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Effect of Splenectomy on the Therapeutic Action of *p*-Aminobenzenesulfonamide on Mice Infected with Hemolytic Streptococcus.

PAUL GROSS, F. B. COOPER AND M. L. PEEBLES. (Introduced by Arthur P. Locke.)

From the Western Pennsylvania Hospital, Institute of Pathology.

Sufficient experimental and clinical data have been reported by various authors, particularly by Colebrook and Kenny,[1, 2] to establish beyond all doubt the therapeutic effectiveness of *p*-aminobenzenesulfonamide and related diazo-sulfonamide compounds (Prontosil and Prontosil, soluble) in hemolytic streptococcal infections.

Ever since the discovery of Prontosil by Domagk,[3] the mechanism of the action of this group of chemicals has been a puzzling question. No evidence of bacteriostatic or bactericidal action of Prontosil had been proved until Colebrook, Buttle and O'Meara[4] demonstrated such action with *p*-aminobenzenesulfonamide against small numbers of hemolytic streptococci in culture medium and in blood. These investigators found Prontosil to be inactive as previously reported, but were able to demonstrate inhibitory effects when Prontosil was reduced with magnesium powder under partial vacuum. Long and Bliss[5] were also able to "activate" Prontosil by reduction with sodium formaldehydesulfoxalate.

As has been pointed out by Colebrook and coworkers,[4] and by Long and Bliss,[5] there is a wide discrepancy between the relatively small bacteriostatic and bactericidal action of the drugs *in vitro* and the excellent *in vivo* results. This discrepancy suggests that the bacteriostatic action may not be an essential factor in the therapeutic results obtained.

Colebrook, *et al.*,[4] suggest that the bactericidal action of the tissues of the whole animal may be important; whereas Long and Bliss[5] believe the phagocytic activity of the polymorphonuclear leukocytes and monocytes plays a paramount rôle in controlling infections caused by the beta-hemolytic streptococcus.

Our own investigations[6] on over 200 mice infected with 2 differ-

1 Colebrook, L., and Kenny, M., *Lancet*, 1936, **1**, 1297.

2 Colebrook, L., and Kenny, M., *Lancet*, 1936, **2**, 1319.

3 Domagk, G., *Deutsche Med. Wchnschr.*, 1935, **61**, 829.

4 Colebrook, L., Buttle, G. A. H., and O'Meara, R. A. Q., *Lancet*, 1936, **2**, 1323.

5 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

ent strains of hemolytic streptococci have shown no greater indication of phagocytosis in treated animals (as demonstrated in smears of peritoneal exudate and heart blood, as well as in sections of the livers and the spleens) than in the untreated controls. We are, therefore, not inclined to accept the hypothesis of Long and Bliss.

Various authors have theorized upon the possible rôle of the reticulo-endothelial system, which, when presumably stimulated by the sulfonamide compounds, supposedly combats streptococcal infections. No experimental evidence has been offered in support or refutation of this concept, except by Bosse,[7] who claims to have demonstrated the lack of protective action of Prontosil against hemolytic streptococcal infections in splenectomized mice. Unfortunately, no details regarding the number of mice employed, or the manner in which the experiment was controlled were given.

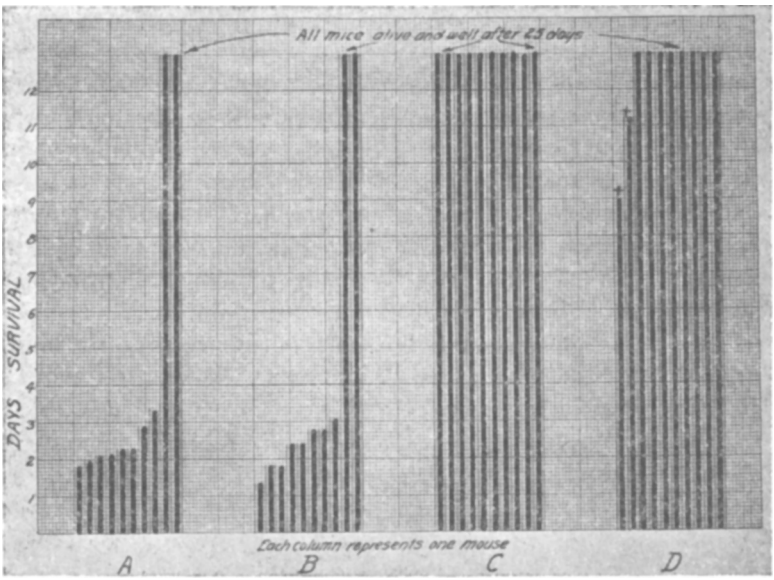

FIG. 1.

Graph of the survival time of mice infected intraperitoneally with the "Stoddard" hemolytic streptococcus (0.5 × 10^{-9} cc. of a 24-hour broth culture).

A. Normal untreated controls.

B. Splenectomized untreated controls.

C. Normal mice treated with 25 mg. *p*-aminobenzenesulfonamide by mouth immediately after infection and every 24 hours for 9 consecutive days.

D. Splenectomized mice treated in a manner identical to those in C. The columns marked + represent mice which at autopsy showed no streptococci in either the peritoneal cavity or the heart blood.

[6] Mellon, R. R., Gross, Paul, and Cooper, F. B., *J. A. M. A.* In press.

[7] Bosse, O. A., *Fortschritte D. Therap.*, 1936, **9**, 540.

The sections of livers and spleens from our several series[6] of mice infected with hemolytic streptococci and treated with Prontosil or *p*-aminobenzenesulfonamide, as well as sections from the untreated controls, failed consistently to show any morphologic evidence of activity on the part of the reticulo-endothelial system. This contradictory finding suggested an investigation of Bosse's claim.

The culture used was the "Stoddard" strain of hemolytic streptococcus in its mucoid phase. Preliminary titrations of a 24-hour broth culture on both normal and splenectomized mice showed no significant difference in their susceptibility and placed the lethal dose at 0.5×10^{-9} cc.

Twenty splenectomized mice which had been allowed to recover from the effects of the operation for a period of 3 to 4 weeks, and 20 normal mice were inoculated intraperitoneally with 0.5×10^{-9} cc. of a 24-hour broth culture. Ten of the normal, and 10 of the splenectomized mice were treated with 25 mg. of *p*-aminobenzenesulfonamide by mouth immediately after infection, and every 24 hours thereafter for 9 consecutive days, after which the treatment was discontinued. The other infected animals, 10 normal, and 10 splenectomized, were not treated, and served as controls.

The normal, as well as the splenectomized controls, showed an 80% mortality rate in 3 days, 2 mice surviving in each group. All mice in the normal treated group survived, whereas there were 2 deaths in the splenectomized treated group—one on the tenth, and one on the twelfth day. At autopsy, these 2 animals showed no streptococci in either the peritoneum or the heart blood. The results are shown graphically in Fig. 1.

Conclusions. 1. *P*-aminobenzenesulfonamide is capable of protecting splenectomized mice against fatal doses of highly virulent hemolytic streptococci. 2. The degree of protection observed in splenectomized mice was identical with that obtained with normal animals.