ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1979, p. 7-13
0066-4804/79/01-0007/07$02.00/0

Vol. 15, No. 1

Mechanism of Aminoglycoside Antibiotic Resistance in Anaerobic Bacteria: *Clostridium perfringens* and *Bacteroides fragilis*

L. E. BRYAN,[1] S. K. KOWAND,[2] AND H. M. VAN DEN ELZEN[2]

Division of Medical Microbiology, University of Calgary, Calgary, Canada T2N 1N4,[1] and Department of Medical Bacteriology, University of Alberta, Edmonton, Canada T6G 2H7[2]

Received for publication 16 August 1978

Cell-free amino acid incorporation using ribosomes from strains of either *Clostridium perfringens* or *Bacteroides fragilis* was shown to be susceptible to inhibition by streptomycin and gentamicin. Ribosomes bound dihydrostreptomycin as effectively as ribosomes from *Escherichia coli*. No inactivation of streptomycin or gentamicin was detected by cell extracts of either anaerobic bacterial species. *B. fragilis*, grown without added hemin, menadione, and fumarate, and *C. perfringens* did not show any time-dependent accumulation of dihydrostreptomycin or gentamicin at concentrations tested. Decreased resistance to aminoglycosides and time-dependent uptake of dihydrostreptomycin at 500 μg/ml was observed with *B. fragilis* grown with hemin, menadione, and fumarate. With the last additions, cytochrome *b* was detected by cytochrome spectra of *B. fragilis*. These results demonstrate that anaerobic bacteria unable to carry out oxygen- or nitrate-dependent electron transport are resistant to streptomycin and gentamicin because of failure to transport aminoglycosides. The induction of fumarate-dependent electron transport in *B. fragilis* is associated with some aminoglycoside transport that is of poor efficiency relative to bacteria with electron transport to oxygen or nitrate.

The strictly anaerobic bacteria *Clostridium perfringens* and *Bacteroides fragilis* are resistant to aminoglycosides. Streptomycin and gentamicin are not effectively transported into facultatively anaerobic bacteria under anaerobic conditions (2, 3). They are transported, although less effectively, if nitrate is provided in place of oxygen as a terminal electron acceptor in *Escherichia coli* and *Pseudomonas aeruginosa* (H. M. Van Den Elzen and L. E. Bryan, unpublished data). Recently a model has been proposed for the entry of aminoglycosides into bacteria (4) which could account for these observations. The model proposes that initial aminoglycoside entry (energy-dependent phase I [4]) is most effectively driven by energy obtained from electron transport using oxygen (or, alternatively, nitrate) as a terminal electron acceptor. This energy is used to create a receptor state for aminoglycoside binding and transport by a component of the cytoplasmic membrane. This component could be a transport carrier utilized by some other solute (or solutes) or, less likely, specific for aminoglycosides. The possibility of this type of carrier seems unlikely, in our opinion, because of the inability to demonstrate transport competition during energy-dependent phase I with a series of compounds structurally related to aminoglycosides and streptomycin. However, these studies do not exclude the use of a "borrowed" carrier, as the list of the potential carriers is enormous. If several low-affinity borrowed carriers exist, competition may not be demonstrable. Recently it has been proposed that the second energy-dependent phase of transport is due to a polyamine transport system (10). However, this phase is not initiated until the aminoglycoside has become bound to ribosomes. Under conditions where the cellular proton-motive force is impaired (e.g. in the uncoupled *E. coli* mutant, NR-70) or enhanced (in certain *uncA* and *uncB* mutants of *E. coli*), the rate of aminoglycoside entry decreases or increases, respectively (4). Aminoglycoside transport seems to respond to the magnitude of the proton-motive force generated by respiration. In addition, initial aminoglycoside entry under most conditions shows a nonsaturation type of kinetics, although saturation can be obtained under certain circumstances (3). Taking these observations into account, we have further suggested that the transport carrier either is inti-

mately coupled to or is part of the membranous complex involved in maintaining the energized membrane state and in coupling energy from electron transport with the membrane adenosine triphosphatase or other membranous sites of energy utilization. If this model is correct, bacteria with absent or limited electron transport systems and relatively inefficient yields of ATP and other membrane energy from this source should be resistant to aminoglycosides. We examined two strains of anaerobic bacteria, *C. perfringens* and *B. fragilis,* to determine their mechanism of aminoglycoside resistance and to see how that resistance fits into the above model of aminoglycoside transport and susceptibility.

MATERIALS AND METHODS

Bacterial strains. *C. perfringens* UACp-1 and *B. fragilis* subsp. *fragilis* UABf-1 were clinical isolates from the University of Alberta Hospital in Edmonton. They were identified by criteria of the *Anaerobe Laboratory of the Virginia Polytechnic Institute and State University Laboratory Manual* (9). *E. coli* K-12 SA1306 and a single-step, high-level streptomycin-resistant derivative (*E. coli* SA1306 Str^r) have been previously described (3). R-factor R71a was obtained from Y. Chabbert, Paris, and *E. coli* JR 76-9 came from Julian Davies, University of Wisconsin, Madison. Properties of other strains have been reported (3, 14).

Media. The medium for most studies was brain infusion broth (Difco) (BHIB) or agar. The medium when supplemented (BHISB) contained menadione (0.5 μg/ml) and hemin (5 μg/ml). In certain experiments, the partly defined medium described by Harris and Reddy (8) was used either with added hemin (4 μg/ml), menadione (4 μg/ml), and fumarate (2 μg/ml) or without these additions.

Aminoglycoside susceptibility testing. Minimal inhibitory concentrations (MICs) were determined by growing strains in BHIB until the absorbance at 500 nm was 0.2. Cell preparations were diluted 50-fold into fresh BHIB containing serial concentrations of gentamicin or streptomycin. These were incubated for 24 h at 37°C in 80% N_2–10% CO_2–10% H_2. The MIC was taken to be the first tube showing no detectable turbidity when examined visually. Disk susceptibility was determined by preparing 7-mm paper disks containing either 75 μg of streptomycin or 50 μg of gentamicin. Strains were grown overnight on the partly defined medium with 1.5% agar either with hemin, menadione, and fumarate or with these compounds deleted. Cells were suspended into the identical broth to a density at 600 nm of 0.05. An inoculum was spread evenly over the surface of agar plates prepared from partly defined medium either with or without hemin, menadione, and fumarate, and the disks were applied. Plates were incubated at 37°C in 80% N_2–10% H_2–10% CO_2 for 24 h. Zone diameters were measured in millimeters. For growth inhibition studies, partly defined medium was used with an atmosphere of 100% CO_2, and pH was controlled throughout growth periods to pH 7.1 ± 0.1.

Cell-free amino acid incorporation studies. *C. perfringens* and *B. fragilis* were grown anaerobically in BHISB at 37°C, and the 30,000 × *g* supernatant (S-30) fraction was prepared from these as described (12). *E. coli* cells were grown aerobically in Trypticase soy broth (BBL). The S-30 fraction was centrifuged at 160,000 × *g* (4°C) for 2 h. The upper one-half of the supernatant was recovered and used as the S-100 fraction. The remainder of the supernatant was discarded. The pellet was suspended in 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.8)–0.06 M NH_4Cl–0.01 M magnesium acetate–6 mM mercaptoethanol and centrifuged at 160,000 × *g* at 4°C for 2 h. The pellet was again collected and suspended as the ribosomal fraction. Polyuridylic acid-directed amino acid incorporation was performed as previously described using [^{14}C]phenylalanine (1).

Ribosomal binding studies. Binding of [^{3}H]dihydrostreptomycin was performed by the equilibrium dialysis method for "native" ribosomes described by Chang and Flaks (5).

Assays for modification of streptomycin and gentamicin. Assays for enzymatic modification of aminoglycosides using radiolabeled substrates were as described by Haas and Dowding (7). Microbiological assays for inactivation were performed as previously described (1).

Accumulation of dihydrostreptomycin and gentamicin. Cultures were grown in 80% N_2–10% CO_2–10% H_2 at 37°C in BHIB or BHISB until a turbidity reading of 0.2 at 600 nm was achieved (Unicam SP1800 spectrometer, 1-cm path length). A 12-ml sample of culture was transferred to a 100-ml bottle with a screw cap containing a rubber diaphragm. This bottle and the culture were flushed with 80% N_2–10% CO_2–10% H_2 and sealed. Bottles were placed in a 37°C water bath and incubated until a turbidity reading of 0.3. Uptake of [^{3}H]dihydrostreptomycin for *B. fragilis* was also performed using cells grown in partly defined medium either with or without hemin, menadione, and fumarate in a 100% CO_2 atmosphere. The pH was controlled to 7.1 ± 0.1 throughout. Otherwise the procedure was identical. An appropriate quantity of either gentamicin and [^{3}H]gentamicin (Amersham/Searle custom synthesis [2]) or dihydrostreptomycin and [^{3}H]dihydrostreptomycin (Amersham/Searle, 3 Ci/mmol) to produce the desired final antibiotic concentration was introduced in a 1-ml volume of broth. Specific activities of labeled antibiotics used were [^{3}H]gentamicin, 20 dpm/ng and [^{3}H]dihydrostreptomycin, 4 dpm/ng. Samples of 1 ml were removed as rapidly as possible after addition of labeled antibiotic and at various intervals thereafter. The turbidity at 600 nm was determined on each sample. Samples were either filtered through 25-mm 0.45-μm cellulose nitrate filters pretreated with cold gentamicin or streptomycin (2, 3) or rapidly centrifuged at top speed in a Beckman Microfuge B. In the latter case, cells were pelleted within 1 min. Filtered cells were washed with three 10-ml fractions of 3% NaCl. Centrifuged, pelleted cells were washed three times by suspending them in 1.5 ml of 3% NaCl and recentrifuging. Filters were dried and counted by liquid scintillation counting in a toluene-based scintillation fluid. Centrifuged cells were resuspended in 0.1 ml of Protosol (New England Nuclear, Montreal, Canada) and counted.

Quinone determinations. Quinones were extracted and chromatographed as described by Newton et al. (13). Reference quinones used were vitamin K_1, vitamin K_6, ubiquinone Q_6, and ubiquinone Q_{10}. A menaquinone-requiring strain of *Bacteroides melaninogenicum* was used to look for naphthoquinones in *B. fragilis* subsp. *fragilis* UABf-1 and *C. perfringens* UACp-1 as described (6).

Cytochrome determinations. *C. perfringens* UACp-1 and *B. fragilis* UABf-1 were grown on BHI agar with added fumarate (0.2%) or in the partly defined medium of Harris and Reddy (8) with and without added hemin, menadione, and fumarate. Cells were scraped from the plates and suspended to a cell density of at least 3×10^{10} cells per ml. Cells were frozen in liquid nitrogen, and the absorbance was determined for air-oxidized relative to sodium dithionite-reduced preparations by using a Unicam SP1800 spectrophotometer. Absorbance was determined by scanning at 1 nm/s from 400 to 630 nm and recording the results on a Cole-Parmer recorder (Cole-Parmer, Chicago, Ill.).

RESULTS

MICs. MICs of streptomycin for *C. perfringens* and *B. fragilis* in BHIB were 1,280 and 640 μg/ml, respectively. Gentamicin MICs were 320 and 1,280 μg/ml for *C. perfringens* and *B. fragilis*, respectively. Interestingly, *C. perfringens* was more resistant to streptomycin than to gentamicin, whereas the reverse was true of *B. fragilis*. In the partly defined medium with no hemin, menadione, and fumarate, 1,000 μg of streptomycin per ml was required to inhibit growth of *B. fragilis* within 90 min. In the same medium with added hemin, menadione, and fumarate, growth was inhibited within 90 min by 100 μg of streptomycin per ml. The deletion of hemin, menadione, and fumarate or hemin and menadione from the partly defined medium altered the susceptibility of *B. fragilis* to streptomycin and gentamicin as tested by disk diffusion. Zone diameters obtained without hemin, menadione, and fumarate for gentamicin were no zone and for streptomycin, 9 mm. With these additions present, the zones increased to 12 and 18 mm, respectively. These compounds did not affect the susceptibility of *C. perfringens* to streptomycin or gentamicin nor *B. fragilis* susceptibility to penicillin, tetracycline, or chloramphenicol when tested by disk diffusion. Thus, under conditions where electron transport to fumarate was present, the susceptibility to gentamicin and streptomycin increased. The addition of 0.4% sodium nitrate did not alter aminoglycoside susceptibility tested by growth inhibition or disk diffusion methods.

Cell-free amino acid incorporation and ribosomal binding studies. The results shown in Table 1 demonstrate that, with polyuridylic acid as mRNA, amino acid incorporation is inhibited by streptomycin or gentamicin using ribosomes from *C. perfringens, B. fragilis*, or *E. coli*. The incorporation of labeled phenylalanine with ribosomes from *B. fragilis* was considerably less active than with the other two bacteria, although the relative inhibition did not differ significantly. As expected, a control using streptomycin-resistant ribosomes of *E. coli* SA1306 Str^r was resistant to inhibition by streptomycin, showing only about 10% inhibition even at a concentration of 100 μg of streptomycin per ml. Table 2 gives the results of $[^3H]$dihydrostreptomycin ribosomal binding obtained by a dialysis method using ribosomes from the two anaerobic strains and from *E. coli*. Approximately 1 molecule of streptomycin was bound per ribosome by ribosomes of *B. fragilis* or *C. perfringens* and by an *E. coli* streptomycin-susceptible strain. On the other hand, ribosomes of an *E. coli* streptomycin-resistant strain bound about 0.02 molecules of streptomycin per ribosome. These results agree with the amino acid incorporation studies demonstrating similar affinity for streptomycin of *E. coli, C. perfringens*, and *B. fragilis* ribosomes.

Modification of streptomycin and gentamicin. Several approaches were taken to look for modification of the aminoglycoside antibiotics by cell-free extracts obtained from *B. fragilis* or *C. perfringens*. In the first case, enzymatic assays using radiolabeled substrates were used to look for acetyl coenzyme A-dependent acetylation or ATP-dependent adenylylation or phosphorylation of streptomycin and gentamicin. Table 3 shows that control strains were capable of carrying out the various reactions, but there was no evidence of acetylation, adenylylation, or phosphorylation by *C. perfringens* or *B. fragilis*. In the event that the inactivation mechanism was different from such mechanisms as the above, cell-free extracts were incubated in the presence of acetyl coenzyme A, ATP, and gentamicin or streptomycin. The residual bioactivity of antibiotic was determined after incubation periods of 6 h. Again, all control strains carried out significant inactivation of the antibiotics with no evidence of inactivation by the cell-free extracts of *B. fragilis* or *C. perfringens*, thus confirming the results of the radioenzymatic assays (Table 4).

Uptake of dihydrostreptomycin and gentamicin. Uptake of tritiated gentamicin or dihydrostreptomycin was determined using both a filter method and a centrifugation method. No difference in uptake was found qualitatively by either method. Figure 1 illustrates the results of uptakes carried out using 100 μg of gentamicin

TABLE 1. *Effect of gentamicin or streptomycin on [^{14}C]phenylalanine incorporation by cell-free systems directed by polyuridylic acid*

Bacterial strain	Antibiotic[a]	Drug concn (μg/ml)	[^{14}C]phenylalanine incorporated (pmol/250 μl)[b]	Inhibition (%)
E. coli K-12 SA1306	None		2,123	
	Gm	0.1	1,140	46
	Gm	1.0	846	60
	Gm	10.0	457	78
	Sm	1.0	971	54
	Sm	10.0	1,143	46
	Sm	100.0	1,083	49
E. coli K-12 SA1306 Str^r	None		2,609	
	Gm	0.1	988	62
	Gm	1.0	733	72
	Gm	10.0	521	80
	Sm	100.0	2,351	10
C. perfringens UACp-1	None		429	
	Gm	0.1	257	40
	Gm	1.0	122	71
	Gm	10.0	53	88
	Sm	1.0	173	60
	Sm	10.0	80	81
	Sm	100.0	49	88
B. fragilis UABf-1	None		74 (65–83)	
	Gm	0.1	37 (34–40)	50
	Gm	1.0	24 (23–25)	68
	Gm	10.0	24 (23–25)	68
	None		53 (47–59)	
	Sm	1.0	28 (25–31)	48
	Sm	10.0	22 (21–23)	58
	Sm	100.0	12 (10–14)	77

[a] Gm, Gentamicin; Sm, streptomycin.
[b] Incorporation was carried out for 30 min. Each assay result is the average of two determinations, except for *B. fragilis*, where results are from four separate experiments. The range of incorporation in the four experiments is given in parentheses for *B. fragilis* because of the low incorporation.

TABLE 2. *Binding of [^{3}H]dihydrostreptomycin by native ribosomes from strains of C. perfringens, B. fragilis*, and *E. coli*

Strain	Δcpm	[^{3}H]DHS bound (nmol)[a]	Ribosomes (nmol)	Ratio ([^{3}H]DHS/ ribosomes)
E. coli SA1306	34,522	233	232	0.96
E. coli SA1306 Str^r	642	4	192	0.02
B. fragilis UABf-1	45,182	335	351	0.92
C. perfringens UACp-1	66,484	390	430	0.91

[a] [^{3}H]dihydrostreptomycin ([^{3}H]DHS) bound after 30 h of dialysis (see the text).

and 1,000 μg of dihydrostreptomycin per ml in BHISB. As seen in the figure, considerable variation occurred in sampling for individual time periods, particularly for dihydrostreptomycin. However, three repetitions of the uptakes at the above concentrations and at lower concentrations did not demonstrate any time-dependent uptake over the 60-min period shown. The general pattern of uptake consisted of initial primary binding, which occurred with both the filter and centrifugation methods, followed by a period in which no net accumulation per unit cell mass occurred. There is no evidence of any of the time-dependent phases of accumulation of gentamicin or dihydrostreptomycin seen for aerobic bacteria (2, 3). In BHISB *B. fragilis* and *C. perfringens* did not accumulate intracellular gentamicin or dihydrostreptomycin even at high

TABLE 3. *Assays for acetylation, adenylylation, or phosphorylation of gentamicin and streptomycin by cell-free extracts of various bacteria using radiolabeled acetyl coenzyme A or ATP*

Strain source of S-30 fraction	Antibiotic[a]	Assay (cpm/mg of protein/15 min)		
		Adenylylation	Phosphorylation	Acetylation
C. perfringens UACp-1	Sm	<100	<100	
	Gm	<100	<100	<100
B. fragilis UABf-1	Sm	<100	<100	
	Gm	<100	<100	<100
E. coli JR76-9	Gm	18,900		
E. coli (R71a)	Sm	234,536		
P. aeruginosa (R931)	Sm		17,915	
P. aeruginosa (R130)	Gm			11,270

[a] Sm, Streptomycin; Gm, gentamicin.

TABLE 4. *Assay of residual bioactivity of streptomycin or gentamicin*

Source of Extract	Antibiotic[a]	Modification	% Bioactivity retained after 6 h of incubation[b]
P. aeruginosa 931 (R931)	Sm	Phosphorylation	13
E. coli SA1306 (R71a)	Sm	Adenylylation	50
E. coli SA1306 (R76-2)	Gm	Adenylylation	24
P. aeruginosa 280 (R130)	Gm	Acetylation	6
C. perfringens UACp-1	Sm	Adenylylation-phosphorylation	100
C. perfringens UACp-1	Sm	Acetylation	100
C. perfringens UACp-1	Gm	Adenylylation-phosphorylation	100
C. perfringens UACp-1	Gm	Acetylation	100
B. fragilis UABf-1	Sm	Adenylylation-phosphorylation	100
B. fragilis UABf-1	Sm	Acetylation	100
B. fragilis UABf-1	Gm	Adenylylation-phosphorylation	100
B. fragilis UABf-1	Gm	Acetylation	100

[a] Sm, Streptomycin; Gm, gentamicin.

[b] Cell extracts were prepared and incubated with antibiotic preparations as described in the text. For each extract a control heated to 80°C for 30 min to destroy enzymatic activity before incubation with the antibiotic was used to evaluate nonenzymatic antibiotic inactivation. Residual bioactivity of streptomycin or gentamicin was determined using *P. aeruginosa* 280 (MIC: gentamicin = 0.2 μg/ml; streptomycin = 2.0 μg/ml). Bioactivity retained is the percentage of activity retained relative to the control preparation.

concentrations of the two drugs. However, as noted above, the growth of *B. fragilis* in partly defined medium was associated with increased susceptibility to streptomycin and gentamicin. This medium also allowed an assessment of the effect of electron transport producing fumarate reduction on dihydrostreptomycin uptake. Time-dependent uptake was present in *B. fragilis* UABf-1 at 500 μg of dihydrostreptomycin per ml when bacteria were grown and the uptake was determined in partly defined medium with added hemin, menadione, and fumarate. In the same medium with no added hemin, menadione, and fumarate, however, no uptake occurred. Thus the presence of the electron transport system causing fumarate reduction produces uptake of dihydrostreptomycin. This finding correlates with the increased susceptibility to streptomycin under the same conditions. It should be noted that the concentrations used for uptake are higher than MICs. This is generally necessary, since uptake is measured over only 1-h periods, whereas under conditions used for MIC determination uptake may occur more slowly for many hours.

Quinone determinations. Respiratory quinones were not demonstrated in *C. perfringens* UACp-1 by our assay system. This finding is in keeping with literature reports that quinones are absent in *C. perfringens*. Napthoquinones were

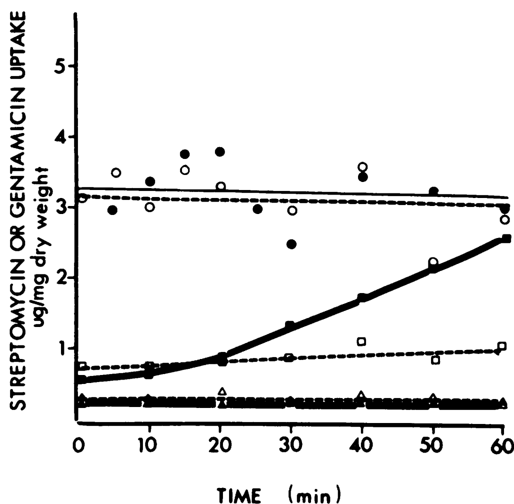

FIG. 1. *Accumulation of [^{3}H]gentamicin or [^{3}H]-dihydrostreptomycin with time by whole cells of B. fragilis and C. perfringens. Symbols: C. perfringens: dihydrostreptomycin, 1,000 μg/ml, BHISB (●——●); gentamicin, 100 μg/ml, BHISB (▲——▲). B. fragilis: dihydrostreptomycin, 1,000 μg/ml, BHISB (○- - - -○); gentamicin, 100 μg/ml, BHISB (△- - - -△); dihydrostreptomycin, 500 μg/ml, partly defined medium with hemin, fumarate, and menadione (■——■); dihydrostreptomycin, 500 μg/ml, partly defined medium with no additions (□- - - -□).*

detected in *B. fragilis* using a napthoquinone-dependent strain of *B. melaninogenicum.*

Cytochrome determinations. No peaks corresponding to cytochrome absorption maxima were present in *C. perfringens* whole cells grown in BHIB or BHISB with 0.2% fumarate. *B. fragilis* cells grown in both BHIB and BHISB had an absorption maximum of 558 nm with another peak at 525 nm. These absorption bands are consistent with the presence of cytochrome *b* as described (8, 11). In addition another smaller absorption maximum was noted at 590 nm. A scheme for electron transport terminating in reduction of fumarate has been proposed for *B. fragilis* (8). When *B. fragilis* cells were grown in partly defined medium with hemin, menadione, and fumarate, similar absorption maxima were obtained. These absorption bands were not detectable when cells were grown in the same medium without hemin, menadione, and fumarate. These findings agree with those of Macy et al. (11).

DISCUSSION

The results presented demonstrate that the strictly anaerobic bacteria *C. perfringens* and *B. fragilis* have ribosomes that are susceptible to the action of streptomycin and gentamicin. There is no evidence that the ribosomal affinity, especially for streptomycin, is reduced below that of *E. coli* ribosomes. The use of cell-free amino acid-incorporating systems demonstrates that such ribosomes function normally in amino acid incorporation. These results also show that there is no evidence of antibiotic modification present in these strains, as measured by radioactive enzymatic and bioactivity assays.

There is no evidence for cytochromes or quinones in *C. perfringens,* either in these studies or in literature reports. These findings show that such bacteria do not carry out cytochrome and quinone-dependent electron transport. Such bacteria should be resistant to aminoglycosides because of their failure to transport aminoglycosides according to the model proposed by our research group. As shown here, there is no time-dependent accumulation of either gentamicin or dihydrostreptomycin at the concentrations used. In view of a susceptible target for the aminoglycosides, no detectable inactivation of these drugs, and absent time-dependent accumulation, resistance must be ascribed to defective entry of streptomycin and gentamicin. The evidence presented is compatible with defective entry being due to the absence of quinone and cytochrome-dependent electron transport and the absence of coupling of energy thus generated to, for example, ATP synthesis.

With *B. fragilis* the situation is more complex, as these bacteria may have both cytochromes and quinones. However, as with *C. perfringens,* there is evidence for a susceptible target and a lack of aminoglycoside inactivation. Unlike *C. perfringens,* some degree of aminoglycoside entry could be demonstrated in the presence of electron transport to fumarate. There is evidence reported which indicates that ATP synthesis may be coupled with electron transport using fumarate as a terminal electron acceptor in *B. fragilis* (11). Such electron transport may involve molecular hydrogen as an electron donor and ferrodoxin or a similar low-redox electron acceptor as the primary electron acceptor. We were able to produce consistent significant increases in susceptibility to gentamicin and streptomycin for *B. fragilis* if hemin, menadione, and fumarate were added to a partly defined medium. Under these conditions cytochromes are synthesized, as are significant amounts of fumarate reductase (11). It is probable that this redox segment participates in proton translocation, which in turn is coupled to ATP synthesis and perhaps other functions. This process appears to be available to drive aminoglycoside entry, albeit poorly, as judged by the enhanced susceptibility and the uptake of streptomycin in partly defined medium with hemin, menadione,

and fumarate but no uptake without these additions. It is not possible to state from our studies what the efficiency of such low-redox-generated energy is relative to that generated using aerobic or nitrate-dependent electron transport. Certainly susceptibility to gentamicin or streptomycin for *B. fragilis* even with supplements is not as great as that found with aerobic bacteria or with bacteria like *Bacteroides ureolyticus*, which carry out nitrate-dependent electron transport (F. L. Jackson, personal communication). However, as pointed out by Macy et al. (11), the efficiency of ATP formation per mole of glucose with *B. fragilis* is small relative to an aerobically grown *E. coli* strain. We conclude that *B. fragilis* is relatively resistant to aminoglycoside because of poor aminoglycoside transport. The extent of aminoglycoside entry is related to the presence and efficiency of electron transport. Under hemin-deficient growth conditions, cytochromes and perhaps other electron transport chain components are absent. Even if cytochromes are found, it is necessary that a satisfactory electron acceptor be present (e.g. fumarate) before significant anaerobic electron transport occurs. When cytochromes and other electron-transport chain components are present, it is likely that the efficiency of coupling energy from these to ATP synthesis is low relative to that for aerobic electron transport. It is probable that the membrane component needed to transport aminoglycosides is not effectively energized by the low-redox proton-translocating segment operative in *B. fragilis* with fumarate reduction.

The findings in these studies do not clarify the nature of the initial aminoglycoside transport site or carrier. However, they confirm the significance of energy generated by an electron transport chain in driving aminoglycoside cell entry.

ACKNOWLEDGMENTS

We are grateful to Bunnie Cielin and Richard Sherburne for their assistance in preparing this manuscript.

This work was supported by Medical Research Council of Canada grant MT4350.

LITERATURE CITED

1. **Bryan, L. E., R. Haraphongse, and H. M. Van Den Elzen.** 1976. Gentamicin resistance in clinical isolates of *Pseudomonas aeruginosa* associated with diminished gentamicin accumulation and no detectable enzymatic modification. J. Antibiot. (Tokyo) **29**:743-753.
2. **Bryan, L. E., and H. M. Van Den Elzen.** 1975. Gentamicin accumulation by sensitive strains of *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiot. (Tokyo) **28**:696-703.
3. **Bryan, L. E., and H. M. Van Den Elzen.** 1976. Streptomycin accumulation in susceptible and resistant strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **9**:928-938.
4. **Bryan, L. E., and H. M. Van Den Elzen.** 1977. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother. **12**:163-177.
5. **Chang, F. N., and J. G. Flaks.** 1972. Binding of dihydrostreptomycin to *Escherichia coli* ribosomes: characteristics and equilibrium of the reaction. Antimicrob. Agents Chemother. **2**:294-307.
6. **Gibbons, R. J., and L. P. Engle.** 1964. Vitamin K compounds in bacteria that are obligate anaerobes. Science **146**:1307-1309.
7. **Haas, M. J., and J. E. Dowding.** 1975. Aminoglycoside-modifying enzymes. Methods Enzymol. **43**:611-628.
8. **Harris, M. A., and C. A. Reddy.** 1977. Hydrogenase activity and the H_2-fumarate electron transport system in *Bacteroides fragilis*. J. Bacteriol. **131**:922-928.
9. **Holdeman, L. V., and W. E. C. Moore.** 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg.
10. **Höltje, J. V.** 1978. Streptomycin uptake via an inducible polyamine transport system in *Escherichia coli*. Eur. J. Biochem. **86**:345-351.
11. **Macy, J., I. Probst, and G. Gottschalk.** 1975. Evidence for cytochrome involvement in fumarate reduction and adenosine 5'-triphosphate synthesis by *Bacteroides fragilis* grown in the presence of hemin. J. Bacteriol. **123**:436-442.
12. **Modolell, J.** 1971. The S-30 system from *Escherichia coli*, p. 1-65. *In* J. A. Last and A. I. Laskin (ed.), Protein biosynthesis in bacterial systems. Marcel Dekker, New York.
13. **Newton, N. A., G. B. Cox, and F. Gibson.** 1972. Function of ubiquinone in *Escherichia coli*. J. Bacteriol. **109**:69-73.
14. **Shahrabadi, M. S., L. E. Bryan, and H. M. Van Den Elzen.** 1975. Further properties of P-2 R-factors of *Pseudomonas aeruginosa* and their relationship to other plasmid groups. Can. J. Microbiol. **21**:592-605.