from 10^{-2} to 10^{-5}, 6 out of 10 receiving the 10^{-6} dilution, and 7 out of 8 receiving the 10^{-7} dilution. The latter dose contained approximately 8 organisms.

Summary. 1. Production of hydrogen sulfide by *E. coli, K. pneumoniae, B. subtilis,* and *P. aeruginosa* in synthetic media containing cysteine or methionine and their derivatives was studied. 2. With the exception of *P. aeruginosa,* all these organisms formed moderate to maximal amounts of hydrogen sulfide from cysteine and cystine. *P. aeruginosa* produced only traces.

3. Only *K. pncumoniae* produced hydrogen sulfide from N-benzoyl-cysteine. This confirms previous observations made with beef infusion agar basic medium.[1] Benzoylation of the alpha amino group of cysteine does not interfere with desulfurization by this organism, although it prevents the reaction in the case of other bacteria studied. This indicates fundamental metabolic differences prevailing in the organisms investigated with respect to this particular reaction. Additional strains of *K. pneumoniae* and other organisms should be tested under similar conditions.

4. None of the organisms formed hydrogen sulfide or mercaptan from S-benzyl-cysteine, in contrast to the results obtained when this substance was added to beef infusion agar. Hydrogen sulfide was not produced by any of the bacteria from cysteic acid, methionine, S-benzyl-homocysteine, or homocystine. 5. A limited number of observations on pigment formation by *P. aeruginosa* in the presence of cysteine, methionine, and their derivatives was made.

6. A strain of *K. pneumoniae* continued to produce large capsules and maintained high virulence for mice after many transfers in a simple synthetic medium.

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A Rapid Method for the Determination of Races of *Shigella dysenteriae* Flexner.

LUIS M. GONZÁLEZ AND P. MORALES OTERO.

From the Department of Bacteriology of the School of Tropical Medicine, San Juan, Puerto Rico.

Several investigators have already isolated the polysaccharide fraction of *Shigella dysenteriae.* Kurauchi[1] and Meyer and Morgan[2] isolated specific polysaccharide from the Shiga organism; Kurauchi[1] from the Flexner and Sonne. Spassky and Dannenfeldt[3] used the precipitin test for the diagnosis of *Sh. dysenteriae* infection, but failed to note the specificity of the precipitin reaction, basing their failure on the extreme complexity of the antigenic apparatus of the dysenteric bacillus.

We have tried to develop a practical method for the typing of races of *Shigella dysenteriae* Flexner by using type specific antibodies capable of reacting with the polysaccharide. Antiserum made with the classical Oxford strains did not give clear cut results. However, when freshly isolated strains were utilized, specific reaction was obtained.

Freshly isolated cultures of *Shigella dysenteriae* Flexner strains, classified as W, V, and Z, were utilized. Rabbits were immunized by intravenous injections of saline suspensions of the organisms killed by formalin. A 3-week course of graded doses of dysenteric bacilli was then given to each rabbit. Injections were applied on 3 consecutive days, followed by a rest period of 4 days before starting the next 3 doses. Eight days after the last inoculation, the ani-

[1] Kurauchi (Report of K. Ando), *J. Immunol.,* 1929, **17**, 555.

[2] Meyer, K., and Morgan, W. T. J., *J. Exp. Path.,* 1935, **16**, 476.

[3] Spassky, N. M., and Dannenfeldt, L. A., *Bul. Biol. et Méd. Exp.,* U. S. S. R., 1939, **7**, 202.

mals were bled from the heart and the serum separated aseptically.

Cultures of *Shigella dysenteriae* were isolated from suspected feces, using the technic described by Hardy *et al.*[4] The organisms can be obtained directly from the Krumwiede triple sugar agar.

The polysaccharide fraction of the dysenteric bacteria was extracted by using Fuller's formamide method.[5] To perform the test, 0.1 cc of the antiserum was placed in a small precipitin test tube, an equal amount of polysaccharide solution carefully laid over the serum and the test tube placed in the incubator at 37°C. The precipitin ring usually appeared from 10 to 15 min, sometimes earlier. The test was then applied to one hundred different cultures that had been previously typed by Watt[6] and classified as follows: 29 as W, 34 as Z, and 35 as V. These were treated by the above procedure and their races determined by the precipitin test. In all instances, the classification corresponded to the typing as determined by agglutination, except in 2 cultures, in which there was a difference in the results. The reason for this exception is not yet clear but is the object of study.

Summary. The polysaccharide fraction is a determining factor in the type and group antigens of *Shigella dysenteriae* Flexner. The precipitin test performed by using the polysaccharide fraction and the specific antiserum is presented as a method for classifying the varieties of the group of *Shigella dysenteriae* Flexner.

Recently isolated cultures are essentially necessary for the preparation of type specific antiserums and for the preparation of type specific antigen.

4 Hardy, A. V., Watt, J., and DeCapito, T., *Pub. Health Rep.*, 1942, **57**, 501.

5 Fuller, A. T., *Brit. J. Exp. Path.*, 1938, **19**, 130.

6 Watt, J., personal communication.

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A Method for Determining the Concentration of Penicillin in Body Fluids and Exudates*

CHARLES H. RAMMELKAMP. (Introduced by Chester S. Keefer.)
From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine.

During a study on the effect of penicillin in certain human infections, it at once became apparent that a sensitive, rapid, and simple method for determining the concentration of penicillin in body fluids was desirable. Previously, Fleming[1] used a serial dilution method with *Staphylococcus aureus* as the test organism. Florey[2] found that by using the Oxford-plate method it was possible to determine the concentration of penicillin in both sterile and contaminated fluids. This method also utilized the staphylococcus as the test organism after preliminary dilutions of the unknown solution. The disadvantages of such a method have been commented on recently by Hobby.[3] Foster[4] has shown that the inhibition of growth of the staphylococcus may be measured turbidmetrically and is a function of the concentration of penicillin. This method requires large amounts of the unknown solution and is not readily applicable to the study of body fluids and exudates.

As a preliminary step in our own investigations numerous organisms were tested for

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1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

2 Florey *et al.*, *Lancet*, 1941, **2**, 177.

3 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 227.

4 Foster, J. W., *J. Biol. Chem.*, 1942, **144**, 285.