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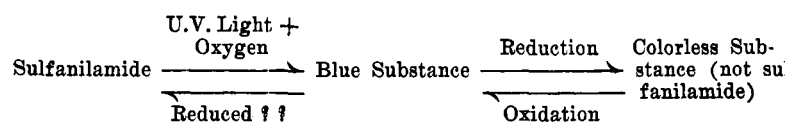
Further study[1] of the blue substance[2] formed by the photoxidation of aqueous sulfanilamide has shown that this substance is the oxidized form of a reversible system:

* 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.



Inasmuch as this substance is present in the blood[3] of patients receiving sulfanilamide, apparently responsible in part for their cyanosis, the possible effect of sulfanilamide in bacterial oxidation-reduction reactions[4, 9] was investigated.

Dubos[5] has shown that bacterial growth can be induced by a lowered oxidation-reduction potential and inhibited by an elevated potential. Fildes[6] similarly induced and inhibited the germination of tetanus spores. Clark, Cohen, and Cannon,[7] later Schmelkes,[8] showed that the addition of small amounts of iodine (or chloro compounds) at frequent intervals had no deleterious effects on a growing culture, provided the electrode-potential was never allowed to rise to the region positive to that of the aërobic cell. The total quantity of iodine added in this way far exceeded the amount necessary to sterilize the culture, when added at one time. Hewitt[4] has pointed out the great difference between the time-potential curves of hemolytic-streptococcal and pneumococcal cultures on the one hand and those of staphylococcal and *C. diphtheriæ* cultures on the other. These metabolic differences may be causally related to the differences in the response of streptococcal and staphylococcal infections to sulfanilamide.

In the present experiments hemolytic streptococci were grown with sulfanilamide under various controlled conditions and the effects on the potentials of bright-platinum electrodes were observed. Because thermodynamic interpretation of these potentials would be extremely hazardous, "it seems preferable to avoid for the present theoretical entanglements and to regard the *observations* as important."[7]

Methods. The apparatus and procedures used in the measurement of potentials were identical with those described by Zinsser and Schoenbach[10] except that the cultures (contained in smaller—20 cc—

[3] Fox, C. L., Jr., and Cline, J. E., to be published.

[4] Hewitt, L. F., *Oxidation-reduction Potentials in Biochem. and Med.*, 1936, 4th ed.

[5] Dubos, R., *J. Exp. Med.*, 1929, **49**, 559, 575.

[6] Fildes, P., *Brit. J. Exp. Path.*, 1929, **10**, 151, 197.

[7] Clark, W. M., *Harvey Lectures*, 1933-34, p. 97.

[8] Schmelkes, F. C., *J. Am. Water Works Assn.*, 1933, **25**, 695.

[9] Michaelis, L., *Oxidation-reduction Potentials*, 1930.

[10] Zinsser, H., and Schoenbach, E. R., *J. Exp. Med.*, 1933, **66**, 207.

flasks) were mixed continually by the Ridley alternate-suction method.[11] No attempt was made to exclude air. Duplicate readings in each culture were taken at suitable intervals.‡

The strain of hemolytic streptococcus, the media, the method of counting bacteria, and the sulfanilamide solutions were the same as those used by Chandler and Janeway.[12] Sulfanilamide-medium always contained 10 mg %. Neopeptone-water was freshly autoclaved and serum added just before each experiment. This medium (25% horse-serum neopeptone-water) is well buffered and repeated tests always gave a final pH of 8.0, except as described in the experiments with 1% glucose. The inoculum used was such as to give an initial count of 25,000 colonies per cc. Cultures were incubated at 37.5°C in a constant-temperature waterbath. Procedures adopted to alter the electrode-potentials are described in the appropriate paragraphs. All experiments have been performed at least twice.

Results. The graphs record typical experiments.

1. Sterile media with and without sulfanilamide showed no difference in the rise of electrode-potential that followed incubation.

2. Comparison between sulfanilamide- and control-cultures (Fig. 1): The Eh of both cultures remained the same for the first 9 hours, but growth only proceeded equally for 6 hours. After 6 hours, the control culture continued to multiply rapidly and its Eh value quickly dropped 55 mv after the eighth hour. On the other hand, little change occurred in either the population or the potential of the sulfanilamide-culture for the next 16 hours. When this culture was observed for a longer period (Fig. 2), after 24-30 hours there was a rapid fall in potential and a profuse multiplication of the organisms. During the next 30-hour period the Eh of the sulfanilamide-culture slowly rose to the initial level, as had the control many hours before.

3. The effect of the size of the inoculum on the electrode-potential (Fig. 2): The Eh of the heavily inoculated culture fell 70 mv to the minimum within 2 hours, whereas the potential of the lightly inoculated cultures remained stationary for the first 9 hours and then fell to a slightly lower minimum in the next 4 hours.

4. The effect of cysteine (Fig. 3): (0.1% neutralized cysteine

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11 Ridley, F., *Brit. J. Exp. Path.*, 1928, **9**, 253.

‡ The electrode-potential of chlorine is here regarded as positive, that of hydrogen as negative. The potential-reading added to the value of the calomel half-cell (+0.337 volt) is referred to as the electrode-potential, Eh.

12 Chandler, C. A., and Janeway, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 179.

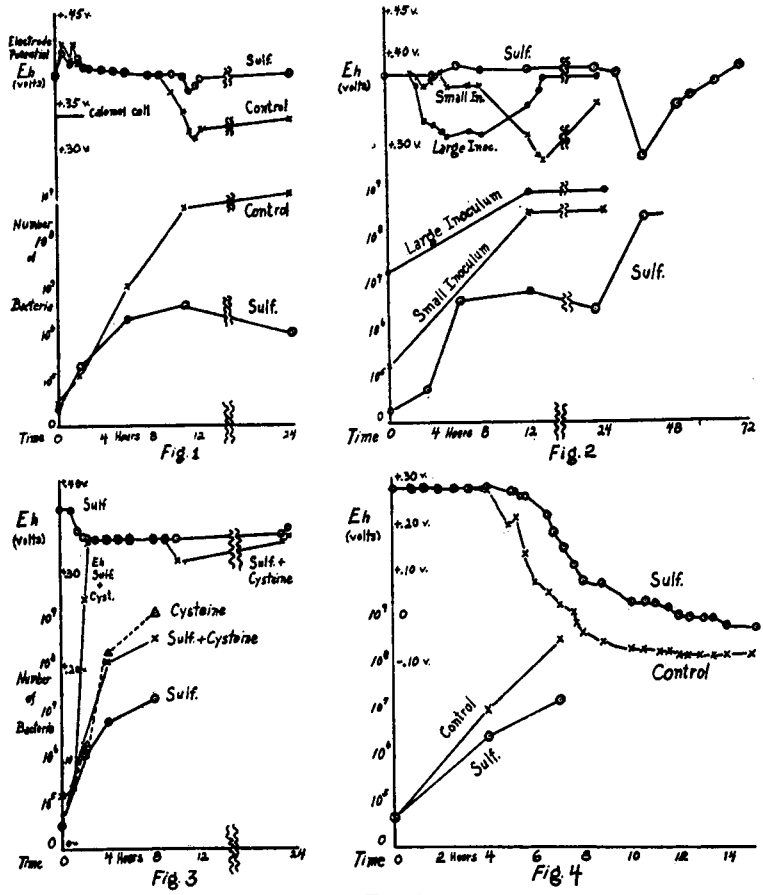

FIG. 1.

Time-potential curves and growth-curves of hemolytic streptococcus, strain S43, under various conditions. Ordinates represent number of bacteria as colonies per cc and electrode-potentials in volts; abscissæ, time in hours.

HCl was used. Potentials were measured in sulfanilamide-cultures with and without cysteine; counts were taken on these as well as on the control culture without sulfanilamide.) The initial potential of the sulfanilamide-cysteine culture was about 300 mv below that of the plain sulfanilamide-culture, but gradually rose to the same Eh level. The growth-curves show a striking reduction in the bacteriostasis by sulfanilamide in the presence of cysteine.

5. Effect of partial anaërobiosis:§ (A 1 cm layer of sterile vaseline was poured over the surface of the cultures, which were not mixed during the experiment). The Eh of both control and sul-

§ The inhibition of sulfanilamide-bacteriostasis by cysteine, anaërobiosis, and catalase is to be reported in greater detail subsequently.

fanilamide-cultures remained stationary for the first 4 hours of growth, but then the potential of the control fell 175 mv in the next 4 hours while multiplication continued at a rapid rate. The potential of the sulfanilamide-culture continued at an elevated level for 2 hours longer before falling 120 mv, while moderate growth occurred.

6. The effect of catalase: (Hewitt[4] has shown that catalase will lower the electrode-potential of a culture, presumably by decomposing H_2O_2[13]) || (0.1 cc of washed horse-erythrocytes were added as a source of catalase at the beginning of the experiment.) The initial potential of the sulfanilamide-catalase culture was 25 mv lower than that of the plain sulfanilamide-culture, remained so for the first 12 hours, but then gradually approached that of the control. Although more marked bacteriostasis occurred in this experiment because the organisms inoculated had been previously grown 18 hours in sulfanilamide, the sulfanilamide-culture containing catalase showed far less bacteriostasis, indicating an antagonism between sulfanilamide and catalase.¶

When liver-catalase (prepared by the method of Batelli and Stern[14]) was added to fully grown cultures the Eh promptly dropped 60 mv.

7. The effect of glucose: With 1% glucose in the media, the usual elevated initial potentials persisted in both cultures throughout a 24-hour period of observation. The pH of the control culture dropped to 5.8, that of the sulfanilamide culture persisted at 8.0. Good bacteriostasis was obtained.

8. The effect of a synthetic medium containing no protein: (Since Lockwood[16] has suggested that sulfanilamide may block the proteolytic enzymes which make it possible for the organism to grow in serum, it seemed worthwhile to study the effect of sulfanilamide in a protein-free synthetic medium, supplied by Rane and Subbarow.[17] The initial Eh of both cultures was about 160 mv below the usual level, but rose during the first 9 hours to the usual initial level,

[13] Broh-Kahn, R. H., and Mirsky, A. I., *J. Bact.*, 1938, **35**, 455; *Nature*, 1938, **142**, 153.

|| Neither the potato-benzidine method of Avery nor fresh starch-iodine paper ever yielded a positive test for H_2O_2 in our cultures.

¶ After this work was done, the report of Main[15] and her associates appeared on the anticatalase effect of sulfanilamide increased by ultraviolet radiation.

[14] Batelli and Stern, *Compt. Rend. Soc. Biol.*, 1934, **57**, 374.

[15] Main, E. R., Shinn, L. E., Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

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

[17] Rane, L., and Subbarow, Y., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 837.

while practically no growth occurred. The Eh of the sulfanilamide-culture remained at this elevated level and bacteriostasis persisted; the Eh of the control culture remained elevated for 5 hours, but by the 25th hour it had fallen 170 mv, and profuse growth had occurred.

Summary. 1. Potentiometric measurements are reported of the electrode potentials of cultures of hemolytic streptococci growing in 25% horse-serum neopeptone-water with and without sulfanilamide. 2. The Eh of aërobic control cultures remained elevated until after active multiplication was well advanced, then rapidly fell about 50 mv. The Eh of aërobic sulfanilamide-cultures remained elevated much longer, but when bacteriostasis ceased, it also fell. 3. With a very large inoculum the Eh fell no more than with a small inoculum, but the change occurred many hours earlier. 4. The addition of cysteine or catalase, or the exclusion of air with vaseline not only lowered the potential of both control and sulfanilamide-cultures, but also definitely reduced the bacteriostatic effectiveness of sulfanilamide. 5. In the presence of 1% glucose, the Eh of both control and sulfanilamide-cultures remained elevated, and sulfanilamide was effective as a bacteriostatic agent. 6. Sulfanilamide bacteriostasis is accompanied by an elevated electrode potential; normal growth by a rapidly falling potential.

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Effects of Light and Darkness on Activity of the Pituitary of the Rat.*

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The effect of light on the time at which sexual maturity is attained and on the sexual cycle of a polyestrous mammal has been studied by Browman[1] and by Hemmingsen and Krarup,[2] but the nature of the change in the secretory activity of the pituitary responsible for these phenomena is as yet incompletely understood. It was with this

* Supported in part by a grant from the Rockefeller Foundation administered by Frederick L. Hisaw.

1 Browman, L. G., *J. Exp. Zool.*, 1937, **75**, 375.

2 Hemmingsen, A. M., and Krarup, N. B., *Det. Kgl. Danske Videnskabernes selskab.*, 1937, **18**, 7.