorganism employed was the Giorgio strain of coagulase positive Staphylococcus aureus, which had been used in this laboratory previously. The organisms were cultured overnight in trypticase soy broth and appropriate dilutions were administered through the catheter. Inocula were enumerated by counting viable bacterial units in

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seriously diluted agar pour plates after a one-day incubation period. The catheter held about 0.1 ml. per inch. An additional 0.5 ml. of diluted experimental culture was injected to dislodge blood clot formation at the tip of the catheter.

Two series of animals were studied:

**Group I**: rabbits with catheters containing sterile saline.

**Group II**: rabbits with catheters containing staphylococci.

Animals were killed by the intravenous injection of 120 mg. of sodium pentobarbital followed by ethyl ether inhalation, if necessary. Postmortem examinations were performed immediately following sacrifice of the rabbit or when the animal was found dead. A terminal blood sample was drawn from the marginal ear vein or the inferior vena cava. Aseptic technique was observed during the postmortem examination. The gross organs were examined and pathological specimens taken. If the animal had not died, the kidneys, liver and spleen were homogenized *in toto* and cultured quantitatively. The spleens were weighed upon removal. One ml. of urine from the bladder was cultured. Viable bacterial units from samples cultured were counted after a 24-hour incubation period.

Special care was taken in examining the heart. The catheter was clamped in the arch of the aorta prior to the removal of the heart. The heart was then carefully examined to locate the tip of the catheter and to detect the location of vegetations. The catheter was removed and its contents expressed and cultured. The vegetations were then incised and a small platinum loopful of material was recovered for gram stain and culture. Where gram stain did not reveal numerous bacteria, two or three sites of the vegetation were cultured. The loop employed carries about 0.001 ml. of liquid.

**RESULTS**

**Group I**: This series of animals had catheters filled with sterile saline placed in their right carotid arteries. The catheters remained *in situ* until the rabbits were sacrificed or died, 18 to 42 days after operation. The catheter tips were found in the region of the aortic valve in six animals and were discovered to be endothelialized along the wall of the aorta in two rabbits (Table 1). Sterile vegetations were present at the aortic valve or on other immediately adjacent tissue surfaces in each of the five of six animals with catheters free in the vascular system (Fig. 1). Among this group only one animal was found dead, the remainder were sacrificed. The single animal that died had gram negative rods cultured from the catheter contents and from the aortic vegetation. Kidney infarctions were noted in 87.5% (7/8) of the rabbits (Fig. 2). Splenic enlargement (> 2 gms) was not noted. No microorganisms were cultured from the heart vegetations, catheter contents, organ homogenates, blood, or urine samples of the remaining animals.

**Group II**: The nine rabbits of this group were cannulated with catheters filled with $10^4$-$10^8$ staphylococci. At postmortem examination, seven of the rabbits had vegetations from which staphylococci were cultured (Fig. 3). One animal had vegetations which, when cultured, grew staphylococci and gram negative organisms as well. The catheter was not recovered from the
| Rabbit no. | Died(O) or lost (X) | Spleen wt. (gms) | Cath contents | Blood | Organ | Vegetation | Gross findings |
|------------|---------------------|------------------|---------------|-------|-------|------------|----------------|
| 96         | X                   | 1.39             | neg           | neg   | kidneys—neg | neg         | Cath tip at aortic valve with veg. Kidney infarcts bilat. |
| 101        | X                   | 1.27             | neg           | neg   | kidneys—neg | neg         | Cath at cusps; valve swollen. Sterile kidney infarcts bilat. |
| 105        | X                   | 1.45             | neg           | neg   | kidneys—neg | neg         | Large veg on cusps & surface of aorta. Liver: chronic passive congestion. Small kidney infarcts bilat. |
| 135        | X                   | 1.54             | neg           | neg   | kidneys—neg | neg         | Cath endothelialized in aorta. Veg in L. ventricle & on papillary muscle. Kidneys normal. |
| 175        | X                   | 1.31             | neg           | neg   | liver—neg spleen—neg urine—neg | neg         | Veg in aortic valve. Small infarct in R. kidney. |
| 176        | X                   | 1.12             | kidneys—neg urine—neg | neg | Vegs in aortic arch & healed veg in valve. Multiple healed kidney infarcts. |
| 181B       | O                   | <2               | contam        | kidneys—neg spleen—neg | contam | Cath tip at aortic valve. Veg at aortic valve. Old scar in L. kidney. |
| 182        | X                   | 0.81             | neg           | kidneys—neg urine—neg | neg | Cath endothelialized in aortic cusps. Healed kidney infarcts. |

* Viable units per ml. or gm.
Fig. 1. Rabbit no. 105. Sterile vegetation on cusps and surface of aorta.

Fig. 2. Rabbit no. 96. Sterile kidney infarcts in an animal with sterile aortic vegetations.
Fig. 3. Rabbit no. 245. Catheter tip at aortic valve. Large numbers of staphylococci were cultured from the vegetation. Areas of myocarditis are indicated by the white arrow.
| Rabbit no. | Days animal followed | Died(O) or sacrificed(X) | Spleen wt. (gms) | Staphylococci recovered* | Vegetation | Gross findings |
|------------|----------------------|--------------------------|------------------|-------------------------|------------|----------------|
| 92         | 4                    | O                        | <2               | 135                     | >10⁶       | Cath tip at aortic valve; cusps filled with veg and thrombus. Kidney infarcts bilat. |
| 155        | 13                   | O                        | 3.39             |                         | >10⁶       | Large veg replacing aortic valve; kidney infarcts and abscesses. |
| 178        | 18                   | O                        |                  |                         | >10⁶       | Large aortic veg; kidney infarcts bilat. |
| 183        | 12                   | O                        |                  |                         | 10⁶        | Small veg on aortic cusps; L. ventricle enlarged; normal kidneys. |
| 184        | 32                   | X                        | 1.26             | neg                     | kidneys—neg urine—neg | No veg. Cath not located. Kidney infaracts bilat. |
| 220        | 1                    | O                        | 1.66             | >10⁶                   | >10⁶       | Fibrous veg in aortic valve. |
| 245        | 3                    | O                        | 4.1              | >10⁶                   | >10⁶       | Cath tip at aortic valve; vegs on aortic cusps and mitral valve; septum mottled. Kidney infarcts & abscesses bilat. |
| 252        | 5                    | O                        |                  | 20                     | >10⁶       | Large veg in aortic valve. Kidney infarcts bilat. |
| 270        | 3                    | O                        | 3.98             | >10⁶                   | >10⁶       | Veg on cusps of aortic valve. Small kidney infarcts. |

* Viable units per ml. or gm.
** Organs not cultured when animal found dead.
heart of the remaining animal; it was probably short and not in the proper position (Table 2).

In the eight infected rabbits, the catheter tips were in propinquity to the aortic valve. The vegetations were located predominantly on the aortic cusps, but were also found on a papillary muscle, the mitral valve, and on other surfaces contiguous with the aortic valve. Evidence of infarction or abscess of the kidneys was present in 78% (7/9) of the animals. Splenic enlargement (>2 gms) was evident in 50% (3/6) of the rabbits whose spleens were weighed. Only three rabbits of this series had terminal blood cultures taken; two were positive. Of the Group II rabbits 89% (8/9) died within the first 18 postoperative days; the single surviving animal was sacrificed on day 32; the catheter was not recovered from this rabbit and endocarditis was not found.

The gross vegetations on the aortic valves were similar to those found in human endocarditis and to those described in previous work on the right side of the heart using this model. The lesions were 1-5 mm., friable, aciform, pale-pink excrescences in the infected rabbits. The lesions in the animals with sterile endocarditis were less friable, flatter, and pale. They distorted the valve to a lesser degree. Lesions occurred in similar positions in both series.

Histological differences existed between the lesions of the animals inoculated with bacteria and those into which sterile saline was injected. Rabbit 181B received a catheter containing sterile saline. Microscopic examination of the lesions revealed bland vegetations consisting of fibrin and rare white cells. Rabbit 245 had large masses of bacteria situated amongst platelets and fibrin; mononuclear cells and fibroblasts were evident, but polymorphonuclear leukocytes were seen only rarely in direct contact with the bacteria. Occasional areas of myocardial necrosis, containing zones of calcification, were noted. Renal lesions were typical of anemic infarcts or abscesses. No glomerulonephritis was observed.

DISCUSSION

A model has been developed for the production of sterile marantic endocarditis and bacterial endocarditis on the left side of the rabbit heart. The technique employed was one that had previously been shown to induce right-sided endocarditis. The present technique has the advantage of permitting the initiation of infection in a normal animal. Techniques employed in the past depend upon severe hemodynamic changes, such as the creation of fistulae between the aorta and the inferior vena cava, or surgical procedures predisposing to aortic insufficiency. Systemic immunologic reactions have been induced. Simulation of high altitude with and without
hemodynamic stress and bacteremia has been employed, as has alteration of lymphatic drainage of the heart, and the injection of large numbers of organisms, and stresses in various other forms.

As anticipated from earlier studies, sterile verrucous lesions were manifest when sterile saline was injected, whereas staphylococcal endocarditis developed when staphylococci were inoculated. The difference in severity between the bacterial and sterile diseases is of interest. Of the animals receiving sterile saline, seven of the eight rabbits were sacrificed and all were alive for at least 18 postoperative days. Conversely, of the eight rabbits with left-sided infective endocarditis, all had died by the 18th postoperative day. Thus the bacterial disease was of greater severity. Emboli to the kidneys occurred in about equal proportions in the two experiments.

It is remarkable that the left-sided endocarditis caused death of the rabbits, whereas right-sided infectious endocarditis rarely resulted in death. The rabbits of group II, in the present study, had catheters filled with staphylococci placed in proximity to their aortic valves. The rabbits of the previously reported study had staphylococci-filled catheters placed in proximity to their tricuspid valves through femoral vein catheterization. The rabbits from the previous study were sacrificed at intervals of 8-41 days; of the 13 animals in that study, eight survived beyond 18 days. Of the eight animals of the present study with infected aortic endocarditis, all died during the eighteen days following operation. Obviously, the course of the disease in the left heart was more fulminant than in the right heart. Systemic dissemination of the staphylococci, as measured by the positive cultures from organ homogenates, was present in the majority of rabbits in both series.

There is no obvious explanation for the fatal outcome of the left-sided as compared with the right-sided staphylococcal endocarditis in rabbits. Although it is possible that cerebral emboli result from left-sided lesions, this would seem unlikely since sterile left-sided endocarditis produces the same frequency of renal emboli and would therefore be expected to produce the same frequency of cerebral emboli; yet, sterile aortic endocarditis did not kill the rabbits.

Another possible explanation for the death of the animals with left-sided infective endocarditis may derive from the fact that lesions of myocarditis were seen which were not found in animals with right-sided disease. It is not clear, however, whether these lesions are peculiar to aortic valve disease or whether they may be a result of more extensive bacterial multiplication leading to more severe bacteremia in aortic disease. Unfortunately, the bacteriological studies carried out so far were not sufficiently detailed to permit comparison of the extent of infection in the two models. The data
do show that at least \(10^6\) staphylococci/gm. were present in the vegetations in both studies. However, the animals with aortic endocarditis had all been dead for some time before examination. Careful culture of the entire vegetation would be required to make more accurate comparisons.

**SUMMARY**

A model has been developed for the production of endocarditis of the aortic valve in rabbits. The technique is similar to that previously employed for producing right heart endocarditis; it depends on the catheterization of the heart through the peripheral vascular system with a polyethylene catheter. Placement of a sterile catheter results in sterile endocarditis. The introduction of staphylococci through the catheter results in infective endocarditis.

Sterile left-sided endocarditis is well tolerated, whereas bacterial endocarditis on the left side of the heart regularly causes death of the animals. This contrasts with the rarity of death due to right heart infective endocarditis reported previously.

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**REFERENCES**

1. Garrison, P. K. and Freedman, L. R.: Experimental endocarditis I: Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of the heart. *Yale J. Biol. Med.*, 1970, 42, 394-410.
2. Smits, H. and Freedman, L. R.: Prolonged venous catheterization as cause of sepsis. *New Engl. J. Med.*, 1967, 276, 1299-1233.
3. Moran, J. M., Atwood, R. F., and Rowe, M. I.: Clinical and bacteriologic study of infections associated with venous cutdowns. *New Engl. J. Med.*, 1965, 272, 554-560.
4. Collins, R. N., Braun, P. A., Zinner, S. H., and Kass, E. H.: Risk of local and systemic infection with polyethylene intravenous catheters. *New Engl. J. Med.*, 1968, 279, 340-343.
5. Lillehei, C. W., Bobb, J., and Visscher, M. D.: Occurrence of endocarditis and valvular deformities in dogs with A-V fistulas. *Ann. Surg.*, 1950, 132, 577-590.
6. Lillehei, C. W., Bobb, J., and Visscher, M. D.: Occurrence of endocarditis and valvular deformities in dogs with A-V fistulas. *Proc. Soc. exp. Biol. (N.Y.)*, 1950, 75, 9-16.
7. Rabens, R. A., Geraci, J. E., Grindlay, S. H., Karlson, A. G., and Edwards, J. E.: Experimental bacterial endocarditis due to *Streptococcus Mitis* I: Method of induction. II: Pathology of valvular and secondary lesions. *Circulation*, 1955, 11, 199-205, 206-214.
8. Lee, S. H. B., Fisher, E. R., and Little, A.: A-V fistulae and bacterial endocarditis. *Surgery*, 1962, 52, 463-467.
9. Angrist, A. and Oka, M.: Pathogenesis of bacterial endocarditis. *J. Amer. med. Ass.*, 1963, 62, 249-252.
10. Walker, W. F. and Hamburger, M.: A study of experimental endocarditis in dogs, I: Production of the disease, its natural history and tissue bacteriology. *J. Lab. clin. Med.*, 1959, 53, 931-941.
11. Kinsella, R. A. and Muether, R. D.: Experimental streptococcal endocarditis. *Arch. intern. Med.*, 1938, 62, 247-270.

12. Highman, B., Roshe, J., and Altland, P. D.: Production of endocarditis and glomerulonephritis by single bacterial injections in dogs with aortic insufficiency. *Fed. Proc.*, 1956, 15, No. 1684, 518-519.

13. McGeown, M.: Bacterial endocarditis. Experimental study of healing. *J. Path. Bact.*, 1954, 67, 179-186.

14. Highman, B. and Altland, P. D.: Effect of altitude and cobalt polycythemia, hypoxia, and cortisone on susceptibility of rats to endocarditis. *Circulation Res.*, 1955, 3, 351-356.

15. Angrist, A., Oka, M., Nakao, K., and Marquiss, J.: Studies in experimental endocarditis: I. Production of valvular lesions by mechanisms not involving infection of sensitivity factors. *Amer. J. Path.*, 1960, 36, 181-191.

16. Miller, A. J., Pick, R., Kline, J. K., and Katz, L. N.: The susceptibility of dogs with chronic impairment of cardiac flow to staphylococcal valvular endocarditis. *Circulation*, 1964, 30, 417-424.

17. MacNeal, W. J., Spence, M. J., and Wasseen, M.: Experimental production of endocarditis lenta. *Amer. J. Path.*, 1939, 15, 695-705.

18. Oka, M., Shirota, A., and Angrist, A.: Experimental endocarditis: Endocrine factors in valve lesions on A-V shunt rats. *Arch. Path.*, 1966, 82, 85-92.

19. Oka, M., Belenky, P., Brodie, S., and Angrist, A.: Studies of bacterial susceptibility of heart valves. *Lab. Invest.*, 1968, 19, 113-121.

20. Nedzel, A. J.: Experimental endocarditis. *Arch. Path.*, 1937, 24, 143-200.

21. Jarcho, S.: Historical milestones: Experimental endocarditis (Wyssokowitsch, 1886). *Amer. J. Cardiol.*, 1969, 24, 876-879.