In 8 animals with diminished blood volume the rate of disappearance of the dye from the blood stream was followed. It was found to be normal. The finding of the diminished blood volume was, therefore, not an error due to delayed mixing of injected dye.

The primary vascular effect of ergotamine tartrate poisoning may be thought to be a generalized vascular spasm. This results in a diminished blood volume, which may occur alone or in association with either gangrene of the tail or vascular hypertension, or both. The localized vascular dilatation which follows bilateral lumbar sympathectomy tends to raise general blood volume. It also makes the tail more susceptible to the gangrene of ergotamine tartrate poisoning.

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Observations on the Mode of Action of Sulfanilamide *in vitro*.

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The bacteriostatic effect of sulfanilamide *in vitro* has been demonstrated by many workers and there is little doubt that it plays an important part in the action of the drug *in vivo*. The experiments reported in this paper were designed to analyze some of the factors that play a rôle in this bacteriostatic action.

Methods. For the sake of uniformity, a single strain was used throughout. This strain, obtained through the courtesy of Dr. Rebecca Lancefield, was a group A, type 6, mucoid, hemolytic streptococcus, designated S 43. Unless otherwise stated, the medium used was neopeptone-water[1] to which horse serum was added to a final concentration of 25%. The concentration of sulfanilamide† was 10 mg %. Bactericidal and phagocytic experiments were performed according to the technics described by Ward[2] and Ward and Lyons.[1] Defibrinated human blood containing no antibody to this particular strain was employed. In the phagocytic

* Work done under tenure of the Edward Hickling Bradford Fellowship.

1 Ward, H. K., and Lyons, C., *J. Exp. Med.*, 1935, **61**, 515.

† Sulfanilamide obtained through the courtesy of the Research Department of the Winthrop Chemical Co.

2 Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.

experiments 2½-hour cultures showing well encapsulated organisms were used. The viable bacteria were estimated by the standard methods of serial dilution (in broth), pour-plates and colony-counts.

Results. In an attempt to confirm the work of Lyons[3] the authors first investigated the effect on strain S 43 of repeated transfer in 1-10,000 sulfanilamide broth. No permanent change was demonstrable in the organism morphologically even after 18 transfers, nor could any evidence of attenuation be found. The strain maintained its original virulence for mice and its ability to grow in whole blood. In fact, in at least one instance, the culture that had been repeatedly transferred in sulfanilamide-broth became *more* resistant to the bactericidal effect of additional sulfanilamide than either the broth-transferred control or the original untransferred culture. In performing these bactericidal experiments various combinations of type-specific immune rabbit serum (.1 cc of 1:20) and sulfanilamide were added to the blood. It was found that the combination of immune serum and sulfanilamide was more bactericidal than either immune serum or sulfanilamide alone. The left-hand columns in Fig. 1 represent these findings graphically. The average number of organisms killed by 0.25 cc of blood in the combined series was 680,000 as compared with 10 organisms with immune serum alone and 9000 with sulfanilamide alone. The columns on the right of Fig. 1 indicate the effect of the repeated transfer of strain S 43 in sulfanilamide-broth. The first set of columns represents the sulfanilamide-transfer series (S.T.S. 43), the second, the broth-trans-

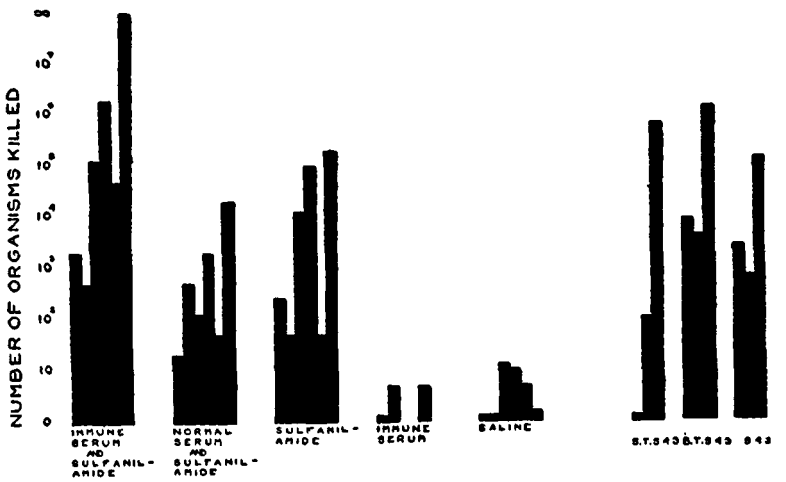


FIG. 1.

[3] Lyons, C., *Ann. Surg.*, 1938, **108**, 813.

fer series (B.T.S. 43), and the third, the original untransferred culture (S 43). From the relative heights of the columns in the 3 series it is apparent that the sulfanilamide-transferred culture showed no diminution in resistance. The fact that in making up dilutions of organisms for inoculating bactericidal tubes sulfanilamide was not added to the diluent may be the reason that the present findings fail to confirm Lyon's results.

The effect of sulfanilamide on phagocytosis was next investigated. In a concentration of 1-10,000 sulfanilamide had no demonstrable effect on phagocytosis but in more dilute solutions (1-40,000 to 1-80,000) phagocytosis was increased markedly. This observation confirms the results of Finklestein and Birkeland.[4] The probable explanation, however, is that sulfanilamide in concentrated solutions, like so many non-specific compounds[5] is somewhat toxic for leukocytes as well as bacteria but in dilute solutions is toxic only for the organisms. Such an explanation would indicate that the effect of sulfanilamide on phagocytosis is not a fundamental one and therefore does not explain its mode of action.

The final set of experiments in this series consisted of observations on the growth-characteristics of strain S 43 when exposed to sulfanilamide over varying periods. Although as previously stated no permanent morphological change was observed as a result of repeated transfer in sulfanilamide, temporary morphological alterations, similar to those noted by Gay and Clark[6] and by Lockwood[7] were noticed during the period of bacteriostasis. Capsules became larger, stained more deeply and remained on the organisms longer than in control cultures. Chains became markedly elongated and often contained as many as 50 cocci.‡

Chart 1 shows the effect of varying the size of the inoculum and Chart 2 the effect of varying the concentration of sulfanilamide with a moderately large inoculum and with a very small inoculum (initial count, not recorded on the chart, was 3 colonies per cc). These results are consistent with those of Lockwood for cultures containing peptone.

Since it was found that specific antibody greatly enhanced the killing power of sulfanilamide in human blood, the effect of this combination on the growth of the organism was determined. Chart

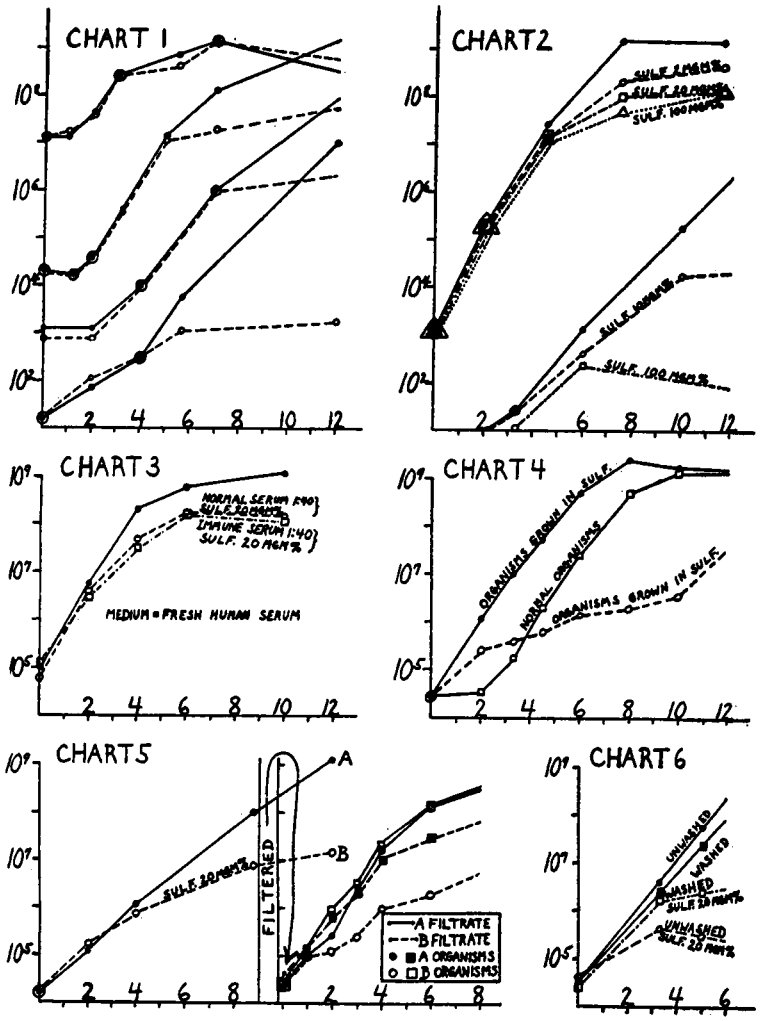
[4] Finklestein, R., and Birkeland, J., *Science*, 1938, **87**, 441.

[5] Tunnicliff, R., *J. Inf. Dis.*, 1931, **48**, 161.

[6] Gay, F. P., and Clark, A. R., *J. Exp. Med.*, 1937, **66**, 535.

[7] Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

‡ Since colony-counts represent the number of chains, rather than number of individual cocci, this may account for some of the apparent bacteriostasis.



Growth-curves of hemolytic streptococcus, strain S43, in 25% horse-serum neopeptone-water (excluding Chart 3). Solid lines represent control cultures, broken lines cultures containing sulfanilamide (10 mg %, unless stated otherwise). Ordinates represent number of colonies per cc; abscissæ, time in hours.

3 illustrates such an experiment in which the culture medium was fresh human serum to which sulfanilamide (20 mg %) and type-specific immune rabbit serum (1:40) were added. Another experiment in which a smaller inoculum was used gave the same results.

In all of these experiments there was a period of several hours during which multiplication proceeded at a normal rate in the presence of sulfanilamide before the bacteriostatic effect became

manifest. Examination of the data of other investigators[7-12] shows such a latent period in every case and Whitby[13] has recently called attention to it. It must be kept in mind in planning and evaluating any investigation on the action of this compound. It is impossible, for example, to draw any conclusions about the effect of sulfanilamide on the oxygen-consumption of bacteria from the work of Chu and Hastings[14] because the period of observation was too short.

The following experiments were designed to show whether an alteration of the organisms or of their environment occurs during the latent period. Chart 4 shows the result of inoculating organisms, grown over night in the presence of sulfanilamide, into control and sulfanilamide-media. It is evident that the growth of such streptococci is inhibited after a very brief period of multiplication in the presence of sulfanilamide, but proceeds at a normal rate in its absence. Chart 5 records the following experiment: Two cultures, one with and one without sulfanilamide, were divided when there was definite bacteriostasis, and a portion of each was passed through a Berkefeld V filter. Each filtrate, sterile on subculture, was immediately divided, and one-half was inoculated from the first and the other from the second of the original cultures. The normal organisms were not affected by the filtrate containing sulfanilamide any more than they would have been by fresh medium containing the drug. The organisms from the sulfanilamide-culture were promptly inhibited in this filtrate, just as in the previous experiment. These results point to an alteration of the organisms themselves rather than of their environment. In order to see how firmly the sulfanilamide is bound to such altered streptococci, organisms grown in the presence of the drug were washed 3 times in plain broth in the course of an hour and inoculated into fresh media. From Chart 6 it is evident that such treatment considerably reduced their susceptibility to the action of the sulfanilamide.

Summary. 1. Repeated transfer of a virulent strain of hemolytic streptococcus in 1-10,000 sulfanilamide-broth does not attenuate the organism under certain experimental conditions. 2. The combined bactericidal action of antibody and sulfanilamide is greater than that

8 Colebrook, L. C., Buttle, G. A. W., and O'Meara, R. Q., *Lancet*, 1936, ii, 1323.
9 Long, P. N., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.
10 Finklestone-Sayliss, H., Paine, G. G., and Patrick, L. B., *Lancet*, 1937, i, 792.
11 Osgood, E. E., and Brownlee, I., *J. A. M. A.*, 1938, **110**, 349.
12 White, H. J., and Parker, J., *J. Bact.*, 1938, **36**, 481.
13 Whitby, L. E. N., *Lancet*, 1938, ii, 1095.
14 Chu, H. I., and Hastings, A. B., *J. Pharm. and Exp. Ther.*, 1938, **63**, 407.

of either one alone. 3. In dilute solutions sulfanilamide promotes phagocytosis, probably nonspecifically. 4. In 25% horse-serum neopeptone-water containing sulfanilamide, hemolytic streptococci form long chains and very large capsules. 5. Organisms previously grown in sulfanilamide are rapidly inhibited in fresh media containing sulfanilamide, whereas normal organisms multiply at the usual rate for several hours in the presence of sulfanilamide before bacteriostasis occurs. Evidence is presented to show that this delay is due to the formation of a loose union between the drug and the organisms rather than to any change brought about in the medium. 6. The bacteriostatic effectiveness of sulfanilamide varies directly with the concentration of the drug and inversely with the size of the inoculum. 7. The addition of specific antibody does not significantly enhance the bacteriostatic effect of the drug in fresh human serum, although it markedly increases the bactericidal effect of sulfanilamide in whole blood. 8. It is suggested that the effectiveness of a given concentration of sulfanilamide depends largely on the number of organisms present at the time when bacteriostasis begins, and this is dependent on the number of organisms inoculated and their rate of multiplication.

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Effect of Sulfanilamide on Electrode-Potential of Hemolytic Streptococcal Cultures.

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Further study[1] of the blue substance[2] formed by the photoxidation of aqueous sulfanilamide has shown that this substance is the oxidized form of a reversible system:

* Work done under tenure of the Moritz Rosenthal Fellowship assisted by the Emanuel Libman Fellowship Fund, Mt. Sinai Hospital, New York City.

† Work done under tenure of DeLamar Medical Student Fellowship, Harvard.

1 Fox, C. L., Jr., Cline, J. E., Ottenberg, R., *J. Pharm. and Exp. Ther.*, in press.

2 Ottenberg, R., and Fox, C. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 479.