EXPERIMENTAL THROMBOTIC BACTERIAL (STREPTOCOCCUS VIRIDANS) ENDOCARDITIS

I. Its Production and Incidence in the Rabbit *

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The primary objective of these experiments was to help resolve the problem of therapeusis in subacute bacterial endocarditis. The more rational and innocuous approach of animal experimentation appealed to us rather than direct evaluation of new expedients in human patients afflicted with the disease. It is true that countless therapeutic measures have been advanced for this disease, which alone attests to the futility of most of them.

For our purpose it is essential that the disease be reproduced consistently in a convenient laboratory animal. This initial report deals with methods and procedures adopted to achieve the greatest possible incidence of the experimental disease in rabbits.

Experimental endocarditis produced by bacteria other than *Streptococcus viridans* isolated from human cases has been reported many times.[1-3] There are also numerous reports of experimental endocarditis produced by traumatizing the endocardium or by the injection of particulate matter in addition to bacterial inoculations.[4-7]

Dreschfeld,[8] in 1887, was the first investigator to reproduce the disease without first resorting to mechanical injury of the cardiac valves. Horder [9] and Rosenow [10] also produced endocardial vegetations by simply injecting intravenously cultures of *Str. viridans*. The most recent work in this direction is the splendid accomplishment of MacNeal, Spence and Wasseen,[11] who demonstrated that it is possible to transmit endocarditis lenta of man to the rabbit by repeated intravenous injections of large amounts of pure cultures in serum-broth.

The technic followed was an adaptation of the pattern suggested by MacNeal and his co-workers.[11] Strains of *Str. viridans* were obtained by blood culture from clinically typical cases of subacute bacterial endocarditis. The organisms were subplanted in meat infusion broth which was occasionally enriched with rabbit plasma. One to 6 cc. of a fresh 24-hour culture was injected daily into an ear vein of a rabbit for 6 days. After an interval of 48 hours, a blood culture was taken and the sequence then repeated. This program was continued for variable periods until repeated positive blood cultures, progressive loss in weight and the development of cardiac murmurs heralded the presence

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of endocardial vegetations. Some animals died spontaneously, others were sacrificed. In some series attempts were made either to prevent or to cure the experimental disease. These results will be reported in a later communication. Necropsies were performed as soon after death as possible. Where vegetations were found, the organisms could be demonstrated histologically with ease, as well as in cultures. Mitral lesions predominated, although aortic, tricuspid and multiostial lesions were also encountered. Gross renal and splenic infarctions were observed as were the typical microscopic, embolic, renal lesions of Löhlein.[12]

The initial experiments were begun in August, 1940. For this series 31 rabbits and five strains of *Str. viridans* were employed. For these probatory experiments the organisms were grown in plain meat infu-

TABLE I

Strain	No. rabbits	No. positive	Per cent positive
M	1	1	100.0
R	6	3	50.0
S	7	1	14.0
H	5	0	0.0
A	2	1	50.0
M and R	10	5	50.0
Total	31	11	35.5

TABLE II

Strain	No. rabbits	No. positive	Per cent positive
S	7	3	42.8
H	8	8	100.0
B	6	4	66.6
Total	21	15	71.4

sion broth and only 1 to 2 cc. of the fresh 24-hour culture was injected. The results of this early experiment are shown in Table I.

For the purpose of this work the results in this series were too desultory and unpredictable. It was felt that the percentage of animals with endocarditis could be improved by stepping-up the virulence of our strains. With this in mind the H and S strains, which incidentally had given the poorer results, were passed through mice and cultured on broth enriched with rabbit plasma. In addition, the daily dose of inoculum was increased to 4 cc. Finally, a recently isolated and, presumably, more virulent strain was singled out and employed. The more helpful response is noted in the experiments summarized in Table II.

The inferences to be drawn from these experiments are that different strains of *Str. viridans* vary in their capacity to produce endocardial lesions in the rabbit, and, further, that this potentiality can be enhanced by passage of weak strains through mice, or by growth in enriched mediums, or both.

This thesis was confirmed in the next series of experiments which were begun in May, 1941. Streptococcal strains H and B, which were permitted to deteriorate by transplanting in plain meat infusion broth, were employed, as were two newly recovered strains of *Str. viridans*, F and G. The results are summarized in Table III.

Thus, endocarditis was produced in only 4 of 15 rabbits with the use of the devitalized strains H and B. On the other hand, fresh, relatively virulent strains F and G produced lesions in 8 of 11 animals.

TABLE III

Strain	No. rabbits	No. positive	Per cent positive
H	7	3	42.8
B	8	1	12.5
F	5	5	100.0
G	6	3	50.0

CONCLUSIONS

1. The rabbit is a suitable and convenient laboratory animal for the experimental production of subacute bacterial endocarditis.
2. A simple technic is outlined for the production of the disease in rabbits.
3. Different strains of *Str. viridans* vary in their ability to produce the experimental disease in the rabbit.
4. The virulence of weak strains of *Str. viridans* can be enhanced by passage through mice and by culturing on enriched mediums.
5. Subacute bacterial endocarditis was produced in from 50 to 100 per cent of rabbits in adequately controlled experiments.

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REFERENCES

1. Blahd, M., Frank, I., and Saphir, O. Experimental endocarditis in dogs. *Arch. Path.*, 1939, **27**, 424–432.
2. Kinsella, R. A., and Muether, R. O. Experimental streptococcic endocarditis. *Arch. Int. Med.*, 1938, **62**, 247–270.
3. Lloyd-Jones, D. M. An Experimental Study of Malignant Endocarditis. In: Perry, C. B. Bacterial Endocarditis. J. Wright & Sons, Ltd., Bristol, 1936, pp. 113–137.
4. Rosenbach, O. Ueber artificielle Herzklappenfehler. *Arch. f. exper. Path. u. Pharmakol.*, 1878, **9**, 1–30.
5. Ribbert, J. Ueber experimentelle Myo- und Endocarditis. *Fortschr. d. Med.*, 1886, **4**, 1–13.
6. Welch, H., Murdock, T. P., and Ferguson, J. A. Subacute bacterial endocarditis produced in rabbits with streptococci that resemble diptheroids. *J. Lab. & Clin. Med.*, 1935–36, **21**, 1264–1273.

7. Friedman, M., Katz, L. N., and Howell, K. Experimental endocarditis due to *Streptococcus viridans*. Biologic factors in its development. *Arch. Int. Med.*, 1938, **61**, 95–118.
8. Dreschfeld. Experimental investigations on the bacterial origin of nonulcerative malignant endocarditis. *Brit. M. J.*, 1887, **2**, 887.
9. Horder, T. J. Report on a study of the micro-organisms associated with rheumatic fever and malignant endocarditis. *Rep. Med. Off. Local Gov. Bd., London*, 1906–07, **36**, appendix B, no. 6, 279–310.
10. Rosenow, E. C. Experimental infectious endocarditis. *J. Infect. Dis.*, 1912, **11**, 210–224.
11. MacNeal, W. J., Spence, M. J., and Wasseen, M. Experimental production of endocarditis lenta. *Am. J. Path.*, 1939, **15**, 695–705.
12. Löhlein, M. Ueber hämorrhagische Nierenaffektionen bei chronischer ulzerözer Endokarditis. *Med. Klin.*, 1910, **6**, 375–379.

DESCRIPTION OF PLATE

PLATE 18

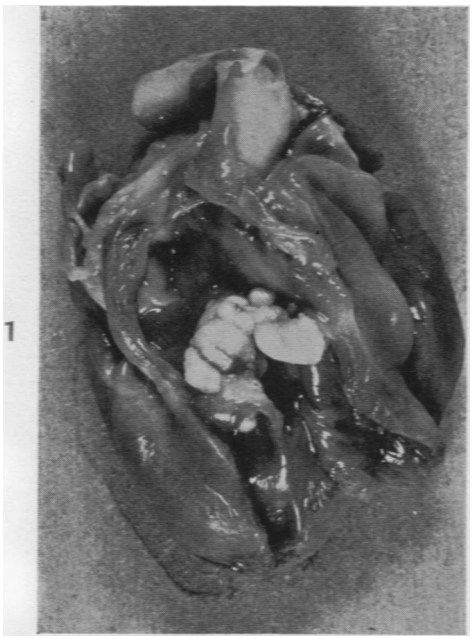
FIG. 1. Rabbit 903. Thrombotic endocarditis of tricuspid valve. This was combined with a mitral lesion. × 1.4.

FIG. 2. Rabbit 903. Thrombotic endocarditis of mitral valve. (See Fig. 1.) × 1.2.

FIG. 3. Rabbit 16. Thrombotic endocarditis of aortic valve. × 1.4.

FIG. 4. Rabbit 631. Infarct in kidney. Heart revealed aortic endocarditis. × 1.6.

FIG. 5. Rabbit 13. Experimental thrombotic endocarditis involving the aortic valve, showing complete destruction of cusps and huge vegetation harboring clumps of streptococci. × 20.



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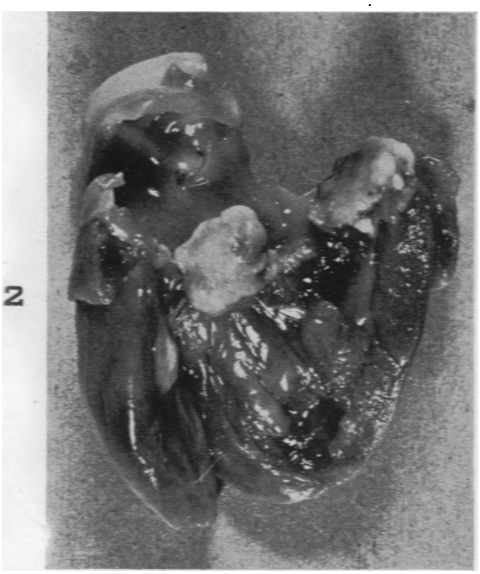

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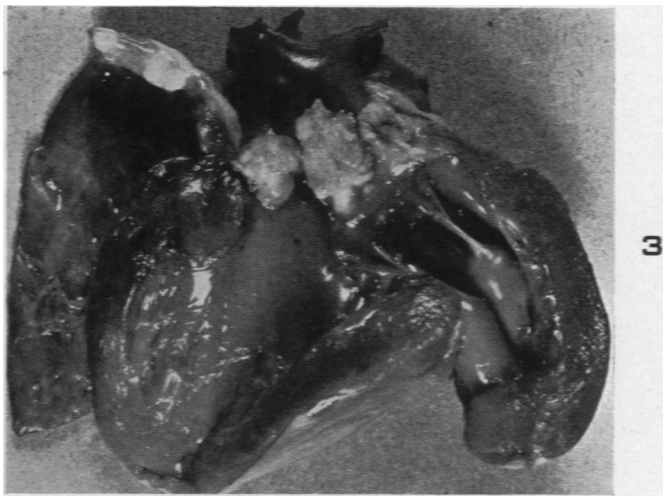

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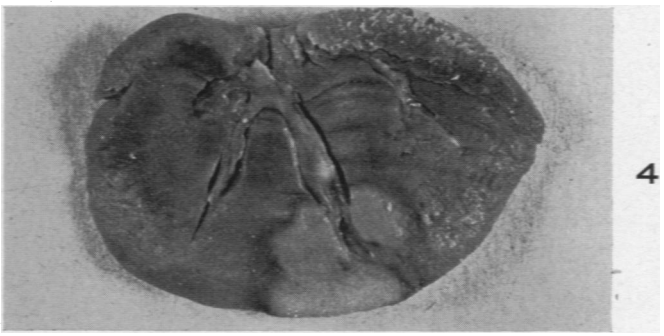

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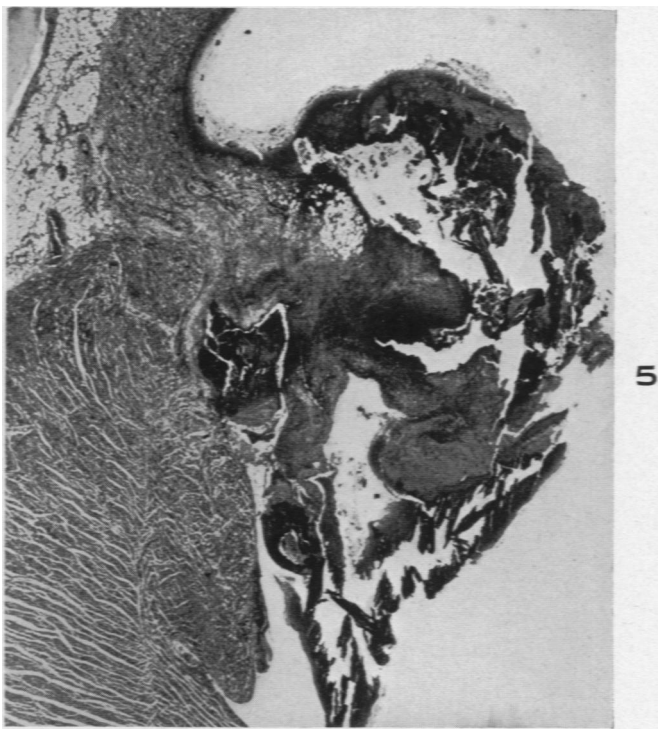

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Experimental Bacterial Endocarditis