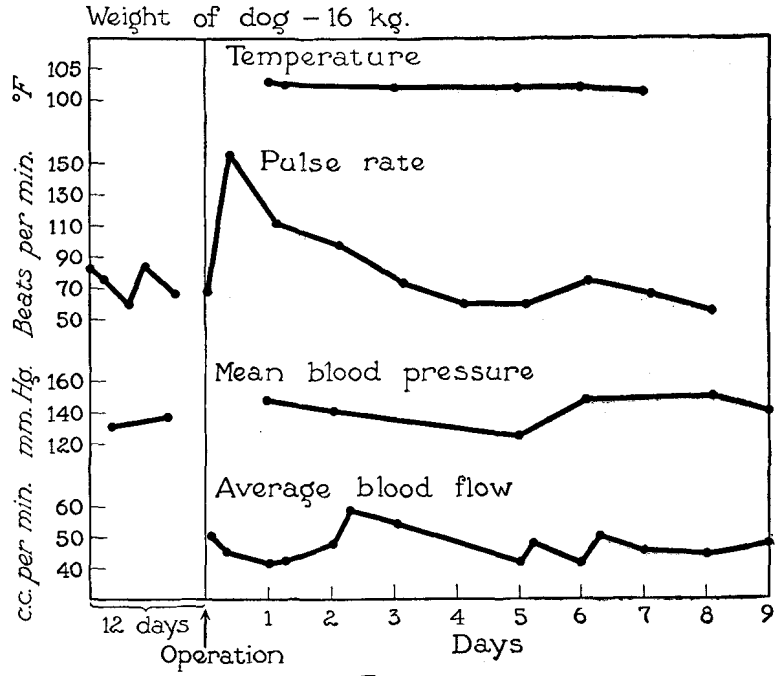

FIG. 2.

Summary of data on rectal temperature, pulse rate, blood pressure and blood flow. Six cat units of digiglusin were given from the second to the sixth post-operative day.

completed in collaboration with Dr. Maurice B. Visscher. The results, which are comparable to those reported here, will appear elsewhere.

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Failure of Sulfanilamide to Prevent Hemolysis, Fibrinolysis, and Production of Erythrogenic Toxin by Hemolytic Streptococci *in vitro*.

ROBERT W. HUNTINGTON, JR.* (Introduced by J. V. Cooke.)

From the Department of Pediatrics, Washington University School of Medicine, the St. Louis Children's Hospital, and the Shriners' Hospital for Crippled Children, St. Louis, Mo.

The mode of action of sulfanilamide in hemolytic-streptococcal infections is still unexplained. The bacteriostatic effect, which can

* Theron Catlin Fellow.

sometimes be demonstrated *in vitro*,[1] might conceivably be but one expression of a profound temporary change in the organism's activities. *In vivo* experiments have suggested that sulfanilamide renders streptococci more readily phagocytized.[2] Thus one might suspect an effect of sulfanilamide on the toxic substances produced by streptococci, and look either for inactivation of these agents or inhibition of their production.

From the finding that bone-marrow cultures preserve their morphology in the presence of copious streptococcal growth if the medium contains minute amounts of sulfanilamide, Osgood[3] suggests that the drug inactivates hemolysin and perhaps other toxic products as well. The trivial antihemolytic effect that he observed with sulfanilamide in blood-agar plates was attributed to impaired diffusion.

In view of Osgood's stimulating hypothesis, it was decided to investigate the action of sulfanilamide on hemolysis and fibrinolysis by broth cultures of hemolytic streptococci. Production of erythrogenic toxin and possible neutralization of this toxin were also studied.

The technical procedures were simple. Sulfanilamide was added to culture medium, hemolytic or fibrinolytic systems, and toxin-dilutions, from a stock solution of 200 mg. % in saline. The solution was sterilized by filtration, without appreciable loss of the drug. The solution was kept in the refrigerator, and, although no precipitation was observed, was warmed before use. The observations recorded were controlled with appropriate dilutions of sulfanilamide, which were found to be devoid of hemolytic, fibrinolytic, or erythrogenic effect.

The 4 streptococcal strains used were, "McGrew," freshly isolated from a child's mastoid, "ML-1A," an old septic-sore-throat strain from the National Institute of Health, the mouse-virulent "Todd," from Dr. H. M. Powell of Eli Lilly Co., and the scarlatinal N. Y. 5.

Concentrations of sulfanilamide of 15 to 40 mg. %, added along with rabbit blood to sugar-free broth, produced a slight delay in hemolysis when the tubes were inoculated. Such tubes showed little hemolysis 2 hours after inoculation, when there was marked hemolysis in the sulfanilamide-free controls. However, at 7 hours or later little difference could be noted. It was interesting that despite gross hemolysis, both sulfanilamide- and control-tubes

[1] Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

[2] Gay, F. P., and Clarke, A., *J. Exp. Med.*, 1937, **66**, 535.

[3] Osgood, E. E., *J. A. M. A.*, 1938, **110**, 319.

showed a few intact red cells in the sediment. The broth was not hemolytic.

Quite similar results were obtained when 16-hour cultures in 0.05% glucose broth were added to suspensions of washed human erythrocytes. After one hour in the waterbath there would be marked hemolysis by the control culture, and little by that grown in sulfanilamide-broth; but during the third hour the latter would rapidly catch up. Sulfanilamide 50 mg. % in the hemolytic system produced a similar effect in some instances, and none at all in others. Using 18-hour filtrates, Dr. Paul Hageman, to whom I am indebted for many unpublished data,[4] was unable to detect any antihemolytic effect of sulfanilamide.

Because turbidity appeared more slowly in sulfanilamide-media, it seemed likely that the slight early inhibition of hemolysis was due to growth-lag. DeKruif and Ireland[5] found that free hemolysin appeared in the medium only during very rapid growth, and was rapidly inactivated as growth slackened. Since sulfanilamide frequently flattens the growth-curve of *Str. hemolyticus,* the trivial effect on hemolysis is perhaps a little surprising. Living marrow-cells, if at all able to acquire tolerance for hemolysin, might detect slight hemolysis-inhibiting effects not readily demonstrable with peripheral erythrocytes. This is a possible explanation of the differences between these results and Osgood's. These findings are in accord with those of Swift, *et al.*,[6] which indicated that sulfanilamide did not inhibit the production of antigenic hemolysin in infected patients.

Fibrinolytic tests were done with the technic of Tillett and Garner.[7] Cultures were grown for 15 hours in 0.05% glucose-broth with and without 20 mg. % sulfanilamide. Each fibrinolysis-tube contained 0.2 cc. oxalated plasma, 0.5 cc. culture, and either 0.8 cc. saline or 0.4 cc. each of saline and stock sulfanilamide-solution. Coagulation was induced with 0.3 cc. of 0.25% calcium-chloride solution. No antifibrinolytic effect of sulfanilamide was noted. The results of a typical experiment are shown in Table I.

The remaining observations were on scarlatinal toxin. The broth used in this laboratory for the production of scarlatinal toxin is made with 0.1% glucose and 1% Parke-Davis "Bacteriological" peptone, in a veal-infusion base, and is autoclaved for 30 minutes to caramelize the glucose. No blood is added. With N. Y. 5 this

4 Hageman, P. O., personal communication.

5 DeKruif, P., and Ireland, P. M., *J. Inf. Dis.*, 1920, **26**, 285.

6 Swift, H. F., Moen, J. K., and Hirst, G. K., *J. A. M. A.*, 1938, **110**, 426.

7 Tillet, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

TABLE I.

Tube	Sulfanilamide solution, cc.	Saline, cc.	Culture	Time of lysis, min.
1	0.0	0.8	ML-1A, pl. broth	20
2	0.0	0.8	" "	15
3	0.0	0.8	" "	30
4	0.0	0.8	" sulf. broth	20
5	0.4	0.4	" pl. broth	15
6	0.0	0.8	Todd, "	60
7	0.0	0.8	" "	75
8	0.0	0.8	" "	90
9	0.4	0.4	" "	75
10	0.0	0.8	McGrew, "	65
11	0.0	0.8	" "	40
12	0.0	0.8	" "	40
13	0.0	0.8	" sulf. broth	70
14	0.4	0.4	" pl. broth	40
15	0.4	0.4	" "	45

pl. broth—without sulfanilamide.
sulf. broth—with 20 mg. % sulfanilamide.
sulfanilamide solution—200 mg. % sulfanilamide in saline.

broth regularly gives a titer of 50,000 STD/cc. A flask with 100 cc. broth and 25 cc. stock sulfanilamide-solution was inoculated with N. Y. 5; the 5-day filtrate was quite as potent as a toxin made in the same lot of broth without sulfanilamide. Children at the Shriners' Hospital were tested with 0.1 cc. of 1:500 and 1:5000 dilutions. Complete parallelism between the 2 toxins was observed in the 10 subjects with positive reactions to the higher dilution and in the 8 additional ones who reacted only to the lower.

For neutralization-tests the plain-broth toxin was diluted in saline with and without sulfanilamide. The tubes were then incubated at 37°C. for 30 minutes, and parallel skin-tests were done with 0.1 cc. amounts. Known positive reactors to toxin were used. Reactions to 1:1000 toxin in 10 mg. % sulfanilamide were studied in 15 subjects, and reactions to 1:5000 toxin in 200 mg. % sulfanilamide in 2 others. All of these reactions were indistinguishable from the controls. Dr. Hageman has found no neutralization of skin-toxin by 0.8% sulfanilamide. These findings are in accord with the clinical impression at this hospital that sulfanilamide is without effect on the purely toxic phase of scarlet fever.

Summary. Sulfanilamide produced a slight delay of hemolysis in blood-broth cultures of hemolytic streptococci. This effect was probably attributable to modification of the growth-curve. When used in concentration equal to or greater than that induced in the body-fluids therapeutically, sulfanilamide was without apparent effect upon fibrinolysis or formation of erythrogenic toxin *in vitro*, and was unable to inactivate small amounts of toxin.