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*

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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.

TABLE I.
In Vitro Test for Concentration of Penicillin.

Sample	Culture	Serial dilutions in broth: 0.2 cc of sample	1:2	1:8	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
Standard penicillin	BB	0	0	0	0	0	0	0	0	+	++
	BAP						0	0	0	++	++
Serum	BB	0	0	0	0	+	++	++	++	++	++
	BAP				0	++	++				

Standard penicillin is an 0.85% solution of sodium chloride containing 20 Florey units per cc.
Serum sample obtained from patient 10 minutes after an intravenous dose of 10,000 Florey units.
Concentration in unknown sample is determined by multiplying the dilution factor by 0.0039.
Culture: BB = blood broth. BAP = blood agar plates. 0 = no visible growth or hemolysis. + and ++ = degrees of hemolysis or growth.
Inoculum: 0.5 cc blood broth containing 1100 streptococci.

their susceptibility to the action of penicillin. It was found that, in general, hemolytic streptococci are from 4 to 16 times more sensitive than staphylococci. An especially sensitive strain of hemolytic streptococcus was therefore chosen as the test organism.†

Methods. The unknown samples of penicillin‡ are stored at 5°C until the time of testing. If the samples are known to be contaminated, sterilization is effected by passing them through a Seitz filter.

To the first 2 tubes of a series of small culture tubes, 0.2 cc of the unknown sample is added. The tubes, with the exception of the first one in the series, contain 0.2 cc of veal infusion broth. From the second tube, then, 0.2 cc of the broth-penicillin sample is removed and serial dilutions are made. In addition, if the solution to be tested is known to contain a very small quantity of penicillin, a further tube containing 0.5 cc of the unknown is added to the test.

A control run with each determination is made up from a standard of penicillin which is stored at 5°C in a solution of 0.85% sodium chloride in a concentration of 20 Florey units per cc. This standard is then treated in a manner similar to the unknown samples.

The test organism is a Group A strain of hemolytic streptococcus obtained from the blood stream of a patient with erysipelas. The appropriate dilution of a 12-hr broth culture is made in veal infusion broth containing 1% erythrocytes so that the final number of organisms varies between 1,000 and 10,000 per cc. The inoculum consists of 0.5 cc of this dilution and is added to each tube as well as to the control series containing dilutions of a known amount of penicillin. The final volume in each instance is 0.7 cc. The cultures are then incubated for 18 hr, following which the tubes are examined for hemolysis. A 3 mm loop of the cultures near the endpoint is streaked on blood agar plates as a check of sterility.

Results. Table I illustrates the results obtained when the test was applied to the serum of a patient 10 min after the intravenous injection of 10,000 Florey units of penicillin. As demonstrated here, 0.0039 Florey unit was required to sterilize the culture. By comparison with the control, then, the serum contained 0.25 Florey unit or 1.25 units per cc.

The above method has been used to test unknown samples of penicillin, whole blood, erythrocytes, urine, spinal fluid, exudates from empyema cavities, and joint fluid. The test has proven to be satisfactory in all instances. Preliminary studies demonstrate that the addition of these various materials does not alter the result of the test. The amount of unknown fluid required is from 0.2 to 0.9 cc which makes the method readily adaptable to clinical studies.

The reliability of the test is well demonstrated by the results obtained in the 30 observations made with the penicillin standard on separate days. In 28 instances the cul-

† Hobby[3] has used the streptococcus to determine the concentration of penicillin.

‡ Penicillin for this study was supplied through the courtesy of Dr. George A. Harrop, Squibb Institute for Medical Research, New Brunswick, N.J.

tures were sterilized by 0.0039 units and in the remaining 2 by 0.0019 units. The test is subject to the error of serial dilution methods in general.

It is important to store all solutions containing penicillin in the ice box until the time of testing since high temperatures result in a loss of activity. In one experiment a solution of penicillin in saline containing 100 Florey units per cc lost all activity within 7 days when placed at 37°C, whereas the same solution stored at ice box temperature retained its antibacterial effect. Urines which contain penicillin have been stored in the ice box for 2 weeks without loss of penicillin potency.

For those fluids known to be contaminated, sterilization may be effected by passage through a Berkefeld or Seitz filter. This procedure is not attended by any appreciable loss of penicillin.

The sensitivity of the test is increased when a small number of organisms and a small total volume of culture media are used. In general, an inoculum of between 100 and 100,000 organisms is advisable. Variations within this range usually do not alter the results of the test. The addition of 1% erythrocytes to the culture media aids in reading the test. The cultures which show no hemolysis are usually sterile.

Conclusions. A method for determining the concentration of penicillin in various body fluids and exudates is described. It is possible by this method to determine 0.0039 Florey unit per 0.2 cc of solution.

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Changes in Body Proportions Produced in Frog Embryos by Supra-Normal Temperatures.

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Several investigators have produced changes in body proportions by varying the conditions of temperature in which development occurs. Hoadley[1] has found that constant exposure to slightly supra-maximal temperatures produced gradations of microcephaly in *Rana pipiens* and *Rana sylvatica larvae*. Huxley[2] and his associates[3] and Gilchrist[4] have found that temperature *gradients,* within the ranges of temperatures normal to development, when properly applied to the developing amphibian egg for short intervals have a stimulative effect which is apparent in the development of the larva. Considering the fluid environment of the egg membranes and of the embryo itself, it is improbable that an embryo in nature would be subjected to a temperature gradient. However, short exposures of the whole egg to temperatures higher than the usual maximum for normal development must frequently occur. Possible long range effects on the developnig embryo of abnormal temperatures due to the heating of the spawning ponds of certain amphibia and to fever in the case of the pregnant mammal present problems in this connection. The present work was undertaken to test the effect on body proportions of short exposures of the whole amphibian egg and embryo to temperatures above the normal.

The eggs of *Hyla regilla,* the Pacific tree frog, were used in this work. An effort was made to obtain the larger clusters of 25 or

1 Hoadley, Leigh, *Growth,* 1938, **2**, 25.

2 Huxley, J. S., *Arch. F. Entwmech.,* 1927, **112**, 480.

3 Dean, I. L., Shaw, M. E., and Tazelaar, M. A., *Brit. J. Exp. Biol.,* 1928, **5**, 309.

4 Gilchrist, Francis G., *J. Exp. Zool.,* 1933, **66**, 15.