Proposed Mechanism for Gentamicin Transport

Keyphrases □ Gentamicin—proposed mechanism for transport across biological membranes □ Transport across biological membranes—gentamicin, proposed mechanism □ Stearyl aldehyde—interaction with gentamicin, used to formulate mechanism for gentamicin transport, effect of sodium bisulfite

Sir:

This communication reports our preliminary work dealing with the biological transport of the water-soluble aminoglycoside antibiotic, gentamicin[1]. This antibiotic does not transport to any significant degree across the membranes of the GI tract, as evidenced by the facts that measurable blood levels of gentamicin are not observed following oral administration to humans and animals and that nearly 98% of the antibiotic is excreted unchanged in the feces[2]. On the other hand, gentamicin has been shown to cross the otic (1) and placental (2) membranes and, in the case of sensitive bacteria, it has been demonstrated that gentamicin crosses the cell membrane and binds to bacterial ribosomes (3).

In view of the variability of gentamicin transport, it seems reasonable to assume that the transport of this antibiotic is dependent, at least in part, upon the physical-chemical properties of the biological interface (*viz.*—membrane). On the basis that the primary amino moieties of gentamicin are capable of interacting with aldehyde compounds *via* Schiff base formation, we hypothesized that biological membrane fatty aldehydes might also react with and subsequently bind gentamicin to the particular membrane, thus constituting a possible major step in the pathway for the transport of this antibiotic across biological membranes. Until recently, cellular lipid aldehydes were assumed to be present *in vivo* only in their bound, cryptic forms (*viz.*—plasmologens) (4). However, more recent work has shown that free fatty aldehydes are normal components of a variety of living systems, including bacteria (5), human placenta (6), mouse muscle (7), human collagen (8), and rat, dog, and bovine heart (9).

To test the validity of the gentamicin aldehyde Schiff base hypothesis, we studied the interaction of gentamicin with stearyl aldehyde, using previously described monolayer techniques (10).

Figure 1 shows the interfacial activity of gentamicin in the presence of insoluble films of stearyl aldehyde as a function of pH. The development of surface pressure, at areas per molecule where stearyl aldehyde alone exhibits no surface pressure, indicates penetration of the aldehyde film by gentamicin in the subphase. It can be seen that an enhancement of gentamicin penetration occurs with increasing pH. At a pH of 6.0, where gentamicin exists essentially in the protonated form, no significant penetration was observed. This enhancement may be explained by the fact that at higher pH values there is a concomitant increase in the fraction of nonprotonated primary amino groups. Furthermore, only these nonprotonated primary amino groups are capable of participating in Schiff base formation (11). Earlier experiments in these laboratories established that gentamicin (1×10^{-3} *M*) exhibited no surface activity at the air–water interface and that gentamicin did not interact with stearyl alcohol and only interacted slightly with stearic acid.

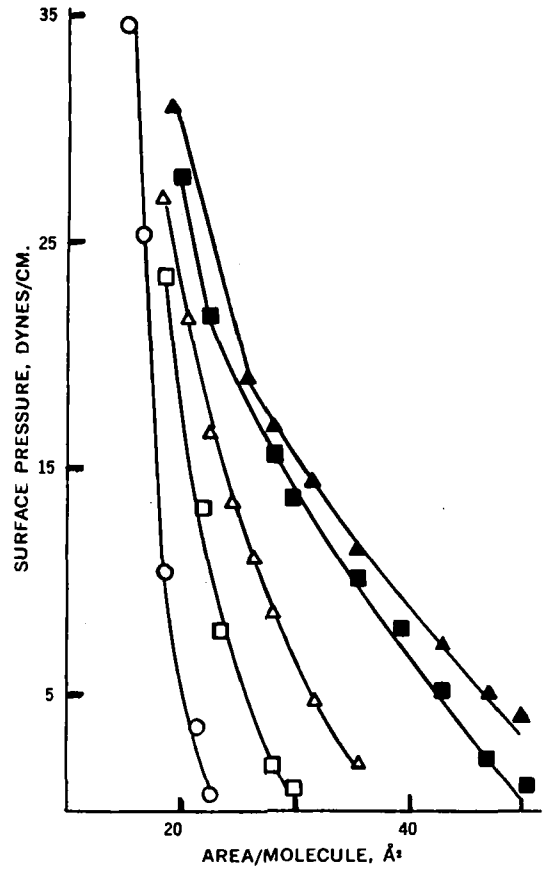

Figure 1—*Surface pressure–surface area curves of stearyl aldehyde alone at pH values of 7.2–8.4* (○) *and in the presence of* 1×10^{-3} M *gentamicin at pH values of 6.0* (○), *7.2* (□), *7.6* (△), *8.0* (■), *and 8.4* (▲).

Figure 2 demonstrates the uptake of gentamicin into an insoluble film of stearyl aldehyde, with and without the addition of 0.05% sodium bisulfite to the subphase. Gentamicin was injected under a film of stearyl aldehyde, and the rate of surface pressure development was followed. A lag period of approximately 4 min. was

Table I—Effect of Sodium Bisulfite on Antibacterial Activity of Gentamicin against *E. coli*

Sodium Bisulfite Concentration, %[a]	—Gentamicin, mcg./Disk— 0.12	0.60
	Zone of Inhibition, mm.[b]	
0	1.5	3.0
0.05	0.2	1.5

[a] Incorporated into agar medium. [b] Measured from edge of disk to edge of zone of inhibition.

[1] Received as a mixture of components (C_1, C_{1a}, and C_2); see D. Cooper *et al.*, *J. Chem. Soc., C*, 1971, 3126.

[2] D. J. Cooper, Smith Kline and French Labs., Swedeland, PA 19479

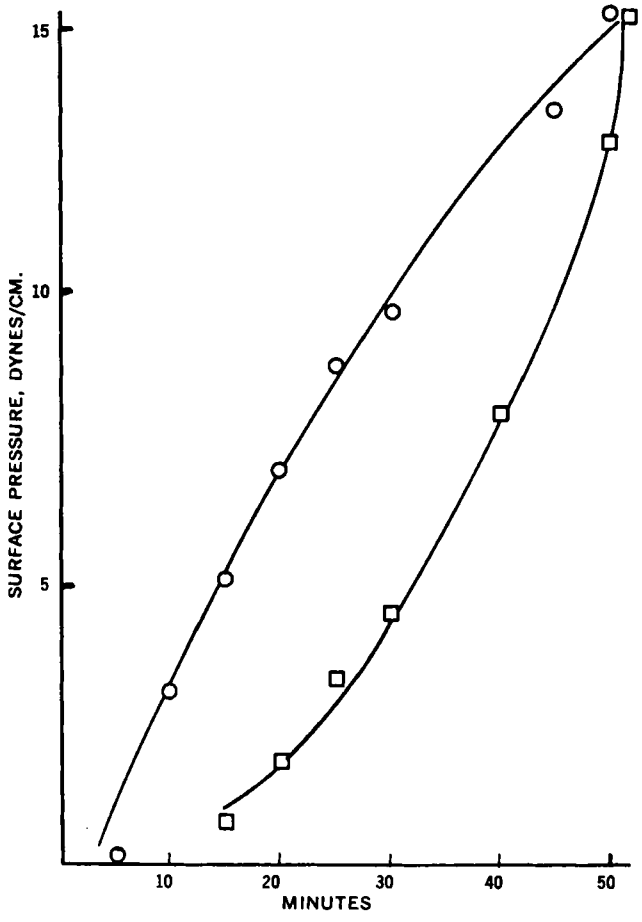

Figure 2—*Rate of surface pressure increase of a stearyl aldehyde film at pH 8.0 in the presence of 1 × 10^{-3} M gentamicin* (○) *and 1 × 10^{-3} M gentamicin with 0.05% sodium bisulfite* (□).

observed when gentamicin was studied in the absence of bisulfite ion.

In the case of a similar experiment conducted with 0.05% sodium bisulfite in the subphase, the previously observed lag period was increased to 14 min. The data clearly show that bisulfite significantly diminishes the interaction of gentamicin with stearyl aldehyde. This may be explained by the fact that bisulfite reacts with aldehydes to form α-hydroxysulfonic acid derivatives (12) which do not form Schiff bases.

The implications of the monolayer studies were extended by studying the effect of the bisulfite ion on the antibacterial activity of gentamicin. A disk-plate method (13), utilizing *Escherichia coli* as the test organism, was employed. Gentamicin-containing filter paper disks were placed on seeded nutrient agar plates containing various concentrations of sodium bisulfite. Following incubation at 37° for 18 hr., the plates were examined and the diameters of the observable zones of inhibition were measured (Table I).

The data indicate that bisulfite ion at 0.05% inhibits the antibacterial activity of gentamicin against *E. coli*. If the mechanisms of interaction of gentamicin at the monolayer and the bacterial membrane are the same, then it appears that bisulfite inhibits gentamicin activity by blocking membrane aldehyde sites which may be necessary for the transport of gentamicin into the bacterial cell.

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Self-Association of Theophylline in Aqueous Solution

Keyphrases □ Theophylline, association in aqueous solution—determination, ultracentrifugation, molecular weights □ Association, theophylline—aqueous solution □ Ultracentrifugation—determination, theophylline self-association, molecular weights

Sir:

In 1957, Guttman and Higuchi (1) concluded that theophylline did not self-associate in water at concentrations from 2.3 × 10^{-3} to 28 × 10^{-3} *M*; partitioned with chloroform–isooctane (9:1) where the theophylline concentration was 1 × 10^{-4} to 12 × 10^{-4} *M*.

In 1971, Ng (2) presented IR evidence for the self-association of theophylline by hydrogen bonding in nonaqueous (deuterochloroform) solutions. Thakkar *et al.* (3) showed, from NMR spectra of aqueous solutions (5 × 10^{-3} to 42 × 10^{-3} *M*), that theophylline self-associates by hydrophobic interactions in water. The present communication presents data, obtained