The Journal of Medical Microbiology

Vol. 14, No. 2

ACQUIRED CROSS RESISTANCE TO AMINOGLYCOSIDES IN GENTAMICIN-SENSITIVE AND GENTAMICIN- RESISTANT STRAINS OF ENTEROBACTERIA

M. J. S. AL-ASADI*, K. TOWNER AND D. GREENWOOD

*Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham
NG7 2UH*

SUMMARY. The development of non-specific resistance to gentamicin, tobramycin and amikacin was studied in 12 clinical isolates of enterobacteria with various patterns of aminoglycoside resistance. Strains were characterised with respect to MIC, transferability of resistance and possession of acetylating and adenylating enzymes.

An increase in aminoglycoside resistance was induced in 10 strains by a single exposure to the concentration of gentamicin, tobramycin or amikacin immediately below the MIC. Such resistance was non-specific; all three aminoglycosides were affected irrespective of which one had been used to induce the increase. Increments in non-specific aminoglycoside resistance were also evoked by exposure of enterobacteria to changing drug concentrations similar to those achieved in plasma during therapy. When strains already resistant to gentamicin or other aminoglycosides were exposed to therapeutically achievable drug concentrations, no further increase in resistance was observed in most cases. This suggests that use of an aminoglycoside to which the organism is resistant, as during "blind" therapy, will not usually compromise subsequent treatment with related antibiotics. The possible relevance of non-specific aminoglycoside resistance to therapy is discussed.

INTRODUCTION

Resistance to aminoglycosides may arise in bacteria by mutation at the ribosomal level, by enzymic modification of the drug or by exclusion of the drug from the bacterial cell. Strains with the latter type of resistance are obtained easily by laboratory habituation procedures (Weinstein *et al.*, 1971;

Received 10 Jul. 1980; accepted 4 Sep. 1980.

* Present address: Al-Asadi Pharmacy, Second Al-Hooria City, Baghdad, Iraq.

Houang and Greenwood, 1977); they do not transfer their resistance, but characteristically exhibit extensive cross resistance within the aminoglycoside group, although not to other antibiotics (Houang and Greenwood, 1977). The clinical importance of this type of resistance has been in doubt since Weinstein *et al.* (1971) showed that laboratory-derived variants exhibited altered growth characteristics and reduced virulence for laboratory animals. However, an increasing number of reports of resistance emerging during therapy with aminoglycosides (Amirak *et al.*, 1977; Mawer and Greenwood, 1977; Meyer, 1977; Seal and Strangeways, 1977) and of resistance in clinical isolates which do not possess aminoglycoside-inactivating enzymes (Bryan, Haraphongse and Van den Elzen, 1976; Phillips, King and Shannon, 1978; Shannon, Phillips and King, 1978) suggest widespread prevalence of this type of resistance in clinical infections.

Previous studies in this laboratory have shown that small but significant increases in aminoglycoside resistance are readily induced by a single exposure of bacteria to concentrations of gentamicin just below the MIC (Mawer and Greenwood, 1978) or by concentrations that transiently rise above the MIC and subsequently fall, as they do during the treatment of patients (Al-Asadi, Greenwood and O'Grady, 1979). Because such resistance is non-specific, exposure of a gentamicin-resistant strain to gentamicin, as may occur in "blind" therapy, may induce a non-specific increase in resistance to other aminoglycosides to which the strain was originally sensitive. Preliminary experiments *in vitro* suggest that such a danger exists (Mawer and Greenwood, 1978).

In the present study we have extended these in-vitro observations by examining the emergence of resistance to gentamicin, tobramycin and amikacin in clinical isolates of enterobacteria that exhibit a variety of aminoglycoside-resistance patterns.

MATERIALS AND METHODS

Bacterial strains. Ten gentamicin-resistant strains (7 *Escherichia coli*, 2 *Proteus mirabilis*, 1 *Providencia stuarti*) were kindly provided by Dr Fiona Drasar and Dr Elizabeth Houang, Department of Medical Microbiology, The London Hospital. Two gentamicin-sensitive strains (*E. coli* and *P. mirabilis*) were laboratory stock cultures originally isolated from urine.

Culture medium. Tris-buffered "complete" medium, (CMT broth, pH 7.2) described by Mawer and Greenwood (1978) was used for all titrations and turbidimetric experiments.

Antibiotics. Gentamicin sulphate (Nicholas Laboratories Ltd, Bath Road, Slough, Berks), tobramycin sulphate (Eli Lilly & Co, Ltd, Kingsclere Road, Basingstoke, Hants) and amikacin sulphate (Mead Johnson Laboratories, Stenford House, Langley, Berks) were standard pharmaceutical preparations obtained from the hospital pharmacy.

Antibiotic titrations. Serial doubling dilutions of aminoglycosides were prepared in 1-ml volumes of CMT broth and one drop of a 1 in 1000 dilution of an overnight broth culture of the appropriate organism was added to each tube (an inoculum of $1-5 \times 10^4$ bacteria/ml). End-points were estimated visually after overnight incubation at 37°C.

Turbidimetric studies. One drop of an overnight broth culture of bacteria was added to each of a series of tubes containing 20 ml of CMT broth containing graded concentrations of aminoglycoside (inoculum = 5×10^5 – 10^6 bacteria/ml). The tubes were incubated in the multi-channel continuous opacity-monitoring device described by Mackintosh *et al.* (1973). In this way the minimum antibacterial concentration (MAC; the concentration causing a deviation

from the normal growth curve) and the MIC (the concentration suppressing growth overnight) were estimated. Cultures growing during the overnight incubation period at the concentration immediately below the MIC were retested in fresh turbidimetric experiments to determine whether an increase of resistance had occurred. Whenever the concentration just below the MIC exceeded that achievable therapeutically, cultures exposed to 8 µg/ml were also retested for increased resistance. Although achievable serum concentrations of amikacin are greater than this, 8 µg/ml was also used for this antibiotic for comparative purposes.

In-vitro model. To investigate the emergence of resistance to aminoglycosides when the antibiotic concentration was continuously changing, the in-vitro model described by Al-Asadi *et al.* (1979) was used. In this device antibiotic is allowed to diffuse across a cellulose-acetate membrane into a bacterial culture. When the peak antibiotic concentration is achieved, the drug is removed by a flow-assisted back-diffusion process. By variation of the drug concentration and the rate of backflow, various drug-exposure profiles can be achieved. In the present experiments antibiotic exposure was commenced when bacterial growth had raised the opacity to 2% of maximum. A peak concentration of aminoglycoside 10 µg/ml was achieved 90 min after the commencement of drug exposure and the concentration subsequently fell to below detectable levels after a further 6 h. The procedure has been described by Al-Asadi *et al.* (1979). Antibiotic assays were performed at appropriate intervals by the well-diffusion method, with *Klebsiella aerogenes* strain NCTC10896 as test organism, to check that the correct drug concentrations were achieved.

Gentamicin-uptake experiments. The method was based on those described by Bryan and Van den Elzen (1976) and Thorbjarnardóttir *et al.* (1978). Bacteria were grown in 9.9 ml of nutrient broth at 37°C for 100 min in a shaking waterbath; 0.1 ml of (³H) gentamicin sulphate (specific activity 143 µCi/ml, 515 µCi/µmol; The Radiochemical Centre, Amersham, Bucks) was added to each culture to give a final concentration of 2.8 nmol/ml (1.31 µg/ml), and incubation was continued. At appropriate intervals, 0.5-ml samples were filtered through 0.45 µm Millipore filters, which had been pretreated by filtering 1 ml of a solution of gentamicin (100 µg/ml) to prevent variations due to different degrees of filter binding of extracellular unincorporated ³H-gentamicin. Filters were washed with 5 ml of 3% (w/v) NaCl, dried for 1 h at 37°C, placed in counting vials with a toluene-based scintillation fluid containing 5 g of PPO (2,5-diphenyloxazole) and 0.1 g of POPOP {1,4-bis-2-(5-phenyl-oxazolyl)-benzene}/L and counted in an Inter-technique SL 3000 scintillation counter. Cell mass was calculated from a predetermined relationship between absorbance and dry weight in which 1 A₆₀₀ unit represents 0.33 mg (dry weight) of cells (Bryan and Van den Elzen, 1976).

Transfer of gentamicin resistance. Overnight broth cultures of the test strains were mixed in equal proportions with *E. coli* K12 strain J53.2 (F⁻, *pro*, *met*, *rif*^r) and incubated in static conditions for 18 h at 37°C. The mixture was then spread (0.1 ml/plate) on Oxoid DST agar containing 4% lysed horse-blood plus gentamicin sulphate 8 µg/ml and rifampicin 100 µg/ml. Colonies on the selective plates were checked for proline and methionine requirement by streaking on minimal salts agar (Vogel and Bonner, 1956) with and without the appropriate amino acids. Experiments in which gentamicin resistance transfer was not observed were repeated with selection for the transfer of other antibiotic resistances expressed in the donor strain. Selection was made on DST agar containing rifampicin 100 µg/ml plus either ampicillin (Beecham) 50 µg/ml, tetracycline (Lederle) 10 µg/ml or sulphamethoxazole (Roche) 250 µg/ml.

Detection of aminoglycoside-inactivating enzymes. The method was based on those of Shannon *et al.* (1978) and Murray and Moellering (1979), with a cellulose phosphate paper-binding system (Benveniste and Davies, 1973). Suspensions of cells grown at 37°C in 50 ml of YE2 broth (Hughes and Meynell, 1977) were harvested by centrifugation at 4500 *g* for 20 min at 4°C and resuspended in 5 ml of buffer (Shannon *et al.*, 1978) containing 10 mM dithiothreitol. The cells were disrupted in an MSE ultrasonic disintegrator (4 × 15-s bursts at an amplitude of 15 microns with 15 s between bursts); during this procedure the tube was kept in a beaker of iced water. The resulting preparations were tested for their ability to bind radioactivity from 1-¹⁴C-acetyl coenzyme A (specific activity 1 µCi/ml; concentration 250 nmol/ml) or 8-¹⁴C-adenosine 5'-triphosphate (specific activity 0.5 µCi/ml; concentration 500 nmol/ml) to gentamicin, tobramycin or amikacin. Radioactive materials were obtained from the Radiochemical Centre,

Amersham, Bucks. Reaction mixtures consisted of 25 μ l of buffer, 25 μ l of radioactive substrate, 25 μ l of gentamicin, tobramycin or amikacin (0.4 mg/ml) and 25 μ l of cell sonicate. Mixtures were incubated at 37°C for 1 h and then pipetted on to 20 $\times$ 20 mm squares of Whatman P81 cellulose phosphate paper. The papers were washed in distilled water, dried and put in scintillation vials for counting as above. All tests were performed in triplicate on at least two occasions.

RESULTS

Characterisation of strains

The 12 strains were characterised by testing (i) their susceptibilities to gentamicin, tobramycin and amikacin in conventional broth titration, (ii) their ability to transfer any resistance detected in MIC tests and (iii) their ability to acetylate and adenylylate each of the three aminoglycosides.

Minimum inhibitory concentrations. MICs of gentamicin, tobramycin and amikacin estimated by conventional broth titration against a bacterial inoculum of $c. 5 \times 10^4$ organisms/ml are shown in table I. Two strains (*E. coli* strain ECSA1 and *P. mirabilis* strain Co0) were sensitive and two (*E. coli* strains F57 and F69) were resistant to all three aminoglycosides. One strain (*P. mirabilis* strain Fb) was resistant to gentamicin only. The remaining seven strains were resistant to gentamicin and tobramycin, but sensitive to amikacin. A considerable spread of MIC values was observed.

Transfer of resistance. The 10 gentamicin-resistant strains were tested for their ability to transfer resistance to *E. coli* K12 strain J53.2. Results are shown in table II. From four strains (*E. coli* strains F57, F69 and Ga and *Prov. stuarti* strain Ge) transfer was not detected. From two additional strains (*E. coli* strains F140 and F189) transfer was not detected when selection was for gentamicin resistance, but transfer of gentamicin resistance was observed when selection was for another antibiotic-resistance marker. All four strains from

TABLE I
Minimum inhibitory concentrations of gentamicin, tobramycin and amikacin for the 12 test strains

Organism and strain no.	Minimum inhibitory concentration (μ g/ml) of		
	gentamicin	tobramycin	amikacin
<i>E. coli</i> ECSA1	2	4	8
<i>E. coli</i> F57	16	16	64
<i>E. coli</i> F69	32	64	128
<i>E. coli</i> F140	64	64	8
<i>E. coli</i> F171	16	32	4
<i>E. coli</i> F170	128	16	1
<i>E. coli</i> F189	256	16	2
<i>E. coli</i> Ga	256	16	8
<i>Prov. stuarti</i> Ge	64	32	4
<i>P. mirabilis</i> Fb	32	4	4
<i>P. mirabilis</i> Fe	128	64	4
<i>P. mirabilis</i> Co0	4	2	8

TABLE II

Transfer of resistance from the 10 gentamicin-resistant strains to Escherichia coli K12 strain J53.2

Donor strain	Selected resistance markers	Resistances transferred
<i>E. coli</i> F57	Gm	ND
<i>E. coli</i> F69	Gm	ND
	Tc	Tc, Ap
<i>E. coli</i> F140	Gm	ND
	Ap	Gm, Ap, Su
<i>E. coli</i> F171	Gm	Gm, Tc, Ap, Su, Cm
<i>E. coli</i> F179	Gm	Gm, Tc, Ap
<i>E. coli</i> F189	Gm	ND
	Ap	Gm, Tc, Ap
<i>E. coli</i> Ga	Gm, Ap, Su	ND
<i>Prov. stuarti</i> Ge	Gm, Tc, Ap, Su	ND
<i>P. mirabilis</i> Fb	Gm	Gm, Cm, Ap
<i>P. mirabilis</i> Fe	Gm	Gm, Su, Ap

Gm = gentamicin, Tc = tetracycline, Ap = ampicillin, Su = sulphonamide, Cm = chloramphenicol, ND = none detected.

which gentamicin-resistance transfer was detected by gentamicin selection (*E. coli* strains F171, F179; *P. mirabilis* strains Fb and Fe) exhibited transfer frequencies 100-fold higher when selection was made for another resistance marker. However, all associated resistances were always co-transferred, no matter which resistance was selected, showing that only one plasmid was involved.

Aminoglycoside-inactivating enzymes. All the gentamicin-resistant strains were examined for acetylating and adenylylating enzymes. Enzymic modification of gentamicin was detected in six of the 10 gentamicin-resistant strains (table III). Four of these strains, including a tobramycin-sensitive strain, seemed able to modify tobramycin, and two amikacin-sensitive strains seemed

TABLE III

Acetylation and adenylylation of aminoglycosides by the 10 gentamicin-resistant strains

Organism and strain no.	Enzymic inactivation of		
	gentamicin	tobramycin	amikacin
<i>E. coli</i> F57	ND	ND	ND
<i>E. coli</i> F69	ND	ND	ND
<i>E. coli</i> F140	ND	ND	ND
<i>E. coli</i> F171	ND	ND	ND
<i>E. coli</i> F179	Ac	ND	ND
<i>E. coli</i> F189	Ac	ND	ND
<i>E. coli</i> Ga	Ac	Ac	ND
<i>Prov. stuarti</i> Ge	Ac	Ac	ND
<i>P. mirabilis</i> Fb	Ac/Ad	(Ad)	(Ad)
<i>P. mirabilis</i> Fe	Ad	Ad	(Ad)

ND = not detected, Ac = acetylation, Ad = adenylylation, ( ) = weak reaction.

to adenylylate amikacin only weakly. However, weak reactions might be due to non-specific binding of the radioactive label in the presence of high concentrations of aminoglycoside (Shannon *et al.*, 1978).

Turbidimetric studies

Typical continuous opacity records of the fully sensitive *E. coli* strain exposed to gentamicin are shown in fig. 1A. From such data the MAC (0.5 µg/ml in fig. 1A) and the MIC (4 µg/ml in fig. 1A) were estimated for each strain (table IV). The inoculum in turbidimetric experiments was about 50-fold higher than that used in conventional MIC titrations and this is reflected in higher MIC values in the turbidimetric system (cf. table I).

The susceptibility of cultures growing during the overnight incubation period at the concentration immediately below the MIC was tested in fresh turbidimetric experiments; results obtained when the *E. coli* culture growing in gentamicin 2 µg/ml in the experiment depicted in fig. 1A was retested in this way are shown in fig. 1B. The culture displayed a striking increase of gentamicin resistance. Experiments with radioactive gentamicin showed that this was accompanied by a marked decrease in the ability of the bacteria to take up the drug (fig. 2).

Similar experiments were made with the other aminoglycosides. Fig. 3 illustrates the results obtained when cultures of *E. coli* strain ECSA1, previously exposed to gentamicin, tobramycin or amikacin, were retested against gentamicin. Results are expressed as the time taken for cultures to grow to an opacity level of 50% of that of a fully grown culture after exposure to various concentrations of gentamicin. Exposure of *E. coli* strain ECSA1 to any of the

TABLE IV

Minimum inhibitory concentrations and minimum antibacterial concentrations of gentamicin, tobramycin and amikacin for the test strains by turbidimetric estimation

Organism and strain no.	Concentration (µg/ml) of					
	gentamicin at		tobramycin at		amikacin at	
	MAC	MIC	MAC	MIC	MAC	MIC
<i>E. coli</i> ECSA1	0.5	4	1	4	0.5	16
<i>E. coli</i> F57	16	32	16	32	32	128
<i>E. coli</i> F69	16	64	32	64	64	128
<i>E. coli</i> F140	8	128	16	128	1	8
<i>E. coli</i> F171	32	256	8	128	1	16
<i>E. coli</i> F179	64	1024	4	32	2	8
<i>E. coli</i> F189	64	1024	8	64	1	8
<i>E. coli</i> Ga	64	512	2	32	2	16
<i>Prov. stuarti</i> Ge	64	512	16	128	1	32
<i>P. mirabilis</i> Fb	4	128	0.5	8	0.5	16
<i>P. mirabilis</i> Fe	64	1024	16	256	2	16
<i>P. mirabilis</i> Co	2	16	1	16	2	64

MAC = minimum antibacterial concentration, MIC = minimum inhibitory concentration (see *Methods*).

FIG. 1.—Continuous opacity records of *E. coli* strain ECSA1 grown in CMT broth in the presence of indicated concentrations of gentamicin. A: inoculum not previously exposed to gentamicin. B: inoculum derived from culture exposed overnight to gentamicin 2 µg/ml in experiment A.

three drugs evoked an increase in gentamicin resistance. The MAC and the MIC were increased, but the effect on the MAC was more pronounced.

Similar experiments were performed for all 12 strains and all three aminoglycosides. The fold increases in the MAC of gentamicin, tobramycin and amikacin after exposure to a concentration of each drug just below the MIC are shown in table V. When the sub-MIC concentration was above 8 µg/ml, cultures exposed to that concentration were tested for increased resistance and these results are also shown in table V. Increases in aminoglycoside resistance were not readily induced in *E. coli* strains F59 and F69, which were resistant to all three aminoglycosides and in which enzyme activity and resistance transfer were not detected. In the remaining strains substantial increments of resistance were detected in cultures exposed to aminoglycoside concentrations just below their respective MICs, but not in cultures of resistant bacteria exposed to aminoglycoside 8 µg/ml.

In-vitro model for varying antibiotic concentrations

Four strains were examined in the diffusion model in which early logarithmic-phase cultures were exposed to changing concentrations of each of the

FIG. 2.—Uptake of radioactive gentamicin by *E. coli* strain ECSA1. A: overnight broth culture not previously exposed to gentamicin. B: culture previously exposed overnight to gentamicin 2 µg/ml.

three aminoglycosides. Bacteria that survived exposure to the drug were tested in the turbidimetric system, as above, to detect any change in susceptibility. The results are summarised in table VI. When an increase in resistance was observed it was generally less than that seen in the corresponding static experiments.

DISCUSSION

In this study, 12 clinical isolates of enterobacteria were chosen to represent bacteria with various patterns and levels of aminoglycoside resistance; they were selected initially on conventional MIC values. Further studies revealed that neither enzymic activity nor resistance transfer could be detected in two strains of *E. coli* (F57 and F69) that were resistant to all three aminoglycosides. In two other strains, resistance transfer was detected only when selection was made with a marker other than gentamicin, which is presumably an example of phenotypic lag (Bryan, Shahrabadi and Van den Elzen, 1974). Other strains produced aminoglycoside-modifying enzymes, but could not transfer their resistance, or transferred resistance that could not be shown to be enzymic in nature. Facilities for testing for phosphorylating enzymes were not available but aminoglycoside phosphotransferases are unusual among gentamicin-resistant enterobacteria (Shannon *et al.*, 1978). These findings suggest

FIG. 3.—Response to gentamicin of *E. coli* strain ECSA1. Time taken for cultures to grow to an opacity level of 50% of maximum in the presence of various concentrations of gentamicin. Inocula were derived from a previously unexposed culture (Δ—Δ) or from cultures exposed overnight to gentamicin 2 µg/ml (□---□), tobramycin 2 µg/ml (●.....●), or amikacin 8 µg/ml (○-.-.-○).

that the relationship of aminoglycoside-inactivating enzymes to bacterial resistance and its transferability need more precise definition.

Comparison between MIC values obtained by conventional titration and by turbidimetric experiments in which the inoculum was 50-fold greater showed an inoculum effect in all cases. This was most marked with some of the gentamicin-resistant strains.

TABLE V

Alterations in aminoglycoside susceptibility induced in the static turbidimetric system by exposure of micro-organisms to gentamicin, tobramycin or amikacin

Organism and strain no.	Exposure to gentamicin (µg/ml)	Fold increase in MAC of			Exposure to tobramycin (µg/ml)	Fold increase in MAC of			Exposure to amikacin (µg/ml)	Fold increase in MAC of		
		gent	tob	amik		gent	tob	amik		gent	tob	amik
<i>E. coli</i> ECSA1	2	4	4	16	2	4	2	16	8	4	4	16
<i>E. coli</i> F57	8	2	0.5	0	8	2	0.5	0	8	2	0.5	0
	16	0	0.5	0	16	0	0.5	0	64	2	2	2
<i>E. coli</i> F69	8	0.5	0.5	0.5	8	0.5	0.5	0	8	0.5	0.25	0.5
	32	2	0	2	32	0	0	0.5	64	0.5	2	0
<i>E. coli</i> F140	8	2	0	4	8	0	0	4	4	8	4	2
	64	16	8	4	64	4	4	8				
<i>E. coli</i> F171	8	0	2	0	8	0	2	0	8	2	8	16
	128	8	16	8	64	4	16	16				
<i>E. coli</i> F179	8	0	2	0	8	2	2	2	4	0	2	4
	512	4	4	2	16	8	4	2				
<i>E. coli</i> F189	8	0	0.5	0	8	2	2	0	4	8	2	8
	512	8	4	4	32	8	4	4				
<i>E. coli</i> Ga	8	0	0	0	8	0.5	2	0	8	0	0	2
	128	4	4	4	16	0	4	2				
<i>Prov. stuarti</i> Ge	8	0	0	0	8	0	0	0	8	2	2	2
	256	8	4	2	64	8	4	8	16	4	4	16
<i>P. mirabilis</i> Fb	8	4	2	2	4	32	8	16	8	32	8	16
	64	16	8	16								
<i>P. mirabilis</i> Fe	8	0	0	0	8	0	2	0.5	8	2	4	2
	512	2	8	2	128	4	8	2				
<i>P. mirabilis</i> Co	8	4	4	16	8	4	4	16	8	2	4	4
									32	2	4	8

MAC = minimum antibacterial concentration, gent = gentamicin, tob = tobramycin, amik = amikacin.

TABLE VI

Alterations in aminoglycoside susceptibility induced in the diffusion model

Organism and strain no.	Culture exposed to	Fold increase in MAC of		
		gentamicin	tobramycin	amikacin
<i>E. coli</i> ECSA1	gentamicin	4	2	4
	tobramycin	4	2	4
	amikacin	2	2	4
<i>E. coli</i> F189	gentamicin	0	2	2
	tobramycin	0	0	2
	amikacin	0	2	2
<i>P. mirabilis</i> Coo	gentamicin	2	4	4
	tobramycin	0.5	0	0
	amikacin	0.5	0	0
<i>P. mirabilis</i> Fb	gentamicin	4	4	4
	tobramycin	2	4	4
	amikacin	8	4	4

The distinction between MAC and MIC was observed in all strains in the present study irrespective of the type or level of aminoglycoside resistance.

The difference between the two values was in all cases shown to be due to the emergence of variants with increased resistance. The increase was non-specific in that it affected all three aminoglycosides no matter which of them had been used to induce the resistance.

It has always been assumed that such laboratory-induced resistance is due to mutation but if so, back-mutations must occur very readily to account for the slow, systematic reversion to sensitivity that is observed (Mawer and Greenwood, 1978). An alternative theory is that laboratory-induced resistance is a phenotypic variation selected by antibiotic exposure. Whatever the mechanism, increases in resistance could be induced in previously sensitive bacteria by concentrations of drug achievable *in vivo*, and experiments in which cultures were exposed to the changing drug concentrations that occur *in vivo* demonstrated that transient exposure of sensitive cultures to aminoglycosides could also generate a more resistant population.

In aminoglycoside-resistant strains, a further increase in resistance was elicited by exposure to sub-MIC drug concentrations, but when such strains were exposed to therapeutically achievable concentrations an increase in resistance was observed only in strains of borderline susceptibility in which the "therapeutic concentration" used (8 µg/ml) had some inhibitory effect on bacterial growth. Thus it appears that the danger of evoking non-specific increases in aminoglycoside resistance by "blind" therapy exists only if the strain involved exhibits a low level of resistance before treatment.

Increases in non-specific resistance induced by amikacin seemed to be particularly marked. However, substantially higher serum concentrations of this antibiotic can be achieved and organisms resistant to amikacin are likely to be resistant to other aminoglycosides so that use of this drug should not pose any special danger of inducing resistance.

The demonstration that resistance can be induced by transient exposure of bacteria to concentrations of aminoglycosides similar to those achieved therapeutically encourages the view that this type of resistance may have some therapeutic relevance. In the patient, antibacterial drugs normally support host-defence mechanisms, and the presence of a small proportion of bacterial cells with increased drug resistance may pose no threat. Furthermore, during therapy the activity of antibacterial drugs is reinforced by regular dosing. Nevertheless, in patients with depleted cellular or humoral defence mechanisms in whom the antibiotic acts without the full force of host support, small increases in resistance may compromise the success of drugs, such as the aminoglycosides, that have a low therapeutic ratio. It is possible, for example, that adaptive resistance contributes to the relative ineffectiveness of gentamicin in neutropaenic patients (Bodey *et al.*, 1972).

Other clinical situations may sometimes provide conditions conducive to the appearance of this kind of resistance, *i.e.*, large bacterial populations relatively protected from host defences and exposed to transient and erratic concentrations of drug. Such conditions may arise in superficial lesions treated with topical agents or in the infected urine of patients with indwelling catheters. Seal and Strangeways (1977) have remarked on the common occurrence of gentamicin-resistant *Pseudomonas aeruginosa* in leg ulcers; these strains were cross resistant to tobramycin and amikacin and did not possess aminoglycoside-inactivating enzymes. Development of non-specific resistance during treatment of catheterised patients has also been reported (Seal and Strangeways, 1975; Mawer and Greenwood, 1977).

The adaptive type of resistance has attracted less attention than the enzymic variety. The present results suggest that it may have therapeutic implications and an attempt has been made to define the conditions that may favour its emergence *in vitro*. It is hoped that these results will pave the way for more clinically orientated studies aimed at establishing the prevalence and importance of this type of resistance in infections.

REFERENCES

- AL-ASADI, M. J. S., GREENWOOD, D. AND O'GRADY, F. 1979. *In vitro* model simulating the form of exposure of bacteria to antimicrobial drugs encountered in infection. *Antimicrob. Agents Chemother.*, **16**, 77.
- AMIRAK, I. D., WILLIAMS, R. J., NOONE, P. AND WILLS, M. R. 1977. Amikacin resistance developing in patient with *Pseudomonas aeruginosa* bronchopneumonia. *Lancet*, **1**, 537.
- BENVENISTE, R. AND DAVIES, J. 1973. Mechanisms of antibiotic resistance in bacteria. *Ann. Rev. Biochem.*, **42**, 471.
- BODEY, G. P., MIDDLEMAN, E., UNSAWADI, T. AND RODRIGUEZ, V. 1972. Infections in cancer patients: results with gentamicin sulfate therapy. *Cancer*, **29**, 1697.
- BRYAN, L. E., HARAPHONGSE, R. AND VAN DEN ELZEN, H. M. 1976. Gentamicin resistance in clinical isolates of *Pseudomonas aeruginosa* associated with diminished gentamicin accumulation and no detectable enzymatic modification. *J. Antibiot., Tokyo*, **29**, 743.
- BRYAN, L. E., SHAHRABADI, M. S. AND VAN DEN ELZEN, H. M. 1974. Gentamicin resistance in *Pseudomonas aeruginosa*: R-factor mediated resistance. *Antimicrob. Agents Chemother.*, **6**, 191.

- BRYAN, L. E. AND VAN DEN ELZEN, H. M. 1976. Streptomycin accumulation in susceptible and resistant strains of *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, **9**, 928.
- HOUANG, E. T. AND GREENWOOD, D. 1977. Aminoglycoside cross-resistance patterns of gentamicin-resistant bacteria. *J. clin. Path.*, **30**, 738.
- HUGHES, C. AND MEYNELL, G. G. 1977. Rapid screening for plasmid DNA. *Molec. gen. Genet.*, **151**, 175.
- MACKINTOSH, I. P., O'GRADY, F., GREENWOOD, D., WATSON, B. W., CRICHTON, T. C., PIPER, R. AND FERRER, A. 1973. A twelve channel bacterial growth monitoring system. *Biomed. Eng.*, **8**, 514.
- MAWER, S. L. AND GREENWOOD, D. 1977. Aminoglycoside resistance emerging during therapy. *Lancet*, **1**, 749.
- MAWER, S. L. AND GREENWOOD, D. 1978. Specific and non-specific resistance to aminoglycosides in *Escherichia coli*. *J. clin. Path.*, **31**, 12.
- MEYER, R. D. 1977. Patterns and mechanisms of emergence of resistance to amikacin. *J. infect. Dis.*, **136**, 449.
- MURRAY, B. E. AND MOELLERING, R. C. 1979. Aminoglycoside-modifying enzymes among clinical isolates of *Acinetobacter calcoaceticus* subsp. *anitratus* (*Herellea vaginicola*): explanation for high-level aminoglycoside resistance. *Antimicrob. Agents Chemother.*, **15**, 190.
- PHILLIPS, I., KING, B. A. AND SHANNON, K. P. 1978. The mechanisms of resistance to aminoglycosides in the genus *Pseudomonas*. *J. Antimicrob. Chemother.*, **4**, 121.
- SEAL, D. V. AND STRANGEWAYS, J. E. M. 1975. Resistant pseudomonads in a neurosurgical unit. *Lancet*, **1**, 48.
- SEAL, D. V. AND STRANGEWAYS, J. E. M. 1977. Aminoglycoside resistance due to mutation. *Lancet*, **1**, 856.
- SHANNON, K. P., PHILLIPS, I. AND KING, B. A. 1978. Aminoglycoside resistance among *Enterobacteriaceae* and *Acinetobacter* species. *J. Antimicrob. Chemother.*, **4**, 131.
- THORBJARNARDÓTTIR, S. H., MAGNUSDÓTTIR, R. A., EGGERTSSON, G., KAGAN, S. A. AND ANDRESSON, O. S. 1978. Mutations determining generalized resistance to aminoglycoside antibiotics in *Escherichia coli*. *Molec. gen. Genet.*, **161**, 89.
- VOGEL, H. J. AND BONNER, D. M. 1956. A convenient growth medium for *Escherichia coli* and some other microorganisms (medium E). *Microb. Genet. Bull.*, **13**, 43.
- WEINSTEIN, M. J., DRUBE, C. G., MOSS, E. L. AND WAITZ, J. A. 1971. Microbiologic studies related to bacterial resistance to gentamicin. *J. infect. Dis.*, (December supplement) **124**, S11.