METHODS OF MEASURING PENICILLIN CONCENTRATIONS IN BODY FLUIDS[1]

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Inhibition of bacterial growth is the basis of the three methods commonly used for determining the potency of penicillin solutions. With the Oxford cup method (Abraham *et al.*, 1941; Foster and Woodruff, 1944), penicillin is allowed to diffuse out into agar plates from small glass cups, and the diameters of the zones of inhibition of bacterial growth are measured. In dilution methods (Foster and Woodruff, 1943; Rammelkamp, 1942), the smallest amount of penicillin which will completely inhibit bacterial growth in solid or liquid media is the standard with which unknowns are compared. In turbidimetric methods, a range is selected in which bacterial growth is proportional to penicillin concentration, and a standard curve is constructed from turbidimetric measurements of the bacterial suspensions.

These methods, developed for the titration of penicillin lots during production, have had to be modified for clinical use because the concentrations in body fluids are much smaller than those which can be most conveniently measured during penicillin manufacture. A search through the literature reveals that heretofore only the dilution method has been used clinically.

In connection with extensive clinical studies, attempts have been made in this laboratory to adapt all three methods to the measurement of penicillin in body fluids. The results of these studies will be presented briefly.

OXFORD CUP METHOD

This method is not satisfactory for clinical use because the concentrations of penicillin, especially in the blood, are usually too small to produce adequate zones of inhibition. In the urine and sometimes in chest and spinal fluids, the cup method may be used, but since blood levels are usually measured simultaneously, it is simpler to employ one procedure for all the determinations. After many attempts with both *Bacillus subtilis* and *Staphylococcus aureus*, we have abandoned the cup method altogether.

DILUTION METHOD

The procedure described by Rammelkamp (1942) has been used widely and is excellent for routine determinations. We have modified the technique somewhat

[1] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

to give a narrower range of dilutions, and consequently greater accuracy. Instead of serial dilutions, tube dilutions[2] are made in the range in which the levels are likely to occur. The unknown and standard samples are added to two rows of ten tubes each in the amounts listed in table 1.

The standard is a solution of 0.1 Unit per ml of penicillin in normal saline, and calculations are based on units per ml rather than on the actual number of units

TABLE 1

Procedure for the dilution method: The unknown specimen is added to a second row of ten tubes in the amounts listed above for the standard penicillin solution.

TUBE	STANDARD PENICILLIN SOLUTION 0.10 U/ML	STANDARD PENICILLIN SOLUTION 0.1 U/ML DILUTED 1:10	Broth	1% R.B.C. + HEM. STREP. IN BROTH
	ml	*ml*	*ml*	*ml*
1	0.5	0	0	1
2	0.4	0	0.1	1
3	0.3	0	0.2	1
4	0.2	0	0.3	1
5	0.1	0	0.4	1
6	0	0.5	0	1
7	0	0.4	0.1	1
8	0	0.3	0.2	1
9	0	0.2	0.3	1
10	0	0.1	0.4	1

TABLE 2

Amount of penicillin per ml measured in each tube with the Rammelkamp method and the present modification

TUBE	RAMMELKAMP METHOD	PRESENT METHOD
~~*U/ml*~~	*U/ml*	*U/ml*
1	0.0195	0.04
2	0.039	0.05
3	0.078	0.07
4	0.156	0.10
5	0.312	0.2
6	0.624	0.4
7	1.25	0.5
8	2.5	0.7
9	5.0	1.0
10	10.0	2.0

in each tube as in the Rammelkamp method. The remainder of the procedure, including the addition of red cells and hemolytic streptococci, the time of incubation, and the determination of the end point, is exactly as described by Rammelkamp.

[2] Serial dilutions, 1:2, 1:4, 1:8, etc. Tube dilutions, 0.5 ml, 0.4 ml, 0.3 ml, etc., in successive tubes.

The concentrations of penicillin in the standard tubes with both the serial dilution method and the present modification are listed in table 2.

The difference in concentration from one tube to the next is much smaller with tube dilutions, and the total range of 0.04 to 2.0 U per ml is more than adequate for most purposes. The only disadvantage of the tube dilution method is that it requires a larger quantity (2 ml) of the unknown specimen, but this is seldom a serious problem. Because it is equally easy to perform and is more accurate, this modification has replaced the serial dilution method in this laboratory.

TURBIDIMETRIC METHOD

Admittedly the most accurate means of determining penicillin concentrations (Foster and Woodruff, 1943), this method has steadily gained in popularity (Joslyn, 1944; Lee *et al.*, 1944). Attempts to adapt turbidimetry to clinical use, however, met with two serious difficulties: (1) Turbidities and colors of body fluids made comparison with standards difficult, and (2) even with highly enriched

TABLE 3

Optical densities (turbidities) of suspensions of hemolytic streptococci in nutrient broth with and without plasma after 4 hours' incubation, showing the marked stimulation of growth produced by the plasma

TUBE	CONCENTRATION OF PENICILLIN	WITH PLASMA	WITHOUT PLASMA
	U/ml		
1	0.010	0.47	0.12
2	0.008	0.54	0.14
3	0.006	0.61	0.16
4	0.004	0.68	0.18
5	0.002	0.75	0.22
6	0	0.80	0.28

nutrient broth there was a marked nonspecific stimulation of growth of the organisms, especially with blood plasma and chest fluid (table 3).

A highly sensitive method was finally developed for determining blood and urine levels. The test organism selected was a Group A hemolytic streptococcus the growth of which was proportional to the concentration of penicillin in a range of 0 to 0.01 U per ml (figure 1). Variations in color were avoided by using only fasting specimens of blood, and errors due to nonspecific growth stimulation were prevented by adding equal amounts of the patient's plasma to each tube. The plasma added to the tubes from which the standard curve was constructed was obtained at a time when there was no penicillin in the blood stream, preferably before treatment was begun. Table 4 lists the amounts of plasma, inoculum, broth, and penicillin added to each tube. Each unknown plasma was tested undiluted and diluted, 1:2, 1:10, and 1:20, so that with concentrations ranging from 0.01 to 1.0 U per ml, one, and often two, of the dilutions could be plotted on the standard curve. The inoculum contained about one million organisms per ml, and turbidities were measured after incubation at 37 C for 4 hours. Urine was

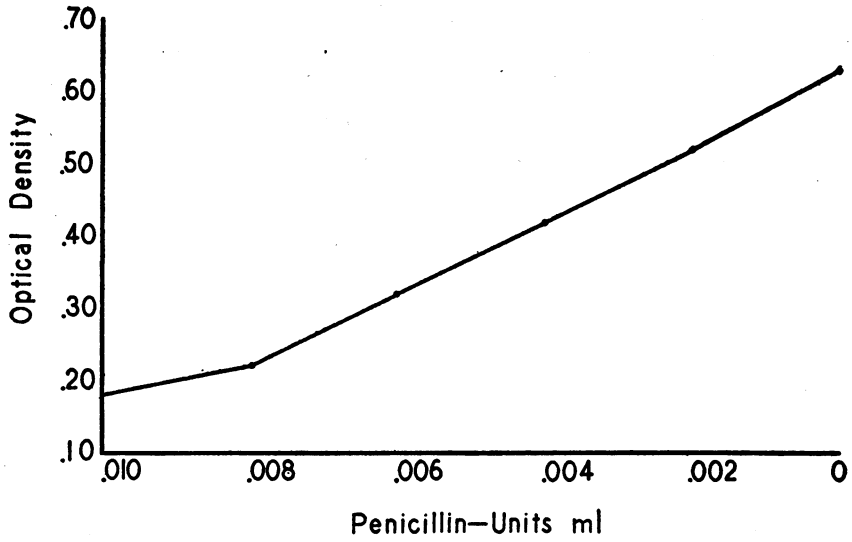

FIG. 1. STANDARD CURVE FOR TURBIDIMETRIC METHOD AFTER 4 HOURS' INCUBATION

TABLE 4

Procedure for turbidimetric method. See text for further explanation

Standard Curve

PENICILLIN 0.1 ML IN BROTH	STERILE BROTH	PLASMA OBTAINED BEFORE TREATMENT	HEM. STREP. IN BROTH	FINAL CONCENTRATION OF PENICILLIN
ml				*U/ml*
0.5	3.0	0.5	1.0	0.010
0.4	3.1	0.5	1.0	0.008
0.3	3.2	0.5	1.0	0.006
0.2	3.3	0.5	1.0	0.004
0.1	3.4	0.5	1.0	0.002
0	3.5	0.5	1.0	0

Determination of Unknown

TUBE	PLASMA OBTAINED BEFORE TREATMENT DILUTED 1:2 IN BROTH	STERILE BROTH	UNKNOWN PLASMA	HEM. STREP. IN BROTH
	ml	*ml*	*ml*	*ml*
1	0	3.5	0.5 undiluted	1.0
2	0.5	3.0	0.5 diluted 1:2	1.0
3	0.9	2.6	0.5 diluted 1:10	1.0
4	0.95	2.55	0.5 diluted 1:20	1.0

diluted 1:100 at the outset, otherwise the procedure was identical with that for blood. Although color variations and growth stimulation were minimal, the results were more uniform if urine samples were also added to the standard tubes.

Plasma samples containing 0.50 U per ml of penicillin were tested repeatedly with both the turbidimetric and dilution methods (table 5).

Errors were consistently under 15 per cent with the turbidimetric method, whereas with the dilution method they were often 20 per cent or more.

DISCUSSION

In comparison with determinations of the sulfonamides, determinations of penicillin concentrations in body fluids are tedious, time-consuming, and relatively inaccurate. However, they are of great aid in establishing and evaluating various modes of administration of penicillin, for they indicate whether an effective concentration is present at any given time.

Of the three methods developed in connection with the production of penicillin, the Oxford cup technique is the least useful clinically because the concentrations in body fluids are usually too small to produce adequate zones of inhibition.

The serial dilution technique is unquestionably the most suitable for routine clinical use. It is relatively simple to perform and sufficiently accurate for most purposes, and the results are clear-cut and reproducible. In the modification

TABLE 5

Results of turbidimetric and dilution tests of a sample of plasma to which penicillin was added in a concentration of 0.5 U/ml.

SAMPLE	TURBIDIMETRIC ERROR		DILUTION ERROR	
1	0.48 U/ml	+4%	0.40 U/ml	−20%
2	0.46 U/ml	−8%	0.40 U/ml	−20%
3	0.50 U/ml	0	0.50 U/ml	0
4	0.45 U/ml	−10%	0.70 U/ml	+40%
5	0.53 U/ml	+6%	0.70 U/ml	+40%
6	0.48 U/ml	−4%	0.40 U/ml	−20%
7	0.50 U/ml	0	0.50 U/ml	0
8	0.52 U/ml	+4%	0.50 U/ml	0
9	0.44 U/ml	−12%	0.40 U/ml	−20%
10	0.50 U/ml	0	0.50 U/ml	0

described in detail in this paper, tube dilutions are substituted for serial dilutions to give greater accuracy in the range of levels which usually occur in blood and urine.

The turbidimetric method developed in this laboratory is highly accurate but impractical for most purposes. It requires a supply of plasma before treatment is begun, and fairly large amounts (2 ml) of plasma obtained in the fasting state are needed for each determination. For studies of the renal clearance of penicillin (Rantz and Kirby, 1944), this method gave the necessary high degree of accuracy and caused no great inconvenience because each experiment required a total of only eight hours. Except for such highly specialized research procedures, the turbidimetric technique will probably be employed very little.

SUMMARY

Attempts to adapt the three most widely used methods of determining penicillin concentrations to clinical use are described.

A modification of the serial dilution technique is presented.

A highly accurate turbidimetric method for determining penicillin concentrations in the blood and urine is described in detail.

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