14. R. C. Gesteland, *Ann. N.Y. Acad. Sci.*, in press.
15. I thank L. M. Beidler for his support and advice and D. Tucker for his technical advice on the twig preparation. Supported by U.S. Public Health Service grant NB 5258-05.

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Persistence of Bacteria in "Protoplast" Form after Apparent Cure of Pyelonephritis in Rats

Abstract. *Appropriate antibiotic treatment of experimental enterococcal pyelonephritis in the rat appears to effect the removal of the infecting organisms from the kidney, as judged by the culturing of kidney homogenates on standard bacteriological media. When the homogenates were cultured on media containing 0.3*M *sucrose, osmotically stabilized, it was demonstrated that bacteria were present in the "protoplast" form, and that this form persisted in the kidney for at least 13 weeks after therapy.*

The course of pyelonephritis may be characterized by frequent recurrences despite seemingly adequate therapy. Furthermore, in many cases, the disease appears to progress histologically despite the absence of bacterial infection as determined by either urine cultures or bacteriological examination of biopsy specimens from the kidney. A possible explanation of these findings is that bacteria persist in the kidney, even after appropriate antibiotic therapy, in a form that cannot be cultured on standard bacteriologic media. It has recently been reported from this laboratory that enterococcal pyelonephritis in the rat can apparently be cured by penicillin therapy (*1*). Experiments were designed to determine if renal persistence of bacteria in the form of "protoplasts" (*2*) occurred.

Ninety-four white, male Wistar rats weighing 100 to 115 g were injected intravenously with 1.0 ml (4.0×10^8 bacteria) of an 18-hour broth culture of *Streptococcus faecalis* (*3*). One day after inoculation, 49 of these rats were started on therapy which consisted of 100,000 units of procaine penicillin injected intramuscularly twice daily for 3 weeks. Groups of treated and untreated rats were killed at intervals during and up to 13 weeks after cessation of treatment, and the kidneys were removed for bacteriological study. Each kidney was divided into three equal pieces; each piece was homogenized and the homogenate was serially diluted in distilled water, 0.85 percent sodium chloride solution, or 0.3*M* sucrose solution in heart infusion broth. For cell counts of viable bacteria portions of the diluted homogenates made with 0.85 percent sodium chloride solution or distilled water were incorporated into pour plates containing blood agar base (Difco). Portions of the suspensions diluted in 0.3*M* sucrose were incorporated into pour plates made with blood agar base that also contained 0.3*M* sucrose. This concentration of sucrose, 0.3*M*, acts as a stabilizing agent for the osmotically fragile "protoplasts." All plates were incubated at 37°C for 48 hours. Several colonies were picked from each plate and the organisms were identified as *S. faecalis* by the following criteria: typical appearance on gram stain, growth in 6.5 percent NaCl in heart infusion broth, growth in and reduction of the dye in *S. faecalis* medium (BBL), negative catalase test and fermentation of lactose, mannitol, glucose and sorbitol.

The results, presented in Table 1, (*4*) indicated that the bacterial population in the kidneys of infected, untreated rats persisted in relatively constant numbers throughout the period of observation. These data do not indicate that significant "protoplast" formation occurred in the untreated animals. In those infected animals which received penicillin treatment, bacteria were not found in the kidneys at the end of therapy when homogenates of these organs were cultured on standard, nonstabilized medium. However, when 0.3*M* sucrose medium was used, "protoplasts" were found in the kidneys of the majority of animals up to 13 weeks after cessation of therapy. It is clear that the bacteria persisted in the tissues of the penicillin-treated animals in the form of "protoplasts," as indicated by the lack of growth in the nonstabilized medium. However, when grown on 0.3*M* sucrose medium, the "protoplasts" reverted to standard bacterial forms as manifested by growth in subculture in the various nonstabilized media used for identification.

Two additional control groups of animals were studied. Twelve normal (uninfected and untreated) rats and eight treated (uninfected but given penicillin for 3 weeks) rats were killed at times corresponding to 1 and 2 weeks after treatment and homogenates of the kidney were cultured. In no instance were bacterial or "protoplast" forms isolated. Thus, "protoplasts" developed only in the kidneys of animals infected with *S. faecalis* and subsequently treated with penicillin. Although penicillin therapy accounted for the conversion of bacterial forms to "protoplasts," it should be recognized that the osmolar concentration of renal tissue and urine is sufficiently high to protect these "protoplasts" from osmotic shock (*5*). This suggests that "protoplasts" might persist in renal tissue for long periods of time. The reason that "protoplasts" persist without reversion to the bacterial form is unknown but there may be a relation to the part played by serum antibody. Muschel *et al.* (*6*) have shown that exposure of bacteria to antibody, complement, and lysozyme can result in "protoplast" formation. Antibacterial antibody has been found in rats with *S. faecalis* pyelonephritis (*7*)

Table 1. Average of the logarithm of the number of bacterial and "protoplast" forms, per gram of kidney, isolated from treated (with penicillin) and untreated pyelonephritic rats. The numbers in parentheses indicate the proportion of kidneys containing organisms. Since all kidneys of untreated animals examined contained bacteria, the fraction is not presented in this group.

Time*	Treated			Untreated		
	No. of rats	Culture medium		No. of rats	Culture medium	
		Standard	0.3*M* sucrose		Standard	0.3*M* sucrose
2 days†	5	2.35 (4/5)	4.39 (5/5)	5	3.86	5.70
8 days†	6	1.02 (3/6)	2.49 (6/6)	6	6.39	6.60
3 wk	6	0.14 (1/12)	3.64 (12/12)	6	6.04	5.91
4 wk	6	0	3.82 (12/12)	6	6.46	6.53
5 wk	6	0	1.95 (6/12)	6	6.18	6.50
6 wk	6	0	2.64 (11/12)	6	5.42	5.83
11 wk	6	0	1.06 (4/12)	6	5.19	5.44
16 wk	8	0	2.01 (12/16)	4	4.92	5.61

* Interval between intravenous infection and sacrifice. † In the 2- and 8-day experiments only alternate kidneys were studied. At subsequent times, both kidneys were examined for bacterial and "protoplast" forms.

and it is possible that this, plus other serum factors, might account for the persistence of "protoplasts" in the kidney.

Host-parasite relationships of "protoplasts" are not well defined. The availability of an experimental model of infection in which "protoplasts" occur during the natural history of the treated disease will now permit study of this important problem.

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References and Notes

1. L. B. Guze, E. G. Hubert, G. M. Kalmanson, *J. Lab. Clin. Med.* **62**, 90 (1963).
2. As used in this paper, "protoplast" refers to an osmotically fragile bacterial cell in which the amount of cell wall present has not been determined.
3. L. B. Guze, B. H. Goldner, G. M. Kalmanson, *Yale J. Biol. Med.* **33**, 372 (1961).
4. Since the results obtained with 0.85 percent sodium chloride solution and distilled water were the same, only those obtained with sodium chloride will be presented.
5. P. Mitchell and J. Moyle, *J. Gen. Microbiol.* **15**, 512 (1956); K. J. Ullrich, K. Kramer, J. W. Boylan, *Progr. Cardiovascular Dis.* **3**, 395 (1961); A. I. Braude, J. Siemienski, I. Jacobs, *Trans. Assoc. Am. Physicians* **74**, 234 (1961).
6. L. H. Muschel, W. F. Carey, L. S. Baron, *J. Immunol.* **82**, 38 (1959); W. F. Carey, L. H. Muschel, L. S. Baron, *ibid.* **84**, 183 (1960).
7. G. M. Kalmanson, E. G. Hubert, L. B. Guze, *Proc. Soc. Exptl. Biol. Med.* **113**, 918 (1963).
8. Supported by grants (AI-02257 and AI-03343) from U.S. Public Health Service.

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Induction of Papillary Growths in the Heart

Abstract. *Papillary growths appeared on the atrial endocardium of dogs at sites where adjacent surfaces presumably came into contact with a reciprocating motion. It is suggested that the resulting friction stimulates the growth of papillae which then act as peduncular ballbearings, reducing the friction.*

While villi, papillary growths, and papillomas are ubiquitous structures in animals, such growths have been induced previously only by using chemical carcinogens or following specific viral infection (*1*). In our experiments circumscribed overgrowths of surface layers appeared in the course of studies designed to evaluate the role of physical forces in the differentiation of vascular and related tissues (*2, 3*).

In nine anesthetized thoracotomized dogs, the left atrial appendage was invaginated to form an epicardial sheath. A 4-ml Silastic (*4*) ball was placed into this sheath and held in place by a suture. This procedure introduced an endocardium-lined mass into the atrium. The animals were killed 10 to 202 days after surgery.

All the growths were found in the left atrium. At 10 days a marked fibroblastic proliferation was seen on the endocardial surface of the mass. In the other eight animals killed 31 to 202 days after surgery, round, slightly raised cauliflower-like growths about 5 mm in diameter were present on the endocardial surfaces of the intra-atrial mass (Fig. 1). Each of these growths was paired with one on the adjacent parietal atrial wall or on the mitral valve. Two or three pairs of such growths were found in the left atrium in each of the animals. The central portion of some of the growth on the atrial wall was saucerized, as if wear had taken place. Some of the masses were deep brown; others were lighter in color. The microscopic structure in both types was similar. The growths were richly vascularized, endothelium-covered excrescences consisting of numerous papillae, some of which were branched (see cover). The endothelial cells on the tips of the papillae were hyperplastic (Fig. 2), and contained vesicles which were periodic acid-Schiff positive and stained with alcian-blue dye. Occasional polymorphonuclear and mast cells, elastin, and small quantities of hemosiderin were present in the stroma of the stalks.

In previous studies at this laboratory, it has been suggested that specific mechanical forces induce definitive changes in the walls of blood vessels. As examples, collagen appears in tissues subjected to tensile forces, elastin at sites of a high rate of change of tension (*5*), cartilage in regions of high rates of change of compression (*3*), and increased cross-sectional areas of blood vessels at sites of increased hydrodynamic drag (*6*).

The motion of the heart probably produced a to-and-fro movement and sliding contact of the intra-atrial mass with adjacent atrial and valvular surfaces. The appearance of papillary growths at such sites suggests that frictional forces may contribute to the genesis of these growths.

A number of data appear to support this hypothesis. For example, the papillomatous proliferation of the intestinal mucosa in ulcerative colitis suggests that frictional action of apposing colonic surfaces may stimulate the formation

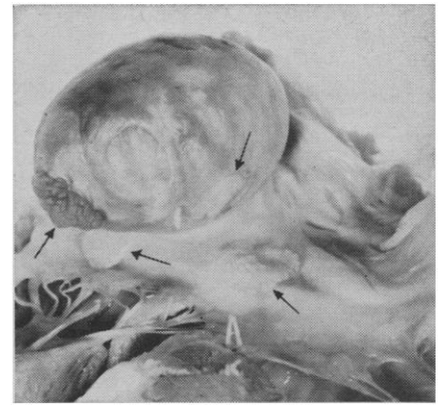

Fig. 1. Growths in the heart of a dog killed 7 months after surgery. The elliptical body is the endocardium lined intra-atrial mass. The mitral valve is at the lower left. Arrows point to the cauliflower-like growths, two on the intra-atrial mass, one on the parietal atrial wall (right), and one on a mitral leaflet.

of these growths. A ring of hypertrophic villi is usually present on the margin of peptic ulcers at which increased frictional forces may be operative. Papillomatous nodules occur on the vocal cords at sites which strike each other irregularly and haphazardly (*7*). Similar mechanisms during the inflammatory stage of rheumatic fever may contribute to the characteristic papillary proliferations on heart valves. Skin tags (*8*) (papilloma molle) are commonly found on the anterolateral surfaces of the neck and trunk at sites where collars and other clothing may produce chronic reciprocating frictional forces.

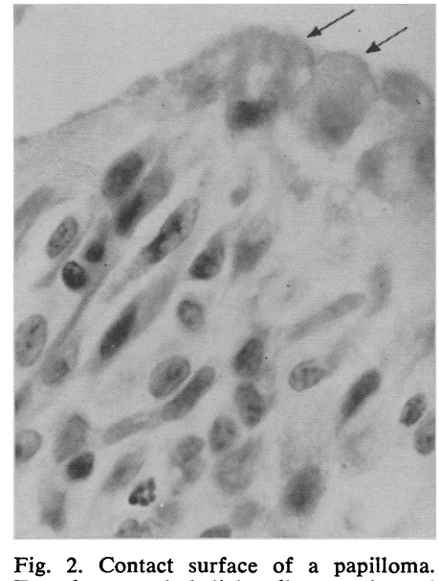

Fig. 2. Contact surface of a papilloma. Two large endothelial cells are situated above the fibroblastic growth (hematoxylin eosin, × 900).