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Failure of Aminoglycoside Antibiotics to Kill Anaerobic, Low-pH, and Resistant Cultures

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INTRODUCTION

Clinical microbiologists are continually aware that although many antiribosomal antibiotics act on ribosomes, most of them are bacteriostatic. The aminoglycosides are almost unique in their bactericidal activity on ribosomes. Here I want to recall some features of the mechanism of antibiotic action on the ribosomes and then turn, with the assurance of someone relatively outside the field, to some data and speculations about why these potent antibiotics are cidal and yet fail to function in certain instances.

(This discussion was first presented as the Sonnenwirth Lecture at the 1987 Annual Meeting of the American Society of Microbiology.)

SITE OF ACTION OF STREPTOMYCIN

A priori, one knows that an antibiotic must reach its target intact and act irreversibly at its target site for it to have lethal effects. Some of the effects of streptomycin on ribosome function rapidly became clear and have often been reviewed (for example, in the standard treatment of antibiotic action by Gale et al. [17]). Briefly, the target was seen early to be the ribosome (35). In particular, mutants either resistant to or dependent on streptomycin were easily derived from *Escherichia coli*, for example, and some were shown to be altered in a specific ribosomal protein (33).

Our own experimental work analyzed somewhat further the effects of aminoglycosides on the physiology of ribosome function. The initial idea was a simple one, suggested from analyses of the distribution of ribosomes in extracts of easily lysed *E. coli*. From the large numbers of ribosomal subunits and polysomes compared with the small number of 70S ribosomes, we inferred that ribosomes periodically cycle through polysomes (29), 30S and 50S subunits periodically forming couples, traversing a messenger ribonucleic acid molecule forming a protein chain, and then dissociating again to rejoin the pool of subunits transiently.

This cycle has been demonstrated (21) and has been much elaborated on. The formulation suggested a simple way to subclassify the action of antiribosomal antibiotics by asking for their effects on the distribution of ribosomes in vivo. It was anticipated that antibiotics which blocked protein synthesis at initiation, for example, would permit ribosomes in polysomes to complete their transit of messenger ribonucleic acid; but with the restart of protein synthesis blocked, the ribosomes would accumulate as free subunits and 70S ribosomes. In a similar way, antibiotics which blocked protein synthesis in the elongation phase would be expected to freeze polysomes as such.

Results with chloramphenicol (20), erythromycin, lincomycin, and a number of other antibiotics were completely consistent with expectation. Ribosomes were indeed frozen for some time in polysomes by a block in the elongation phase. In contrast, streptomycin and other aminoglycosides affected ribosomes in a much greater number of ways (slowing down elongation, provoking miscoding, etc.), but had a primary action at initiation (27, 28). As predicted, polysomes and subunits were depleted progressively, with the concomitant accumulation of an odd species of 70S ribosomes that were essentially "dead" (Fig. 1). A rather similar inference was made by Wallace and Davis (36).

The accumulation of 70S ribosomes was clearly consistent with the primary action of streptomycin at the initiation of protein synthesis and with the resistance of certain mutants modified in their ribosomes. The target of streptomycin action was therefore reasonably well understood.

However, if the action of antibiotics was "understood," one might expect to account for their action in detail. In particular, it should be possible to understand why individual antibiotics are bactericidal or bacteriostatic. The model we had set up failed this requirement: the action of chloramphenicol and many other antibiotics that block protein synthesis is reversible (and thus, bacteriostatic), but the action of streptomycin and many other aminoglycosides is irreversible (and thus, bactericidal). Why should this be? We were left with a puzzle.

The reason why streptomycin acts so irreversibly in susceptible cells proves unexpectedly to be intimately related to the reason why it fails to act in some cases. The connection is as follows.

CASES WHEN STREPTOMYCIN ACTION FAILS

We know of a number of puzzling instances when streptomycin does not work. Ignoring the clinically important but mechanistically obvious case of inactivation of aminoglycosides by modifying enzymes, the cases of failure (4) include:

(i) cells with resistant ribosomes; (ii) cells with susceptible ribosomes, but treated with chloramphenicol (before or along with streptomycin); (iii) cells grown at low pH; and (iv) cells cultured anaerobically. This is a strange assortment of conditions. The first two seem easily related to ribosome function; in cells with resistant ribosomes, the target resists the drug, and in cells treated with chloramphenicol, one can imagine that bound chloramphenicol (allosterically?) prevents effective subsequent binding of aminoglycosides to ribosomes. But what about the other two conditions? Is there a connection?

At one time it seemed possible that the failure was related to changes in the protein-synthetic machinery that occur, for example, in cells cultured anaerobically (25). However, cultures of *E. coli* incubated in 95% nitrogen–5% carbon dioxide instantaneously become resistant to aminoglycosides, yet protein synthesis is highly susceptible to streptomycin in extracts of cells grown anaerobically for many generations (and even tested under anaerobic conditions). Rather than having their protein-synthetic machinery modified, the anaerobic cultures proved to have an unexpected feature: the uptake of streptomycin into cells is defective (3–5).

Is this the reason for failures in the other instances? It seems possible that some uptake mechanisms might be very different at low pH, but why should the blockage of protein synthesis by chloramphenicol, much less a mutation to resistance in ribosomes, affect the transport of a drug into cells? Nevertheless, in those cases as well, it turns out that the transport of streptomycin fails. Thus, if we want to understand completely how streptomycin kills, and how it fails to act in a number of cases, we must understand how it enters cells.

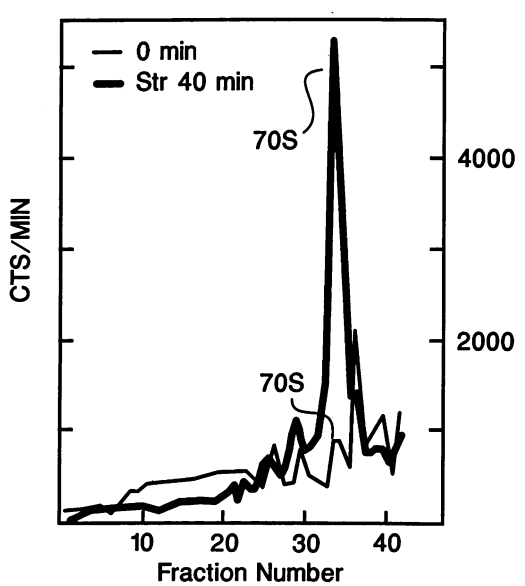

FIG. 1. Depletion of polyribosomes and accumulation of 70S particles in streptomycin (Str)-treated *E. coli*. Ribosomes were prelabeled with [^{3}H]uridine. Streptomycin was added at time zero, and, both then and 40 min later, ribosomes and polysomes were extracted from portions of the cells and fractionated in sucrose gradients. Data from Luzzato et al. (27) are used with permission of Academic Press.

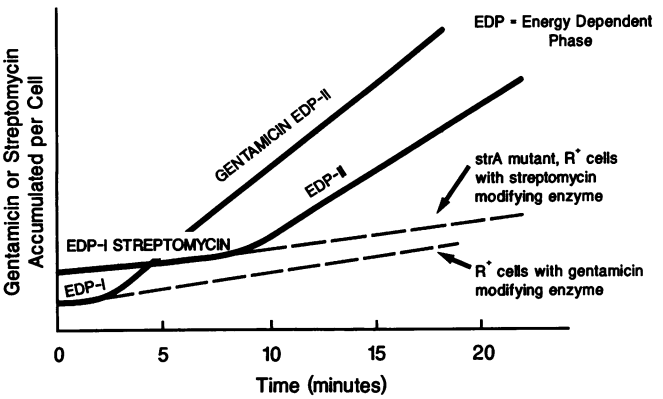

FIG. 2. Kinetics of uptake of gentamicin and streptomycin. R^+ cells contain a plasmid that encodes an inactivating enzyme. Data from Bryan (3) are used with permission of Cambridge University Press.

ODDNESS OF STREPTOMYCIN UPTAKE AS STANDARD TRANSPORT

The classic studies of streptomycin uptake are those of Bryan (3). He has shown that entry of streptomycin into cells exhibits three phases (Fig. 2). The first is essentially instantaneous and is correlated with ionic binding of the drug to negatively charged membrane sites. The second and third are both energy dependent (EDP-I and EDP-II), the slow second phase succeeded by a much more rapid third phase. It is this third phase which selectively fails in resistant cells. Thus, one must analyze an uptake mechanism with odd kinetics.

Streptomycin uptake also shows other unusual features. (i) Especially notable is that uptake is irreversible (13, 32): internalized drug is not released from cells when the streptomycin in the external medium is removed, nor does internalized drug exchange with additional streptomycin added to the medium. (ii) The drug is nevertheless apparently not held in cells by covalent attachment to a cellular component, for it is released when the permeability barrier is breached by organic solvents such as toluene (32). (iii) Although streptomycin is selectively taken up into susceptible cells, a specific carrier has not been found (see reference 4). (iv) Unlike other energy-dependent accumulation of solutes, membrane vesicles are incapable of taking up streptomycin (6, 7, 32); only vesicles containing cytoplasm have been shown to transport drug (7).

These characteristics of aminoglycoside uptake are clearly strange and exclude all standard mechanisms of bacterial transport. In particular, consider three hypotheses regarding why uptake of the drug is irreversible.

Hypothesis 1. Streptomycin uptake might be coupled to transport dependent on a binding protein. Such a mechanism would be consistent with the energy dependence of uptake, but in all other cases to date, the solute that is transported, unlike streptomycin, equilibrates with the external medium (and has usually been shown to enter membrane vesicles in the presence of a metabolizable energy source). Usual forms of binding protein-dependent transport are thus excluded.

Hypothesis 2. Streptomycin in the cell might not be free as it is in solution. If it were bound to some kind of "sink," that would drive the uptake in a progressive way. A likely candidate for such a sink is the ribosomes, as suggested by Kogut et al. (24) and Bryan (3, 4, 7), since streptomycin binds to 30S ribosomes at one strong affinity site (10) and

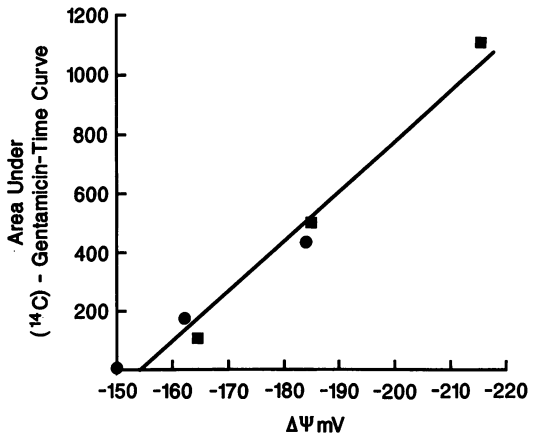

FIG. 3. Relation between ΔΨ (stimulated to varying extents by addition of dicyclohexylcarbodiimide) and the uptake of gentamicin. Gentamicin uptake was estimated by integrating the transport levels over time. Reproduced from reference 14 with permission. See reference 14 for further experimental details.

many weaker ones. This binding, however, is not irreversible, since streptomycin is freely released after toluene treatment, when ribosomes remain in the cells (and, in control cells untreated with streptomycin, can still form protein). Also, as long as binding is ionic in nature, internal streptomycin would still be expected to exchange freely with streptomycin added to the medium. In a relevant case, Mg^{2+} ions bind and are concentrated by the negatively charged phosphates of nucleic acids and nucleotides, with an affinity comparable to that of streptomycin. As expected for normal transport, part of the internal Mg^{2+} pool is free at any time, and internal Mg^{2+} is freely exchangeable with Mg^{2+} added to media. In contrast, streptomycin, contrary to expectation, is neither released nor exchanged from cells. An ionic "ribosome sink" cannot simply resolve the apparent conflict with thermodynamics.

Hypothesis 3. Streptomycin might be covalently modified (e.g., phosphorylated) as it enters the cell. In analogy to other uptake systems, this mechanism would concentrate the drug irreversibly. However, the drug can be recovered from killed cells in completely active and unmodified form. Thus, covalent modification is an unlikely explanation of irreversible uptake.

Since uptake does not proceed by any of the usual mechanisms, one is led to infer that streptomycin is taken into bacteria by a different transport mechanism.

How might streptomycin be taken up? It seems unlikely that bacteria have evolved a specialized mechanism to concentrate cidal antibiotics irreversibly. Rather, one is led to look for some mechanism of uptake of normal molecules, perhaps incompletely understood, that is somehow being scavenged by streptomycin.

One clue to the way aminoglycoside transport might work comes from studies of the relation of streptomycin uptake to energy production in cells. A role for energy was first analyzed by Bryan and Van den Elzen (9). Then it was realized that uptake specifically required the electrical potential gradient (or "proton motive force" [PMF]) across the cell membrane (8). The requirement for membrane potential in antibacterial activity (11) was further specified when it was found that uptake requires a threshhold value of PMF (7, 30). Above that value, uptake of streptomycin is proportional to the increase in potential (7); Figure 3 shows an analysis of the similar dose response observed for uptake of gentamicin (14).

In the jargon of the transport field, these results mean that the uptake of streptomycin is electrically "gated." From the kinetics of Fig. 2, the formation of the "gate" may somehow require time.

The microbiological literature indeed contains other instances in which bacterial transport is gated in a similar way. They include polyamine and arginine transport, which are much less studied but show kinetic phases similar to those shown in Fig. 2 (6, 7). (Those studies [6, 7] also show that puromycin treatment, which promotes uptake of streptomycin into resistant cells, as discussed below, has the same effect in promoting the uptake of polyamines and arginine!) Also included is deoxyribonucleic acid (DNA) uptake, which shows a similar requirement for PMF both during transformation and during the penetration of T4 DNA from infecting bacteriophage (19, 26).

The very uptake of DNA is itself difficult to understand as a form of transport in bacteria. Standard textbooks of microbiology usually point out that bacteria normally take up only small molecules; "endocytosis" of large molecules is a property of eucaryotic cells. But the same books also point out that many species of bacteria can be transformed by DNA, scarcely a small molecule.

Polyamines and arginine share with streptomycin a high positive charge (in fact, streptomycin is itself a polyamine), and DNA also has a strong charge, though negative. This suggests one possibility for a nonstandard type of transport into bacteria:

Hypothesis 4. Streptomycin uptake occurs by an unusual system designed for the irreversible uptake of certain highly charged molecules and characterized by a threshhold for PMF, an accelerated energy-dependent phase, and a lack of a demonstrated specific carrier.

How far can such an hypothesis take us in extending the formulation of streptomycin action on bacteria?

RIBOSOMES AND "PORES"

Pores involved in the uptake of streptomycin must be very peculiar. If they were ordinary "holes," drug should come out of cells as easily as it goes in. Indeed, this is precisely what happens when holes are actually made in the membrane by toluene. Instead, since uptake of streptomycin is irreversible, any pores involved in its transport are one-way.

The notion that some kind of pores and changes in membrane permeability are associated with the lethal action of aminoglycosides is not new: in fact, it was the original suggestion for the mode of action of the drugs (1). Furthermore, Bryan has shown that streptomycin uptake is diminished by mutations that render ribosomes insensitive to the drug (3, 4). Thus, a connection among ribosomes, uptake, and changes in permeability has seemed indubitable. What has been elusive is the mechanism.

Bryan has incorporated most of his findings in an especially comprehensive treatment of this problem (5, 7; it includes many of the questions discussed here). He treats the possibility that streptomycin may after all be taken up by a normal transport mechanism: an energy-driven process ("uniport") mediated by a carrier. In his formulation, the possibility of both a quinone-related carrier and a multiplicity of carrier proteins is considered for a first stage in uptake. Ribosomes would be involved in a second and equally critical stage of the process, with the interesting suggestion

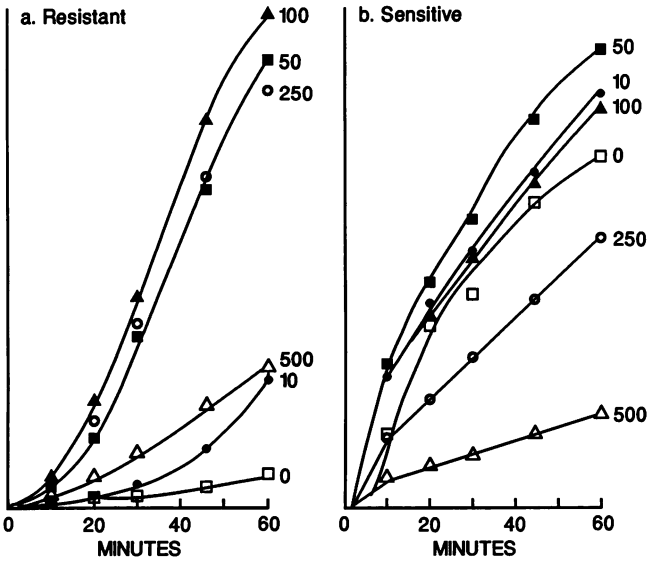

FIG. 4. Uptake of dihydrostreptomycin by susceptible and resistant *E. coli* B cells as a function of concentration of puromycin. Uptake was measured as the net amount of radioactive drug bound to cells collected and washed on a nitrocellulose membrane, after subtraction of zero-time control values. (a) Uptake by a resistant strain; (b) uptake by a susceptible strain. Cells were exposed to [^{3}H]dihydrostreptomycin, and the concentrations of puromycin (in micrograms per milliliter) are indicated on the graphs. Data from reference 22 are used with permission of Elsevier Science Publishers.

that the ribosomes would remove drug molecules from the membrane-bound carrier system and bring them into the cytoplasm. Progressively, though it is not clear how, holes would form that permit extensive leakage of small and even large components, helping to account for lethality.

This model takes into account the requirements for oxidative metabolism, phased uptake, and ribosome involvement. Normal transport followed by "transfer" of antibiotic to ribosomes can also account for the net concentration of aminoglycoside inside the cell, because the ionic interaction of drug and ribosome is strong. However, as discussed earlier, this mechanism is inadequate to explain why uptake is irreversible or why internalized drug cannot exchange with drug added to the medium.

Mechanisms that suggest direct interaction with a susceptible ribosome as an obligate intermediate in uptake have also become less appealing on other grounds. In particular, a direct role for ribosomes has traditionally been based on the finding that bacteria with resistant ribosomes take up antibiotic poorly and that even susceptible strains take up much less streptomycin if they are also treated with chloramphenicol. However, it had been clear for some time that uptake in resistant strains, though low, showed some of the characteristics of that in susceptible strains (particularly energy dependence [9]). Then some totally unexpected results of Hurwitz et al. (22) (Fig. 4) indicated that resistant cells also have an intrinsic capacity to take up streptomycin at rapid rates. They demonstrated that resistant strains could take up as much streptomycin as did susceptible ones if the cultures were pretreated with appropriate levels of puromycin.

Since cells with resistant ribosomes can take up large quantities of streptomycin, the high-affinity site on susceptible ribosomes is apparently not required for uptake. On the other hand, if interaction with low-affinity sites on any ribosomes is sufficient, one cannot explain why susceptible ribosomes usually promote much more rapid uptake. Thus, the previous view had to change drastically. Ribosomes are somehow involved in the uptake of streptomycin, but the activity or metabolism of ribosomes is more critical than their susceptibility or resistance to the drug. Low levels of puromycin potentiate uptake in a resistant strain, but high levels inhibit the transport, which suggests that an appropriate level of ribosome activity is required. As for chloramphenicol, it may block uptake by changing the susceptibility of ribosomes (for example, by prevention of ribosomal dissociation to a susceptible 30S stage [22]). But stopping the activity of ribosomes might instead prevent cycling required for uptake.

According to the hypothesis for streptomycin uptake that we are considering here, rather than acting as a sink, ribosomes might somehow function in the formation of the gate or pore associated with transport. Davis et al. (12) have considered how streptomycin action could be linked to membrane permeability phenomena and have proposed the interesting notion that it involves bits of proteins that are membrane bound. These would be fragments of proteins that are ordinarily secreted. In the presence of streptomycin, susceptible strains would make only short polypeptides with miscoded amino acids (12). These would have their usual affinity for the membrane, in which they could conceivably form a weak point.

As an alternative possibility, polysomes at the membrane may be involved. They are probably always involved in forming transient pores for the secretion of nascent protein. These could become magnified when the rate of formation of initiation complexes and short peptides is increased. This particular alternative has the advantage that the pores might exist only transiently, consistent with the irreversibility of uptake.

According to either formulation of "induced pores," puromycin at low levels would produce increased rates of interaction of both streptomycin-susceptible and streptomycin-resistant ribosomes at membrane sites, but the dose response to puromycin of the two types of ribosomes would be different, as observed by Hurwitz et al. (22), by affecting the rate of either the ribosome cycle or production of "damaging" polypeptides.

This type of mechanism can reconcile apparent differences in the rates of uptake and killing. At one extreme, in cells with resistant ribosomes, EDP-II uptake can occur without any subsequent killing (22). At the other extreme, in susceptible cells, cells die (lose the ability to form colonies) before the major influx of streptomycin or efflux of K^+ ions (22). In that case, because streptomycin is taken up irreversibly, the initial amount transported is apparently sufficient to be lethal even after cells are diluted out of medium containing the drug.

According to the model of an interaction of the cycling ribosomes with transport, ribosomes that are modified by mutation might alter transport processes. Recently, several fascinating mutants of exactly this type have been reported. For example, a mutation in protein export can be suppressed by a second mutation in the gene for ribosomal protein S15 (15). Mutations in other elements of the protein-synthetic machinery, such as initiation factor IF2 (34), can also suppress secretion-defective mutations.

The ideas of how ribosomes can help form one-way "endocytic" pores in susceptible bacteria are still somewhat vague, but they are consistent with both the observations of modulation of secretion by mutations in ribosomes and the early observations that uptake of streptomycin is initiated with the formation of some kind of pores large enough to

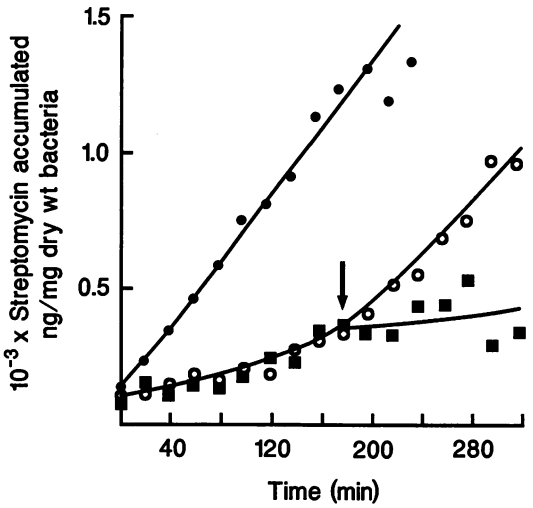

FIG. 5. Streptomycin accumulation in chemostat cultures under aerobic and anaerobic conditions. *E. coli* grown in a fortified minimal medium were grown aerobically or with continuous flushing with nitrogen to achieve anaerobiosis. Streptomycin was added to growing cells, and uptake was measured essentially as in the legend to Fig. 4. Symbols: Accumulation under aerobic conditions (●); under anaerobic conditions with an energy poison added (■) at the time indicated by the arrow or without such addition (○). Data are used with permission of the Society for General Microbiology (31).

permit the efflux of small metabolites and ions such as K^+ (1, 12).

ESCAPE OF CELLS AT LOW pH, IN ANAEROBIOSIS, AND IN OTHER CONDITIONS

How far can the present models take us in understanding other cases in which aminoglycoside action fails?

The failure of streptomycin action at low pH is explicable by the uptake mechanism proposed. At low pH, the PMF falls, and so, as several groups have pointed out (7, 11, 14), transport will become much poorer, lowering the effective intracellular concentration of the drug.

With respect to the failure of streptomycin action in anaerobic cultures, recent experiments have once again forced a change in thinking. The notion long current was that anaerobic cultures could take up streptomycin only to low final levels. In short-term cultures, this is patently true: for example, streptomycin-dependent bacterial strains stop growth when transferred to anaerobic conditions, and "phenotypic suppression" of several alleles by streptomycin (18) requires very high levels of drug in anaerobic cultures. Bryan et al. (5), however, showed that anaerobic cultures of *Bacteroides fragilis* can take up streptomycin in certain conditions, and *E. coli* grown anaerobically can also take up much larger quantities of the drug after an initial refractory period (31) (Fig. 5).

Bryan has discussed the possibility that anaerobic cultures may simply have a reduced rate of electron transport, effectively lowering the PMF required for uptake. This idea remains attractive, but if PMF were indeed lower in anaerobic cells, the induction of uptake after a lag (Fig. 5) would be hard to explain. In addition, some investigators have found that the PMFs of anaerobic and aerobic cultures are comparable. Thus, the effect of anaerobiosis may be more complex. In the present context, one can suggest that pores may require time to form in anaerobic cells. This would be true, for example, if the membrane structure is immediately modified (to affect secretion?) in anaerobic conditions. More possibly, pore formation might require an oxidative insult that would of course occur at a much lower frequency anaerobically. Such a mechanism has been shown to occur during the formation in fungal cells of polyene-dependent pores (2). It might be that streptomycin uptake in anaerobic cultures, even after prolonged incubations, could, like irreversible damage by polyenes, be counteracted by added catalase.

Such a mechanism could help to rationalize still other puzzling phenomena associated with streptomycin action. For example, *E. coli* cells infected with phage T6 provide another case, not mentioned thus far, in which streptomycin fails to inhibit protein synthesis strongly (16). Phage development proceeds essentially normally, although the synthesis of lysozyme late in infection is strongly inhibited. It may be that early in phage infection, when host protein synthesis stops and there is very little protein secreted from the cells, the paucity of membrane-bound polysomes results in a paucity of potential pores for streptomycin entry. Late in infection, when the plasma membrane begins to become leaky, the drug would enter cells more easily, and as a consequence the formation of proteins like lysozyme would be largely blocked.

PRACTICAL CONSEQUENCES: CAN ANTIBIOTIC IRREVERSIBILITY BE CONTROLLED?

Whether the particular formulation of the hypothesis for uptake proposed here is right or wrong, the special characteristics of streptomycin transport are critical in understanding its lethality. The early experiments on the site of action of streptomycin remain persuasive. This is especially true in light of the experiments of Hurwitz et al. (Fig. 4), which showed that internal streptomycin can be brought to very high levels in both susceptible and resistant bacteria, but even then only the bacteria with susceptible ribosomes are killed. However necessary membrane pores may be for killing, they remain secondary to the effect on ribosomes.

On the other hand, the killing action of the drug obviously requires its irreversible uptake. For this reason, the special uptake characteristics may well be the reason why aminoglycosides are cidal, whereas chloramphenicol, which is reversibly taken up by a more conventional transport mechanism, is bacteriostatic.

Following this line of supposition, one can imagine two ways in which antiribosomal antibiotics might be changed in their spectrum or their static/cidal character:

(i) The uptake of aminoglycosides could be increased, in all cultures by manipulations of PMF and in anaerobic cultures in particular by conjugating the drugs with a molecule that would not affect drug activity but would confer a "normal" uptake mechanism (for example, glucose).

(ii) Chloramphenicol or other static antibiotics might become lethal if they were attached to a highly charged molecule (a DNA oligonucleotide?) that could be taken up into bacteria by the irreversible mechanism.

Of course, it is also possible that such substitutions would destroy antibiotic activity or that the "normal" uptake mechanism for chloramphenicol would override any uptake based on a covalently attached substituent. But in a review one benefits from the privilege of speculation. What is undeniable is that the blockage of aminoglycoside action in anaerobic cultures is linked to the very strange mechanism of uptake of such drugs, and an understanding of that process is very likely to have practical consequences.

ACKNOWLEDGMENT

Alex Sonnenwirth was my close friend and colleague, and it was in discussions with him that the puzzle of streptomycin action and its odd failures repeatedly came up. It would be more satisfying to honor his memory with facts rather than speculations on these issues, but at least it seems fitting to try to be provocative about the prospects of understanding these problems as a source of potential benefit.

LITERATURE CITED

1. **Anand, N., and B. D. Davis.** 1960. Damage by streptomycin to the cell membrane of Escherichia coli. Nature (London) **185:** 22–33.
2. **Brajtburg, J., S. Elberg, D. R. Schwartz, A. Vertut-Croquin, D. Schlessinger, G. S. Kobayashi, and G. Medoff.** Involvement of oxidative damage in erythrocyte lysis induced by amphotericin B. Antimicrob. Agents Chemother. **27:**172–176.
3. **Bryan, L. E.** 1982. Bacterial resistance and susceptibility to chemotherapeutic agents, p. 135–160. Cambridge University Press, Cambridge.
4. **Bryan, L. E.** 1984. Mechanisms of action of aminoglycoside antibiotics, p. 17–36. *In* R. K. Root and M. A. Sande (ed.), New dimensions in antimicrobial therapy. Churchill Livingstone, New York.
5. **Bryan, L. E., S. K. Kowand, and H. M. Van Den Elzen.** 1979. Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob. Agents Chemother. **15:**7–13.
6. **Bryan, L. E., and S. Kwan.** 1981. Mechanisms of aminoglycoside resistance of anaerobic bacteria and facultative bacteria grown anaerobically. J. Antimicrob. Chemother. **8**(Suppl. D): 1–8.
7. **Bryan, L. E., and S. Kwan.** 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrob. Agents Chemother. **23:**835–845.
8. **Bryan, L. E., T. Nicas, B. W. Holloway, and C. Crowther.** 1980. Aminoglycoside-resistant mutation of *Pseudomonas aeruginosa* defective in cytochrome c_{552} and nitrate reductase. Antimicrob. Agents Chemother. **17:**71–79.
9. **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.
10. **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.
11. **Damper, P. D., and W. Epstein.** 1981. Role of the membrane potential in bacterial resistance to aminoglycoside antibiotics. Antimicrob. Agents Chemother. **20:**803–808.
12. **Davis, B. D., L. Chen, and P. C. Tai.** 1986. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. Proc. Natl. Acad. Sci. USA **83:** 6164–6168.
13. **Dickie, P., L. E. Bryan, and M. A. Pickard.** 1978. Effect of enzymatic adenylylation on dihydrostreptomicin accumulation in *Escherichia coli* carrying an R-factor: model explaining aminoglycoside resistance by inactivating mechanisms. Antimicrob. Agents Chemother. **14:**569–580.
14. **Eisenberg, E. S., J. J. Mandel, H. R. Kaback, and M. H. Miller.** 1984. Quantitative association between electrical potential across the cytoplasmic membrane and early gentamicin uptake and killing in *Staphylococcus aureus*. J. Bacteriol. **157:**863–867.
15. **Ferro-Novick, S., and J. Beckwith.** 1985. Genetic evidence for the coupling of the synthesis and secretion of proteins in *Escherichia coli*, p. 38–43. *In* M. J. Gething (ed.), Protein transport and secretion. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Freda, C. E., and S. S. Cohen.** 1966. Streptomycin and infection of *Escherichia coli* by T6r$^+$ bacteriophage. J. Bacteriol. **92:**1670–1679.
17. **Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring.** 1981. The molecular basis of antibiotic action, p. 270–295. Wiley, London.
18. **Gorini, L.** 1974. Streptomycin and misreading of the genetic code, p. 791–803. *In* M. Nomura et al. (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Grinius, L.** 1982. Protonmotive-force-dependent DNA transport across bacterial membranes, p. 129–132. *In* A. N. Martinosi (ed.), Membranes and transport, vol. 2. Plenum Publishing Corp., New York.
20. **Gurgo, C., D. Apirion, and D. Schlessinger.** 1969. Polyribosome metabolism in *Escherichia coli* reacted with chloramphenicol, neomycin, spectinomycin or tetracycline. J. Mol. Biol. **45:** 205–220.
21. **Guthrie, C., and M. Nomura.** 1968. Initiation of protein synthesis: a critical test of the 30S subunit model. Nature (London) **219:**232–238.
22. **Hurwitz, C., C. B. Braun, and C. L. Rosano.** 1981. Role of ribosome recycling in uptake of dihydrostreptomycin by sensitive and resistant *Escherichia coli*. Biochim. Biophys. Acta **652:**168–176.
23. **Kaback, H.** 1986. The lac permease of *Escherichia coli*: a jewel in the membrane, p. 83–95. *In* F. Alvarado and C. H. van Os (ed.), Ion-gradient coupled nonelectrolyte transport. INSERM Symposium no. 26. Elsevier Science Publishers, B. V., Amsterdam.
24. **Kogut, M., J. Lightbown, and P. Isaacson.** 1965. Streptomycin action and anaerobiosis. J. Gen. Microbiol. **39:**155–164.
25. **Kwan, C. N., D. Apirion, and D. Schlessinger.** 1968. Anaerobiosis induced changes in an isoleucyl-tRNA and the 50S ribosomes of Escherichia coli. Biochemistry **4:**427–433.
26. **Labadan, B., and E. B. Goldberg.** 1982. DNA transport across bacterial membranes, p. 133–138. *In* A. N. Martinosi (ed.), Membranes and transport, vol. 2. Plenum Publishing Corp., New York.
27. **Luzzatto, L., D. Apirion, and D. Schlessinger.** 1969. Polyribosome depletion and blockage of the ribosome cycle by streptomycin in *Escherichia coli*. J. Mol. Biol. **42:**315–335.
28. **Luzzatto, L., D. Apirion, and D. Schlessinger.** 1969. Streptomycin action: greater inhibition of *Escherichia coli* ribosome function with exogenous than with endogenous messenger ribonucleic acid. J. Bacteriol. **99:**206–209.
29. **Mangiarotti, G., and D. Schlessinger.** 1966. Polyribosome metabolism in *Escherichia coli*. I. Extraction of polyribosomes and ribosomal subunits from fragile growing *Escherichia coli*. J. Mol. Biol. **28:**123–143.
30. **Mates, S. M., E. S. Eisenberg, L. J. Mandel, L. Patel, and R. H. Kaback.** 1982. Membrane potential and gentamicin uptake in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **79:** 6693–6697.
31. **Muir, M. E., M. Ballasteros, and B. J. Wallace.** 1985. Respiration, growth rate and the accumulation of streptomycin in *Escherichia coli*. J. Gen. Microbiol. **131:**2573–2579.
32. **Nickols, W. W., and S. N. Young.** 1985. Respiration-dependent uptake of dihydrostreptomycin by *Escherichia coli*. Biochem. J. **228:**505–512.
33. **Ozaki, M., S. Mizushima, and M. Nomura.** 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in *E. coli*. Nature (London) **222:**333–339.
34. **Shiba, K., K. Ito, Y. Nakamura, J. Dondon, and M. Grunberg-Manago.** 1986. Altered translation initiation factor 2 in the cold-sensitive ssyG mutant affects protein export in *Escherichia coli*. EMBO J. **5:**3001–3006.
35. **Spotts, C. R., and R. Y. Stanier.** 1981. Mechanism of streptomycin action on bacteria: a unitary hypothesis. Nature (London) **192:**633–637.
36. **Wallace, B. J., and B. D. Davis.** 1973. Cyclic blockade of initiation sites by streptomycin-damaged ribosomes in *Escherichia coli*: an explanation for dominance of sensitivity. J. Mol. Biol. **75:**377–390.