Privine, on the basis of this method of comparing the effectiveness of vasoconstrictors, is not comparable in potency to epinephrine. Dilutions of Privine greater than 1:10,000 killed almost the same percentage of animals as did cocaine alone. Concentrations of 1:10,000 and 1:1000 killed respectively 38% and 40% more than did the cocaine alone. The assumption of a synergism between cocaine and Privine would explain this greater mortality with the higher concentrations of Privine, although it might also be explained by the assumption that a 1:1,000 concentration of Privine caused local vascular damage or depression with a resultant dilation so that the absorption of the cocaine was actually favored. Privine in the first 4 concentrations employed (see Table I) protected a total of 3 animals from convulsions, a result of debatable significance in view of the unchanged mortality rates in those 4 groups and the insignificantly changed average time before the onset of convulsions.

The injection of 1 cc of 1:250 (4 mg) Privine per kg killed none of 10 animals; 1 cc of 1:100 (10 mg) Privine per kg killed all of 10 animals. The LD_{50} for Privine, which we did not determine, would be intermediate between those 2 doses. Baylac[6] reported the same limits for epinephrine. Animals killed by Privine exhibited that acute pulmonary edema usually seen in animals killed by epinephrine.

Summary. 1. Privine, on the basis of the method employed for comparison, is a less effective vasoconstrictor than epinephrine. 2. The LD_{50} of Privine for guinea pigs is intermediate between 1 cc of 1:250 (4 mg) and 1 cc of 1:100 (10 mg) per kilogram. 3. Actively growing guinea pigs of less than 300 g are more sensitive to a given toxic dose of cocaine in g per kg than are mature or less actively growing guinea pigs of more than 300 g. 4. The toxicity, after subcutaneous injection, of a dose of cocaine in g per kg may be decreased by decreasing the concentration of the solution used for injection; this has been previously reported by other investigators.

[6] Baylac, J., *Arch. med. de Toulouse*, 1905, **11**, 245.

14870

Method for Testing *In Vitro* Resistance of Group A Hemolytic Streptococci to Sulfonamides.*

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A suitable medium for testing sulfonamide susceptibility of Group A streptococci should meet certain requirements: it should promote good growth of all freshly isolated strains; it should promote growth from a uniform and measurable inoculum; and it should be practically free of substances antagonistic to sulfonamide action. Most media lack the last mentioned qualities. Furthermore, the relative capacity of different strains to multiply *in vitro* in the presence of sulfonamides is determined not only by inherent differences in their drug susceptibility but also by the size of the inoculum as well as by the character of the medium.

This paper describes a test in which the above mentioned criteria are fulfilled. It demonstrates the inhibition of streptococcal growth by various concentrations of sodium sulfadiazine in a semi-synthetic, semi-solid medium reinforced with serum.

The semi-synthetic medium was developed

* The opinions expressed in this paper are those of the author and do not necessarily represent those of the Navy Department.

TABLE I.
Dilution Schedule for 24 Tests.

	Control	1 mg	5 mg	25 mg	125 mg
Solution I (basal medium)	33.0	33.0	33.0	33.0	33.0
Solution V (growth factors, vitamins, and minerals)	2.5	2.5	2.5	2.5	2.5
Solution VI R or VI H	2.5	2.5	2.5	2.5	2.5
Serum	5.0	5.0	5.0	5.0	5.0
Saline (0.85% NaCl)	2.0	1.5	1.75	0.75	0.75
SSD* 0.1%	—	0.5	—	—	—
1.0%	—	—	0.25	1.25	—
5.0%	—	—	—	—	1.25
Solution VII	5.0	5.0	5.0	5.0	5.0

* SSD—Sodium sulfadiazine solutions.
All measurements are in cc.

by Roe and Adams[1] for the growth of pneumococci. It supports adequate growth of streptococci when supplemented with normal rabbit serum or with normal horse serum and xanthine. The inoculation technic was adopted from that of Ward and Rudd[2] who employed it to differentiate growth characteristics of streptococcal colonies in a semi-solid medium.

Preparation of Stock Solutions. Solution I: (Basal Medium) Caseine hydrolysate (10% solution, SMACO "vitamine free"), 200 cc; Cystine, 150 mg (dissolved in 10 cc N HCl); Tryptophane, 20 mg; KCl, 3.0 g; Na_2HPO_4 (anhydrous), 3.0 g; and $MgSO_4 \cdot 7H_2O$, 0.5 g. The ingredients are mixed in about 800 cc of distilled water; the pH is adjusted to 7.5; and the volume is made up to 900 cc. The mixture is autoclaved at 10 lbs for 10 minutes, filtered through paper, and reautoclaved.

Solution II: (Vitamins) Biotin, 0.015 mg; nicotinic acid, 15.0 mg; pyridoxin, 15.0 mg; calcium pantothenate, 60.0 mg; thiamine chloride, 15.0 mg; riboflavine, 7.0 mg; adenine sulfate, 150.0 mg; and uracil, 150.0 mg. These are dissolved in about 75 cc of distilled water; the pH adjusted to 7.2; the volume made up to 100 cc with distilled water; and the final solution sterilized by filtration.

Solution III: (Minerals) $CuSO_4 \cdot 5H_2O$, 50 mg; $ZnSO_4 \cdot 7H_2O$, 50 mg; $FeSO_4 \cdot 7H_2O$, 50 mg; $MnCl_2 \cdot 4\ H_2O$, 20 mg; and concentrated HCl, 1.0 cc; distilled water to 100 cc. This solution does not require sterilization.

Solution IV: (Growth factors) Glutamine,† 200 mg; dextrose, 2 g; Solution III, 2 cc; $CaCl_2 \cdot 2H_2O$, 10 mg; asparagin, 100 mg; choline chloride, 10 mg; and distilled water, 40 cc. Sterilized by filtration.

Solution V: (Vitamins, minerals and growth factors) Solution II, 8 cc, and Solution IV, 42 cc.

Solution VI R: $NaHCO_3$ (sterilized by autoclaving in dry test tubes), 0.2 g; sterile distilled water, 10 cc; and thioglycollic acid (10% solution, sterilized by boiling), 0.2 cc. This solution is used when the medium is to be made with rabbit serum.

Solution VI H: $NaHCO_3$ (prepared as in Solution VI R), 0.2 g; sterile distilled water, 8 cc; sterile 10% thioglycollic acid, 0.2 cc; and xanthine solution (100 mg xanthine per 100 cc N/20 NaOH, sterilized by filtration), 2 cc. This solution is used when the medium is to be made with horse serum.

Solution VII: (Agar base) Bacto agar 1.5 g is added to 100 cc of Solution I which is then heated until the agar is dissolved. After the reaction is adjusted to pH 7.5, the solution is heated until a granular precipitate forms. It is then cleared by filtering through absorbent cotton, distributed in convenient amounts and autoclaved.

Stock sodium sulfadiazine solutions containing respectively 0.1 g, 1.0 g, and 5.0 g of drug per 100 cc in distilled water are sterilized by immersion in boiling water for 10

[1] Roe, A. S., and Adams, M. H., abstracted in *J. Bact.*, 1944, **47**, 26. The present author wishes to express his appreciation for permission to use this medium before full details of its composition have been published.

[2] Ward, H. K., and Rudd, G. V., *Australian J. Exp. Biol. and M. Sc.*, 1938, **16**, 181.

† Glutamine should be kept in a desiccator over calcium chloride in the refrigerator at 4°C.

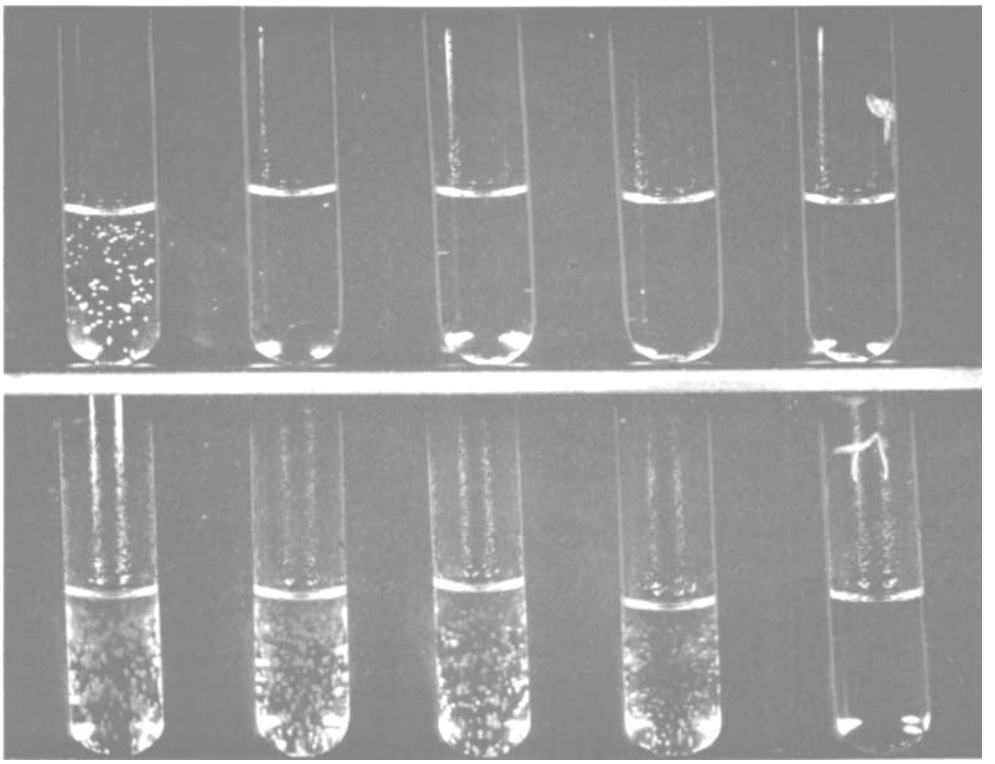

FIG. 1.
Sulfonamide Resistance Tests.
Top: A fully susceptible strain. Result "0." Bottom: A highly resistant strain. Result "25." The tubes from left to right contain respectively 0, 1, 5, 25, and 125 mg of sodium sulfadiazine per 100 cc of medium.

minutes.

Solutions VI R and VI H must be prepared immediately before use. All of the others can be made in advance and stored in the ice box.

Technic. To perform the test, sets of the various solutions are mixed in each of 5 sterile, suitably sized Erlenmeyer flasks according to the appended dilution schedule (or in fractions or multiples thereof, depending on the number of strains to be tested). Solutions I, V, VI R and rabbit serum, or VI H and horse serum, saline and sodium sulfadiazine are mixed in the flasks which are then placed in a 48°C water bath. Solution VII is melted in the autoclave and is placed in the same water bath. When all solutions have reached the proper temperature, the indicated quantity of Solution VII is added to each flask by means of warmed pipettes; and the final solutions are then distributed in 2.0 cc amounts in sterile 13 x 100 mm test tubes. Thus 5 tubes contain the test media for a single strain and each contains respectively a concentration of 0, 1, 5, 25, and 125 mg of sodium sulfadiazine per 100 cc of the material.

Strains to be tested are grown overnight in 2.0 cc of medium composed of 80% of Solution I, 5% of Solution V, and either 5% of Solution VI R plus 10% of rabbit serum or 5% of Solution VI H and 10% of horse serum, according to the serum to be employed the following day for the final test.

A small loopful (the loop is made by winding 26-gauge platinum wire around an 18-gauge needle) of this overnight culture is transferred to 2.0 cc of Solution I in a test tube. This dilute cell suspension is agitated sharply 30 times, and a loopful of it is transferred to each of the 5 tubes constituting the test. The loop is flamed between inoculations. The inoculum is considered to be of satisfactory size if 10 to 100 colonies grow in the tube

which contains no sulfonamide. If the number of colonies is not within these limits the test must be repeated with a more suitable inoculum.

The tubes are incubated at 37°C for 18 to 24 hours; and the test is read in terms of the concentration of sodium sulfadiazine in the highest tube in which visible growth occurs. If this be in the control tube only the result is "0"; if in all tubes except that containing 125 mg of the drug per 100 cc of medium the result is "25." Tests showing values of 0 and 25 are shown in Fig. 1.

Discussion. Results have been reproducible with a variation of plus or minus one tube. Strains have been kept on blood agar slants in the ice box for at least one month without undergoing a change in their *in vitro* resistance to sulfadiazine.

To obtain consistent results the same sulfonamide and serum from the same species must be used. Sodium sulfathiazole has given readings about one tube lower than sodium sulfadiazine; and medium made with rabbit serum has given readings about one tube higher than that made with horse serum and xanthine.

The semi-synthetic part of the medium is doubtless unnecessarily complex, because several of its constituents are contributed in sufficient quantity by the added serum. No attempt has, as yet, been made to eliminate these superfluous materials or to reduce the medium to its simplest form.

Many strains of hemolytic streptococci are inhibited by as little as 0.2 mg of sodium sulfadiazine per 100 cc of the medium, which indicates that the content of substances antagonistic to sulfonamide action is very low.

No success has attended attempts to refine the test in such a manner that lesser degrees of sulfonamide resistance might be demonstrated. The intervals between the concentrations of drug in the tubes (multiples of 5) have been chosen deliberately; and the good correlation which exists between the findings of the test and clinical observations in the field[3] suggests that it is sufficiently precise to be of practical value.

Summary. A technic for testing the resistance of Group A hemolytic streptococci to sulfonamides is presented. The test has been found to be sufficiently precise to indicate clinically significant differences in sulfonamide susceptibility.

[3] Wilson, A. T., and Coburn, A. F., to be published.

14871

Competition of Antigens in Isoimmunization by Pregnancy.*

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When an experimental animal is injected with a mixture of two substances, one a good antigen and the other a poor antigen, the antigenicity of the weaker antigen is often suppressed.[1] The purpose of the present communication is to report some observations in man of this phenomenon, known as competition of antigens.

The properties A and B are good antigens in man, as revealed by observations made following inadvertent transfusions of blood of an incompatible group.[2] On the other hand, only 1 in 25 to 50 Rh-negative individuals respond to transfusions of Rh-positive blood, or pregnancy with an Rh-positive fetus, by

* Aided by a grant from the United Hospital Fund of New York City.

[1] *Cf.* Landsteiner, K., *The Specificity of Serological Reactions*, 2d edition, p. 104, Harvard Univ. Press, Boston, 1945.

[2] Wiener, A. S., *Blood Groups and Transfusions*, p. 123, 3rd ed., C. C. Thomas, Springfield, Ill., 1943.