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Simple Method for Elimination of Aminoglycosides from Serum to Permit Bioassay of Other Antimicrobial Agents

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Cellulose phosphate powder can be added to serum to selectively bind aminoglycosides by ionic interaction without binding or inactivation of the penicillins, the cephalosporins, clindamycin, vancomycin, chloramphenicol, or trimethoprim. This simple, rapid technique permits the measurement of non-aminoglycoside antibiotics in the presence of aminoglycosides.

Aminoglycosides are often used concomitantly with other antimicrobials to treat life-threatening infection. The indications for routine determination of serum levels of antibiotics other than aminoglycosides are unclear. There may be circumstances where assay of serum or body fluid (e.g., cerebrospinal fluid) for agents used in combinations with aminoglycosides may be indicated, particularly to assess adequacy of treatment of meningitis, pulmonary infection, or bacteremia.

Measurement of one component of several antimicrobials used in combination can be difficult with microbiological assays. To measure other antibiotics in the presence of aminoglycosides, several techniques have been described including (i) radioenzymatic assay (3), (ii) high-pressure liquid chromatography (6), and (iii) the following microbioassays that (a) use organisms resistant to aminoglycosides but sensitive to other antibiotics (4), (b) shift the pH of the media to the acidic range to obviate the activity of aminoglycosides (5), or (c) that incorporate sodium polyanetholsulfonate into the medium to selectively inhibit aminoglycosides (2). Some of these techniques are quite specific and, consequently, are only useful for certain non-aminoglycoside antimicrobials. Others, like the microbiological methods, necessitate either modifications to existing methods or preparation of special medium.

The purpose of this work is to report a simpler, far more rapid, and perhaps more generally applicable method for specifically inactivating aminoglycosides by using cellulose phosphate powder. This technique permits measurement of other antimicrobial agents without the need for preparing special media or agar plates, or changing the microbiological methods a laboratory may be currently using.

A microbioassay by the agar diffusion technique was used with several standard test organisms (7). Both *Klebsiella pneumoniae* and *Bacillus globigii* were used in aminoglycoside assays, *Bacillus globigii* for the penicillins, vancomycin and clindamycin, and *Micrococcus lysodicticus* for the cephalosporins. Trimethoprim levels were determined by a microbiological assay described by Bushby (1).

To inactivate aminoglycosides we used the following procedure: to polystyrene tubes (12 by 75 mm) (Falcon Plastics, Oxnard, Calif.), we added cellulose phosphate powder (ICN Biochemicals, Cleveland, Ohio) to a depth of approximately 6 mm, which corresponds to the first scored line on the tube and is approximately 50 mg. To this we added 0.5 ml of the serum sample to be inactivated, vortexed, and immediately centrifuged at 2,000 × *g* for 5 min. A portion of the supernatant was then taken for measurement by the appropriate microbiological assay.

Using this system, we determined if the cellulose phosphate would selectively bind aminoglycosides by adding serum samples of the following aminoglycosides at several concentrations up to 100 μg/ml: tobramycin, amikacin, kanamycin, gentamicin, sisomicin, netilmicin, and samples of several non-aminoglycoside antibiotics at serum concentrations that were anticipated clinically.

No aminoglycoside could be detected in the serum treated with cellulose powder as described at concentrations ranging from 1 μg to as high as 100 μg of aminoglycoside per ml of serum. Such data indicate that 1 mg of cellulose phosphate has the capacity to bind at least 1 μg of aminoglycoside antibiotic.

The diameter of the zones of inhibition in millimeters, which is indicative of the microbiological activity of the antibiotic, of various standards of several non-aminoglycoside anti-

biotics with and without cellulose phosphate, is summarized in Table 1. No significant differences in zone sizes with and without cellulose phosphate were observed for any of the antibiotics, which included the penicillins: carbenicillin, penicillin G, dicloxacillin, and oxacillin; the cephalosporins: cefazolin, cephalothin, and cefoxitin; vancomycin; chloramphenicol; trimethoprim; and clindamycin.

TABLE 1. *Effect of cellulose phosphate powder on the microbiological activity of non-aminoglycoside antimicrobial agents*

Representative antimicrobial agents	Concn of antimicrobial agent (μg/ml of serum)	Zone size of inhibition (mm)	
		Without cellulose phosphate	With cellulose phosphate
Penicillins			
Carbenicillin	400	26	25
	100	18.5	18
	25	13.8	13.2
Oxacillin	100	33	34
	25	25	25
	6.25	11.5	11.5
Penicillin G	400	24	22.8
	100	19	18.5
	25	16.5	15.5
Dicloxacillin	100	34	34
	25	26	27
	6.25	21.8	21
Cephalosporins			
Cefazolin	200	14.7	14.8
	50	11.2	11.4
	12.5	7.5	7.0
Cephalothin	200	19	22
	50	12.8	14.8
Cefoxitin	200	15.7	16
	50	9.7	10.7
	25	7.4	7.8
Miscellaneous			
Vancomycin	100	16.5	17
	25	12.4	12.2
	6.25	8.5	8.1
Chloramphenicol	50	13	12
	12.5	6.5	7.1
Trimethoprim	10	17.2	16.8
	1.5	14.2	13.6
Clindamycin	10	11.5	11.5
	5	8.7	8.7

Aminoglycoside antibiotics exist as positively charged molecules at the pH range from 5 to 8, which includes the pH of human serum. This cationic property has been effectively used in radioenzymatic assays to separate radiolabeled aminoglycosides from complex reaction mixtures by binding to negatively charged phosphocellulose paper (P. Stevens and L. S. Young, p. 64–72, *Microbiology-1975*, American Society for Microbiology, Washington, D.C., 1976). With this principle we have used cellulose phosphate powder to selectively bind and inactivate aminoglycosides by ionic interaction, thereby permitting the measurement of non-aminoglycoside antimicrobial agents by microbiological techniques. This method would appear particularly advantageous in combinations of three antimicrobials, e.g., an aminoglycoside, a β-lactam antibiotic, and chloramphenicol, when one desires to measure chloramphenicol. In such a case, this technique could be used to bind the aminoglycoside, a β-lactamase could inactivate the β-lactam antibiotic, and chloramphenicol could be measured microbiologically by using a sensitive indicator ("seed") organism.

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LITERATURE CITED

1. **Bushby, S. R. M.** 1973. Trimethoprim-sulfamethoxazole: in vitro microbiological aspects. J. Infect. Dis. 128 (Suppl.):S442–S462.
2. **Edberg, S. C., C. J. Bottenbley, and K. Gam.** 1976. Use of sodium polyanethol sulfonate to selectively inhibit aminoglycoside and polymyxin antibiotics in a rapid blood level antibiotic assay. Antimicrob. Agents Chemother. 9:414–417.
3. **Lietman, P. S., T. J. White, and W. V. Shaw.** 1976. Chloramphenicol: an enzymological microassay. Antimicrob. Agents Chemother. 10:347–353.
4. **Louie, T. J., F. P. Tally, J. G. Bartlett, and S. L. Gorbach.** 1976. Rapid microbiological assay for chloramphenicol and tetracyclines. Antimicrob. Agents Chemother. 9:874–878.
5. **Sabath, L. D., J. I. Casey, P. A. Ruch, L. L. Stumpf, and M. Finland.** 1971. Rapid microassay of gentamicin, kanamycin, neomycin, streptomycin and vancomycin in serum or plasma. J. Lab. Clin. Med. 78:457–463.
6. **White, E. R., M. A. Carroll, J. E. Zarembo, and A. D. Bender.** 1975. Reverse phase high speed liquid chromatography of antibiotics. J. Antibiot. 28:205–214.
7. **Winters, R. E., K. D. Litwack, and W. L. Hewitt.** 1971. Relation between dose and levels of gentamicin in blood. J. Infect. Dis. 124 (Suppl.):S90–S95.