Infective endocarditis (IE) in humans may occur in patients with previously damaged native valves (native valve endocarditis or NVE) or, less frequently, in patients with prosthetic valves (prosthetic valve endocarditis or PVE) (14). Viridans group streptococci and staphylococci are most often isolated from blood cultures in NVE and PVE, respectively, whereas Coxiella burnetii, the agent of Q fever, is the primary etiologic agent of culture-negative IE. The most widely used experimental models of IE are the rabbit (1, 5, 10) and rat (13) models, in which cardiac valve lesions are induced by a polyethylene catheter introduced into the left ventricle (10, 13). Since the intracardiac catheter has a considerable influence on the ability of bacteria to colonize the endocardium (2, 3, 8, 9, 11, 16), these models more closely mimic the pathogenesis of PVE. Also, the intracardiac catheter decreases the efficacy of the antibiotic therapy used for either therapeutic or prophylactic purposes (6, 9, 12, 15). Our goal was to develop an experimental model of IE that more closely mimics the pathogenesis of PVE.

**We present a new experimental model of *Staphylococcus aureus* infective endocarditis in guinea pigs. Permanent aortic valve damage was produced by electrocoagulation after catheterization of the right carotid artery, which allowed avoidance of the intracardiac catheter to produce cardiac vegetations. Our model closely mimics pathological mechanisms of native valve endocarditis.**

**Production of aortic valve damage.** Male Hartley GP (weight, 500 to 600 g) were anesthetized by a 1-ml intramuscular injection of a mixture of ketamine hydrochloride (25 mg/ml; Panpharma, Fougeres, France) and chlorpromazine (2.5 mg/ml; Specia, Paris, France). Polyethylene tubing (Seldicath [1.0-mm outer diameter]; Plastimed, St-Leu-La-Forêt, France) and chlorpromazine (2.5 mg/ml; Specia, Paris, France) were introduced into the right carotid artery, which was exposed through an anterior incision. The catheter was then withdrawn by 2 cm so as to place its tip above the aortic valve, and a metal wire was introduced into the catheter so that it protruded from its distal tip by 1 cm. The aortic valve was damaged by three electrocoagulations with an electrocoagulator (Coagulasem; Dolley SA, Montroge, France). The catheter and its wire were then removed. Valve coagulation was obtained within 20 min, with a mortality rate from the operation and anesthesia of less than 10%. Surviving animals showed few abnormalities and fed normally.

**Assessment of aortic valve damage.** Valve lesions were assessed by macroscopic and histologic examination of heart valves. Aortic valves were excised and prepared for light microscopy. They were fixed in 10% formal saline, paraffin embedded, and sectioned at 5 μm. They were stained with hematoxylin-phloxin-safron. Valve lesions were first assessed in 19 animals sacrificed immediately or up to 30 days following valve electrocoagulation. Macroscopic valve lesions were obtained in 15 (79.9%) of 19 GP. Histologic examination of aortic valves removed within 15 days following electrocoagulation confirmed endothelial lesions and showed a nonbacterial thrombotic vegetation (NBTV) on the endothelial surface (Fig. 1D). In GP sacrificed later, the endothelial surface was almost completely repaired and the NBTV was no longer present.

Aortic regurgitation was assessed in six GP by an angiographic technique before and after valve electrocoagulation, i.e., injection of a 2-ml bolus of iodine contrast medium (Hexabrix 320; Laboratoire Guerbet, Aulnay/Bois, France) through the carotid artery catheter after confirmation of the placement of the catheter tip just above the aortic valve. Aortic regurgitation was demonstrated only in valves which had been electrocoagulated, with massive opacification of the left ventricle (Fig. 1A and B).

**Production of IE.** Thirty-five GP were infected with *S. aureus* 1 to 11 days following valve electrocoagulation. After the animals were anesthetized, an *S. aureus* (ATCC 29213) suspension (0.5 ml, i.e., 8 × 10^7 to 2 × 10^8 CFU) was injected through the femoral vein exposed through an anterior incision. The body temperature was monitored during the challenge. Blood cultures were performed by intracardiac puncture at the time of death or sacrifice of the animals. The presence of IE was confirmed by macroscopic and histologic examination of heart valves as described above and by culturing of cardiac vegetations. *S. aureus* injection resulted in elevation of the body temperature over 40°C within the first 24 h in all animals. IE could not be assessed in four (11.4%) of the animals because of early death. Pathological examination of heart valves did not find infectious valve involvement in 12 (38.7%) of the remaining 31 GP and confirmed IE in 19 (61.3%) of the animals.

GP with IE remained febrile until death, which occurred...
within the first 8 days postinfection in 17 of 19 GP. The remaining two animals were moribund and were sacrificed on day 8 of the experiment. In all GP with IE, *S. aureus* was cultured from the blood and the cardiac vegetations. Yellowish, adherent vegetations (Fig. 1C) approximately 1 to 5 mm wide were found on gross examination of heart valves. IE was confirmed on histologic examination, showing large infectious vegetations with clusters of bacteria in fibrin layers (Fig. 1E).

Eleven of the 12 GP without IE survived the *S. aureus* challenge and were sacrificed 3 to 30 days later. In these animals, fever resolved within the first week postinfection. Blood cultures performed at the time of sacrifice were negative in 11 of the 12 GP. Valve lesions due to electrocoagulation were found in only 1 of the 12 GP without IE. Bacterial vegetations were not found either on macroscopic examination of heart valves or on histologic preparations.

**Pathologic examination of other GP organs.** Specimens from the lungs, the liver, the spleen, and the kidneys were prepared for light microscopy as described above. Histologic findings on other organs are summarized in Table 1. Abscesses were present mostly in GP with IE, predominantly in the myocardium (89.5% of animals) and kidneys (84.2%) and to a lesser extent in the liver (47.3%), spleen (36.8%), and lungs (10.5%).

**Quantitative microbiologic analysis.** Bacterial titers per gram of tissue were determined in cardiac vegetations from 5 animals with IE and in spleen specimens from 13 animals with IE and 11 without IE. Organ tissue fragments were crushed in tryptic soy broth (Pasteur, Paris, France), and 10-fold serial dilutions of each were inoculated onto tryptic soy agar plates. Bacterial titers were determined in terms of CFU and analyzed by using Student’s *t* test. The mean bacterial count determined in cardiac vegetations ± the standard deviation was $5.1 \times 10^9 \pm 5.7 \times 10^9$ CFU/g of tissue. The mean bacterial counts in spleen specimens were $1.81 \times 10^3 \pm 2.23 \times 10^3$ CFU/g of tissue in GP with IE ($n = 13$) and $5.36 \times 10^3 \pm 8.97 \times 10^3$ CFU/g in GP without IE ($n = 11$) ($\alpha < 0.02$; Student’s *t* test).

*S. aureus infection in controls.* Six nonoperated GP were challenged with *S. aureus* as controls. All animals became febrile after *S. aureus* challenge, but the fever resolved within the first week. Blood cultures performed at the time of sacrifice, 1 month following *S. aureus* challenge, were sterile. Pathologic examination of heart valves did not reveal the presence of IE. Abscesses were not found in the other organs removed.

The experiments described here were conducted to establish whether it is possible to produce IE in GP without using intravascular devices. Permanent heart valve damage was induced by an aortic valve electrocoagulation procedure which was easy to reproduce and should also be applicable to other animal models, including rabbits or rats. Aortic regurgitation after valve electrocoagulation was documented by angiography, and pathological examination of electrocoagulated heart valves revealed the presence of permanent damage and the formation of thrombotic vegetation. However, both the NBTV and the aortic regurgitation were no longer present 1 month after electrocoagulation, suggesting that GP have a remarkable capacity to repair damaged valves. All animals with IE died within 8 days following *S. aureus* challenge, which is compara-
ble to the survival time previously reported (5). IE was confirmed by macroscopic and histologic examination of the heart valves and by culture of \textit{S. aureus} from the vegetations, with bacterial numbers within cardiac vegetations comparable to those obtained in rabbits and rats (5, 7, 10, 13). Vegetations were, however, smaller than those usually observed in animals with intracardiac catheters. Persistent bacteremia was demonstrated in GP with IE, along with abscesses predominantly in the kidneys and the myocardium, in keeping with experimental data on rabbits or rats (4, 5, 10, 13). Spleen bacterial counts were significantly (approximately 4 log\textsubscript{10}) lower in GP without IE than in GP with IE, indicating that most of the bacteria had been cleared in the former animals, which were no longer bacteremic. No valve lesions due to electrocoagulation were found in most of the animals without IE, suggesting that the valve electrocoagulation process had not been effective, which may explain, in most cases, the lack of IE development following \textit{S. aureus} injection. Both the NBTV and the \textit{S. aureus} vegetation in the hearts of GP bore a striking resemblance to human IE, and embolic phenomena in GP with IE were consistent with pathologic data on humans (14).

In conclusion, the model described herein closely reproduces the pathophysiology of \textit{S. aureus} NVE. By avoiding the use of an intracardiac foreign body to produce the NBTV, this experimental model may allow more relevant investigations of pathophysiologic mechanisms and antibiotic therapy and prophylaxis of NVE. Experiments intended to establish the applicability of the GP model to Q fever endocarditis are under way.

We thank J. S. Dumler and R. Birtles for reading the manuscript.

REFERENCES