With the concentration of calcium constant, any increase in coagulation time must be due to a decrease in the prothrombin of the plasma or to interfering factors.

The inhibitory action of excess calcium chloride is probably not due to salt action, but specifically to the calcium ion itself as Aggeler and Lucia[6] suggested. This behavior of calcium deserves more careful study especially to determine the nature of its anticoagulant action. It does not appear to be an antithrombic action, since calcium in this concentration does not inhibit the action of thrombin.

10358

Influence of Sulfanilamide and Related Compounds Upon Oxidation-Reduction Potentials of the Hemolytic Streptococcus.*

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(Introduced by F. P. Gay.)

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In an effort to secure information as to the mechanism of the action of sulfanilamide upon the streptococcus we have measured the effect of this drug and related compounds upon the oxidation-reduction potentials of the hemolytic streptococcus. An important relation between bacteriostasis and oxidation-reduction levels was pointed out by Dubos,[1] who noted that the growth of the streptococcus and the pneumococcus was inhibited if the Eh of the system was maintained above a critical level by the introduction of suitable dyestuffs, as oxidized indophenols.

The "Holman" strain of hemolytic streptococcus (Gay) was adopted for this investigation. Freshly seeded cultures which contained 280,000 organisms per cc at the beginning of the experiment, were observed over a period of growth for 72 hours. Beef-infusion broth with 1% peptone (Bacto), pH 7.4-7.6, was used throughout. The sulfanilamide was supplied by the Winthrop Chemical Company.

Potentials were measured against 26 gauge platinum-coil electrodes. All cultures and the calomel half-cell were kept at 37.0 ± 0.5°C. A Leeds and Northrup, Type No. 7660, vacuum-tube po-

6 Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 11.

* Aided by a grant from the Dr. Philip Hanson Hiss, Jr., Memorial Fund.

1 Dubos, R., *J. Exp. Med.*, 1929, **49**, 575.

tentiometer was used for most of the measurements, in some cases the Leeds and Northrup recording potentiometer being utilized. Suitable switch-arrangements enabled readings to be made upon 8 tubes at once. Control tubes containing no drug were set up and measured simultaneously with the sulfanilamide-containing tubes, the potentials of such control preparations being regarded as base-lines for any one experiment.

Uninoculated sterile broth containing sulfanilamide exhibited higher oxidation-reduction potentials than broth without the drug. This difference was in the region of 50 millivolts. (Fig. 1.) From this it can be seen that the drug will change the potential of sterile media. The time-potential curves obtained for streptococcal cultures were consistently higher when they contained sulfanilamide in a concentration of 1:10,000. This elevation was detectable immediately and reached a maximum at about the tenth hour. Maximal levels were about 80 to 100 mv more positive than cultures without the drug and this difference was maintained for 72 hours.

Since this difference in potential could be ascribed to a diminished growth rate of the organism, and possibly to less reduction, sulfanil-

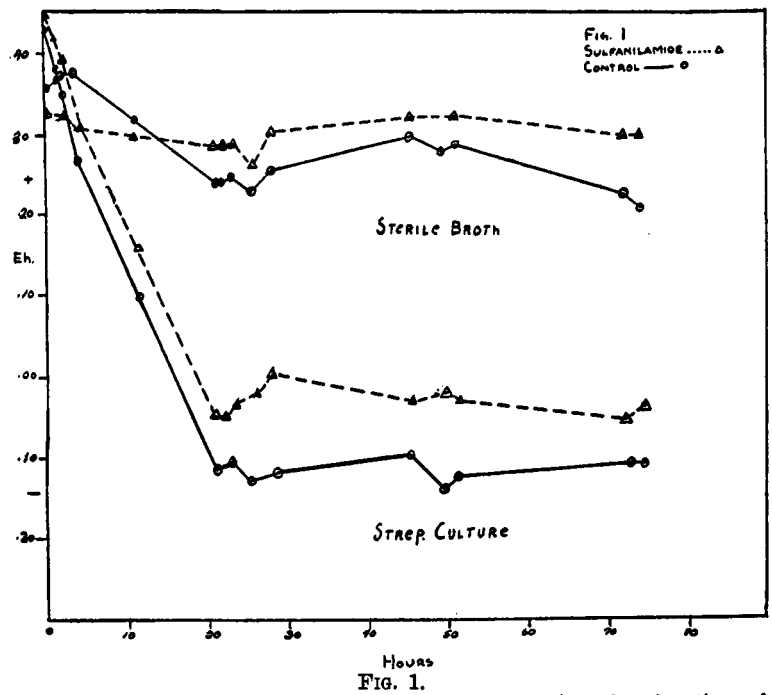

FIG. 1.

Time-potential curves of 2 typical experiments showing the elevation of Eh produced by sulfanilamide.

amide was added to cultures which had grown for 21 hours. This caused an immediate rise in E.M.F., some cultures rising as much as 210 mv, others less than 50. Experiments in which the cocci were grown in 1% or 10% rabbit-serum broth yielded similar results, although the differences in potential were not as marked. The addition of 1% M/15 phosphate buffer, pH 7.44, failed to change the effects of the sulfanilamide upon the culture-potentials.

These findings suggested that the elevations in potential induced by sulfanilamide might be decreased or even eliminated by anaërobic cultivation of the streptococci or by the addition of reducing agents to the culture. Such proved to be the case, for there was no difference between the potentials of anaërobic cultures that contained or did not contain sulfanilamide; neither was there any difference in potential in cultures grown in 0.05% cysteine broth. In such media sulfanilamide, as shown by plate counts, was non-bacteriostatic.

Several compounds related to sulfanilamide with and without therapeutic action were studied for their effects upon the oxidation-reduction potential-curves of growing streptococci. The results are summarized in Table I.

TABLE I.

Compound	Bacteriostatic action at conc. of 1:10,000	Effect on culture potential
Aniline	None	None
Benzenesulfonamide	"	"
p-Acetyl sulfanilamide	"	"
Na sulfanilyl sulfanilate	"	"
Disodium 4-sulphamido-phenyl-2-azo-7 acetylamino-1-hydroxynaphthalene-3,6,disulphonate ("Prontosil")	"	"
p-Aminobenzenesulfonamide	Present	Raises potential
2-sulfanilamido pyridine	"	" "
Na formaldehyde sulphoxalate sulfanilamide	"	" "

Prontosil is inactive *in vitro* unless it is reduced to the sulfanilamide before addition to the culture (Feinstone, Bliss, Ott, and Long[2]). This would account for the lack of potential increase encountered with this drug.

Of the series studied only those compounds which are agreed to possess bacteriostatic activity increased the oxidation-reduction potential of hemolytic streptococci over the normal level. This rise in Eh was not primarily dependent upon the number of living organisms as the drug also raises the potential of sterile broth or of previously grown cultures immediately upon addition of the compound.

2 Feinstone, W. H., Bliss, E., Ott, E., Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

This effect could be eliminated when increased reducing-conditions are maintained in the culture. This might explain the resistance of anaërobic streptococci to sulfanilamide (Colebrook and Purdie[3]), but will not account for the alleged action of the drug upon sporulating anaërobes (Spray[4]).

The means whereby sulfanilamide produces these elevations in potential are at present obscure. It has been suggested by Main, Shinn, and Mellon[5] that the drug interferes with catalase-activity and that an accumulation of peroxides follows. However, this fails to account for the rise of potential occurring in plain broth. That sulfanilamide itself may poise the system at some critical Eh is doubtful and our attempts to measure its Eo, if any exists, have been unsuccessful.

A third possibility, which we are inclined to accept, is that sulfanilamide inactivates enzymic systems in combination with sulfhydryl or other groups normally responsible for the attainment of highly negative reduction-potentials. Such groups are presumably also present in the peptone-broth, the reduction-potential of which is rendered more positive by the drug. This hypothesis is under investigation.

The fact that sulfanilamide maintains a high Eh level in the medium, be it blood, broth, etc., may account for some of the observed properties of its therapeutic action. Lockwood[6] finds that peptone inhibits its bacteriostatic action. Peptones contain reducing agents which, as we have shown, will antagonize the oxidative effects of the drug. Lockwood, Coburn, and Stokinger[7] reported the lack of good therapeutic results when using sulfanilamide in closed, necrotic lesions as compared with diffuse inflammations. The decreased local Eh and anaërobiosis will partially account for this.

On this basis also we might expect sulfanilamide to enhance the action of humoral antibacterial factors, as reported by Branham and Rosenthal[8] for the meningococcus and pneumococcus, for Tillett and Stock[9] found that the bactericidal action of normal human serum upon the streptococcus operated at an optimum if reducing conditions were avoided.

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3 Colebrook, L., and Purdie, A. W., *Lancet*, 1937, **2**, 1237.

4 Spray, R. S., *J. Lab. Clin. Med.*, 1938, **23**, 609.

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

6 Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155.

7 Lockwood, J. S., Coburn, A., and Stokinger, H. E., *J. A. M. A.*, 1938, **111**, 2259.

8 Branham, S., and Rosenthal, S. M., *U. S. Pub. Health Reports*, 1937, **52**, 685.

9 Tillett, W. S., and Stock, C., *J. Exp. Med.*, 1937, **66**, 617.

Summary. The addition of sulfanilamide to sterile broth or to cultures of the hemolytic streptococcus is accompanied by an elevation of the oxidation-reduction potential. This rise can be eliminated when the cocci are grown anaërobically or in cysteine broth. The addition of serum will slightly diminish this effect. Compounds related to sulfanilamide but lacking in bacteriostatic action are without effect upon the culture potentials. The relation of these potential effects to the action of the drug in streptococcal infections is discussed.

10359 P

Heteroplastic Transplantation of the Hypophysis Between Different Species of Ambystoma.*

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The differences in metamorphic activity of the axolotl *(Ambystoma mexicanum)* and the related species, *A. tigrinum,* are well known. That the thyroid of *A. mexicanum* will metamorphose thyroidless frog tadpoles although not able to metamorphose the axolotl itself was shown by Swingle.[1] It is also known through the work of Uhlenhuth and Schwartzbach[2] and others that the axolotl can be metamorphosed by anterior pituitary substances. The establishment of the pituitary's influence upon the thyroid-releasing mechanism makes it probable that the difficulty is in this relationship between these glands. The question arises as to which gland is primarily different. Witschi[3] joined axolotl and tigrinum embryos in parabiosis. These pairs metamorphosed when they were normal but did not when the tigrinum was hypophysectomized, thus showing that the pituitary gland of the tigrinum was responsible for the metamorphosis. The tigrinum thyroid was, however, present and must be considered. Bytinski-Salz[4] interchanged the pituitaries of these 2 species at the orthotopic position. His results showed no effects of the interchange. He concluded that the *Ambystoma tigrinum* with

* This work has had aid from the Graduate School Research Fund of the University of Minnesota.

1 Swingle, W. W., *J. Exp. Zool.*, 1922, **36**, 397.

2 Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

3 Witschi, E., *Anat. Rec.*, 1933, **55**, Suppl., 88.

4 Bytinski-Salz, H., *J. Exp. Zool.*, 1935, **72**, 51.