in this paper, in the same solvent, had no effect.

Summary. Vitamin K produces lowering of blood pressure in rats rendered hypertensive by silk perinephritis. The hydroquinone compound Synkayvite shows no such effect. The trial of vitamin K in human hypertension is being considered.

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Filter Paper Disc Modification of the Oxford Cup Penicillin Determination.*

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At the present time biological assay methods are the sole means of determining penicillin potency. A number of such methods are in use in various laboratories;[1] however, the Oxford cup method,[2] with several modifications,[3] has continued to be regarded as a procedure in which a commensurate accuracy and speed may be obtained with a minimum of labor. The following filter paper disc modification should reduce the labor and time involved in setting up this test, while the ease with which extra replicates may be run should increase the test's accuracy.

The principal modification lies in the use of a thick filter paper disc saturated with the penicillin sample, substituted for the sample-containing small cylinder used in the Oxford cup method. These discs may be conveniently set up on the seeded plates at a rate of about 6 per minute, a rate considerably more rapid than that in the original method. Another advantage of the discs is that the test plates may be manipulated freely to facilitate reading. Experience with a large number of tests has indicated that the zones of inhibition obtained with the discs are more consistent and more sensitive to variations in the penicillin content of the sample than the zones obtained with the cup method. This improvement may be due to a more consistent contact of the penicillin solution with the agar and to a more even diffusion from the disc.

The test organism, *Staphylococcus aureus H*† (Oxford strain), tends to produce a somewhat granular growth in nutrient broth. With the use of peptone broth as the seeding medium, a more diffuse growth is obtained and this in turn effects a more even seeding of the test plates.

Hobby[4] has criticized the plate method as having disadvantages arising from discrepancies in the depth and dryness of the agar and in the lag phase of the test organism. An attempt was made to control these factors by the use of a stabilized culture adapted to the seeding medium, and through a more complete standardization of the preparation and the treatment of the seeded plates.

Aside from the above modifications, the following procedure conforms closely to that of the Oxford method.

Procedure. The test organism, *Staphylococcus aureus H*, was transferred from an agar slant twice through peptone broth‡ for 24-hour

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1 Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1943, **46**, 187.

2 Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, 1941, **2**, 177.

3 Foster, J. W., and Woodruff, H. B., *J. Biol. Chem.*, 1943, **148**, 723.

† Culture furnished through the courtesy of Dr. J. W. Foster, Merck & Co., Rahway, N.J.

4 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

‡ 1% peptone, 0.5% NaCl.

TABLE I.
Zone Formation with Standard Penicillin.

Units in solution	Diameter of zones in mm	Avg zone size in mm	Units penicillin/ml: Mean value	Stand. dev.	Prob. error
4	32 32 32 31 32 33 32 32 32	32.1 ± .335	4.058	1.072	± .258
2	30 30 29 30 30 31 29 30 30	29.9 ± .3	2.01	0.347	± .083
1.5	28 28 28 29 29 29 29 29 30	28.8 ± .15	1.418	0.306	± .073
1.0	28 28 28 27 28 28 28 28 28	27.9 ± .074	1.02	0.084	± .02
.75	25 27 27 27 27 27 26 27 27	26.7 ± .16	.726	0.104	± .025
.5	25 25 25 24 25 26 26 26 26	25.3 ± .15	.521	0.071	± .017
.25	20 20 21 20 21 21 21 22 23	21.0 ± .226	.249	0.038	± .009
.125	15 16 18 16 18 18 18 18 18	17.2 ± .272	.121	0.028	± .007

growth periods at 37°C. The second transfer was held at 5°C for 16 to 18 hours.

Ten ml fresh nutrient agar was pipetted into uniform, flat-bottomed petri dishes and incubated for 16 to 18 hours at 37°C. These plates were held in the refrigerator for at least 1 hour.

Each plate was flooded with 1 ml of refrigerated culture. The excess was removed with a capillary pipette and the plates dried for 1 hour at 37°C (using wooden racks which supported the top half of the petri dish above the bottom half so that there was about ½-inch clearance). The plates were stored in the refrigerator, inverted, at least 1 hour.

Filter paper discs§ were sterilized by dry heat and immersed in the test fluid until saturated (30 seconds). They were then removed from the fluid with sterile forceps and, after gently shaking off the excess, were placed on the seeded plates. Special care should be taken to set the discs in place without smearing the surface of the agar. Three filter papers, evenly spaced, may be placed on each plate. For greater accuracy, plates may be run in triplicate. One of the discs on each plate may be a standard penicillin control.

The plates were incubated, not inverted, at 37°C for 14 hours (placed on wooden block to avoid excess condensation).

With a diffuse light a clear, well-defined zone of inhibition will appear around each disc if the test fluid contains more than 1/16 units of penicillin. By comparing the diameters of the zones to a standard curve of a known sample, the concentration of penicillin may be determined.

Results. Table I shows an example of the results obtained by the disc method on a standard sample of penicillin.‖ This sample, treated turbidimetrically with a photoelectric colorimeter against growth in nutrient broth, produced complete inhibition of *Staphylococcus aureus H* at a concentration of just 0.02 units of penicillin per ml.

An examination of the data indicates the disc modification has materially improved the ±25% accuracy usually claimed for the Oxford method. The aforementioned results are quite typical of the probable error obtained in a considerable number of tests on commercial samples of penicillin. The sample should be diluted to approximately 1 unit per ml for the best results. For example, the above sample, containing 77 units per mg, showed a probable error of ±1.54 units per mg where a 1 unit per ml dilution was used and ±5 units per mg with a 4 unit dilution. Other investigators working with the Oxford method also have found that penicillin samples in the range of 1 unit per ml give the most exact readings.[1,2,5]

The disc method has been found to work equally well on blood serum, spinal fluid, and urine. As in the original Oxford method, no filtration of the sample is necessary under ordinary circumstances.

Penicillin samples containing more than

§ No. 470, ½ inch diameter, Schleicher and Schuell Company, Inc., New York.

‖ Penicillin standard furnished by Merck & Co., Rahway, N.J.

5 Atkinson, N., *Aus. J. Exp. Biol. and Med. Sc.*, 1943, **21**, 127.

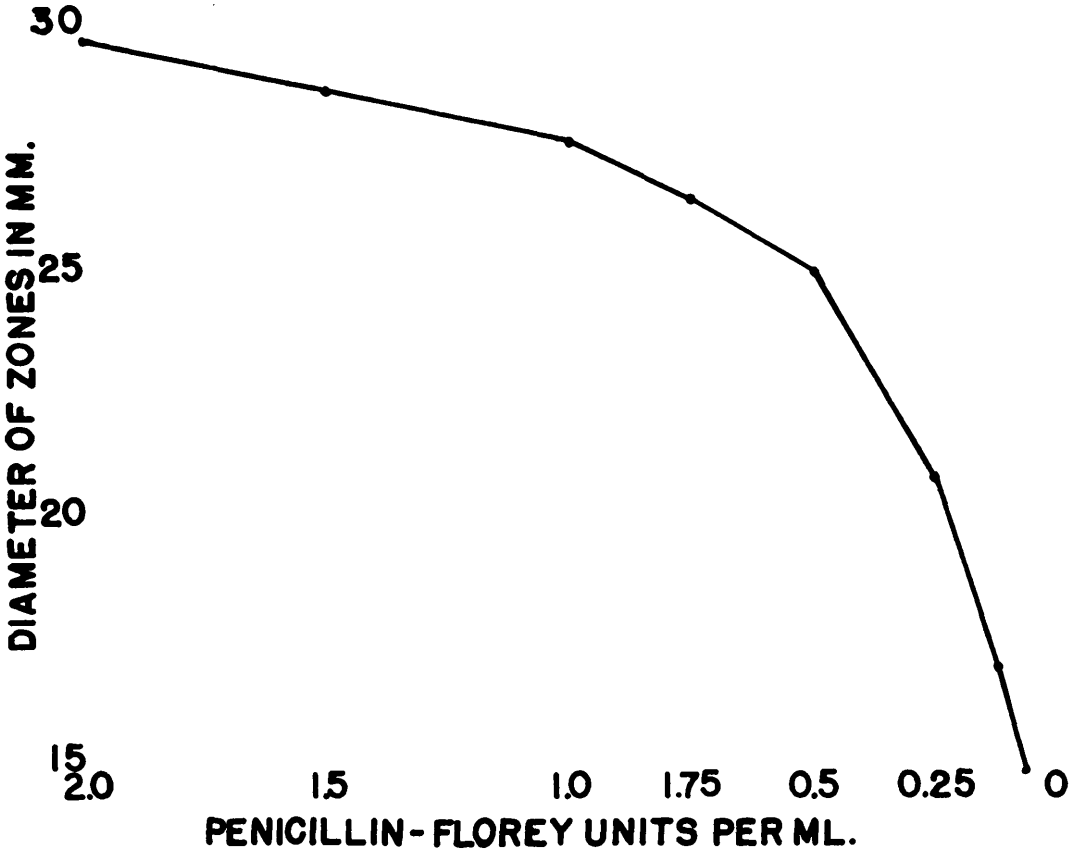

FIG. 1.

4 units per ml can be measured with the disc method, but with no great accuracy. Those containing as little as 1/16 units per ml produce a slight zone around the disc (diameter approximately 15 mm). Solutions of lower concentration cannot be measured by this method, but may be measured by the tube dilution method[6] or its modification.[7]

Fig. 1 shows a standard curve obtained when the diameters of the zones of inhibition are plotted against the concentration of penicillin in Florey units.

[6] Foster, J. W., *J. Biol. Chem.*, 1942, **144**, 285.

[7] Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.