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The Mechanism of Colicin E1 Action

DAVID S. FEINGOLD

Departments of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02115, and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Summary. Uncouplers of oxidative phosphorylation such as cyanide carbonyl m-chlorophenylhydrazone (CCCP) foster proton (H^+) permeability of bacterial and other membranes; counter cation movement is required for the effect of the uncoupler on proton permeability to be manifested maximally. Treatment of *Escherichia coli* with colicin E1 had little effect on the proton impermeability of the bacteria after challenge with an acid load; however, the subsequent addition of CCCP revealed that colicin E1-treated cells became much more permeable to protons than control cells. Thus the colicin caused specific rather than generalized alterations in the membrane permeability properties, allowing the movement of some cations but not H^+. This cation permeability was confirmed directly with studies of ^{42}K leakage on treatment with colicin E1. N,N′-dicyclohexylcarbodiimide blocked the fall in ATP levels usually associated with colicin E1 action, but did not block the K^+ leakage, the inhibition of protein and nucleic acid synthesis, or the lethal effect of the colicin, suggesting that reduced availability of ATP is not the cause of these colicin E1-induced effects. The possible primary action of the colicin E1 molecule is discussed in relation to these data.

Although the mechanism by which the bacteriocin, colicin E1, kills sensitive *Escherichia coli* is not understood, several biochemical events of interest are known to accompany the lethal action of this colicin and the other colicin proteins which appear to act by similar mechanisms – colicins A, I, and K (Nomura, 1963; Levinsohn, Konisky & Nomura, 1968; Nagel de Zwaig, 1969). The synthesis of protein, DNA, and RNA cease promptly and simultaneously (Jacob, Siminovitch & Wollman, 1952; Nomura, 1963); most active transport across the membrane stops (Luria, 1964; Nomura & Maeda, 1965); ATP levels fall dramatically (Levinthal & Levinthal, *unpublished data* cited in Luria, 1964; Fields & Luria, 1969*a*; Hirata, Fukui & Ishikawa, 1969); and, while the dissimilation of glucose continues, pyruvate accumulates and leaks out of the bacteria along with some of the intermediates in the glycolytic pathway (Fields & Luria, 1969*b*). The colicin E1-treated bacteria do not become generally permeable since o-nitrophenyl-

β-D-galactoside does not leak in and α-methyl-D-glucoside can be concentrated within the treated organisms (Fields & Luria, 1969*a*). K^+ ions, however, which are usually retained avidly by the cells, are lost to the medium on addition of colicin E1 (Luria, 1964) or colicin K (Nomura & Maeda, 1965; Hirata *et al.*, 1969). The usual interpretation of these data is that the colicin proteins of this class interfere with the supply of energy in the affected bacteria.

As pointed out by Fields and Luria (1969*a*, *b*), there are several possible mechanisms of colicin E1 action which would explain many of the phenomena observed (*see* subsequent discussion). The studies to be reported here were initiated to define the primary lethal event caused by colicin E1, presumably at the cytoplasmic membrane, and to determine the interrelationships of the lowered ATP levels, the block in biosynthesis, and the altered permeability characteristics of the membrane. The proton and K^+ permeability of colicin E1-treated bacteria were examined using the techniques described by Mitchell and Moyle (1967) and by Harold and Baarda (1968), and the changes in ATP levels on treating with colicin E1 under various conditions were determined. These studies have led to the following conclusions: the membranes of treated cells cannot retain K^+ but remain relatively impermeable to protons; a fall in ATP levels is not central to the action of the colicin; K^+ leakage and inhibition of protein and nucleic acid synthesis both occur in colicin-treated bacteria under conditions in which ATP levels rise rather than fall.

The interpretation of these studies rests on an understanding of the action of two compounds which were studied in association with colicin E1 treatment. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) is an inhibitor of oxidative phosphorylation, which apparently acts by specifically fostering proton permeability across biologic membranes (Heytler & Prichard, 1962; Mitchell, 1966); using this compound, we demonstrated both proton impermeability and K^+ permeability of colicin-treated cells. Dicyclohexylcarbodiimide (DCCD) is a membrane-reactive agent that has recently been shown to be a potent inhibitor of membrane-bound ATPase (Harold & Baarda, 1969); in the presence of this agent, ATP levels rise rather than fall on treatment (and killing) with colicin E1.

Materials and Methods

Bacteria, Media and Cultural Conditions

E. coli M72 is a colicin E1-sensitive, tryptophan-requiring K-12 strain from the collection of S. E. Luria. *E. coli* M42 is a colicin E1-tolerant amber mutant isolated from the wild type by Mr. Terek Schwartz. Cells were grown in LB broth consisting

of 1% tryptone, 0.5% yeast extract, and 0.5% NaCl adjusted to pH 7.2 with NaOH The minimal medium used was the phosphate-buffered medium A of Davis and Mingiol (1950) with glucose or glycerol as the carbon source; L-tryptophan was added at 50 μg/m and vitamin B_1 at 0.5 μg/ml. The phosphate buffer varied in the ratios of Na and K salts as indicated in the particular experimental protocols. The *Streptococcus faecalis* was a clinical isolate from the Diagnostic Bacteriology Laboratory of the Beth Israel Hospital. Cultures were incubated at 37 °C with vigorous rotary shaking.

Colicin E1 Production and Purification

Colicin E1 was produced and purified by a modification of the method of Schwartz and Helsinki (*personal communication*). The colicin-producing strain *E. coli* Y 20 (col E1) was grown from a small inoculum in 12 1-liter batches of broth in 4-liter flasks with vigorous aeration. At a Klett reading of approximately 110 (green filter), mitomysin C was added at 1 μg/ml, and the cultures were incubated for an additional 16 hr. The bacteria, collected by centrifugation, were suspended in 50 ml of 1 M NaCl buffered at pH 7.0 with 0.01 M potassium phosphate. After agitation at very low speed for 30 min in a Waring Blendor, the mixture was centrifuged for 10 min at $12{,}000 \times g$; the pellet was resuspended in the buffered saline and the extraction procedure was repeated twice; the pooled supernatants contained the colicin activity. The precipitate on going from 40 to 60% ammonium sulfate at 4 °C was taken up in 10 ml of 0.85% NaCl buffered at pH 7.0 with 0.01 M potassium phosphate buffer and dialyzed against this buffer until most of the precipitate dissolved. Further purification was performed by sequential DEAE-Sephadex and CM-Sephadex column chromatography in the cold. The DEAE-Sephadex column was equilibrated and developed using the saline-phosphate buffer. The active fractions, assayed by placing a drop on a lawn of sensitive bacteria on agar, were pooled, dialyzed against distilled water, lyophilized, dissolved in 10 ml of 0.05 M sodium borate buffer, pH 9.5, and applied to the CM-Sephadex column. This column was developed with 500 ml of the borate buffer and a linear gradient of 0.3 M KCl. The pooled active fractions were again dialyzed against water and lyophilized. The final product contained 3×10^{12} killing units/mg protein, and, when taken up in water, contained 1×10^{13} killing units/ml when assayed by the technique of Fields and Luria (1969*a*). Small aliquots were kept frozen at −70 °C.

Experiments Measuring pH Changes

Batches (100-ml) of growing cells in broth or minimal medium at 1 to 5×10^8/ml were filtered on a 142-mm diameter, 0.45-μ pore-size membrane filter (Millipore Filter Corp.), and washed extensively on the filter. The filtering and washing procedure took about 3 min. The cells were then scraped from the filter with a spatula and suspended in 10 ml of diluent. In the experiments for pH measurement, the wash solution was usually 50 mM KCl and the diluent 50 mM KCl containing 3.0×10^{-4} M Tris or phosphate buffer. The bacterial suspension at about 1×10^9/ml was transferred to a small beaker, agitated with a magnetic stirrer and capable of accommodating the electrodes of the pH meter. The pH was recorded constantly; the output of the pH meter (Radiometer) was 10 mV/pH unit, and the recorder (Varian Associates) was adjusted such that full deflection of the scale occurred from approximately pH 7.0 to 6.0.

^{42}K Experiments

The bacteria were grown for at least three generations either in minimal Na phosphate-buffered medium with 1 mM KCl or in LB broth, each containing about 200 μc ^{42}K/10 ml. Subsequent manipulation of these cells including washing and resuspension was always done with K^+-free solutions; in the presence of external K^+, a rapid K^+ exchange occurred releasing radioactivity to the medium. Cell suspensions for determination of the per cent of ^{42}K released to the medium were filtered through 0.45-μ pore-size membrane filters; the filtrate and the whole cell suspension were then counted in a Nuclear-Chicago Gamma Counter.

ATP Assay

ATP was assayed using dried firefly lanterns (Sigma) by a modification of the method of Stanley and Williams (1969) in which photon production was measured directly in a scintillation counter. In brief, samples to be assayed for ATP were heated to 100 °C for 10 min or brought to 10% perchloric acid (PCA) at 0 °C. The acid samples were neutralized with 5 N KOH and centrifuged before use. Samples (0.1 ml) were added directly to scintillation vials containing 0.2 ml of glycylglycine buffer, pH 7.4, and 1.65 ml of water. The reaction was initiated by addition of 0.05 ml of the enzyme (10 mg/ml, pH 7.4 in 0.05 M K_3AsO_4 and 0.02 M $MgSO_4$), and exactly 10 sec later the photon emission was counted over exactly 12 sec in a Packard Tricarb scintillation counter operating with the photomultipliers out-of-coincidence, at 100% amplification and at a setting of 60 to 65 divisions. Fig. 1 shows a typical calibration curve using standard solutions in ATP in 10% PCA; the assay was linear on a full logarithmic scale.

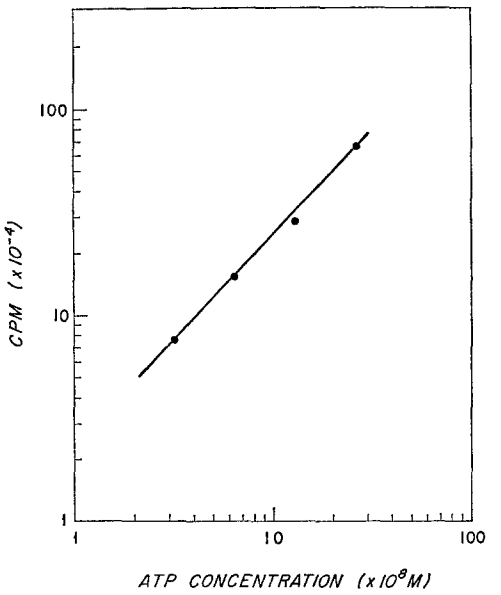

Fig. 1. Standard ATP solutions were acidified with perchloric acid, neutralized, and assayed as described in Materials and Methods

Synthesis of Protein and RNA

Incorporation of ^{14}C-leucine and ^{3}H-uracil into protein and RNA after treatment with colicin E1 was determined by measuring the incorporation of radioactivity into cold 5% trichloroacetic acid (TCA)-insoluble material. Samples (0.5 ml) of the bacterial cell suspensions were added to 2.5 ml of LB broth containing 1 μc of either ^{14}C-leucine or ^{3}H-uracil. A 1-ml portion was immediately removed and added to 1 ml of cold 10% TCA; the remainder was incubated for 10 min at 37 °C before another 1-ml amount was brought to 5% TCA. The acid precipitates were collected on membrane filters, washed well with cold 5% TCA, and the radioactivity present determined in a Beckman liquid scintillation counter.

Chemicals

CCCP and DCCD were purchased from Calbiochem. ^{42}K was obtained from Isoserve Corp. (Cambridge, Mass.). ^{14}C-leucine and ^{3}H-uracil were products of New England Nuclear Corp. All other chemicals were reagent grade purchased commercially.

Results

Effect of Colicin E1 on Response of E. coli *to an Acid Load and to CCCP*

HCl was added to a thick, lightly buffered (3×10^{-4} M Tris or phosphate buffer in 50 mM KCl) bacterial suspension to bring the pH from 6.8 to about 6.2. The probe measures the pH of the medium; if H^+ enters the bacteria, the pH will increase at a rate depending on the rate of entry of the protons. Figs. 2 and 3 depict typical experiments in which colicin E1-sensitive bacteria were exposed to heat-inactivated or potent colicin E1; at the times indicated, 100-ml samples of the treated or control cells were filtered, washed and resuspended as previously described. After initial resuspension in the lightly buffered medium, there was little change in pH in the absence of an energy source; if glucose was added (not shown), there was a steady fall in pH, presumably representing the excretion of organic acid, which was similar in control and colicin E1-treated bacteria. On adding HCl (5–10 μliters, 0.05 N), the pH fell and then stabilized after an initial rise. In the control bacterial suspensions (Figs. 2A, 3A & 3B), there was often a slow increase in pH with time (e.g., Fig. 3B), whereas the pH of the colicin-treated cell suspensions (Figs. 2B–D & 3C–E) tended to be more stable or show a slight further drop in pH (e.g., Fig. 3C). This was not a constant finding, however, and may reflect an increased acid secretion which occurs in colicin-treated bacteria. In the experiment shown in Fig. 4, colicin E1 was added after concentrating 100 ml of bacteria and lowering the pH with an acid load. Here, also, about 1 min after addition of the colicin, a slight but persistent fall in pH occurred (Fig. 4B) that was

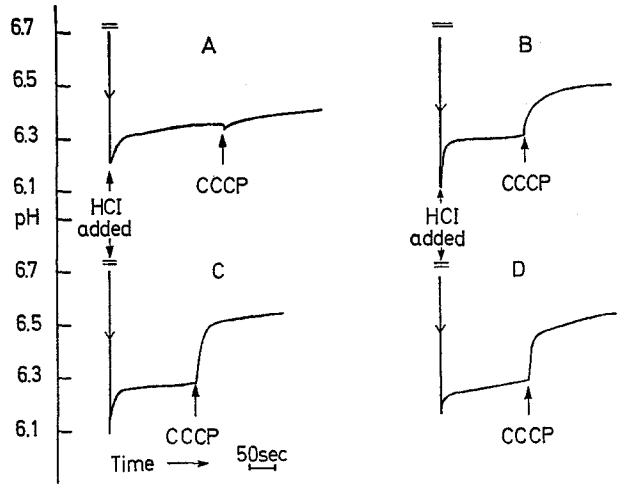

Fig. 2A–D. Batches (100 ml) of *E. coli* M72 growing in LB broth at 2×10^8/ml were treated as follows: (A) heat-inactivated (10 min at 100 °C) colicin E1 [20 killing units (KU)/cell] for 30 min; (B) colicin E1 (20 KU/cell) for 2 min; (C) colicin E1 (20 KU/cell) for 10 min; and (D) colicin E1 (20 KU/cell) for 30 min. After the treatment period, viable counts were performed, and the cultures were filtered, washed with 200 ml of 50 mM KCl and resuspended in 10 ml of 50 mM KCl in 3×10^{-4} M phosphate buffer, pH 6.8. Five µliters of 0.05 N HCl was added to lower the pH to about 6.2, and pH changes were recorded before and after the addition of CCCP at 1.7×10^{-6} M. Viable counts at 0 min $= 1.1 \times 10^9$/ml; after incubation (A) 1.9×10^9/ml, (B) 1.2×10^9/ml, (C) 1.7×10^6/ml, and (D) 10^6/ml

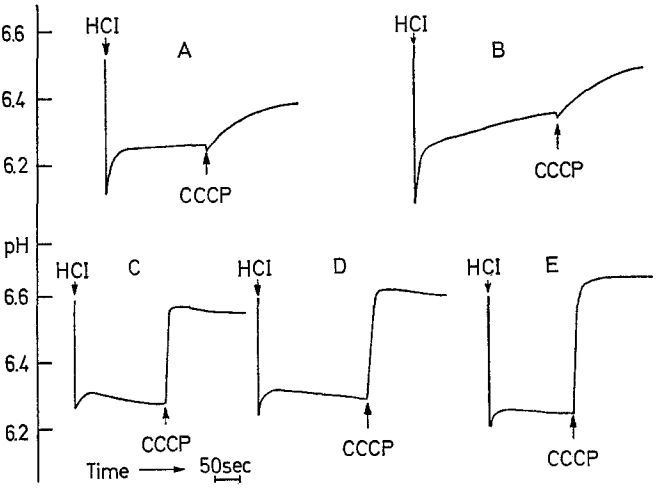

Fig. 3A–E. The protocol was identical to that describing Fig. 2 except that the final cell suspensions were 3.1×10^9/ml in 3×10^{-4} M Tris buffer, pH 6.8. A and B were suspensions of bacteria previously exposed to heat-activated colicin E1 (40 KU/cell). C, D and E were treated with colicin E1 (40 KU/cell) for 30 sec, 2 min, and 10 min, respectively, before filtration

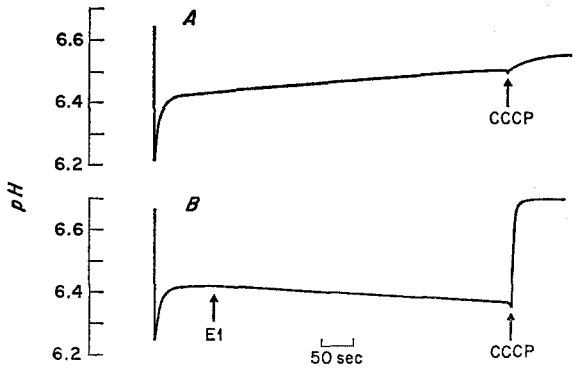

Fig. 4 A and B. The bacteria were filtered and resuspended at 3×10^9/ml in 3×10^{-4} M Tris buffer, pH 6.8, as described. After lowering the pH to about 6.3 with HCl, colicin E1 was added at the point indicated at 50 KU/cell (B). After 7 min, CCCP was added (1.7×10^{-6} M)

not seen in the control cells (Fig. 4A) or in a colicin-tolerant mutant treated with colicin E1. In summary, no major difference in H^+ permeability was observed in the treated and untreated cells, although a perceptible increase in acid secretion often occurred after addition of the colicin. Hirata and his co-workers (1969) reported a colicin K-stimulated excretion of alkaline ion from *E. coli*; this has not been with colicin E1 under the conditions of these experiments.

The addition of CCCP revealed a significant difference between the treated and untreated bacteria. At a concentration of 1.7×10^{-6} M, the pH in the control suspensions either remained unchanged (Fig. 2A) or increased gradually (Figs. 3A, 3B & 4A). In the colicin-treated bacterial suspensions, the addition of CCCP resulted in a precipitous rise in pH. This was a consistent finding and was not seen after treating the colicin-tolerant cells (*E. coli* M42) with colicin E1 or after killing the *E. coli* M72 with colicin E2 – a colicin with a different mechanism of lethal action. The CCCP effect on colicin E1-treated bacteria was similar when the cells were suspended in 50 mM NaCl or a mixture of Na^+, K^+, and Mg^{++} salts. Also, if the cells were first filtered and concentrated in the lightly buffered KCl, and then treated with colicin E1 after lowering the pH with HCl (Fig. 4), the response to the uncoupler was qualitatively similar.

The most reasonable interpretation of these results is that the uncoupler does foster H^+ permeability in the treated and untreated bacteria. Since, in the absence of an energy source, cellular cations could not move out to compensate for the entry of protons in the control bacteria, the pH rise was only slight or moderate. The membranes of the colicin E1-treated

bacteria were apparently more permeable; the inward flow of H^+ fostered by CCCP was not impeded by the development of an electrical gradient, and, hence, the precipitous pH rise was seen as the H^+ ions equilibrated across the membrane. Since K^+ is the major intracellular cation, it was presumed that the colicin E1-treated bacterial membranes were permeable to K^+. Similar interpretations have been offered for the effect of uncouplers on proton translocation across mitochondrial membranes (Mitchell & Moyle, 1967) and membranes of *S. faecalis* (Harold & Baarda, 1968). In these systems, a modest stimulation of proton permeability by the uncoupler was markedly accelerated by pretreatment with valinomycin, a macrocyclic antibiotic which fosters K^+ permeability. Fig. 5 depicts an experiment with *S. faecalis*, valinomycin and CCCP similar to one described by Harold and Baarda (1968). Qualitatively, the effects of valinomycin on the response of *S. faecalis* to CCCP were similar to those caused on *E. coli* by pretreatment with colicin E1. In other words, pretreatment of *E. coli* with colicin E1 or of *S. faecalis* with valinomycin facilitates the translocation of protons by allowing a cation to move in the opposite direction. Valinomycin cannot penetrate to *E. coli* membranes unless the bacteria are first exposed to ethylenediaminetetraacetate (EDTA); under these conditions the effect of valinomycin on *E. coli* is similar to that caused by colicin E1 (Pavlasova & Harold, 1969).

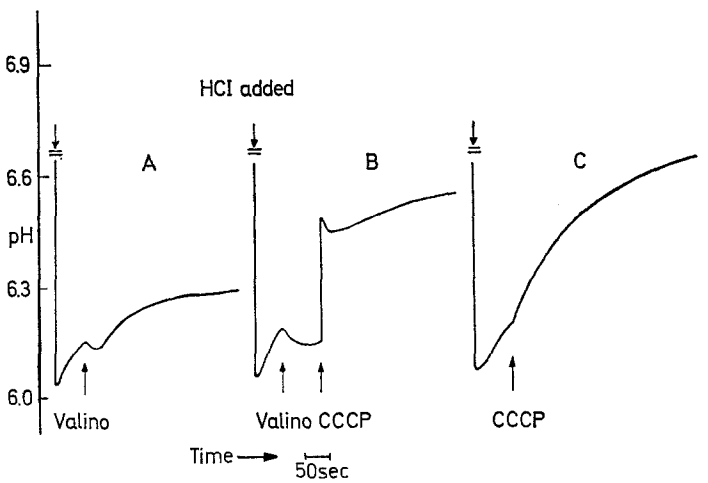

Fig. 5 A—C. *S. faecalis* were grown to about 3×10^8/ml in LB broth at 37 °C. The organisms were filtered, washed and resuspended in 50 mM KCl in 3×10^{-4} M Tris buffer, pH 6.8, at about 2×10^9/ml as described in Methods. After lowering the pH with HCl, the pH changes accompanying the following treatments were recorded: (A) valinomycin (5 μg/ml); (B) valinomycin (5 μg/ml) and then CCCP (1.7×10^{-6} M); and (C) CCCP (1.7×10^{-6} M)

^{42}K Leakage Caused by Colicin E1

The cells, loaded with ^{42}K and suspended in K^+-free medium, retain this cation avidly. In Fig. 6, the release of ^{42}K with time after addition of colicin E1 at a multiplicity of 10 killing units/bacterium is plotted. The K^+ loss is prompt and extensive. In the experiment depicted, within 8 min more than 90% of the intracellular ^{42}K is in the medium in the treated cells (more than 99% in some experiments); the controls lose ^{42}K only slowly. Luria (1964) has previously reported a rapid K^+ efflux caused by colicin E1, and Nomura and Maeda (1965) and Hirata *et al.* (1969) have noted a similar phenomenon with colicin K.

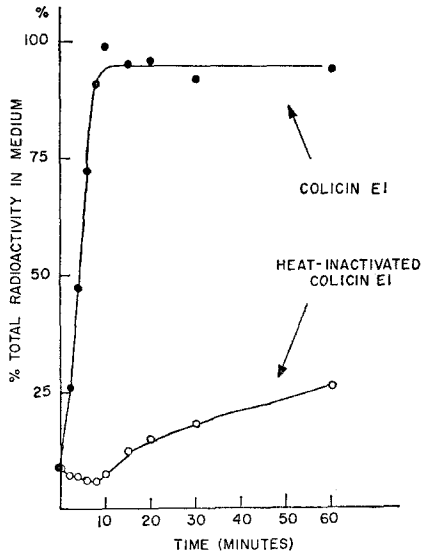

Fig. 6. *E. coli* M72 were loaded with ^{42}K as described in Methods and suspended at 1×10^8/ml in 50 ml of Na-phosphate buffered medium A. At 0 min, 25 ml was treated with colicin E1 (•—•) at 10 KU/cell and 25 ml was treated with heat-inactivated colicin E1 (○—○). At the times indicated, 2.0-ml samples were taken for filtration to determine the per cent ^{42}K released from the bacteria

Effect of Colicin E1 on ATP Levels: Influence of DCCD

In our hands, the fall in ATP levels on treating *E. coli* M72 growing in minimal medium on glycerol with E1 at a multiplicity of 20 killing units/cell averaged 72% within 5 min (six experiments with a range of 86 to 60%). In cells growing with glucose as the carbon source, the fall in ATP was less impressive, averaging only 39% (six experiments with a range of 58% to 18%). Presumably the net ATP synthesis during metabolism with glucose

Table 1. *Effect of colicin E1 on ATP concentration and viability in control and DCCD-treated* E. coli[a]

Time after addition of colicin E1 (min)	Control *E. coli*		DCCD-treated *E. coli*	
	ATP concn.[b]	Viable count/ml	ATP concn.[b]	Viable count/ml
0	67	6.7×10^8	59	5.5×10^8
2	26	—	65	—
5	24	—	92	—
30	20	2.9×10^7	146	2.3×10^7

[a] *E. coli* M72 were grown to about 5×10^8/ml in minimal medium with glycerol as the carbon source. The culture was divided and half was treated with DCCD at a final concentration of 10^{-4} M for 30 min at 37 °C. Colicin E1 was added at 10 KU/cell.

[b] Expressed as pmoles/10^8 bacteria.

as the carbon source is greater than that with glycerol, and more successful in replenishing the depleted stores.

Levinsohn *et al.* (1968) have shown that colicin I causes only about 50% reduction in the incorporation of ^{32}P into ATP in *E. coli.* Since colicin E1 rapidly stops the major ATP-consuming reactions (active transport and macromolecule biosynthesis), the fall in ATP levels seen with apparent continued synthesis is somewhat surprising. One plausible hypothesis is that the colicin directly activates a membrane-bound ATPase or that such an ATPase is indirectly activated, possibly by the loss of ions from the cells. If this is the case, DCCD, a potent inhibitor of membrane-bound ATPase (but not of the soluble enzyme), might be expected to block the fall in ATP caused by colicin E1 treatment. Table 1 shows a typical experiment. In the presence of 10^{-4} M DCCD, the ATP levels rose instead of falling coincident with the bactericidal action of the colicin. Since the bactericidal effect is determined after dilution, plating on agar, and overnight incubation for the generation of colonies from individual viable organisms, it was possible that the lethal action of the colicin did not occur until the concentration of DCCD was decreased by dilution. Thus it was of interest to examine the effect of pretreatment with DCCD on colicin E1-induced K^+ leakage and macromolecule synthesis, as well as ATP levels. Table 2 depicts the effect of colicin E1 action on the leakage of ^{42}K and the synthesis of protein and RNA in bacteria pretreated with DCCD. Extensive ^{42}K leakage and inhibition of incorporation of uracil and leucine into acid-insoluble material was caused by the colicin in spite of high levels of ATP. Thus it would appear that low ATP levels *per se* are not responsible for the effects of

Table 2. *Effect of colicin E1 on ATP concentration, ^{42}K leakage, RNA synthesis, and protein synthesis in DCCD-treated* E. coli[a]

Time after addition of colicin E1 (min)	ATP concn.[b]	% of total ^{42}K in medium	Incorporation of label into acid-insoluble fraction (% of control)	
			^{3}H-uracil	^{14}C-leucine
0	195	0	—	—
5	270	96 (8)	—	—
30	300 (220)[c]	>99 (20)	6 (100)	3 (100)

[a] *E. coli* M72 were grown to about 5×10^8/ml in minimal medium with glycerol as the carbon source. DCCD was added to a final concentration of 10^{-4} M, and the cells were incubated for 30 min at 37 °C before addition of colicin E1 at 20 KU/ml; control cells were treated with heat-inactivated colicin E1.

[b] Expressed as pmoles/10^8 bacteria.

[c] Values in parenthesis represent results with control cells.

colicin E1 although the presence of DCCD may interfere with the utilization of ATP.

Cellular ATP levels can be drastically reduced by washing the bacteria with 0.1 M Tris buffer, pH 8.0. When this was done to *E. coli* M72, they responded normally to an acid load and CCCP, although ATP levels were even lower than those observed in colicin E1 treatment. Thus high ATP levels induced by DCCD did not prevent K^+ leakage caused by the colicin, and low cellular ATP levels *per se* did not result in membranes which were leaky to cations as measured by response to CCCP following an acid load.

Discussion

The lethal action of the colicin proteins is consistent with single-hit killing kinetics (Nomura, 1963), meaning that each colicin molecule has a definite probability of killing a sensitive bacterium. An explanation of the mechanism of colicin E1 action must explain how one or a few lesions in the cell membrane result in a complete block in the biosynthesis of nucleic acids and proteins and in the cessation of most active transport. Nomura (1964) has suggested that an amplification of the effect of the colicin molecule occurs, possibly by some unknown spread of the effect throughout the membrane. Alternatively, however, a localized lesion in the cytoplasmic membrane can readily have generalized effects if the lesion results in dissipation of an essential concentration gradient by imposing a conduit of some type in the membrane. For example, according to Mitchell (1967), there is a gradient of protons or an electrochemical gradient across

biological membranes which is the driving force for the formation of high-energy phosphate bonds. Harold and his collaborators have demonstrated the association of the development of proton permeability of membranes and the loss of active transport by these membranes (Harold & Baarda, 1968). They suggest, in accordance with the chemiosmotic theory of Mitchell, that "a proton gradient across the membrane is directly involved in energy coupling or in the maintenance of an energized membrane conformation". Uncouplers of oxidative phosphorylation foster proton permeability resulting in collapse of the gradient. Fields and Luria (1969*b*) suggested that colicin E1, like uncouplers, may result in the dissipation of such a proton gradient. It is with this in mind that we examined the effect of colicin E1-treatment on the response of *E. coli* to an imposed acid load, and we find no evidence that the colicin abolishes H^+ impermeability as opposed to CCCP which does allow H^+ to enter the cells.

Whereas the responses of the normal *E. coli* and the colicin E1-treated bacteria to an acid load were quite similar, the subsequent effect of adding a potent uncoupler, CCCP, was dramatically different in the control and treated bacteria. This difference is best explained by a markedly increased cation permeability of the bacteria after exposure to colicin E1; presumably these bacteria can respond to the CCCP-induced influx of H^+ by an efflux of cations resulting in maximal collapse of the proton gradient. We have confirmed the previously cited observations that treatment with colicin E1 and colicin K result in an early and extensive leakage of K^+ from the bacteria. It is not yet clear whether colicin E1 also fosters the permeability to other cations, or whether the effect on K^+ permeability is a primary action of the colicin E1 protein or a secondary manifestation of colicin action.

The observations that the activity of colicin E1 resulted in markedly diminished cellular levels of ATP raised the possibility that many of the phenomena associated with the action of the colicin result from a restricted supply of ATP. Our experiments on the influence of DCCD pretreatment on colicin E1 action imply that low ATP levels *per se* are not the cause of either the leakage of K^+ from the cells or the cessation of macromolecule synthesis. In the presence of DCCD, ATP levels increased rather than decreased on treatment with colicin E1; however, prompt K^+ efflux and inhibition of protein and nucleic acid synthesis occurred even in the presence of large amounts of ATP. Since this inhibitor of membrane-bound ATPases abolishes the fall in ATP levels usually associated with colicin E1 action, it suggests that activation of a membrane-bound ATPase may be responsible for the fall usually observed. This activation could reflect a direct effect

of the colicin protein on the membrane or a secondary activation of ATPases related to the loss of cations from the bacterial cells.

The nature of the lesion which abolishes the K^+-retaining properties of the membrane is not clear. The similarity of the effect of colicin E1 and that of valinomycin on the response of *E. coli* to CCCP suggests that the colicin may act like one of these macrocyclic ionophore antibiotics, fostering K^+ (and possibly other cation) permeability by acting as membrane carrier molecules for the ion (Pressman, Harris, Jagger & Johnson, 1967). Whether the single lesion in the ability of the membranes to retain cations could have the multiple effects seen is problematical. Alternatively, colicin E1, or a product generated by the colicin, may result in conformational changes in the proteins or phospholipids of the cytoplasmic membrane. These changes may be allosteric in nature, and a model has been suggested to explain how such changes may be spread throughout the membrane (Changeux & Thiery, 1967).

When oxygen is strictly excluded from the reaction mixture, colicin E1 action is inhibited (Levinthal & Levinthal, *unpublished data* cited in Luria, 1964). Any theory for the action of the colicin should take this observed oxygen dependence into consideration. Thus, one should consider the hypothesis that colicin E1 is bactericidal by an oxidative mechanism similar to that which may be operative in the intracellular killing of bacteria in phagocytic cells. Recently, several bactericidal systems, some present in white blood cells, have been described; these involve the generation of H_2O_2 which, often through mediation of a peroxide and a halide, results in oxidative damage to the cell surface (Klebanoff, 1967, 1968; McRipley & Sbarra, 1967; Miller, 1969; Cline & Lehrer, 1969; Baehner, Gilman & Karnovsky, 1970). Oxidative membrane damage to lipids is manifest by attack on unsaturated fatty acids (May & McCay, 1968; Dormandy, 1969). Hence, the colicin may cause oxidative damage to the membrane; possibly this could occur with local stimulation of an enzyme such as NADPH oxidase or an amino acid oxidase which results in H_2O_2 production and the local evolution of potent oxidizing systems which attack membrane lipids. The autocatalytic nature of oxidation reactions may spread (amplify) a localized effect of the colicin. Such a theory, however, would be difficult to reconcile with the observed trypsin reversibility of colicin E1 action (Nomura & Nakamura, 1962) unless one assumed that the synthetic abilities of the bacteria were capable of repairing the colicin-induced oxidative membrane damage for a significant period of time after initiation of the colicin action, and that during this period digestion of the colicin from the cell surface by trypsin abolishes the destructive action.

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