Comparative Study of the Events Associated with Colicin Induction

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Colicinogenic factors ColI and ColV, which have been shown to behave as sex factors, could not be induced with mitomycin C. In contrast, the $ColE_1$, $ColE_2$, and $ColE_3$ factors, which do not exhibit any fertility factor characteristics, are inducible by this agent. The induced production of colicins E_1, E_2, and E_3 was accompanied by a loss in viability at a concentration of mitomycin C which was bacteriostatic to noncolicinogenic cells or to cells carrying the ColV or ColI factors. The loss in viability accompanying the mitomycin C induction of the $ColE_1$, $ColE_2$, or $ColE_3$ factors also occurred when colicin synthesis was blocked by chloramphenicol or amino acid starvation. However, chloramphenicol was able to block the loss of viability of a recipient cell after mitomycin C induction of a newly acquired Col factor if the antibiotic was present throughout the mating period. No detectable internal colicin or colicin precursor could be demonstrated during the lag period prior to the appearance of colicin outside the cell 20 to 30 min after the addition of mitomycin C. If chloramphenicol was present during the lag period following the addition of mitomycin C, colicin synthesis began immediately after the removal of these antibiotics. The synthesis of tryptophan synthetase and induced β-galactosidase proceeded normally throughout the lag period and well into the period of colicin production. Regulation of β-galactosidase synthesis did not seem to be profoundly affected during the lag period subsequent to mitomycin C addition. Induced colicin synthesis, like bacterial or induced prophage protein synthesis, was subject to inhibition by virulent phage infection.

Colicins are antibiotic proteins, produced by certain strains of *Enterobacteriaceae*, which are lethal for other related enteric bacteria. The ability to synthesize colicin depends upon the presence of a colicinogenic factor (Col factor). Certain Col factors are stable, nonessential genetic elements of plasmid nature (12) composed of deoxyribonucleic acid (4). Colicinogeny confers immunity to the host towards the homologous colicin in addition to the potential to synthesize colicin.

Col factors have been postulated to be defective fertility factors (16), defective lysogenic-phage genomes (15), and defective virulent-phage genomes (6), with little consideration given to the existence of physiological differences among various Col factors. Col factors differ in their ability to determine fertility properties (9), in their phage exclusion characteristics (21), and in their molecular weights (Roth and Helinski, Proc. Natl. Acad. Sci. U.S., *in press*). Colicins have historically been categorized on the basis of cross-immunity, serological cross-reactivity, and common cell-receptor sites as determined

by the mutation to multiple resistance to colicins (5). They have also been shown to have markedly different modes of action (14). Thus, there appears to be an extensive spectrum in the genetic, physiological, and physical characteristics of different Col factors and in the structure and function of various colicins.

The synthesis of certain colicins may be induced by agents such as ultraviolet light (15) and mitomycin C (8), as well as by thymine starvation (18). No extensive comparison of the potential or physiology of induction of various colicins has been carried out, however. In the present study, colicinogenic factors of varying fertility characteristics were examined in *Escherichia coli* for inducibility by bacteriostatic levels of mitomycin C, and some of the physiological events accompanying the induction of inducible colicins were established.

MATERIALS AND METHODS

Organisms. The bacterial strains employed in this study and their relevant characteristics are described in Table 1. In all cases, control experiments were per-

TABLE 1. *Bacterial strains*[a]

Strain	Requirements	Source of Col factor	Resistance
YS40	His, Pro		Sm
YS40V^r	His, Pro		Sm, V
YS40V^{r}T6^r	His, Pro		Sm, V, T6
YS40Az^{r}I^r	His, Pro		Az, Sm, I
YS40(ColE$_1$)	His, Pro	ColE$_1$ from K-30	Sm
W3110			
W3110Sm^r			Sm
W3110(ColE$_1$)		ColE$_1$ from K-30	
W3110(ColE$_2$)		ColE$_2$ from *Shigella* sp. P9	
W3110Sm^{r}E^r(ColI, ColE$_3$)		ColI and ColE$_3$ from CA38	Sm, E$_1$, E$_2$, E$_3$
W3110Sm^{r}E^r(ColE$_3$)		ColE$_3$ from CA38	Sm, E$_1$, E$_2$, E$_3$
W3110Sm^r(ColI)		ColI from *Shigella* sp. P9	Sm
W3110Sm^r(ColV)		ColV from K-94	Sm
JC411(ColE$_2$)	Leu, His Arg, Met	ColE$_2$ from *Shigella* sp. P9	Sm
Salmonella typhimurium LT2 (*cysD36* ColI)	Cys	ColI from *Shigella* sp. P9	

[a] Unless designated otherwise all strains are *Escherichia coli*. Abbreviations: His, histidine; Pro, proline; Leu, leucine; Arg, arginine; Met, methionine; Cys, cysteine; Sm, streptomycin; Az, azide; T6, phage T6; I, colicin I; V, colicin V; E$_1$, colicin E$_1$; E$_2$, colicin E$_2$; and E$_3$, colicin E$_3$.

formed with strains isogenic for all chromosomal characteristics and differing only in the absence of the colicinogenic factor. Strain W3110Sm^{r}E^r(ColE$_3$) was prepared by transferring ColE$_3$ from CA38(ColI, ColE$_3$) (obtained from P. Fredericq) and removing ColI by acridine orange treatment as previously described (9). *Salmonella typhimurium* LT2 *cysD36*(ColI) was kindly provided by M. Nomura.

Media. All induction experiments were performed with Casamino Acids-supplemented minimal medium containing (per liter): Na$_2$HPO$_4$, 7 g; KH$_2$PO$_4$, 3 g; NH$_4$Cl, 1 g; dextrose, 4 g; MgSO$_4$, 10^{-4}M FeCl$_3$, 10^{-6}M; and Casamino Acids (Difco), 3 g. Nutrient broth was Antibiotic Medium 3 (Difco). Hard nutrient agar was prepared with Antibiotic Medium 3 supplemented with 1.5% agar. Soft nutrient agar contained 0.65% agar. When necessary, streptomycin was added at a final concentration of 250 μg/ml.

Lacunae counts and colicin assays. Lacunae counts, a measure of the number of individual cells producing colicin, were performed according to the procedure of Ozeki et al. (15), with *E. coli* YS40V^r as the indicator strain. The quantitation of colicin was carried out by treating a colicinogenic culture with chloroform, serially diluting the culture in nutrient broth, and spotting a drop of each dilution on a nutrient agar plate freshly seeded with 10^7 indicator bacteria (*E. coli* YS40V^r) in 5 ml of soft nutrient agar. The number of colicin units per milliliter was defined as the highest dilution which gave a clear zone of inhibition of growth of the indicator bacteria. The double-layer technique of Fredericq (5) was used to determine the percentage of colicinogenic cells in a bacterial population.

Standard growth and induction conditions. Cultures were inoculated and grown overnight in minimal Casamino Acids medium in a water bath (37 C). To

initiate an induction experiment, the overnight culture was diluted to approximately 10^8 cells/ml with the supplemented minimal medium; 25 ml of the diluted culture was transferred to a 125-ml flask and placed in a rotary shaker (37 C). When the culture reached a bacterial concentration of approximately 5 × 10^8 cells/ml, mitomycin C (Nutritional Biochemicals Corp., Cleveland, Ohio) was added and incubation was continued at 37 C for the time required in each experiment.

Cell lysis. The lysis technique used was a modification of the lysozyme-ethylenediaminetetraacetic acid (EDTA)-sodium dodecyl sulfate technique. To 2.0 ml of culture to be lysed were added 1.8 ml of water, 0.2 ml of 0.07 M EDTA, and 0.08 ml of a 10 mg/ml lysozyme solution (Nutritional Biochemicals). After incubation for 5 min at 0 C, 0.04 ml of a 25% solution of sodium dodecyl sulfate was added. The preparation was diluted for assay immediately upon lysis, to minimize denaturation of colicin.

Enzyme assays. The tryptophan synthetase assay used was a modification of the method of Yanofsky (22), which measures the disappearance of indole due to conversion to tryptophan. Of the cells to be assayed, 1 ml was treated with a drop of toluene, stirred on a Vortex mixer, incubated for 10 min at 37 C, and allowed to settle in an ice bath; 0.5 ml of a reaction mixture containing 5 × 10^{-4} M glutathione, 8 × 10^{-2} M serine, 1.6 × 10^{-4} M pyridoxal phosphate, and 5 × 10^{-4} M indole in 0.1 M potassium phosphate buffer (pH 7.8) was added to 0.5 ml of toluene-treated cells. After incubation for 1 hr at 37 C, the reaction was stopped with 0.2 ml of 1 N NaOH, and the remaining indole was extracted in 2.0 ml of toluene. The amount of indole present in 1 ml of the toluene extract was determined by the *p*-dimethylaminobenzaldehyde color reaction described by Yanofsky (22).

β-Galactosidase was assayed by a modification of the method of Rickenberg et al. (17). Toluene-treated cells (0.2 ml) were diluted with 2.8 ml of 0.05 M sodium phosphate buffer (pH 7.0); 0.5 ml of a 5 mg/ml solution of o-nitrophenyl-β-D-galactoside (Calbiochem) was added at 37 C, and the reaction was allowed to proceed until suitable color had developed. The reaction was stopped by the addition of 2.0 ml of 1 N sodium carbonate, and the absorbancy was determined with a 420-mμ filter in a Klett-Summerson colorimeter.

Enzyme assays are reported in arbitrary units and given as the average of duplicate determinations.

RESULTS

Inducibility of Col factors of varying fertility properties. Strains possessing Col factors of varying degrees of fertility were grown to 5×10^8 cells per ml in the minimal Casamino Acids medium, and one-half of each culture was exposed to mitomycin C (0.2 μg/ml). Under these conditions, there was little or no stimulation of colicin I or colicin V synthesis due to the presence of mitomycin C (Table 2). In contrast, the production of colicins E_1, E_2, and E_3 was increased 100 to 1,000 times by the inducing conditions. In a separate experiment, colicin E_3 was shown to induce to the same level from strains W3110-(ColE₃) and W3110(ColI, ColE₃).

Mitomycin C induction as a lethal event for ColE₂. Ultraviolet light and temperature-sensitive induction of colicin E_2 have both been reported to be lethal phenomena (10, 15).

As illustrated in Fig. 1, concentrations of mitomycin C bacteriostatic for noncolicinogenic cells caused lethal induction of colicin E_2. Colicinogenic and noncolicinogenic cultures were grown to 5×10^8 cells/ml and exposed to mitomycin C (0.2 μg/ml). At the times shown, the cultures were assayed for viable cells and lacunae. At the
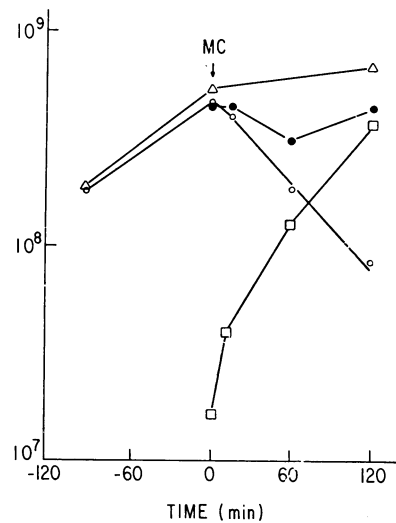

FIG. 1. *Correlation between loss of viability and lacunae production during mitomycin C (MC) induction. MC (0.2 μg/ml) was added to cultures of Escherichia coli W3110 and W3110(ColE₂) at the time indicated by the arrow. (○) Viable cells (per milliliter) of W3110(ColE₂); (△) viable cells of W3110; (□) lacunae from W3110(ColE₂); (●) lacunae plus viable cells of W3110(ColE₂).*

same time, samples of the induced W3110(ColE₂) culture were removed, washed on a membrane filter, resuspended in growth medium at 37 C, and assayed for colicin by the dilution assay 2 hr after the addition of mitomycin C. The majority of colicin was bound to the cells under these conditions, and less than 10% of the colicin activity was removed by filtration. A 2-hr period following mitomycin C addition before assaying was chosen to ensure maximal colicin production for each sample. Bacteria carrying ColE₂ were committed to the inability to give rise to a colony with first-order kinetics when exposed to mitomycin C at a concentration bacteriostatic to the control noncolicinogenic bacteria. The decrease in viable cells was accompanied by a directly proportional increase in lacunae, or individual cells producing colicin, indicating that each cell committed to death became induced for the synthesis of colicin. The potential to produce lacunae and colicin increased throughout the induction period (Table 3).

Correlation of induction of colicin synthesis and lethality for various Col factors. ColE₁, ColE₂, and ColE₃ strains produced relatively large amounts of their respective colicins after exposure to mitomycin C, whereas ColI and ColV cultures were not induced under the conditions employed in these experiments. To deter-

TABLE 2. *Inducibility of various colicins[a]*

Escherichia coli strain	Without mitomycin C	With mitomycin C (0.2 μg/ml)
W3110(ColV)	8	8
W3110(ColI)	1	1
W3110(ColE₁)	20	15,000
W3110(ColE₂)	800	80,000
W3110(ColI, ColE₃)	2,000[b]	200,000[b]

[a] Each culture was divided into two portions, one of which was exposed to mitomycin C. Both were then incubated for an additional 2 hr at 37 C and then assayed for colicin production (units per milliliter).

[b] Assay of W3110(ColI, ColE₃) on a colicin E-resistant indicator strain showed less than 1 unit of colicin I in both uninduced and induced cultures.

mine whether lethality is a common consequence of some aspect of the induction process for all of the inducible Col factors, colicinogenic and non-colicinogenic cultures were exposed to mitomycin C, and viable counts were carried out to determine the kinetics of commitment to death of colicinogenic bacteria. The viability curves (Fig. 2) confirm that the inducibility of the various colicins examined (Table 2) is reflected in the rates of kill upon exposure to a concentration of mitomycin C which is bacteriostatic to noncolicinogenic control cultures. In a separate experiment, the loss of viability of W3110(ColI, ColE₃) and W3110(ColE₃) due to mitomycin C induction was shown to be identical.

Separation of loss of viability and induced colicin

TABLE 3. *Relationship between lacunae production and potential for colicin production after mitomycin C induction of the ColE₂ factor*[a]

Time of removal of mitomycin C	No. of lacunae/ml	Colicin activity 120 min after addition of mitomycin C
min		*units/ml*
0	0.14 × 10⁸	100
15	0.40 × 10⁸	1,500
60	1.24 × 10⁸	3,000
120	3.60 × 10⁸	4.000

[a] At the indicated times after addition of mitomycin C (0.2 µg/ml), 3.0-ml samples were washed on a membrane filter and suspended in fresh medium. Lacunae were assayed on 1-ml portions. The remaining 2-ml portion was placed in a water bath (37 C) and assayed for colicin production 120 min after the addition of mitomycin C.
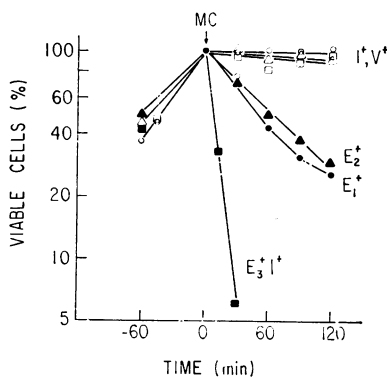

FIG. 2. *Induced commitment to death of cells carrying different Col factors. Cultures were induced with MC (0.2 µg/ml). W3110 (○); W3110(ColI) (△); W3110(ColV) (□); W3110(ColE₁) (●); W3110 (ColE₂) (▲); W3110(ColI, ColE₃) (■).*

production. Ozeki et al. (15) suggested that the production of colicin was responsible for the death of the induced cell. If this were the case, exposure of inducible colicinogenic bacteria to mitomycin C in the presence of chloramphenicol should protect the host from death. Figure 3 shows that cultures carrying ColE₁, ColE₂, or ColE₃ died in the presence of chloramphenicol after the addition of mitomycin C. Under these conditions, there was no detectable increase in colicin titer. Mitomycin C in the presence of chloramphenicol did not cause the death of non-colicinogenic bacteria. The synthesis of colicin, therefore, is not responsible for the loss of viability. Some other aspect of Col factor induction that is independent of colicin production must cause the commitment to death of the induced cell.

Requirement for protein synthesis by the host-Col factor complex for susceptibility to induction. Although protein synthesis does not appear to be necessary for induction, as defined by commitment to death, in a host which has been carrying the ColE₁, ColE₂, or ColE₃ factor, it is possible that protein synthesis must take place after the acquisition of a newly transferred Col factor for induction to occur. It was possible to examine this question by transferring the ColE₂ factor from a streptomycin-sensitive donor into a noncolicino-
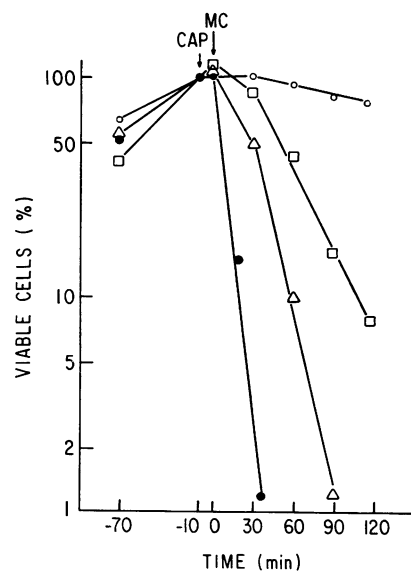

FIG. 3. *Induction of colicinogenic bacteria in the absence of protein synthesis. Chloramphenicol (CAP; 50 µg/ml) was added at the time indicated, and the cultures were then treated with MC (0.1 µg/ml). Control, W3110 (○); YS40(ColE₁) (□); W3110(ColE₂) (△); W3110(ColI, ColE₃) (●).*

genic, streptomycin-resistant recipient in the
presence of chloramphenicol, by use of the ColI-
promoted ColE$_2$-transfer system of Stocker et al.
(20). The source of ColE$_2$ was W3110(ColE$_2$).
S. typhimurium LT2 cysD36(ColI) provided the
ColI factor for promotion of transfer of ColE$_2$
to the W3110Sm^r recipient. The mating mixture
was then exposed to mitomycin C in the presence
of chloramphenicol, and the viability of the re-
cipients was determined by plating on streptomy-
cin-containing plates after vortexing and dilut-
ing the mating mixture. As seen in Fig. 4, if pro-
tein synthesis was allowed to occur during and
subsequent to transfer of the factor to its new
host, the newly acquired factor was inducible,
causing a commitment to death of the recipient
cell. When chloramphenicol was present through-
out mating and during the period of exposure to
mitomycin C, cell death did not occur, despite
the fact that more than 90% of the recipient cells
received the ColE$_2$ factor. Transfer of the ColE$_2$
factor under these conditions was measured after
separating the mating pairs by vortexing, dilu-
tion, and counterselecting the donor of the ColE$_2$
factor with streptomycin. Although protein syn-
thesis is not necessary for induction (as defined by
commitment to death) of a stable Col factor, a
chloramphenicol-sensitive step is necessary for
the susceptibility of a newly acquired Col factor
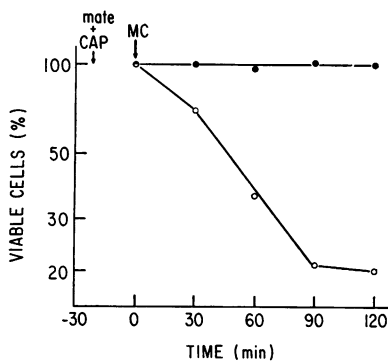to mitomycin C induction.

*Lack of a detectable pool of internal colicin or
colicin precursor during the lag period.* Following
induction of colicins E$_2$ and E$_3$ by mitomycin C,
there is a lag period of 30 and 20 min, respectively,
before the titer of colicin as measured in the dilu-
tion assay begins to increase. The assay tech-
nique employed, however, measures only the
appearance of extracellular colicin. The time lag
in appearance of colicin following induction may
represent the time required for colicin synthesis,
the time required for activation of an inactive
colicin precursor, the time required for transport
of newly synthesized colicin through the cell
membrane, or some combination of these factors.
To determine whether an increase in internal
colicin occurs during the lag period, cultures
singly colicinogenic for ColE$_2$ and ColE$_3$ were
induced with mitomycin C and assayed at various
times to determine external colicin. At the same
time, samples were lysed and assayed to deter-
mine internal plus external colicin. The lack of
any detectable increase in internal colicin prior
to the appearance of colicin activity on the chloro-
formed cells is illustrated in Fig. 5. Despite the
loss in activity due to the lysis procedure, it is
evident that a significantly large pool of active
colicin is not built up during the lag period.

In the early period following induction, an
inactive precursor of colicin may be synthesized
and converted to an active form at the end of the
apparent lag period. To test this possibility, mito-
mycin C was added to cultures of ColE$_2$$^+$ and
ColE$_3$$^+$ cells, and then chloramphenicol was

FIG. 4. *Protein-synthesis requirement for the induc-
tion of a newly acquired colicinogenic factor. Transfer
of the ColE$_2$ factor from Escherichia coli W3110(ColE$_2$)
to W3110Sm^r was allowed to proceed for 20 min in the
presence and absence of chloramphenicol (CAP; 50
μg/ml). CAP was added 20 sec after mixing the donor
and recipient cells in order to allow effective mating
pairs to form. Mitomycin C (0.1 μg/ml) was then added
to both mating mixtures, and the viability of recipient
cells was followed by plating in the presence of strepto-
mycin. Salmonella typhimurium LT2 cysD36(ColI) ×
W3110(ColE$_2$) × W3110Sm^r + CAP (●);
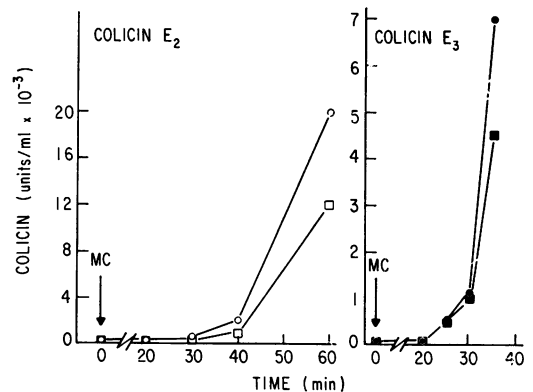LT2 cysD36(ColI) × W3110(ColE$_2$) × W3110Sm^r
(○).*

FIG. 5. *Lack of an internal pool of colicin after
induction. Cultures of Escherichia coli W3110(ColE$_2$)
and W3110(ColI, ColE$_3$) were induced with mitomycin
C (0.2 μg/ml). At the times indicated, one portion was
chloroformed and assayed for external colicin produc-
tion, and another portion was lysed and assayed to
determine internal and external colicin. W3110(ColE$_2$)
(○); lysed W3110(ColE$_2$) (□); W3110(ColI, ColE$_3$)
(●); lysed W3110(ColI, ColE$_3$) (■).*

added at various times to samples of each cul-
ture. The samples were allowed to incubate for a
total of 90 min after mitomycin C addition before
assay. There was no increase in colicin titer for
either colicin E_2 or E_3 after the addition of chlor-
amphenicol, regardless of the time of addition
during the lag period. These results indicate that
either a large pool of inactive precursor did not
accumulate, or its conversion to an active colicin
molecule was prevented by blocking protein syn-
thesis.

*Effect of inhibition of protein synthesis on the lag
period.* As shown above, induction as defined by
loss of viability in $ColE_2^+$ and $ColE_3^+$ cultures
occurs under conditions in which colicin synthesis
is prevented. It was of interest to determine
whether the events occurring during the lag period
leading to colicin production require protein
synthesis. Mitomycin C induction of colicinogenic
cultures was allowed to proceed in the presence of
chloramphenicol. Mitomycin C and chloram-
phenicol were removed by filtration, the cells
were resuspended in fresh medium, and the
kinetics of colicin production were followed by
assaying for colicin activity at 5-min intervals
after removal of these agents. Control colicino-
genic cultures were induced in the same fashion
without chloramphenicol. Both colicin E_2 and
E_3 synthesis began with little or no apparent lag
after the removal of chloramphenicol (Fig. 6),
whereas control cultures showed the charac-
teristic lag previously described.

In a similar experiment, an amino acid-re-
quiring $ColE_2^+$ strain was induced under condi-
tions of amino acid starvation to prevent protein
synthesis. The kinetics of colicin production
following restoration of the required amino
acid were similar to those of the previously
described experiment, again indicating that pro-
tein synthesis is not involved in the events
occurring early in the lag period which lead to
the subsequent production of colicin.

*Synthesis of bacterial enzymes during the coli-
colicin lag period.* $ColE_2$- and $ColE_3$-containing
cultures and a noncolicinogenic strain were
grown for two generations in the minimal Casa-
mino Acids medium supplemented with 2×10^{-3}
M isopropylthiogalactoside (IPTG) to derepress
β-galactosidase synthesis, exposed to mitomycin
C, and assayed for colicin, tryptophan synthe-
tase, and β-galactosidase production at the times
indicated in Fig. 7. The synthesis of both enzymes
continued at a rate similar to the control non-
colicinogenic culture throughout the lag period
and well into the period of colicin production,
diminishing when the rate of synthesis of colicin
also began to decrease.
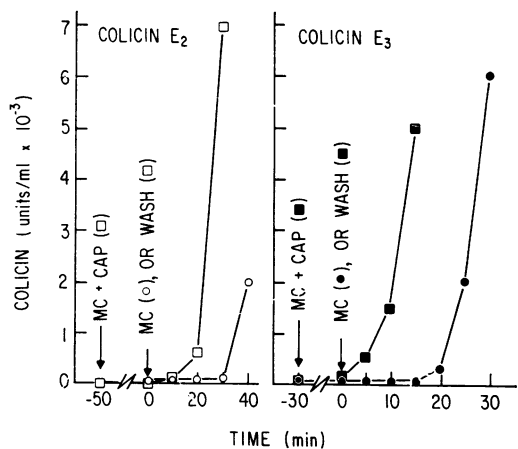
Although the induction of $ColE_2^+$ and $ColE_3^+$

FIG. 6. *Kinetics of induced colicin production after
removal of chloramphenicol. Cultures of Escherichia
coli W3110(ColE₂) and W3110(ColI, ColE₃) were pre-
pared for induction in the usual fashion, and were di-
vided into two portions. The first was induced with mito-
mycin C (0.2 µg/ml) at time zero, and colicin assays
were performed as indicated. Chloramphenicol (50
µg/ml) and mitomycin C (0.2 µg/ml) were added to
the second portion at time −50 min (ColE₂⁺) and −30
min (ColE₃⁺). These cultures were washed on membrane
filters and suspended in fresh medium at time zero, and
the synthesis of colicin was determined at various times.
W3110(ColE₂) + MC (○); W3110(ColE₂) + MC +
CAP (□); W3110(ColI, ColE₃) + MC (●); W3110
(ColI, ColE₃) + MC + CAP (■).*

cultures does not prevent synthesis of at least
certain host enzymes during the lag period, it
could interfere with the regulation of protein
synthesis in the host. To examine this question,
the colicinogenic cultures, grown with glucose
as a carbon source, were assayed for β-galactosi-
dase activity following colicin induction, and
were also tested for their ability to respond to
IPTG during the period after exposure of the
cultures to mitomycin C. β-Galactosidase syn-
thesis could be induced by IPTG during the lag
period of both colicin E_2 and E_3 induction. In
addition, induction of either colicin by mito-
mycin C did not result in a release of β-galactosi-
dase repression in the absence of its specific
inducer.

Inhibition of colicin production by virulent phage.
It is well established that bacterial protein
synthesis stops almost immediately after infec-
tion with T-even phages. Similarly, lysogenic
phage induction can be prevented by infection
with a T-even phage (19). To determine whether
colicin induction is susceptible to virulent phage
shut-off, $ColE_2^+$ and $ColE_3^+$ cultures were
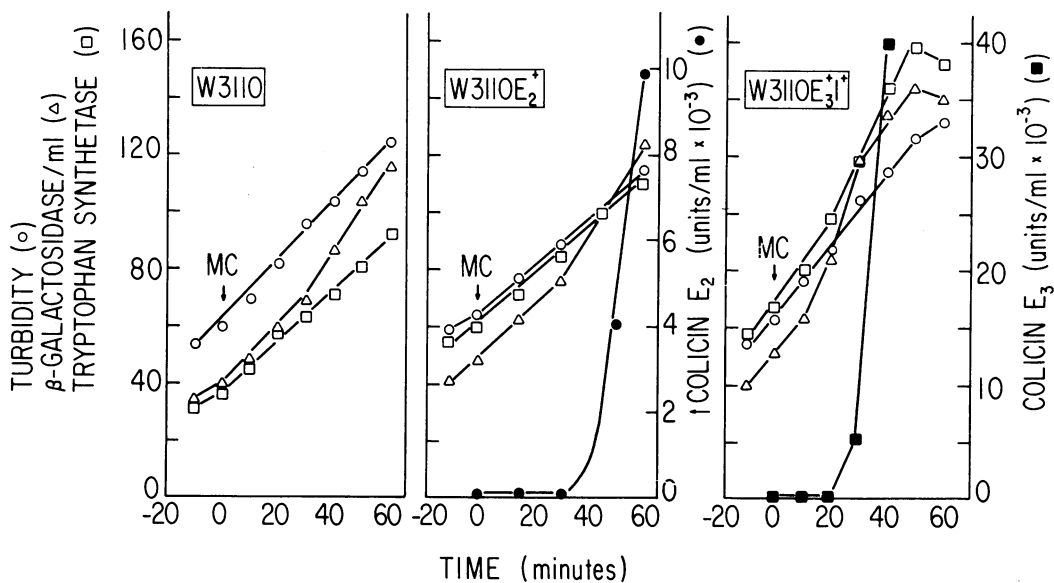induced with mitomycin C, and samples were
infected at various times with phage T6 at a

FIG. 7. *Synthesis of bacterial enzymes during induction. Mitomycin C (0.2 µg/ml) was added to cultures of W3110, W3110(ColE₂), and W3110(ColI, ColE₃) as indicated by the arrows. Culture turbidity (○); β-galactosidase activity (△); tryptophan synthetase activity (□); colicin E₂ activity (●); colicin E₃ activity (■).*

multiplicity of 10. The samples were allowed to incubate for a total of 90 min after the addition of mitomycin C, and were assayed for colicin production on a phage-resistant indicator. The results of these experiments showed that both colicin E_2 and E_3 synthesis were shut off immediately after virulent phage infection throughout the entire induction period.

DISCUSSION

The experiments described above clearly indicate that the colicinogenic factors ColV and ColI employed in these studies are physiologically quite distinct from Col factors $ColE_1$, $ColE_2$, and $ColE_3$. ColV and ColI are not induced with respect to colicin production after exposure to mitomycin C. Colicinogenic factors $ColE_1$, $ColE_2$, and $ColE_3$, however, are significantly induced by this agent. Genetic analysis has shown that ColV is intimately associated with a fertility factor (9). ColI has been demonstrated to be a highly efficient fertility factor following recent entry into a new host (3). In contrast to the marked ability of ColV and ColI to act as fertility agents in *E. coli*, $ColE_1$ is not capable of promoting either its own transfer or the transfer of chromosomal markers to noncolicinogenic recipients (9, 13). Its transfer to a noncolicinogenic cell is dependent upon the presence of fertility factors such as the F factor, ColV, or ColI. $ColE_2$ is also unable to

promote its own transfer or the transfer of any other genetic material (*unpublished data*). Transfer of $ColE_2$ can be carried out with high efficiency, as described above, by a modification of the ColI transfer system of Stocker et al. (20). However, Hfr- or F⁺-promoted transfer of $ColE_2$ is quite low, in contrast to the very effective transfer of $ColE_1$ in the presence of these fertility factors (12, 13). $ColE_3$ is similarly unable to promote its own transfer and appears to be transferred only at a very low frequency by fertility factors present in the same cell (*unpublished data*). Thus, those Col factors (ColV, ColI) which behave as sex factors appear to be uninducible by mitomycin C under the conditions used in this study, and do not render a cell significantly more sensitive to mitomycin C induced-killing than noncolicinogenic bacteria. $ColE_1$, $ColE_2$, and $ColE_3$, which cannot serve as effective fertility agents, are highly inducible. In addition, cells possessing these factors are readily killed by concentrations of mitomycin C that do not kill noncolicinogenic bacteria. The difference in response to mitomycin C of these two classes of Col factors may well reflect fundamental differences in the processes responsible for the regulation of the replication and phenotypic expression of these distinct nonchromosomal genetic elements. It should be pointed out that, although the results described here with mitomycin C distinguish between the Col factors

ColI and ColV, on the one hand, and ColE$_1$, ColE$_2$, and ColE$_3$, on the other, ultraviolet light induction of an *E. coli* strain carrying ColI has been reported to produce an increase in both the number of lacunae and the colicin I titer (3).

Ozeki et al. (15) demonstrated that ultraviolet induction of an *S. typhimurium* strain carrying ColE$_2$ produces an increase in the number of lacunae present in the cell population. They also demonstrated by micromanipulation techniques that single cells of *S. typhimurium* that have produced colicin after ultraviolet-light induction are not able to divide, and on this basis concluded that induction is a lethal event. Ben Gurion (2) reported that, despite the increase in lacunae after irradiation, ColE$_2^+$ cells grown in minimal medium do not show an increased sensitivity to ultraviolet-induced killing. In her studies, colicinogenic cells grown in broth, in contrast, showed a marked increase in sensitivity to ultraviolet-light irradiation, although there did not appear to be any significant difference in lacunae production after irradiation of colicinogenic cells grown in poor and rich media. In contrast to the results of Ben Gurion, Amati (1) reported that cells carrying colicinogenic factors ColI, ColE$_1$, and ColE$_2$, when grown in broth, did not show an increased sensitivity to ultraviolet light. Kohiyama and Nomura (10) isolated a temperature-sensitive chromosomal mutant of an *E. coli* ColE$_2^+$ strain in which increased temperatures caused colicin induction and a loss of viability. They did not report, however, the effect of elevated temperatures on the loss of viability of the mutant strain after curing of the ColE$_2$ factor. With a system in which the question of lethality due to induction is not obscured by death of the host due to the action of the inducing agent on the cell, the experiments described above indicate that mitomycin C induction of strains possessing ColE$_1$, ColE$_2$, or ColE$_3$ is accompanied by death of the host bacterium.

Ozeki et al. (15) suggested that the production of colicin was the cause of death of the host bacterium. It is evident from the above data, however, that chloramphenicol is able to prevent the production of colicins E$_1$, E$_2$, and E$_3$ but not the loss of viability following the addition of mitomycin C to colicinogenic bacteria. Commitment to death is, therefore, not dependent on the synthesis of colicin, or presumably any other protein, after the addition of mitomycin C. The commitment to cell death must result from some event in the induction sequence not requiring protein synthesis. This result is quite similar to the results described by Lieb (11), demonstrating

that heat induction of certain temperature-inducible λ phage mutants could cause death of the host, despite inhibition of protein synthesis by chloramphenicol.

The induction of colicinogenic factors does not cause a sudden cessation of host function. Bacterial enzyme synthesis appears to proceed quite normally during the lag period, and even during much of the period of colicin synthesis. Therefore, the death of the host does not appear to be caused by a Col factor-induced shut-off of host-specific protein synthesis during the lag period. The lack of any significant effect of induced colicin production on β-galactosidase repression or induction during the lag period suggests that regulation of the synthesis of at least certain host proteins is not affected during the lag period and is likewise probably not the cause of cell death.

Our observation that a cell containing a newly acquired Col factor could not be induced if deprived of the opportunity to synthesize protein prior to exposure to mitomycin C is surprising, since it suggests that Col factor-directed synthesis of protein may be required for the initiation of induction. The presence of the non-induced Col factor confers immunity on the host cell, indicating that the noninduced Col factor is certainly not completely dormant physiologically. It is not unlikely that the non-induced factor directs the synthesis of a protein whose activity is required to initiate induction, while not being transcribed in the noninduced state for postinduction proteins such as colicin. Such a protein normally may play a role in the replication or maintenance of the Col factor. Green (7) proposed that cells lysogenic for the temperature inducible λ prophage described above must synthesize an "inducing protein" in the repressed state to permit prophage susceptibility to thermal induction. It is also possible that acquisition of a Col factor induces the synthesis of a protein determined by the bacterial chromosome and required for the maintenance of the factor and also for the initiation of induction.

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