phyrin biosynthesis may be of value both in preparing models for the study of human porphyrias (*13*) and for investigating the mechanisms for regulation of porphyrin biosynthesis.

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Colicin-Tolerant Mutants of Escherichia coli: Resistance of Membranes to Colicin E1

Abstract. *Colicin E1 blocks proline accumulation by membrane vesicles prepared from wild-type sensitive* Escherichia coli. *Two classes of mutant cells are unaffected by colicin. Vesicles from colicin-resistant strains are sensitive to colicin E1, whereas vesicles from colicin-tolerant strains are unaffected by colicin E1. These results suggest that the colicin E1 receptor is on the cell membrane and that colicin-tolerant strains have altered membranes while colicin-resistant strains have altered cell walls.*

Although all colicins appear to be small macromolecules (predominantly proteins) which are adsorbed to and remain on the surface of the cell which is being affected, the mode of action of colicins differs. Some affect DNA metabolism, others ribosomal function, and still others oxidative phosphorylation (*2*, *1*). Colicin E1, with which our studies are concerned, appears to affect oxidative metabolism and active transport in *Escherichia coli* (*3*).

Mutants of *Escherichia coli* which are no longer sensitive to colicin E1 fall into two classes: (i) colicin-resistant mutants which have lost the ability to adsorb the colicin onto the cell surface and are therefore not killed by the action of colicin, and (ii) colicin-tolerant mutants which, although they retain the adsorption receptors for colicin, are not killed by colicin (*1*, *4*). The distinction between these two classes of mutants on the basis of adsorption of colicin (along with additional properties of colicin-tolerant mutants, such as increased sensitivity to lipid-active detergents) has led to the hypothesis that colicin-resistant mutants have altered cell walls such that the cell-wall binding sites will no longer fix the colicin, and that colicin-tolerant mutants have altered cell membranes through which the colicin cannot transmit its lethal action to the intracellular target (*1*, *4*). The experiments reported here with isolated subcellular membrane vesicles (*5*) provide direct support for the distinction between resistant (wall) and tolerant (membrane) mutants, and also show that the cellular binding site for colicin E1 is on the cell membrane and not the cell wall. The closed membrane vesicles lack most of the cell wall material, DNA, RNA, and the soluble enzymes of the original cell, but they are still capable of several membrane-associated functions, such as active

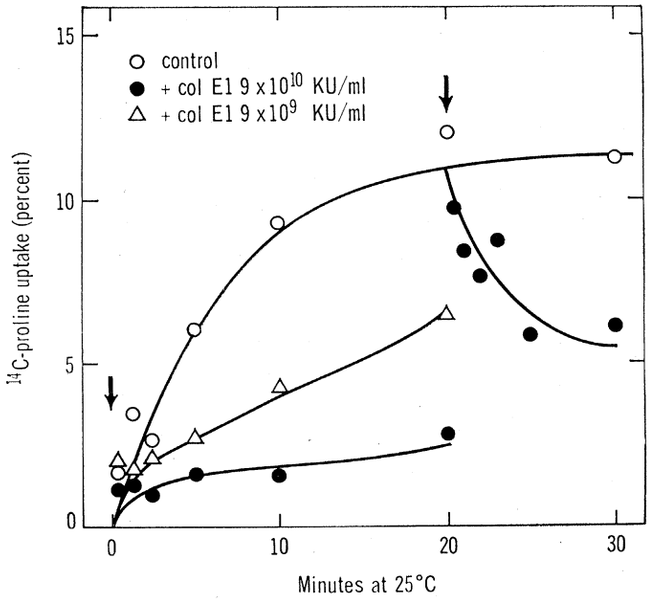

Fig. 1. Effect of colicin E1 on [^{14}C]proline accumulation by isolated membrane vesicles from the colicin-sensitive strain of *E. coli*. Colicin E1 at either 9×10^9 or 9×10^{10} killing units (KU) per milliliter was added to membrane vesicles (2 mg/ml, dry weight) from *E. coli* strain K-12 either 1 minute before or 20 minutes after the addition of [^{14}C]proline. The fraction of [^{14}C]-proline in the vesicles (on the filters) was determined by counting direct samples of the reaction mixture. Colicin titers in "killing units per milliliter" were determined by standard methods (*6*).

transport of proline against a concentration gradient (*5*).

The experiment illustrated in Fig. 1 shows that colicin E1 affects the uptake of proline by membrane preparations from wild-type colicin-sensitive *E. coli* K-12. Membranes were prepared according to Kaback (*5*), suspended in 0.5*M* potassium phosphate (*p*H 6.6) containing 14 m*M* glucose and 10 m*M* $MgSO_4$, and stored at −70°C until used. Just before the experiment, the membrane preparation was thawed at room temperature, homogenized for 1 minute in the cold, and incubated for 15 minutes at 25°C. [^{14}C]Proline (New England Nuclear, 180 mc/mmole; 0.5 μc/ml) was added, and 0.05-ml samples were removed from time to time, diluted to 2 ml in the suspension medium without proline, and filtered rapidly through Millipore (0.45 μm) filters. The filters were not washed, but glued to planchets and counted in a Nuclear Chicago gas-flow counter. Colicin E1 was prepared from the colicinogenic strain of *Salmonella typhimurium* LT2 (col E1) (obtained from R. C. Clowes) by the mitomycin induction procedure of Maeda and Nomura (*6*). After cells and debris were removed by low speed centrifugation, the crude colicin preparation was used without further purification.

When colicin E1 is added just before the [^{14}C]proline, the accumulation of proline by the membrane vesicles is inhibited (Fig. 1). The degree of inhibition is dependent on the concentration of colicin added. When colicin E1 is added after proline accumulation in the vesicles has reached equilibrium (at 20 minutes), the [^{14}C]proline which has been concentrated in the vesicles is rapidly released into the medium. Similar results are obtained with another colicin that inhibits energy metabolism, colicin K (data not shown).

Having shown that colicin E1 acts on isolated membrane preparations from the sensitive *E. coli* strain, we turned to vesicles prepared from colicin-resistant and colicin-tolerant mutants (*7*). When colicin E1 is added to vesicles prepared from the resistant mutant 20 minutes after the addition of [^{14}C]proline, the radioactivity is rapidly lost from the membranes. Both the extent of loss and the kinetics of loss are quite comparable to those obtained with membranes from the sensitive strain (Fig. 2). However, when colicin E1 is added to vesicles from the tolerant mutant, very little effect is found on proline accumulation. As a control for the experiments with membrane vesicles, intact cells were harvested, washed, and resuspended in the same incubation mixture used with the vesicles. [^{14}C]Proline was added, and accumulation of radioactivity was measured by the usual filter procedure (Fig. 2, D–F). The addition of colicin to the sensitive cells results in loss of radioactive proline, but there is little effect of colicin on either the resistant or the tolerant cells. The results in Fig. 2 show (i) that both the cells and the subcellular membrane vesicles from the colicin-sensitive strain are affected by colicin E1; (ii) that the vesicles but not the cells of the resistant mutant are affected; and (iii) that neither the cells nor the vesicles from the tolerant mutant are affected by colicin.

One might question whether the [^{14}C]proline accumulated by the vesicles

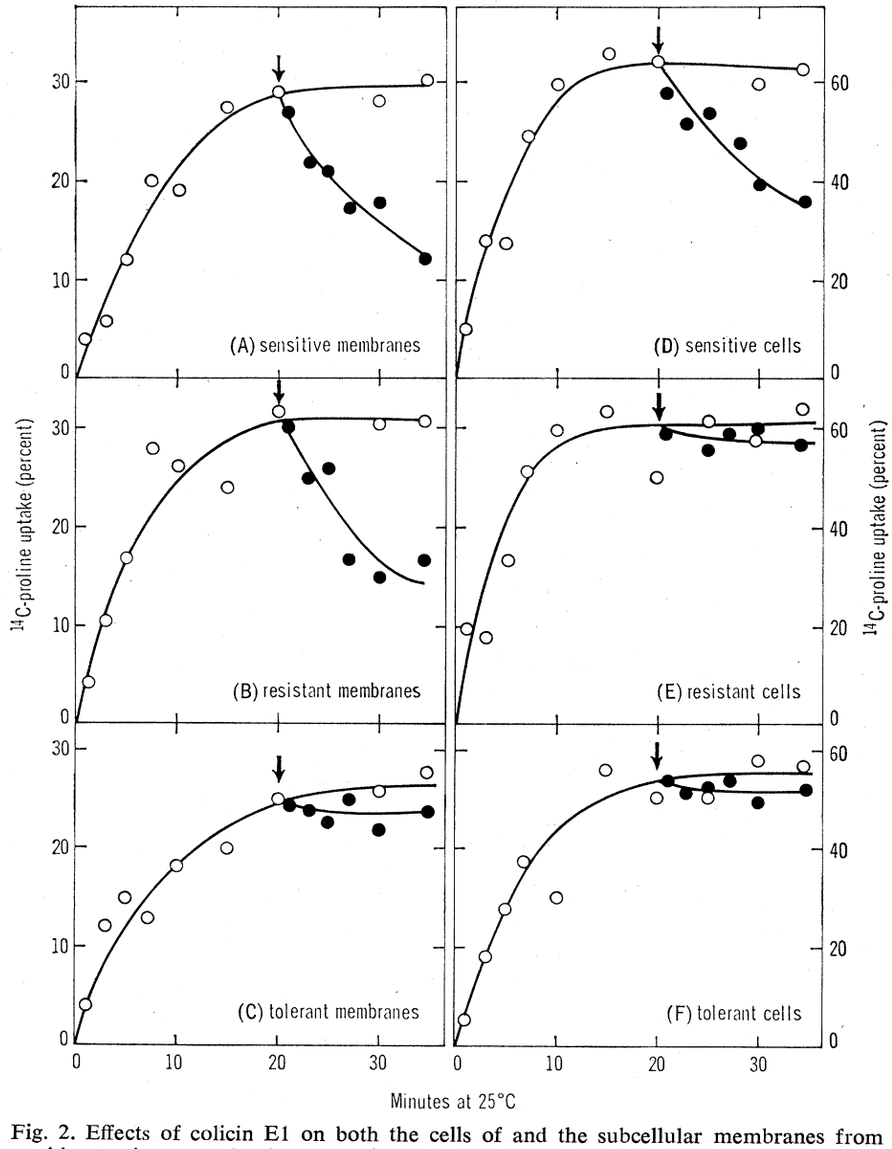

Fig. 2. Effects of colicin E1 on both the cells of and the subcellular membranes from sensitive, resistant, and tolerant strains of *E. coli*. Control (open circles) and colicin-treated samples (closed circles). Colicin E1 was added at 9×10^9 killing units per milliliter to 1 mg (dry weight) of cells per milliliter or at 9×10^{10} killing units per milliliter to 10 mg (dry weight) of vesicles per milliliter, 20 minutes after the addition of [^{14}C]proline. Since the vesicles retain about 15 percent of the mass of the cells (*5*, and our measurements), the multiplicity of killing units per vesicle is one-seventh of the multiplicity of killing units per cell, on the assumption of one vesicle per cell. If more than one vesicle is produced per cell, the ratio of relative multiplicities with vesicles and with cells will be even lower. In this experiment, the sensitive strain was *E. coli* B and not K-12. However, the response to colicin E1 of cells and membranes of *E. coli* B is very similar to that with *E. coli* K-12 (compare Figs. 1 and 2).

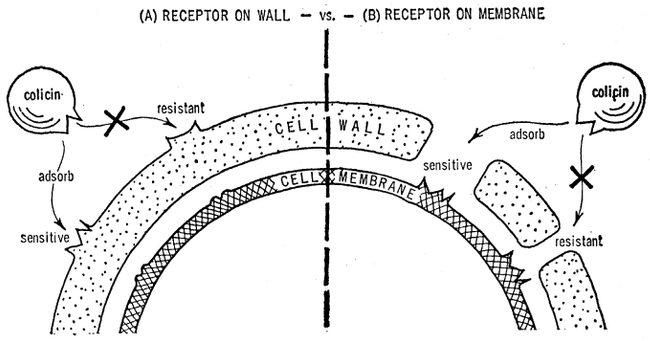

Fig. 3. Models for the colicin E1 receptor and the nature of resistant mutants. The experiments support model B against model A.

remains as free proline or whether it is incorporated into proteins. Kaback and Stadtman (*5*) showed that more than 80 percent of the ^{14}C accumulated by the membrane vesicles could be extracted in a form chromatographically identical with free [^{14}C]proline. In our control experiments more than 85 percent of the [^{14}C]proline accumulated by the membranes remains soluble in cold trichloroacetic acid.

We conclude from the sensitivity of proline transport in subcellular membrane vesicles to colicin E1 that the receptor sites for the colicin are on the cell membrane (Fig. 3B) and not on the cell wall (Fig. 3A). The previously standard model (*1*) for the receptor on the wall (Fig. 3A) is based on the existence of the class of mutants that are resistant to the otherwise unrelated colicins E1, E2, and E3 and to bacteriophage BF23. However, if the phage receptor on the cell wall includes the molecular configuration which we have diagrammatically represented as a wide or narrow channel in Fig. 3B, this would account for the experimental fact that resistant mutants adsorb neither the phage nor the colicins. In our model, the resistance of the resistant mutant to colicin E1 is not due to a change in the shape of the receptor (Fig. 3A) but due to a change in the cell wall which blocks access to the membrane receptor (Fig. 3B). When the wall is enzymatically removed, as in the preparation of membrane vesicles, then the colicin E1 receptor is again accessible. Variations on the model in Fig. 3B are readily devised, but this model has the virtue of predicting the existence of still additional classes of mutants with altered sensitivity and adsorption properties. Some may have already been found by Hill and Holland (*4*), and one specific class which is predicted by the model would be resistant and unable to adsorb colicin E1 owing to a change in the membrane receptor (which would still be present on the isolated membranes). The characterization of other classes of colicin-resistant and tolerant mutants through studies of isolated membranes and the physical isolation of the product of the *tol C* gene from the membranes are reasonable goals.

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Nest Parasitism, Productivity, and Clutch Size in Purple Martins

Abstract. *Mean maximum nestling weight of purple martins decreased with increase in brood size from three to five. Martins in the absence of acarine nest parasites produced young heavier than parasitized young of the same brood size; in addition, unparasitized nestlings tended to reach a maximum weight equivalent to that of young in parasitized broods of one less member. Modal brood size for parasitized and unparasitized martins was four, but there was a significant trend toward production of broods of five by mite-free birds, and of broods of three by parasitized parents. This suggests a potentially important role for nest parasitism in the determination of clutch size in martins and other birds.*

Analysis of data from two colonies of the purple martin, *Progne subis* L., has demonstrated a significant decrease in productivity that accompanies nest parasitism by blood-feeding mites (*1*). The evidence indicates that nest parasitism may be important in natural selection of avian clutch size, although this possibility has been largely ignored in discussions of clutch size (*2*).

Two martin colonies were established in the spring of 1964 in Lawrence, Kansas, and are still being maintained. Situated 10 m apart, each colony consists of 12 apartments constructed to allow daily examination of contents and removal of nestlings for weighing and other purposes. Shortly after establishment, both colonies became infested with mites, chiefly the martin mite, *Dermanyssus prognephilus* Ewing (*3*). Dipterans, reportedly significant nest parasites of martins (*4*), did not appear in our colonies.

Martin mites taken from the colonies were maintained in laboratory culture (*5*), with the domestic fowl as host. Two-week-old chicks introduced as hosts generally died within a relatively short time, frequently in as little as 3 hours. Cause of death is not yet known, but it seems more likely to be due to toxicity of the mite saliva than to exsanguination by the mites in so short a