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Effects of Divalent Cations on Binding of Aminoglycoside Antibiotics to Human Serum Proteins and to Bacteria

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Binding of gentamicin and related deoxystreptamine-containing aminoglycoside antibiotics to proteins in human serum can vary significantly with changes in experimental conditions. The concentrations of divalent cations are important variables, and binding increases progressively as the concentrations of calcium and magnesium decrease. Maximal binding of deoxystreptamine-containing aminoglycosides to human serum is approximately 70% in the absence of divalent cations. The binding of ^{3}H-labeled gentamicin to *Pseudomonas aeruginosa* increases and its bactericidal activity against *P. aeruginosa* is enhanced in the absence of divalent cations. In contrast, binding of ^{3}H-labeled gentamicin to *Escherichia coli* and bactericidal activity against *E. coli* do not vary significantly in the presence and in the absence of divalent cations. Interference with uptake of gentamicin provides a plausible explanation for the observation that the minimal inhibitory concentration of gentamicin for *P. aeruginosa* increases as the concentration of calcium or magnesium in bacteriological media increases. Although significant binding of deoxystreptamine-containing aminoglycosides to plasma proteins does not occur under normal physiological conditions in man, the possibility remains that variations in protein binding of these aminoglycosides might be significant under pathological conditions.

The binding of an antibiotic to proteins or to other macromolecules in body fluids can reduce its effective concentration, decrease its antibacterial activity and modify its pharmacokinetics or toxicity (16, 19, 24). Most studies designed to measure protein binding of aminoglycosides by direct methods have shown little or no binding of these antibiotics to serum proteins (3, 10, 23), but significant protein binding of neomycin has been reported (25). The observation that renal clearances of aminoglycosides are similar to the glomerular filtration rate in man also suggest that protein binding of aminoglycosides is not physiologically important (11). During a reinvestigation of the binding of gentamicin to serum proteins, we observed significant protein binding of gentamicin by equilibrium dialysis (C. H. Ramirez-Ronda, R. K. Holmes, and J. P. Sanford., J. Clin. Invest. **53**:63a, abstr. 236, 1974). In further studies to reconcile these results with the published observations cited above, we have found that the concentrations of divalent cations, especially magnesium, are critically important in determining whether or not the aminoglycosides bind to serum proteins. Our initial experiments, as well as the study showing significant protein binding of neomycin cited above, were performed in the absence of divalent cations. In contrast, at the concentrations of magnesium and calcium present in serum or extracellular fluid, binding of aminoglycosides to serum proteins is minimal.

In earlier studies, concentrations of divalent cations were also shown to affect the susceptibility to gentamicin of some gram-negative bacteria (8, 9, 28). For example, in *Pseudomonas aeruginosa* but not in *Escherichia coli*, resistance to gentamicin is enhanced by high concentrations of magnesium or calcium in the medium used for susceptibility testing (9). Taken together, the effects of divalent cations on bacterial susceptibility to gentamicin and on the binding of aminoglycosides to serum proteins led us to test the hypothesis that the enhanced in vitro resistance of *P. aeruginosa* to gentamicin in the presence of high concentrations of magnesium or calcium ions is associated with decreased binding of the aminoglycosides to the bacteria. Information concerning the possible importance of binding of aminoglycosides to envelope components of bacterial cells as an initial step in uptake of these antibiotics is limited.

In the present report observations on the

effects of the divalent cations magnesium and calcium on binding of aminoglycoside antibiotics to bacteria as well as to serum proteins are presented. Although the relevance of these effects to the activity of aminoglycosides in vivo is not yet established, our data provide a plausible explanation for the very important effects of divalent cations on in vitro tests of susceptibility of *Pseudomonas aeruginosa* strains to aminoglycoside antibiotics. (A preliminary report of this work was presented to the Southern Section of the American Federation for Clinical Research, New Orleans, La., 31 January to 1 February 1975.)

MATERIALS AND METHODS

Bacteria and cultures. The strains of *P. aeruginosa* and *E. coli* used in our experiments were isolated from clinical specimens submitted to the bacteriology laboratory at Parkland Memorial Hospital, Dallas, Tex. Both strains were susceptible to less that 1.25 μg of gentamicin per ml. Bacteria were stored as lyophilized cultures and were routinely grown in Trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.) at 37 C with rotary shaking. The minimal medium described by Norris and Campbell (21) was modified by adjusting the concentrations of $MgSO_4$ and of $CaCl_2$ as indicated in the text. Viable counts were obtained by spreading samples of suitable dilutions of cultures on the surface of Trypticase soy agar (BBL) in petri dishes and counting colonies after incubation for 18 h at 37 C.

Antibiotics and chemicals. Gentamicin sulfate, gentamicins C1, C1a, C2, and sisomicin were provided by G. H. Wagman (Schering Corp., Bloomfield, N.J.); tobramycin was from H. R. Black (Lilly Laboratory for Clinical Research, Indianapolis, Ind.); and kanamycin and amikacin (BB-K8) were from K. E. Price (Bristol Laboratories, Syracuse, N.Y.). A sample of gentamicin sulfate was radioisotopically labeled with tritium by Amersham/Searle (Arlington Heights, Ill.), and the ^{3}H-labeled gentamicin was purified by gel filtration on Sephadex G-10 as described previously (20). The specific activity of the purified ^{3}H-labeled gentamicin was 0.8 Ci/mg (20a). The ^{3}H-labeled gentamicin was diluted with nonradioactive gentamicin to give lower specific activities as required for experiments described in the text. Concentrations of all aminoglycosides are expressed as μg of free base per ml.

Tris(hydroxymethyl)aminomethane (Tris) was from Sigma (St. Louis, Mo.). All other chemicals were reagent grade and were purchased commercially.

Assays for aminoglycoside antibiotics. Three types of methods were used to measure the concentrations of aminoglycoside antibiotics in this study: (i) enzymatic assay with gentamicin adenylyltransferase (13) or kanamycin acetyltransferase (12); (ii) microbiological assay with *Bacillus subtilis* ATCC 6633 as the indicator strain (1); and (iii) measurement of radioactivity in ^{3}H-labeled gentamicin by liquid scintillation spectrometry. Observed counts were corrected by use of internal standards for differences in quenching of tritium in buffer, in serum, and in serum ultrafiltrate.

Serum. Pooled human serum was prepared from clotted whole blood obtained from healthy volunteers who had not recently received antimicrobial agents or other drugs. An ultrafiltrate of pooled human serum was prepared by using a PM-10 membrane filter (Amicon Corp., Lexington, Mass.). Serum and serum ultrafiltrate were sterilized by passage through membrane filters with 0.45-μm average pore diameter (Millipore Corp., Bedford, Mass.) and were stored at 4 C. The concentration of calcium in the pooled serum was 2.33 mM and in the serum ultrafiltrate was 1.57 mM. The concentration of magnesium in the pooled serum was 0.71 mM and in the ultrafiltrate was 0.51 mM.

Measurements of binding of aminoglycoside antibiotics to human serum. Our procedure for equilibrium dialysis was based on the method of Klotz (17) as modified by Kunin (18). In most experiments, 1-ml samples of pooled human serum were placed in sections of no. 8 (3/8-inch diameter) dialysis tubing (Union Carbide Co., Chicago, Ill.) tied at both ends, and the specimens were suspended in 15-ml volumes of buffer (0.05 M Tris-Cl, pH 7.4, containing 0.15 M NaCl plus calcium and magnesium at concentrations as indicated) in test tubes (20 by 150 mm) with screw caps. In some experiments, the Tris buffer was replaced with 15 ml of serum ultrafiltrate previously adjusted to a pH of 7.4 to 7.5 with CO_2 or with 15 ml of 0.05 M potassium phosphate buffer at pH 7.4. Aminoglycoside antibiotics were usually added to the buffer but in some experiments were added to the serum specimens, and dialysis was carried out at 37 C for 48 h with the tubes agitated in a rotating drum (Model TC-7, New Brunswick Scientific Co., New Brunswick, N.J.). Control experiments documented that equilibrium was achieved in this period of time. Volumes of serum and buffer were recorded at the completion of dialysis, and samples of each were stored at 4 C until assays for aminoglycosides were performed. The volumes of the serum specimens after dialysis were usually between 1.3 ml and 1.6 ml. If the concentration of an aminoglycoside in serum is designated A_t (total aminoglycoside concentration) and the concentration in buffer is designated A_f (free aminoglycoside concentration), then the concentration of bound aminoglycoside A_b is equal to $(A_t - A_f)$. The amount of aminoglycoside bound per ml of undiluted serum ($A_b[corr]$) was estimated by multiplying A_b by V_f/V_i, the ratio of the final volume to the initial volume of the serum specimen. The percentage of binding for each aminoglycoside was calculated from the following formula:

$$\% \text{ bound} = \frac{A_b\,(corr)}{A_b(corr) + A_f} \times 100$$

Measurements of the binding of gentamicin to serum were also performed by gel filtration by the method of Hummel and Dreyer (14) by using 1-ml samples of serum and a column (47 cm by 1.8 cm^2) of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala,

Sweden) equilibrated with 0.05 M Tris-Cl buffer, pH 7.4, containing 0.15 M NaCl and 3 μg of gentamicin per ml. Fractions of eluate (1 ml) were collected, and gentamicin concentrations were determined by enzymatic assay (13) or by measurement of radioactivity in ^{3}H-labeled gentamicin.

Measurements of binding of gentamicin to serum were also carried out by ultrafiltration as described by Bennett and Kirby (2) and were performed with the cooperation of W. M. M. Kirby in his laboratory. In these experiments, assays of gentamicin in specimens of serum and of ultrafiltrate were performed by all three methods described above.

Binding of ^{3}H-labeled gentamicin to bacteria. Cultures in 250-ml Erlenmeyer flasks containing 50 ml of minimal medium without added Mg^{2+} or Ca^{2+} were inoculated with 0.5 ml of *E. coli* or *P. aeruginosa* from stationary-phase cultures in Trypticase soy broth and were incubated for 18 h at 37 C with rotary shaking at 240 rpm. Inocula were transferred into fresh samples of minimal medium lacking Ca^{2+} and Mg^{2+}, and the subcultures were incubated until they were growing exponentially (absorbance at 590 nm = 0.1). To 15-ml samples of these cultures in sterile, 50-ml Erlenmeyer flasks, Ca^{2+}, Mg^{2+} or both were added at the desired concentrations, and immediately thereafter ^{3}H-labeled gentamicin (30,000 counts per min per μg) was added at a final concentration of 5 μg/ml. Incubation at 37 C was continued, and 1.0-ml samples were removed immediately and after 2, 5, 60, and 120 min. Bacteria in the samples were collected on sterile membrane filters (0.45-μm average pore diameter, Millipore Corp., Bedford, Mass.), and the filtrates were saved. The bacteria were washed with six successive 1-ml samples of sterile minimal broth containing Ca^{2+} and Mg^{2+} at concentrations corresponding to the cultures sampled. The filters were dried, placed in vials containing 9.5 ml of Aquasol (New England Nuclear, Boston, Mass.) and 0.5 ml of minimal medium, and counted in a Beckman LS-100C scintillation counter. Samples (0.5 ml) of the culture filtrates and of the pooled filtrates from the washing steps were counted in a similar manner. The amounts of ^{3}H-labeled gentamicin associated with the bacteria, with the culture filtrates, and with the wash filtrates were determined, and the percentage of ^{3}H-labeled gentamicin bound to the bacteria in each sample was calculated. Control experiments with uninoculated medium containing ^{3}H-labeled gentamicin showed that nonspecific binding of ^{3}H-labeled gentamicin to the filters was less than 0.5%.

RESULTS

Binding of aminoglycosides to serum proteins. When binding of gentamicin to serum proteins was studied by equilibrium dialysis, the observed binding was found to be dependent on the composition of the dialysis buffer (Table 1). When an ultrafiltrate of human serum was used, only 19% binding was observed. In contrast, 58 to 65% binding was observed in Tris-Cl buffer or sodium phosphate buffer, and this binding was not affected by variations in concentrations of NaCl from 0 to 200 mM (data not shown). Intermediate levels of binding were observed when mixtures of Tris-Cl buffer with serum ultrafiltrate were used. Addition of ethylenediaminetetraacetic acid (EDTA) to serum ultrafiltrate enhanced the binding of gentamicin to serum, but addition of $CaCl_2$ and $MgSO_4$ to Tris buffer inhibited binding. These data indicate that binding of gentamicin to serum proteins does occur under certain conditions in vitro and suggest that the concentration of divalent cations is an important factor affecting such binding.

Binding to serum proteins in the absence of divalent cations is a general property of aminoglycoside antibiotics and was observed with all aminoglycosides tested (Table 2). These data are representative of results obtained with each aminoglycoside tested at several concentrations comparable to the range of blood levels that can be achieved in man during therapy with these drugs. To eliminate the possibility that the observed binding could reflect an artifact of the equilibrium dialysis method, the binding of gentamicin to serum proteins in Tris-Cl buffer without divalent cations was confirmed by gel filtration experiments (Fig. 1A). In addition, experiments with ^{3}H-labeled gentamicin were performed both by equilibrium dialysis (data not shown) and by gel filtration (Fig. 1B) to rule out the possibility that apparent binding might

TABLE 1. *Differences in binding of gentamicin to human serum in equilibrium dialysis experiments with various dialysis buffers*

Buffer[a]	Concn of gentamicin[b] (μg/ml)		Percentage bound[b]
	Free	Bound (corr)	
UF	4.61	1.11	19
TS	4.86	9.01	65
NaP	3.60	4.98	58
UF:TS (2:1)	4.60	1.23	21
UF:TS (1:2)	4.10	1.97	32
UF + 10 mM EDTA	3.86	9.16	71
TS + 1 mM $MgSO_4$ + 2.5 mM $CaCl_2$	5.08	0	0

[a] UF, Ultrafiltrate of human serum adjusted to pH 7.4 with CO_2; TS, 50 mM, pH 7.4, Tris-Cl buffer containing 0.15 M NaCl; NaP, 50 mM, pH 7.4, sodium phosphate buffer containing 0.15 M NaCl.

[b] The concentration of gentamicin added to each buffer before dialysis was approximately 4.7 μg/ml. Assays were performed by the enzymatic method (13). All data are averages from experiments performed in triplicate. See text for experimental design and calculations.

be due to an artifact of the enzymatic assay for gentamicin with specimens differing in protein content. Similar results were obtained by both assay methods, confirming that binding of gentamicin to serum proteins occurs under these conditions.

Experiments to determine the binding of gentamicin to serum proteins as a function of the concentrations of Mg^{2+} and Ca^{2+} during equilibrium dialysis are summarized in Table 3. On a molar basis, Mg^{2+} is much more effective than Ca^{2+} in interfering with the binding of gentamicin to serum. At physiological concentrations of Mg^{2+} (1.0 mM) and Ca^{2+} (2.5 mM) binding of gentamicin to serum proteins is completely inhibited.

TABLE 2. *Binding of aminoglycosides to human serum determined by equilibrium dialysis*[a]

Aminoglycoside	Concn (μg/ml)[b]		Percentage bound
	Free	Bound (corr)	
Gentamicin	1.05	2.72	72
Gentamicin C1	0.88	4.11	82
Gentamicin C1a	0.89	3.44	79
Gentamicin C2	0.86	3.87	82
Tobramycin	0.76	2.31	75
Sisomicin	0.56	3.33	85
Kanamycin	7.20	8.60	54
Amikacin (BB-K8)	6.00	13.28	69

[a] In 0.05 M, pH 7.4, Tris-Cl buffer. Data represent averages from experiments performed in triplicate. Assays were performed by enzymatic methods (12, 13).

[b] The initial concentrations of antibiotics in the dialysis buffers were approximately 1 μg/ml for gentamicin, gentamicins C1, C1a, and C2, tobramycin, and sisomicin, and 7 μg/ml for kanamycin and amikacin.

Measurements of binding of gentamicin to serum proteins by the vacuum ultrafiltration method of Bennett and Kirby (2) support the conclusion that gentamicin does not bind significantly to human serum under normal physiological conditions (Table 4). In these experiments, assays of gentamicin were performed by all three methods described above with concordant results.

Binding of aminoglycosides to bacteria. After addition of ^{3}H-labeled gentamicin to cultures of *P. aeruginosa* in minimal medium, binding of gentamicin to the bacteria occurs rapidly. Maximal binding is observed within minutes, but binding is strongly inhibited by divalent cations (Fig. 2). In cultures of *P. aeruginosa* containing 5 μg of ^{3}H-labeled gentamicin per ml but lacking divalent cations, up to 30% of the gentamicin is bound within 2 min. As the concentration of Mg^{2+} increases from 0 mM to 1.0 mM, binding of ^{3}H-labeled gentamicin to

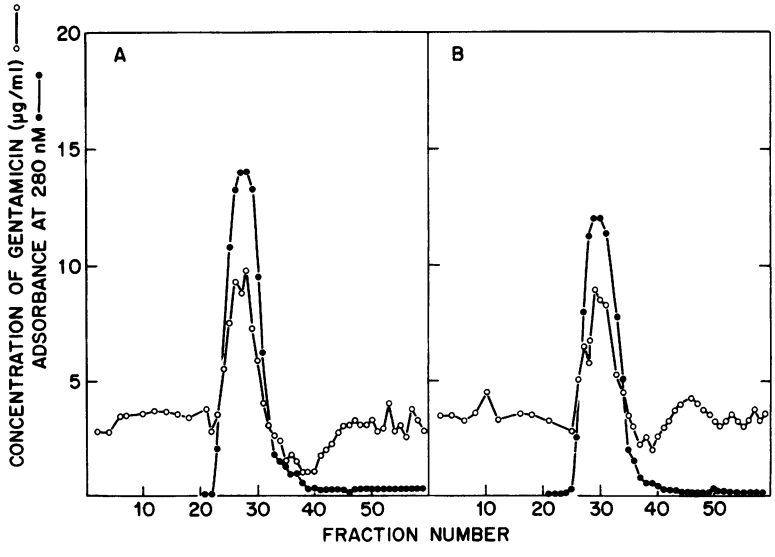

FIG. 1. *Binding of gentamicin to serum proteins demonstrated by gel filtration on Sephadex G-25. Concentrations of gentamicin (○) were determined by enzymatic assay (A) or by measurement of radioactivity in ^{3}H-labeled gentamicin (B). The large peak in the absorbance profile at 280 nm (●) represents serum proteins excluded by Sephadex G-25 that appear at the void volume of the column. Protein binding of gentamicin is responsible for increased concentrations of gentamicin coincident with the protein peak and reduced concentrations of gentamicin in the fractions eluting after the protein peak. The data in (A) and (B) are from separate experiments by using the same column of Sephadex G-25.*

P. aeruginosa decreases about 10-fold. Similar but less striking inhibition of the binding to gentamicin to *P. aeruginosa* is observed in the presence of Ca^{2+}.

Binding of ^{3}H-labeled gentamicin was measured and was compared with killing of bacteria in cultures of *P. aeruginosa* and of *E. coli* growing in minimal medium with and without Mg^{2+} and Ca^{2+} (Table 5). These and other data not presented show that the percentage of ^{3}H-labeled gentamicin that binds to *E. coli* is relatively independent of Mg^{2+} or Ca^{2+} concentration and is significantly less than that bound to *P. aeruginosa* in the absence of divalent cations. Survival of *E. coli* and of *P. aeruginosa* after incubation for 2 h in the presence of 5 μg of gentamicin per ml is similar when no divalent cations are present (2.5%). Addition of Ca^{2+} or Mg^{2+} or both increases survival of *P. aeruginosa* to 54% or greater but does not protect *E. coli* from the bactericidal action of gentamicin. The

TABLE 3. *Effects of Ca^{2+} and Mg^{2+} concentrations on the binding of gentamicin to human serum*

Divalent cations[a] (mM)		Gentamicin[b] (μg/ml)		Percentage bound[b]
Mg^{2+}	Ca^{2+}	Free	Bound (corr)	
1.0	2.5	5.08	0	0
1.0	0	5.21	0.03	6
0.5	0	5.06	0.79	14
0.25	0	4.58	7.44	62
0	2.5	4.69	1.59	25
0	1.25	4.98	3.85	44
0	0.675	4.53	4.30	48
0	0	4.22	8.53	67
0.5	2.5	4.87	2.70	35
0.25	2.5	4.17	11.00	72
1.0	1.25	5.56	1.57	22
1.0	0.675	5.99	0.14	2.3

[a] In 0.05 M, pH 7.4, Tris-Cl buffer containing 0.15 M NaCl.

[b] The concentration of gentamicin added to each buffer before dialysis was approximately 5.1 μg/ml. Assays were performed by the enzymatic method (13). Data represent averages from experiments performed in triplicate. Experiments were performed by equilibrium dialysis as described in text.

TABLE 4. *Measurements of binding of gentamicin to normal human serum determined by the vacuum ultrafiltration method*

Expt no.	Assay method	Gentamicin (μg/ml)[a]		Percentage bound
		Free	Bound	
1	Microbiological	9.39	1.31	12
	Enzymatic	10.5	1.00	9
	^{3}H-labeled gentamicin	8.58	2.08	20
2	Microbiological	9.24	0.56	6
	Enzymatic	10.2	1.60	14
	^{3}H-labeled gentamicin	9.38	2.29	20

[a] Gentamicin was added to the pooled human serum at an initial concentration of 10 μg/ml.

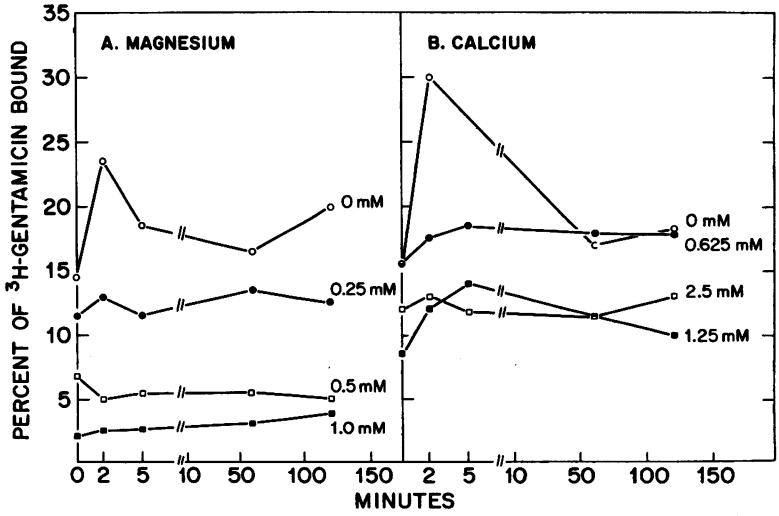

FIG. 2. *Effects of magnesium and calcium on binding of ^{3}H-labeled gentamicin to P. aeruginosa. Experiments were performed as described in text by using ^{3}H-labeled gentamicin at an initial concentration of 5 μg/ml. The percentage of ^{3}H-labeled gentamicin bound to a standardized inoculum of P. aeruginosa was determined at the times indicated in minimal medium supplemented with Mg^{2+} or Ca^{2+} at concentrations indicated in the figure. Maximal binding occurred rapidly, and maximal binding decreased progressively with increasing concentrations of magnesium or calcium.*

TABLE 5. *Effects of calcium and magnesium on binding and on antibacterial activity of gentamicin with P. aeruginosa and with E. coli*

Divalent cation (mM)		*P. aeruginosa*		*E. coli*	
Calcium	Magnesium	Binding[a]	Survival[b]	Binding[a]	Survival[b]
0	0	20.3	2.5	4.4	2.4
0	1.0	4.8	54	6.9	1.2
2.5	0	12.4	54	6.4	0.5
2.5	1.0	2.9	62	NT[c]	NT[c]

[a] Expressed as percent of ^{3}H-labeled gentamicin bound to bacteria at 2 h. Total ^{3}H-labeled gentamicin was 5 μg/ml in the cultures.

[b] Expressed as percentage viability at 2 h. Initial viable counts were 1.28×10^8/ml for *P. aeruginosa* and 1.51×10^8/ml for *E. coli*.

[c] NT, Not tested.

increased resistance of *P. aeruginosa* to gentamicin in the presence of divalent cations is therefore correlated with decreased binding of gentamicin by the bacteria under these conditions.

DISCUSSION

Previous investigations have demonstrated that the susceptibility of *P. aeruginosa* to gentamicin is influenced by the concentrations of magnesium and calcium in bacteriological media (8, 9, 28). Gilbert and associates found that the average minimal inhibitory concentration of gentamicin for *P. aeruginosa* increased from 1.8 μg/ml to 5.6 μg/ml as the magnesium concentration increased from 0.12 mM to 1.9 mM in the presence of 0.17 mM calcium. Zimelis and Jackson studied the bactericidal effects of gentamicin on *P. aeruginosa* and found that the addition of calcium permitted growth of *P. aeruginosa* in the presence of an otherwise lethal concentration of the drug (28). The effects of divalent cations on the actions of gentamicin have not been seen in *E. coli* and *Klebsiella pneumoniae*. Our data confirm previous observations that divalent cations can increase the phenotypic resistance of *P. aeruginosa* but not of *E. coli* to gentamicin. In addition, we have shown that the enhanced resistance of *P. aeruginosa* in the presence of Ca^{2+} or Mg^{2+} is associated with decreased uptake of gentamicin by the bacteria. Although this decreased uptake provides a reasonable explanation for the enhanced resistance of *P. aeruginosa* to gentamicin in the presence of divalent cations, the mechanisms for inhibition of uptake by divalent cations remain to be established.

Our data show that maximal binding of gentamicin to *P. aeruginosa* occurs very rapidly and suggest that the initial binding is most likely to the cell wall. It is therefore possible that antagonism of the activity of aminoglycosides by divalent cations is due to effects of these cations on the bacterial cell wall. The cell wall of *P. aeruginosa* contains calcium, magnesium, and other cations that appear to link polysaccharide subunits and contribute to the structural integrity of the cell wall (5, 6, 7). When *P. aeruginosa* is exposed to EDTA, polysaccharide is released from cell wall and susceptibility of the bacteria to several antibiotics including penicillin and ampicillin increases (27). The influence of EDTA on susceptibility of *P. aeruginosa* to penicillins can be reversed by addition of calcium and magnesium, and this enhanced susceptibility of EDTA-treated cells is seen with *P. aeruginosa* but not with *E. coli* (4, 22). These similarities in the effects of EDTA and of divalent cations on the susceptibility of *P. aeruginosa* to penicillins and to aminoglycosides suggest that disruption of cell wall integrity in the absence of divalent cations may increase the accessibility of the cellular targets to these antibiotics. Although little data have been published concerning the mechanisms for uptake and internalization of aminoglycosides by *P. aeruginosa*, Bryan and his colleagues have recently undertaken a detailed investigation of this problem (L. E. Bryan, M. S. Shahrabadi, and H. M. Van Den Elzen., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 166, 1974).

Although binding of aminoglycosides to serum proteins does not appear to be pharmacologically important in normal man, our data show clearly that significant protein binding of aminoglycosides can occur under appropriate conditions in vitro. With some aminoglycosides such as gentamicin, the pharmacokinetic responses of individual patients to standard dosage regimens are notoriously unpredictable (15, 23, 26). The possibility that significant binding of aminoglycosides to serum or to tissue proteins might occur under pathological conditions in man and might help to determine variations in the pharmacokinetics or in the antibacterial effectiveness of gentamicin in individual patients should therefore be considered and deserves investigation in appropriate clinical situations.

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