The enigma of streptomycin transport

Professor Davis recently reviewed in this Journal (Davis, 1988) his stimulating hypothesis concerning the transport of streptomycin (and dihydrostreptomycin which is believed to behave biochemically in an identical manner to streptomycin) into bacterial cells (Davis, Chen & Tai, 1986; Davis, 1987). In this hypothesis, Davis proposes that mis-translated proteins accumulate in the cytoplasm (Davis, 1987, 1988). However it is here that the 'channel' hypothesis runs into difficulties (Nichols, 1987).

It is perhaps useful to summarize the key features of the 'channel' hypothesis. Davis (1987, 1988) ascribes particular importance to some observations made more than 25 years ago (Dubin, Hancock & Davis, 1963). One of the earliest events that occurs coincidently with, or shortly after, initiation of rapid energy-dependent uptake of streptomycin by Escherichia coli is the loss of K⁺ and nucleotides from the cell cytoplasm into the external medium (Dubin et al., 1963). Davis (1987, 1988) interprets these observations as being the result of the cytoplasmic membrane becoming generally leaky; that is, possessing channels through which the K⁺ and nucleotides diffuse. This is proposed to be due to one of the established actions of streptomycin, that of causing mis-translation during protein synthesis, occurring at ribosomes synthesizing membrane or exported proteins. Specifically, Davis et al. (1986) have suggested that these mis-translated proteins accumulate in the cytoplasmic membrane and cause generalized leakiness there. This proposed generalized membrane leakiness is also taken to explain the onset of the rapid, respiration-dependent transport of streptomycin into the cytoplasm. Positively-charged molecules of streptomycin are proposed to diffuse inward, in response to the electric potential gradient (Damper & Epstein, 1981), through the transmembrane aqueous channels responsible for the generalized leakiness (Davis, 1987, 1988).

The problem is that one would not expect channels that link the two aqueous compart-
ments on either side of the bacterial cytoplasmic membrane to display irreversible kinetics of dihydrostreptomycin transport. This opinion derives from the generally-accepted properties of transport channels. In the absence of any 'gating' (see below), aqueous channels would be expected to behave like the well-studied porin-mediated pores of Gram-negative bacterial outer membranes (Nikaido, 1985; Nakae, 1986; Benz, 1985) that allow transmembrane diffusion equally easily in both directions. Indeed it is a necessary characteristic for a transport pathway to be called a 'channel' that forward and reverse permeabilities be equal for any particular solute (Hille, 1984). There are two simple modifications that can be made. First, one channel hypothesis to allow one to postulate the existence of different forward and reverse kinetics. First the channel might be electrically 'gated' in such a way that below a certain magnitude of the membrane potential the gate would close, and transport would stop. However under conditions in which dihydrostreptomycin transport was kinetically irreversible in E. coli, the membrane potential was not significantly different from the control value which was measured shortly after exposure to the antibiotic (Goss, Spicer & Nichols, 1988). Secondly, one could postulate some kind of cyclic chemical gating (Scarborough, 1985). However this would be more akin to the chemical-reaction-linked transport of dihydrostreptomycin that was recently speculatively suggested as an alternative to the channel hypothesis (Nichols, 1987).

There is one feature of a thermodynamically passive transport process (i.e. one that is not accompanied by an energy-yielding chemical reaction) that would allow transport kinetics to be highly asymmetrical: that is, if there were an obligatory binding site somewhere within the transport pathway, with binding and dissociation of the diffusing molecule at the two sides (cytoplasmic/periplasmic) of the binding site being governed by different kinetic constants. Such a model has no limits to its possible kinetic asymmetry: it can act as a molecular valve (Krupka & Deves, 1979; Stein, 1986). The process mediated by such a transport system would not, however, be termed diffusion through a channel (Hille, 1984) but facilitated diffusion (Stein, 1986) via a carrier (Harold, 1986b). This mechanism is quite distinct from the channel mechanism (Harold, 1986c).

There are two further objections to the channel hypothesis of Davis (1987, 1988) that derive from the following reasoning, rather than being wholly from direct experimental or theoretical evidence. The first point is that the proposed channels have a high degree of specificity, but for a few unrelated solutes; they are supposed to allow transmembrane diffusion of nucleotides and K+ as well as streptomycin, but not other substances (Davis, 1987). Indeed, implicit in the model proposed by Davis et al. (1986) is the assumption that the channels do not allow transmembrane diffusion of H+ ions, since this would result in H+ -equilibration across the cytoplasmic membrane—thereby decreasing to near zero the size of the membrane potential which is the driving force for dihydrostreptomycin transport (Damper & Epstein, 1981). It seems unlikely that a range of mis-read proteins incorporated into the cytoplasmic membrane should all show such identical and unusual specificity. The second polemical criticism is as follows. I have argued above that irreversibility is a rather special property of a transport system, even though thermodynamically passive but kinetically irreversible transport systems can exist (Krupka & Deves, 1979). If one accepts that the property of irreversibility is unusual, then it is again unlikely that a wide range of randomly mis-read membrane proteins should all possess this particular unusual property.

One final criticism can be made of the hypothesis that mis-read proteins cause generalized leakiness of the cytoplasmic membrane. That is that certain E. coli mutants apparently fill themselves with nonsense proteins, some of which are apparently membrane proteins, and yet retain viability and grow, albeit slowly (Gorini & Kataja, 1964; Gale et al., 1981). This is inconsistent with the general view that an intact cytoplasmic membrane, with its impermeability-dependent energy transduction (Harold, 1986a) and homeostatic (Booth, 1985) functions, is necessary for life to be maintained (e.g. see Mitchell, 1970; Nicholls, 1982).

So far this article has been critical. To be more constructive, what are the alternative hypotheses for how streptomycin might cross the bacterial cytoplasmic membrane during the rapid energy-dependent uptake phase (EDP-II; Bryan & Van den Elzen, 1977)? and how is this transport initiated by early events dependent on the binding of a small amount of streptomycin to a few ribosomes?

As stated at the beginning of the article, I believe that Davis' (1987) hypothesis for the early events in streptomycin action agrees well
with the evidence available. However, in contrast to the generalized leakiness hypothesis, I suggest that early mis-translation on ribosomes synthesizing membrane proteins gives rise to a 'rogue' transport system that consists of one, or a few, specific mis-read proteins. I further suggest that the transport process consists of a cycle involving a carrier-based mechanism (Harold, 1986c; and see Scarborough, 1985, for a general model), because then the kinetic irreversibility of transport would be easily explained (Stein, 1986). A chemical reaction as part of the transport cycle has been suggested (Nichols, 1987), but there is no evidence for this; indeed ATP, for example, appears not to be involved (Goss et al., 1988). On the other hand, it has not been entirely discounted that the transport cycle could be coupled to an oxidation/reduction reaction, in respiring bacteria at least, and this would be consistent with many data on the involvement of electron transfer in dihydrostreptomycin transport (Bryan & Van den Elzen, 1977; Arrow & Taber, 1986). However, such an oxidation/reduction reaction would be difficult to envisage in cells growing anaerobically without an electron acceptor, where nevertheless dihydrostreptomycin transport does eventually occur (Muir, Baillesteros & Wallace, 1985).

The transport system that most resembles that for dihydrostreptomycin is the one for the uptake of the polyamine spermidine (Tabor & Tabor, 1966; Holtje, 1978). Spermidine transport into the cytoplasm of E. coli is a kinetically irreversible process (Tabor & Tabor, 1966), as is dihydrostreptomycin transport (Nichols & Young, 1985). Nevertheless, in their review Taber et al. (1987) rejected the likelihood of identity between the two transport systems, despite this kinetic similarity and despite the physical similarity of the two substrates (i.e. in their carriage of multiple positive charges). This subject would probably bear further experimental study.

I conclude that, with the rejection of the hypothesis that a wide range of mis-translated membrane proteins form transmembrane aqueous channels (Davis et al., 1986; Davis, 1987, 1988), there is still no good explanation of how streptomycin enters the cytoplasm during the rapid energy-dependent (EDP-II) uptake phase. In mitigation of this unsatisfactory state of affairs, let me reiterate a quotation that headed an earlier review of this subject (Hancock, 1981). "If history repeats itself, streptomycin has a few more tricks up its sleeve for us" (Brock, 1966). I suspect that this applies as much in 1989 as it did in 1966 and 1981.

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Prevention of late haematogenous infection in major prosthetic joints

As with other prosthetic implants such as heart valves and ventriculo-atrial shunts, infection of prosthetic joints is one of the most serious complications with major clinical and economic consequences. The reported mortality rate varies from 2.7% (Buchholz et al., 1981) to as high as 18% (Ahlberg, Carlsson & Lindberg, 1978) and in survivors it commonly leads to prolonged morbidity and/or joint revision (Stinchfield et al., 1980; Buchholz et al., 1981). It has been arbitrarily classified as early if it occurs within three months of insertion of the joint (Little, 1983). Infection within this time scale is generally considered to be due to implantation of bacteria during the perioperative period. Infection after three months is classified as late; this is still often due to intraoperative contamination, but the importance of bloodstream infection is being increasingly appreciated (Lattimer et al., 1979; Little, 1983; Brause, 1986).

Much attention has gone into reducing the incidence of intraoperative contamination largely by using perioperative prophylactic antibiotics and operating in an ultraclean air environment (Erin, 1985; Lidwell et al., 1987; Nelson, 1987). With such procedures the