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EXPERIMENTAL ENDOCARDITIS III: NATURAL HISTORY OF CATHETER INDUCED STAPHYLOCOCCAL ENDOCARDITIS FOLLOWING CATHETER REMOVAL

INTRODUCTION

Techniques have been described which allow the regular production of sterile or bacterial endocarditis on either side of the heart.^{1,2} The method involves passing a sterile polyethylene catheter from the peripheral vascular system into either the left or right side of the heart. The lesions involved the tricuspid and aortic valves predominantly. If sterile saline was introduced through the catheter, sterile marantic endocarditis occurred; the inoculation of staphylococci resulted in staphylococcal endocarditis. The studies to date described the course of infection with the catheter in place.

The work reported in this paper was undertaken to elucidate the natural history of bacterial endocarditis, established in the manner previously reported, when the catheter was removed.

MATERIALS AND METHODS

The materials and methods for the production of right and left-sided bacterial endocarditis have been described in detail in previous publications.^{1,2} In brief, polyethylene catheters were inserted into the heart via the peripheral vascular system. To reach the region of the tricuspid valve, the left femoral vein was exposed and a catheter passed into it for a distance of about 11-11½ inches. On the left side, a catheter was inserted through an incision in the right carotid artery for a distance of about 3½ inches so that its tip remained in proximity to the cusps of the aortic valve. Staphylococci were inoculated through the catheter to produce infectious endocarditis.

At varying intervals the animals were reanaesthetized and the wound created for catheter introduction reopened. The distal end of the catheter was located and the catheter withdrawn. The carotid artery, proximal to the incision, was ligated with silk; the femoral vein was not tied. The skin wound was then closed with silk. Rigorous sterile technique was not followed and no wound infections were encountered. The contents of the catheter were expressed and quantitatively cultured. Because the catheters were not *in situ* at autopsy, their placement was verified in early trials by x-ray. In addition, proper placement of the catheter in the heart usually resulted in transmission of pulsations along the fluid column in the catheter. The catheter placement procedures took 20-30 minutes; catheter removal took 5-10 minutes.

The microorganisms used in these experiments, a strain of *staphylococcus aureus*, were the same as those used previously by this laboratory in work with this model of

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** Professor of Medicine. Supported by U. S. Public Health Service Career Development Award No. 6K3-HD 22, 587, National Institute of Child Health and Human Development and by Research Grant No. 4767 from the Institute of Allergy and Infectious Disease, U. S. Public Health Service.

Received for publication 22 June 1970.

bacterial endocarditis. The methods of preparation and introduction of the staphylococci have been described in previous publications.^{1,2}

The animals were divided into two experimental groups:

Group I: Rabbits with catheters containing staphylococci placed near the tricuspid valve. The catheters remained *in situ* for from 2-14 days and the animals were sacrificed 3-6 days after the catheter was withdrawn. The total time elapsed from catheterization to bacteriological study was from 5 to 19 days.

Group II: Rabbits with catheters containing staphylococci placed near the aortic valve. The catheters were left in place for from 3-13 days; the total time between initial catheterization and bacteriological study ranged from 3-17 days.

A description of the procedure for postmortem examination of the experimental animals following death or sacrifice has been given previously.^{1,2} The kidneys, spleen, and liver were inspected and quantitatively cultured along with samples of urine and blood. The spleen was weighed.

As related in earlier papers, special attention was given the examination of the heart which was opened in order to locate vegetations. The vegetations were incised and a small platinum loopful of the material recovered for gram stain and culture. The loop holds approximately 0.001 ml. of liquid; therefore O colonies indicates less than 1,000 viable units per gram. Where gram stain did not demonstrate numerous bacteria, two or three sites of the vegetations were cultured.

RESULTS

Group I: These rabbits had catheters containing staphylococci placed in the region of their tricuspid valves. The catheters were withdrawn from the animals several days prior to sacrifice. This series consisted of 18 rabbits. At postmortem examination, 15 animals had sterile vegetations, and three had vegetations from which staphylococci were cultured (Fig. 1). The numbers of viable bacterial units recovered from the vegetations ranged from 3 to 41 colonies per 0.001 ml. loopful. Vegetations occurred on the cusps of the tricuspid valve, the papillary muscles, and on the adjacent tissue surfaces in the atria and ventricles. Their size bore no relation to the finding of organisms. Grossly, they varied from large, friable, grayish masses to small, flat, firm, pale excrescences (Tables 1 & 2).

None of the animals with infected vegetations showed evidence of splenic enlargement (> 2 gms); two rabbits with sterile vegetations did have splenomegaly. The cultures of the organ homogenates and other samples taken were only sporadically positive. When positive they grew only very small numbers of organisms. Positive terminal blood cultures were present in only two rabbits, one with septic and one with sterile vegetations; in each case the numbers of colonies grown were small, four and nine. Removed catheters were usually found to contain $> 10^6$ colonies of staphylococci per ml.

The catheters were left *in situ* for from 2 to 14 days. The animals were sacrificed from three to six days after the catheter was withdrawn. The dis-

TABLE 1. GROUP I: RIGHT HEART CATHETERIZATION WITH CATHETERS CONTAINING 10^4 - 10^8 STAPHYLOCOCCI

Rabbit no.	Days cath in place	Total days	Spleen wt. (gms)	Sacrificed	Gross findings
Animals with infected vegetation:					
122	4	7	1.06	X	Small veg. near tricuspid valve.
133	5	11	0.8	X	Veg. on r. atrial wall.
169	14	17	1.56	X	Veg. on tricuspid valve and r. ventricular wall.
Animals with sterile vegetations:					
110	5	8	2.91	X	Questionable veg.
114	5	9	1.79	X	Questionable veg. on tricuspid valve.
115	3	7	1.58	X	Veg. on tricuspid cusps and on r. atrial wall.
117	3	9	1.11	X	Small veg. on r. atrial wall.
120	2	7	1.77	X	Veg. on r. atrial wall and on papillary muscle.
124	6	10	1.65	X	Friable veg. on tricuspid valve and wall of r. atrium and r. ventricle.
125	6	10	1.90	X	Large veg. in r. atrium just above valve.
126	6	10	2.2	X	Small veg. in r. atrium.
129	5	11	1.07	X	Veg. on tricuspid valve and wall of r. atrium.
134	5	11	1.05	X	R. atrial veg.
143	14	18	1.2	X	Veg. on r. atrial wall.
144	13	19	1.12	X	Small atrial veg.; scarring of one tricuspid valve leaflet.
146	10	15	1.9	X	Large atrial veg.
158	7	10	0.71	X	Small atrial veg.
167	14	17	1.75	X	Atrial veg.

TABLE 2. GROUP I: RIGHT HEART CATHETERIZATION WITH CATHETERS CONTAINING 10^4 - 10^8 STAPHYLOCOCCI. BACTERIOLOGIC FINDINGS: STAPHYLOCOCCI RECOVERED (Viable Units per ml. or gm.)

Rabbit no.	Cath contents	R. kidney	L. kidney	Liver	Spleen	Blood	Urine	Vegetation
Animals with infected vegetations:								
122	$>10^8$	3	6	6	0	0	0	10×10^8
133	$>10^8$	0	1	0	0	18	10	41×10^8
169	$>10^8$	0	24	0	0	0	10	$3 \times 10^8; 7 \times 10^8$
Animals with sterile vegetations:								
110	$>10^8$	0	0	0	0	0	0	0
114	$>10^8$	0	0	0	0	0	0	0
115	$>10^8$	0	0	0	0	0	0	0
117	$>10^8$	0	0	0	0	0	0	0
120	0	0	0	10^8	0	0	<10	0
124	$>10^8$	0	0	0	0	8	<10	0
125	$>10^8$	10^8	10^8	0	0	0	<10	0
126	$>10^8$	10^8	0	10^4	10^8	0	<10	0
129	$>10^8$	0	0	0	0	0	0	0
134	$>10^8$	0	0	0	0	0	0	0
143	$>10^8$	samples contaminated					0	0
144	$>10^8$	samples contaminated					0	0
146	$>10^8$	0	0	0	0	0	0	0
158	$>10^8$	samples contaminated					0	0
167	$>10^8$	0	0	0	0	0	0	0

TABLE 3. GROUP II: LEFT HEART CATHETERIZATION WITH CATHETERS CONTAINING 10^8 - 10^9 STAPHYLOCOCCI

Rabbit no.	Days cath in place	Total days	Spleen wt. (gms)	Died or sacrificed (O) (X)	Gross findings
Animals with infected vegetations:					
237	4	12	1.27	X	Veg. at base of septum. Kidney infarcts bilat.
238	3	5		O	L. ventricle and aortic cusps filled with veg.
248	4	7	2	O	Aortic valves filled with veg. Kidney infarcts and abscesses.
253	5	9	2.88	O	Friable veg. filling aortic cusps. Kidney abscesses. Lung infarct.
254	3	13	1.09	X	Small veg. above cusps of aortic valve.
272	3	9	2.6	X	Large veg. in aortic cusps.
Animals with sterile vegetations:					
206	3	17	1.16	X	Aorta wrinkled around valves; no veg. or endocarditis. Old kidney infarcts. Hemorrhagic lungs.
226	3	7	0.67	X	Veg. on left septum. Kidney infarcts.
235	5	7	1.39	X	Small friable vegs. on cusps and wall of aorta.
239	3	7	1.29	X	Two vegs. on aortic wall just above cusps.
244	4	10	2	X	Veg. inside aortic cusps. Small l. kidney infarct.
249	4	10	1.8	X	Endothelialized veg. in single aortic cusp.
250	6	12	1.26	X	Friable vegs. in valve cusps; endothelialized veg. in carotid artery.
256	3	13	2.47	X	Aortic veg.

TABLE 4. GROUP II: LEFT HEART CATHETERIZATION WITH CATHETERS CONTAINING 10^4 - 10^8 STAPHYLOCOCCI. BACTERIOLOGIC FINDINGS: STAPHYLOCOCCI RECOVERED (Viable Units per ml. or gm.)

Rabbit no.	Cath contents	R. kidney	L. kidney	Liver	Spleen	Blood	Urine	Vegetation
Animals with infected vegetations:								
237	10^4	0	0	0	0	0	0	63×10^8
238	$>10^8$							$>10^8$
248	$>10^8$							$>10^8$
253	$>10^8$							$>10^8$
254	$>10^8$							$>10^8$
272	$>10^8$	$>10^8$	$>10^8$	$>10^8$		5:4	<10	$>10^8$
Animals with sterile vegetations:								
206	$>10^8$	0	0	0	0	0	10	0
226	$>10^8$							0
235	$>10^8$							0
239	$>10^8$	0	0	0	0	0	0	0
244	$>10^8$	0	0	0	0	0	0	0
249	$>10^8$	0	0	0	0	0	0	0
250	10^4	50×10^4	9×10^4	3×10^4	1×10^4	0	0	0
256	10^4	0	0	0	0	0	0	0

tribution of animals throughout these time ranges is shown in Fig. 2. Bacteriological studies, therefore, were carried out from 5 to 19 days after the initial procedure. None of the rabbits died.

Group II: These rabbits had catheters containing staphylococci placed in proximity to their aortic valves; the catheters were withdrawn three to six days after placement. Of 14 rabbits, six had vegetations from which staphylococci were recovered and eight had sterile vegetations (fig. 3). The numbers of viable bacterial units cultured from these vegetations ranged from 63 to 1000 colonies per 0.001 ml. loop of material. The vegetations were most often found on the cusps of the aortic valve and on the wall of the aorta above and the ventricular surface below. Grossly, they varied from large friable masses, usually found in rabbits with septic vegetations, to small firm, endothelialized excrescences, confined to a single cusp (Tables 3 & 4).

Splenic enlargement (>2 gms) was noted in three of six rabbits with in-

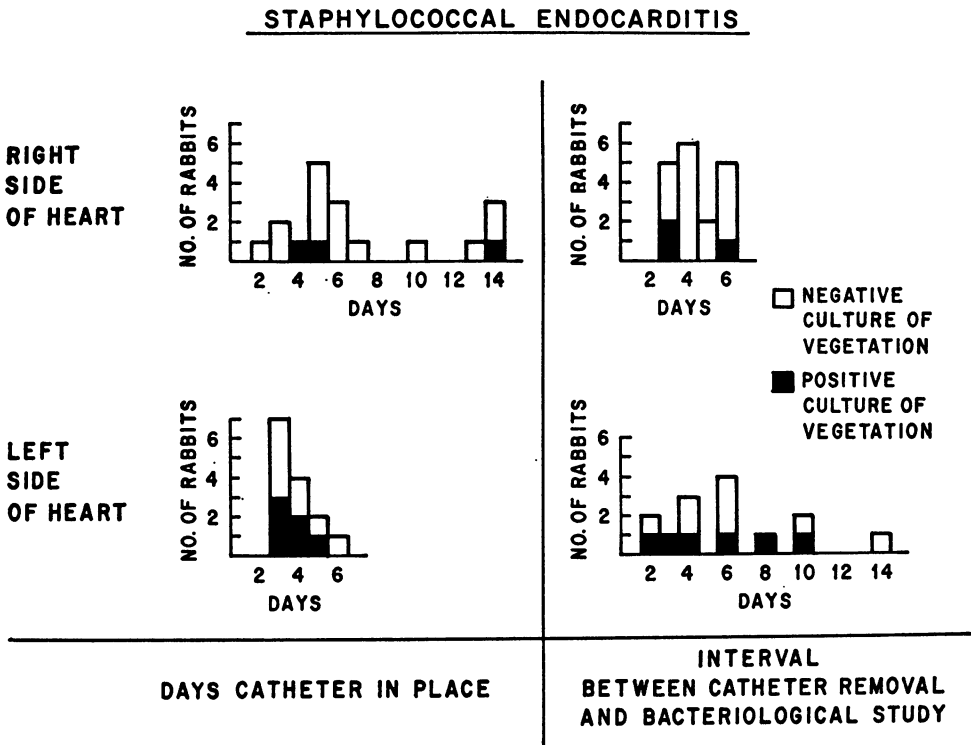


FIG. 2. Distribution of rabbits within intervals indicated.

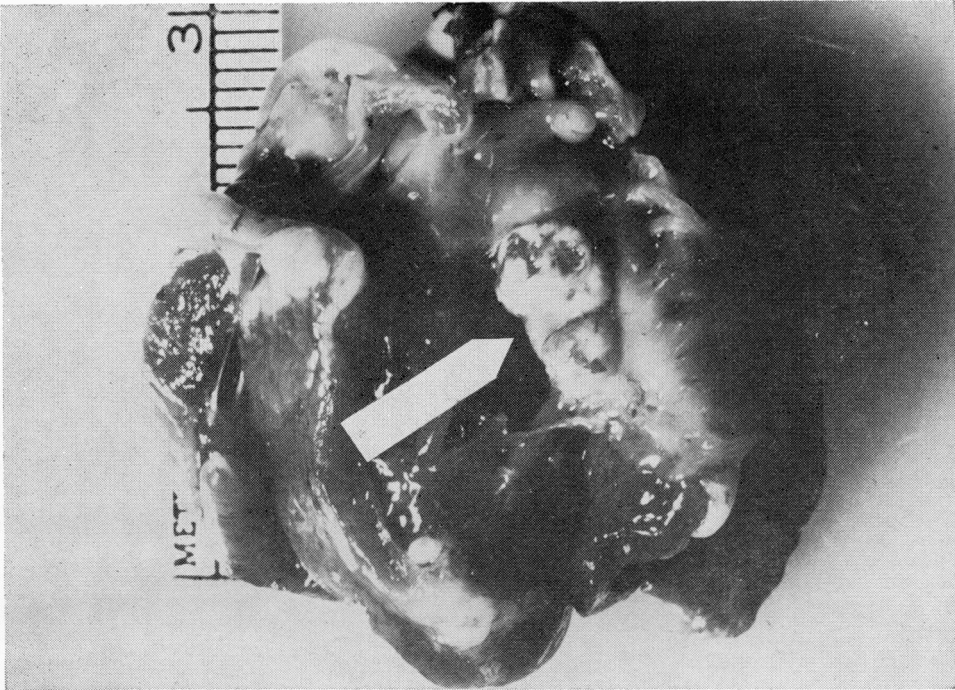


FIG. 1. Rabbit no. 169. Infected vegetation on tricuspid valve and r. ventricular wall. Small numbers of organisms were recovered from the vegetation.

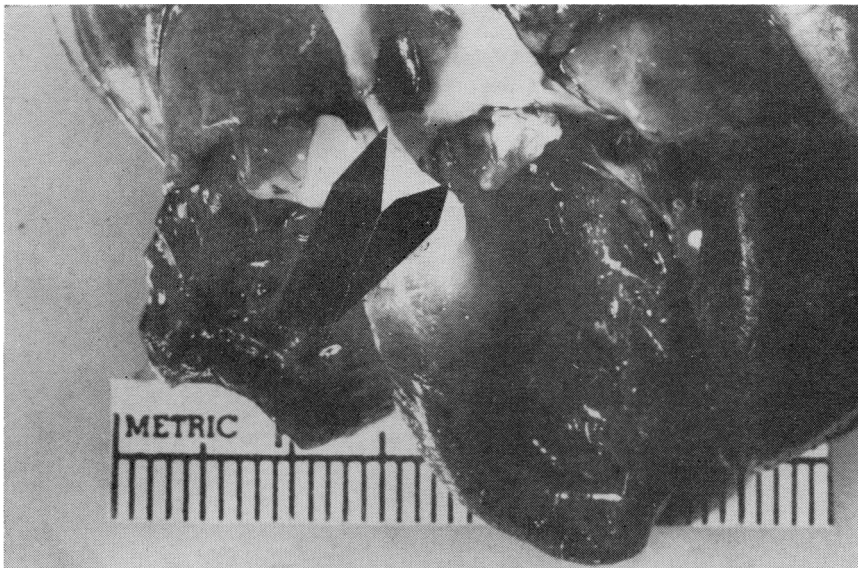


FIG. 3. Rabbit no. 272. Vegetation in aortic cusps from which large numbers of staphylococci were cultured.

fectured vegetations, but in only one rabbit with sterile vegetations. Cultures of the organ homogenates of three of the animals with septic vegetations yielded only one set of positive cultures. Terminal blood cultures of three rabbits with infected vegetations yielded one positive result. Kidney infarcts or abscesses occurred in equal proportions in rabbits with infected and sterile vegetations; they were noted in three of six rabbits with septic vegetations and in four of eight with sterile vegetations.

In the animals that were found to have infected vegetations, the catheter remained in position from three to five days and the animals were sacrificed or died from two to ten days after catheter removal; the total experimental time ranged from 5 to 13 days. The animals with sterile vegetations had catheters in position from three to six days and were sacrificed at intervals from 2 to 14 days after catheter withdrawal; the elapsed time from catheterization to postmortem examination was from 7 to 17 days. The distribution of numbers of rabbits within these time intervals may be ascertained from Fig. 2. Half the rabbits with infected vegetations died; none of the rabbits with sterile vegetations died.

DISCUSSION

In previous experiments it was demonstrated that as long as catheters containing staphylococci remained in the heart, infected vegetations would be encountered at postmortem examination.^{1,2} The experiments presented in this paper elucidate the consequences of catheter removal on the natural history of this model infection. When catheters were removed from the right side of the heart, the rabbits seemed capable of promptly sterilizing the vegetations. In the few animals in which septic vegetations were discovered, the number of viable bacterial units within the vegetation was small. In contrast, after catheters were withdrawn from the aortic region, 43% (6/14) of the rabbits displayed infected vegetations containing large numbers of viable bacterial units. Evaluation of animals with left and right heart catheterizations shows that the groups were generally comparable, i.e. the length of catheterization and the elapsed interval following removal of the catheter were similar in the two groups. If anything, the animals with right-sided endocarditis had catheters in place longer and were examined after a shorter interval following removal of the catheter, as compared with the animals with left-sided endocarditis. Thus the animals with right-sided endocarditis had a somewhat longer period in which to establish infections and were examined at a time when the infections were less likely to have healed. Nevertheless, an enhanced ability to effectively sterilize bacterial endocarditis in the right heart as contrasted with the left heart was apparent.

Indeed, once the catheters were withdrawn, some suggestion of healing

of the lesions appeared in many of the animals that had undergone right heart catheterization; the lesions were smaller and firmer than those previously reported when catheters remained *in situ* until sacrifice.¹ This resolution was not evident as often in animals whose left hearts had contained catheters. Mortality was encountered only in animals with infected aortic vegetations, and not found at all in animals that had undergone right-sided catheterization.

It might have been expected that a critical time would have emerged beyond which, if the catheter remained *in situ* and were subsequently removed, a self-sustaining disease would be induced. No such specific duration for right heart catheterization became apparent. Even animals from which catheters were removed after two weeks were able to sterilize the vegetations. Although a critical time could not be defined in animals subjected to left heart catheterization, an irreversible point may be established after as few as three days of catheter implantation in some animals.

It is noteworthy that with only two to three days of cannulization, even in the absence of continuing infectious disease, gross sterile vegetations were already extant and were evident for as long as 14 days afterwards. The rapidity with which sterile lesions were established was equally evident on both sides of the heart.

The ability of the animals to effectively sterilize right-sided bacterial endocarditis has its analogue in clinical medicine. It is known that a relatively small proportion of cases of bacterial endocarditis involves the right heart exclusively.⁵⁻⁶ Indeed, less than 2% of cases are believed to involve the tricuspid valve alone.⁶⁻⁸ The disparity is, to a large extent, attributed to the greater frequency of pre-existing valvular disease in the left heart. In view of the results of this study, the emphasis on pre-existing lesions may need redirection. Even when damage inducing susceptibility to endocarditis is equal on both sides of the heart, as in the present study, infection on the right side was not maintained. Those factors responsible for the different course of infection in the right and left sides of the heart are not known. The difference in physical and metabolic environment may be important in explaining the different distribution of infectious valvular disease in man as it has been in explaining the distribution of non-infectious endocarditis.⁹

Prior to the advent of antimicrobial chemotherapy, the mortality rate in hospitals for patients having bacterial endocarditis approached 80%;¹⁰ the attainment of the "bacteria-free stage" ranged from 0-3%.^{11,12} Subsequently, the utilization of appropriate therapy substantially reduced the mortality figures.^{13,14} The belief that the conquest of this formidable disease was at hand proved unfounded. Today mortality figures remain in the range of 17-35%.^{15,16} Furthermore, with the advent of cardiac surgical procedures in-

volving the placing of valves and other prostheses, the inability to chemically sterilize postoperative endocarditis has become a taxing problem. For this group mortality rates have again climbed to the 50-75% range.¹⁶⁻²¹ The model infection employed in the present studies may be useful in assessing the efficacy of antimicrobial agents in the treatment of infective endocarditis.

SUMMARY

Previous studies described a technique for the induction of bacterial endocarditis in rabbits that depended upon the presence of a catheter containing staphylococci in either the left or right side of the heart. The present study was undertaken to follow the natural history of these infections after removal of the catheter. Following removal of the catheter, the rabbits with right heart endocarditis were able to sterilize their vegetations more effectively than animals with left heart endocarditis. Of 18 rabbits with right-sided endocarditis, three had bacteria in low numbers recovered from the vegetations, whereas, six out of 14 animals with aortic endocarditis had large numbers of bacteria recovered.

Thus with the conditions for establishing bacterial endocarditis the same on both sides of the heart, infections healed more rapidly on the right side than on the left side.

ACKNOWLEDGMENT

The authors are indebted to Mrs. Mary Lou Breitenstein and Miss Kristen McLane for their expert technical assistance.

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