supports and suspended in a large Pyrex glass cylinder. The end of the tubing passes out through an aluminum cover held on the cylinder by means of metal strips passing underneath. Glass tubes are attached to the cellulose tubing where it passes through rubber stoppers in the aluminum top. This

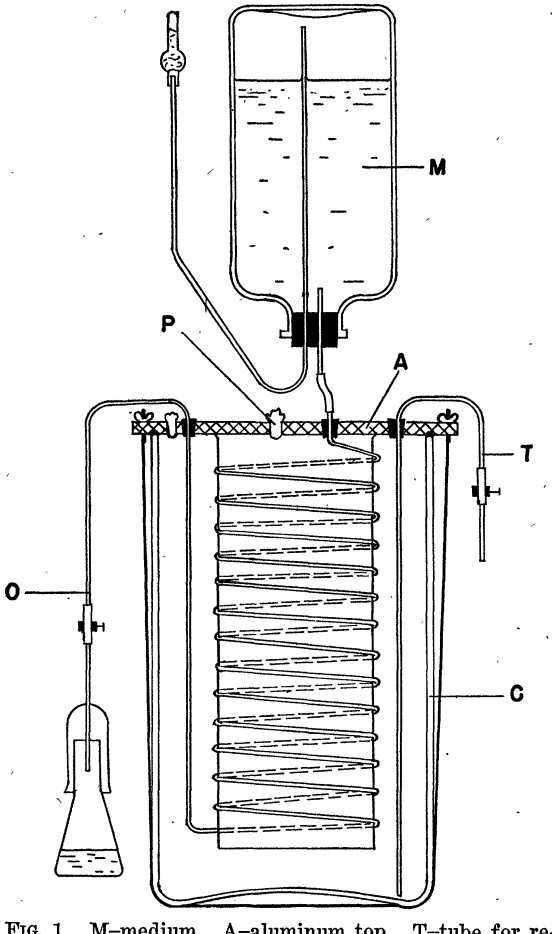

FIG. 1. M–medium. A–aluminum top. T–tube for removing seepage liquid. O–outlet tube. P–cotton plug. C-glass cylinder.

part of the apparatus is covered with a cloth hood, the outlets wrapped and sterilized in the autoclave.

A three-gallon bottle of sterile medium was prepared with an outlet assembly. It was then attached by a rubber tube to the cellulose tube inlet and supported so the medium would flow down into the cellulose tubing. The outside of the tubing was sprayed with a suspension of spores by reaching through the extra holes in the top. All necessary precautions were taken to prevent contamination.

A special atomizer was constructed by increasing the length of the two outlet pipes of a common DeVilbiss atomizer to 12 inches. A small Erlenmeyer flask was used in place of the regular fluid container. Sterile air was obtained by forcing air through water and then through a column of sterile cotton. The whole atomizer was constructed so that it could be autoclaved.

After inoculating with *Penicillium notatum*, the fungus grew readily and in two or three days the tubing was covered with mycelium. Usually the medium in the tubing was changed each day by draining through the outlet tube. The fungus continued rapid development and was covered with spores on the fourth or fifth day. When growth was well established, the medium was allowed to drop from the outlet tube at varying rates, usually about a liter per day. On the sixth to eighth day after inoculation, depending upon conditions, penicillin could be detected in the run-off medium. The penicillin titre increased within a few days so that it equaled or surpassed that of a flask culture. Unfortunately, the experiment could not be carried past the sixteenth day because leaks developed in the cellulose tubing owing to the action of the fungus.

An attempt was made to overcome the breakdown of the tubing by securing a thicker tube of cellulose and by the use of other materials. Thicker tubes of small diameter were not available. Cellulose nitrate films were found to be much more resistant to the fungus action. Attempts to coat the cellulose tubing with a cellulose nitrate film, either inside or outside, failed to give good results. Porcelain tubes with and without a coating of cellulose nitrate have so far failed to give the desired results, although they have overcome the problem of disintegration of the tubing.

Bacillus brevis was grown in this apparatus for three weeks without any apparent breakdown of the cellulose tubing.

The authors believe the apparatus as described can become a useful laboratory tool for studying certain organisms and that if a better tubing can be secured, it may be adaptable to the production of antibiotics and toxins.

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THE ESTIMATION OF PENICILLIN IN BODY FLUIDS

DURING the course of numerous investigations requiring the determination of penicillin activity in various body fluids, the need for a simple method of estimating penicillin levels of a lower order than that readily measured with accuracy by the agar cup plate method became apparent.

A 4-hour turbidimetric assay employing *Staphylococcus aureus* was investigated but was found of little value with blood serums because of an apparent stimulation of the microorganisms by the presence

of the serum. The turbidimetric assay method, because of the erratic readings obtained, was abandoned in favor of the serial dilution method with a lengthened period of incubation.

Various methods for estimating the concentration of penicillin in body fluids have appeared in the recent literature. The method which seemed to possess the most merit was that of Rammelkamp,[1] or modifications thereof, in which a beta hemolytic streptococcus is employed in preference to staphylococci because of its greater sensitivity to penicillin. In order to increase the sensitivity, however, as well as the ease of determining the endpoint, washed red blood cells were used in the test. This necessitated establishing a source of washed cells and the use of young, freshly prepared cultures of the organism to prevent the addition of preformed lysin which interferes with the endpoint.

A large number of various species of bacteria were studied in an effort to determine whether an organism existed which was highly sensitive to penicillin yet could be grown and maintained on common laboratory media with a minimum of effort and which would give sharp and reproducible endpoints. The organism finally selected was a strain of *Bacillus subtilis*[2] obtained from the Northern Regional Research Laboratory. This organism grows luxuriantly at 30° C with a diffuse turbidity, while at 37° C growth consists of a pellicle with the medium below the pellicle remaining clear. Its resistance to penicillin is of the same order as most strains of hemolytic streptococci requiring as little as 0.0085 units per ml to inhibit a 1:100 dilution of a broth culture. This sensitivity is maintained over long periods of time without the necessity of repeated transfers. Cultures maintained at room temperature in screw-capped bottles were tested over an interval of six months with no demonstrable loss of sensitivity. Since then thousands of determinations have been made with satisfactory results.

Technique of the test: One-half ml amounts of broth[3] are placed in Wassermann-tubes and serial dilutions by halves made by adding one-half ml of the fluid being tested to one of the tubes and carrying one-half ml in serial dilution for as many tubes as necessary. The first tube in the series contains one-half ml of the material under test only. A standard is prepared for comparison by diluting a known potency penicillin (reference standard) to one-unit per ml in broth. This one-unit standard is diluted exactly as above in serial dilution by halves. One and one-half ml of a 1:100 dilution of the test organism in broth is then added and all tubes incubated at 37° C over night. The last tube in which no growth occurs is taken as the endpoint. This is usually very sharp, inasmuch as one tube will be absolutely clear while the next one in the series will have the typical pellicle of *B. subtilis* on the surface of the media.

The concentration of penicillin in the unknown is then determined by comparing the endpoint of the unknown with that of the standard. An example is given in Table 1.

TABLE 1

Fluid	Tube No.								
	1	2	3	4	5	6	7	8	9
Standard	0	0	0	0	0	0	+	+	+
Serum	0	0	0	0	0	0	0	+	+
Urine 1:10 ...	0	0	0	0	+	+	+	+	+

In the example in Table 1, the standard caused complete inhibition in the sixth tube. Since this represents one unit, the serum tested contains twice this amount, or two units, while the urine which caused complete inhibition in the fourth tube and had a primary dilution of 1:10 contains 0.25 units × 10 or 2.5 units. The test as described here can be used to determine potencies as low as 0.03 unit per ml. To determine potencies lower than this it is necessary to vary the dilution series of both standard and unknown.

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[1] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.

[2] Some other strains of *B. subtilis* have been found to be considerably less sensitive.

[3] Mimeograph: Methods Used by the Food and Drug Administration for the Assay of Penicillin—Revised, January, 1945.

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