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Author(s)	Shiba, Shigeru; Terawaki, Asaharu; Taguchi, Tetsuo et al.
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Studies on the Effect of Mitomycin C on Nucleic Acid Metabolism in *Escherichia coli* Strain B*

SHIGERU SHIBA, ASAHARU TERAWAKI, TETSUO TAGUCHI AND JUNICHI KAWAMATA

*Surgical Division, Department of Clinical Research, and the Department of Chemotherapy,
Research Institute for Microbial Diseases, Osaka University*

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SUMMARY

By using an exponentially growing culture of *E. coli* strain B, the action of mitomycin C was studied in terms of its effect on the growth and the biosynthesis of protein, RNA and particularly DNA of the organism. The results were as follows:

1. When growth of *E. coli* strain B was measured by the optical density, it was found that the organism grew exponentially (or normally) up to 90 minutes incubation even in the presence of 0.02-0.1 $\mu\text{g./ml.}$ of mitomycin C and after that time, the rate of growth gradually decreased. The viability of the culture, on the other hand, was found to be strikingly reduced. Thus, after 90 minutes incubation in the presence of 0.02-0.1 $\mu\text{g./ml.}$ of the antibiotic, the number of viable cells was only about 5 per cent of the value at the start of incubation.

2. Determination of the content of protein, RNA and DNA of the culture during 90 minutes incubation revealed that 0.02-0.1 $\mu\text{g./ml.}$ of mitomycin C completely inhibited the biosynthesis of DNA by the organism while that of protein and of RNA were entirely unaffected.

3. Similar effects on DNA synthesis of the antibiotic was observed in culture of *E. coli* strain B even after repeated washings of the cells with mitomycin C-free medium which had been in contact with the antibiotic in the medium lacking glucose.

4. The inhibitory effect of mitomycin C on DNA synthesis was found to be reduced to some extent by adding baker's yeast extract to the test medium.

INTRODUCTION

Mitomycin C (abbreviated as MC) is a new antibiotic isolated by Wakaki *et al.* (1956) from *Streptomyces caespitosus*. Since 1956 many papers have appeared on it, all of which suggest that the antibiotic has a remarkable effect on various transplantable animal tumors and that it can be regarded as one of the most effective antitumor substances (Shiba, 1957; Taguchi, 1957; Sugiura, 1958; Teranaka, 1958; Usubuchi, 1958). It has also been reported by Hata *et al.* (1956) that the antibiotic exerts a strong antibacterial activity against not only gram-positive and gram-negative bacteria but even against some kind of viruses.

It is one of the characteristic features of MC that it possesses a marked antimicrobial activity besides the antitumor activity. This suggests that the same mechanism of the antibiotic is involved in its antimicrobial and antitumor activities and further that analysis of the former may become an important clue for elucidating the yet unstudied intrinsic action of the antibiotic on tumor cells.

Therefore, attempts have been made to reveal the biochemical mechanism involved in the antibacterial activity using strains of *E. coli*.

In this paper are reported the results of studies of the effect of MC on the growth, the biosynthesis of protein and of nucleic acids in *E. coli* strain B.

Materials and Methods

Organisms: *E. coli* B and *E. coli* 15 T⁻ were used throughout the work. These strains were obtained from the collection of this Institute and maintained on slants of nutrient agar.

Preparation of cell suspensions: A small amount of bacterial culture was transferred to Glucose-Simmons' medium (PH 7.0) and incubated at 35°C with shaking at 120 strokes per minute for 12-16 hours. Two ml. of this culture was transferred to 150 ml. of a fresh Glucose-Simmons' medium and incubated again at 35°C with shaking for 2.5 hours. The exponential growing culture were then centrifuged and the cells were collected. The cells obtained were diluted with Glucose-Simmons' medium to a final optical density 0.085-0.090 at 660 m μ . The cell suspension was then dispensed in a T-shaped tube (20 mm. in diameter) similar to that devised by Monod *et al.* (1949).

To the diluted cell suspension 1 ml. of appropriately diluted MC in a warm Glucose-Simmons' medium finally added. As a control, 1 ml. of Glucose-Simmons' medium was added to a similar cell suspension. Both the cell suspension containing MC and the control were then incubated at 35°C with shaking. During the incubation, determinations were made, at intervals of growth and protein and nucleic acid content. When necessary, viable cell counts were also made. The number of viable cells in the suspension was found to be 0.8×10^8 per ml.

Assay of growth: Growth was measured as an optical density at 660 m μ in the Coleman Jr. Spectrophotometer. For determination of viability, aliquots of the whole culture properly diluted with physiological saline were plated on agar, and the colonies counted after incubation at 37°C for 16 hours.

Assay of nucleic acids and protein: Nucleic acids were extracted with perchloric acid according to the method of Schneider (1946). Two ml. of the whole culture was mixed with an equal volume of 6% perchloric acid solution and kept in an ice-bath for 40 minutes. Then the precipitate was washed twice with 3% perchloric acid, centrifuged, and the nucleic acids in the precipitate were extracted into 2 ml. of 6% perchloric acid, by heating the sample at 90°C for 15 minutes. After centrifugation, the nucleic acids in a 1 ml. aliquot of the supernatant were measured by the orcinol reaction for RNA (Ceriotti, 1955) and diphenylamine reaction for DNA (Burton, 1956) respectively.

The protein content of the whole culture was determined by the Folin reaction, according to Earl *et al.* (1949) using a 0.5 ml. aliquot of the culture.

Measurements of adaptive formation of β -galactosidase: 2.5 hour cultures of *E. coli* were centrifuged, washed twice with physiological saline and suspended in the medium lacking glucose to final optical density of 0.085-0.090 in a tube of 20 mm. diameter. The prepared cells suspension were divided into two parts.

To one part was added 1 ml. of MC solution and, to the other, 1 ml. of sterile distilled water. The tubes were then incubated at 35°C with shaking for 15 minutes and at the end of the incubation were centrifuged and the cells washed. The washed cells were used for experiments.

Adaptive formation of β -galactosidase was determined manometrically. For assaying the adaptive formation of β -galactosidase, each flask contained 1 ml. of cell suspension, 0.4 ml. of M/15 phosphate buffer (PH 7.0), 0.2 ml. of M/40 lactose, and 0.2 ml. of distilled water or MC solution.

MC: MC used was obtained from Kyowa Fermentation Industry Co., Ltd., Tokyo. (lot number 102.)

Yeast extract: Baker's yeast extract prepared by Oriental Yeast Co., Ltd. was used.

RESULTS

1. Effect of MC on the growth and viability in *E. coli* B

Growth curves of exponentially growing culture of *E. coli* B in the presence and absence of MC are presented in Fig. 1. In MC-free medium the turbidity

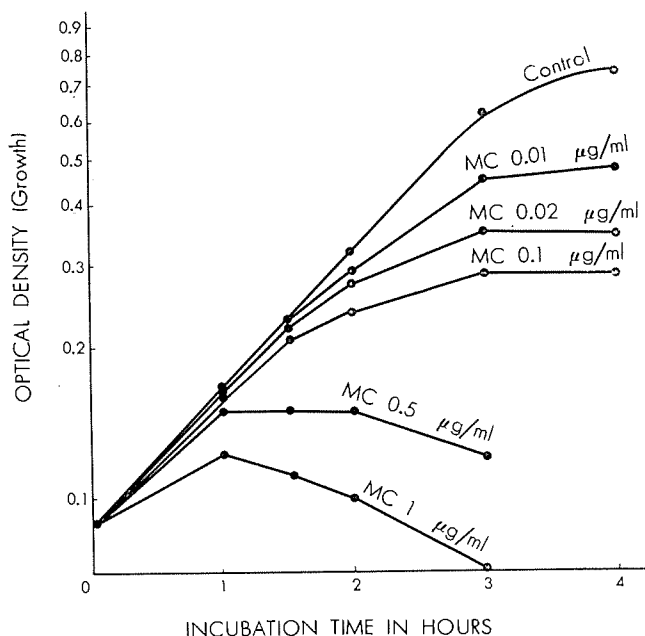


Fig. 1. Effect of MC on growth of *E. coli* B in log phase.

Cells in the log phase of growth were diluted with Glucose-Simmons' medium, giving an initial titer of 0.8×10^7 cells/ml., mixed with MC, and incubated at 37°C. Growth was followed by measurement of the optical density at 660 m μ at one hour intervals in Jr. Coleman Spectrophotometer.

increased exponentially during the first 3 hours. In the presence of 1.0 μ g./ml. MC, the increase of turbidity ceased after 30 minutes. In the medium containing

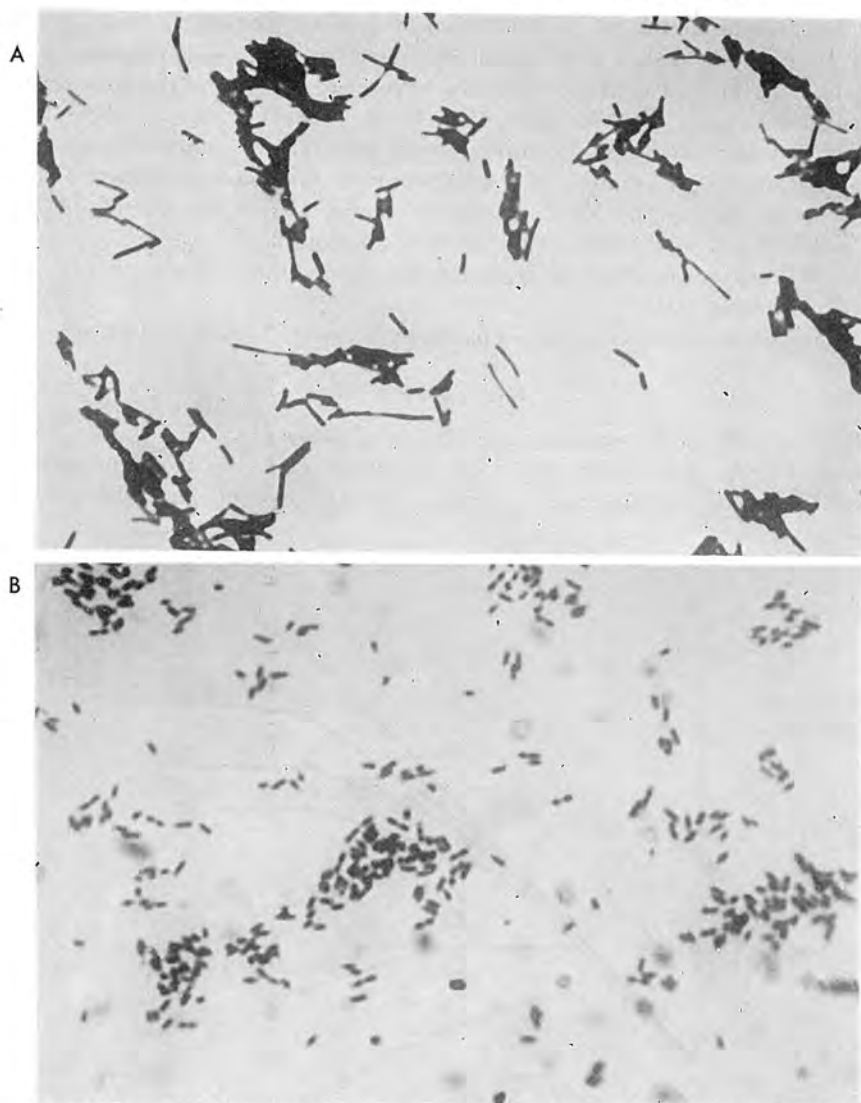


Fig. 2. Log phase *E. coli* B cells incubated for 90 Min. at 37°C in the presence (A) and absence (B) of MC were stained by Pfeiffer solution.

0.1-0.02 $\mu\text{g./ml.}$ of MC, the increase in turbidity continued during the first 90 minutes and, afterwards, the increase was markedly suppressed. Even the presence of 0.01 $\mu\text{g./ml.}$ of MC induced a slight inhibition of the growth after 90 minutes, but 0.001 $\mu\text{g./ml.}$ of MC caused no inhibition.

Results of viable cell counts under the same condition are presented in Table 1. The numbers of viable cells of the control were 0.8×10^8 cells/ml. at the start, and 2.6×10^8 after 90 minutes incubation (about 3 times the initial value). The increase in viability was parallel to the turbidity (O.D.) which

TABLE 1. EFFECT OF MC ON VIABILITY OF *E. coli* B

MC in suspension $\mu\text{g./ml}$	incubation time	viable count/ml
0	0	0.8×10^8
	90	2.6×10^8
0.001	0	0.8×10^8
	90	2.9×10^8
0.01	0	0.8×10^8
	90	6×10^6
0.02	0	0.8×10^8
	90	2.8×10^6
0.05	0	0.8×10^8
	90	1.8×10^6
0.1	0	0.8×10^8
	90	1.2×10^6

A log phase broth culture of *E. coli* B was mixed with MC at zero time, incubated and shaken at 35°C . The viable cell count was measured by plating on nutrient agar.

increased from 0.9 to 0.26 during 90 minutes. From these results, it is deduced that the division time of *E. coli* B in Glucose-Simmons' medium was about 60 minutes in this experiment. On the other hand in MC-containing medium the viable cell count was less than 5% after 90 minutes: 1.2×10^6 cells/ml. at 0.1 $\mu\text{g./ml}$. and 2.5×10^6 cells/ml. at 0.02 $\mu\text{g./ml}$. of MC. At 0.01 $\mu\text{g./ml}$. of MC, which is the minimum concentration to inhibit growth, the viable cell count decreased to 10% of the initial value. No decrease in viability was seen on addition of 0.001 $\mu\text{g./ml}$. of MC.

After Pfeiffer staining, the cells treated with 0.1 $\mu\text{g./ml}$. of MC for 90 minutes were found to be much more elongated than those in the control culture as shown in Fig. 2 (a,b).

II. Effect of MC on the protein and nucleic acid synthesis in *E. coli* B

From the preceding experiment, it was shown that the growth of *E. coli* B as measured by the turbidity was not suppressed after 90 minutes incubation in the presence of 0.02 $\mu\text{g./ml}$. of MC, whereas the viable cell count was markedly decreased. These results may suggest that so-called "unbalanced growth" (Cohen, 1954) occurs in the growth of the organism in the presence of MC. Therefore the following experiments were performed on the effect of MC on the protein and nucleic acid synthesis in *E. coli* B.

i) Effect of MC on *E. coli* B in the growing state: Aliquots were taken from a culture at 30 minutes intervals for analysis. The results are shown in Table 2 and Fig. 3. The amounts of protein and nucleic acid were determined in 0.5 ml. and 1.0 ml. aliquot respectively. In the control culture, the growth and DNA, RNA and protein synthesis proceeded at a similar rate. In the presence of MC

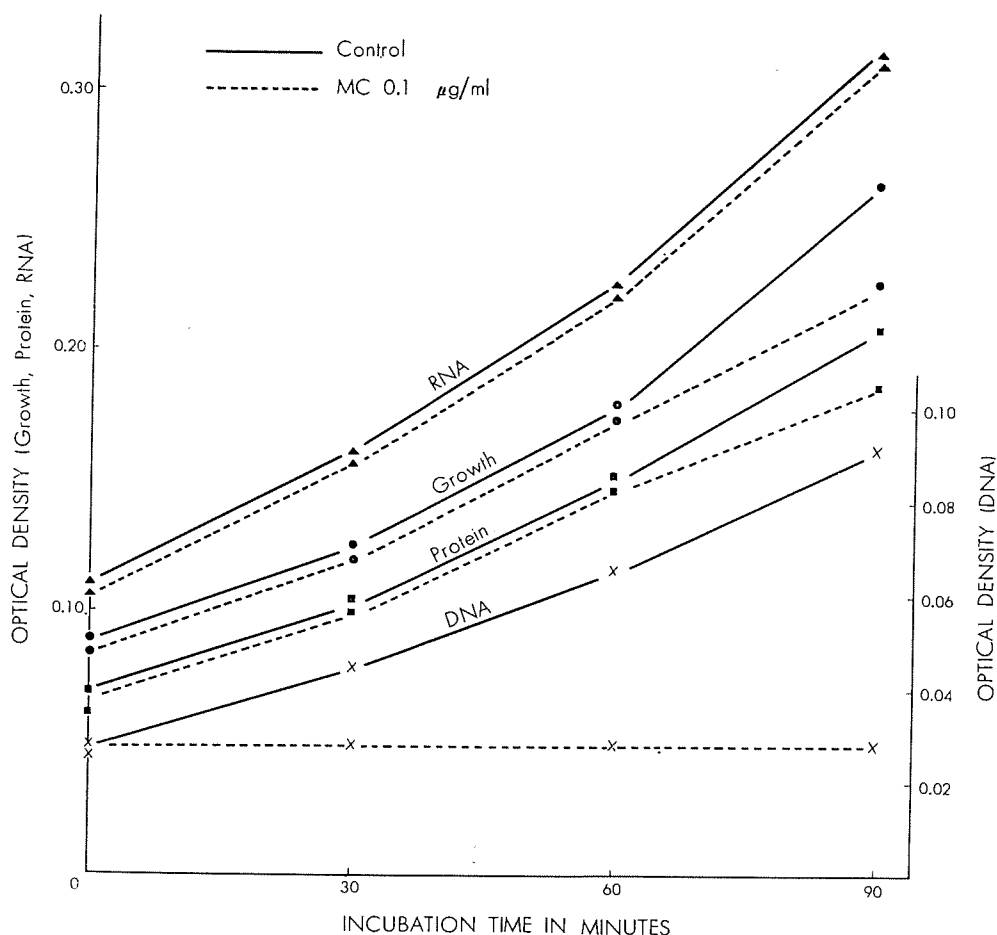


Fig. 3. Effect of MC on growth, protein and nucleic acids synthesis in *E. coli* B. 2.5 ml. of the samples were taken at 30 min. intervals for assay from a culture in the log phase of growth of *E. coli* B shaken continuously at 35°C.

at a concentration of 0.1 $\mu\text{g./ml.}$, (which does not inhibit the growth during 90 minutes incubation) the synthesis of RNA and protein proceeded at the similar rate to that of the control, while DNA synthesis, was completely inhibited immediately after the addition of the antibiotic. Such an inhibition of DNA synthesis took place even in the presence of 0.02 $\mu\text{g./ml.}$ of MC. One hundredth of a microgram per ml. of MC also produced about 60% inhibition. Inhibition of RNA and protein synthesis was obtained in the presence of 1 $\mu\text{g./ml.}$ of MC, a concentration sufficient to prevent the growth of the organism.

ii) Irreversibility of MC-induced inhibition in *E. coli* B in the growing state: After being in contact with MC in a culture at 35°C for 15 minutes, cells were harvested by centrifugation, washed twice with physiological saline and then transferred to an equal volume of Glucose-Simmons' medium. The washed cells

TABLE 2. EFFECT OF MC ON THE GROWTH, PROTEIN, AND NUCLEIC ACID OF *E. COLI* B.

MC in suspension ($\mu\text{g.}/\text{ml.}$)	Time incubated (min)	Turbidity $E_{660} \text{ m}\mu$	Protein Folin reaction $E_{660} \text{ m}\mu$	RNA orcinol reaction $E_{660} \text{ m}\mu$	DNA piperidine reaction $E_{660} \text{ m}\mu$
0	0	0.090	0.070	0.110	0.030
	30	0.125	0.105	0.160	0.045
	60	0.180	0.150	0.225	0.065
	90	0.265	0.210	0.315	0.090
0.01	0	0.090	0.075	0.115	0.035
	30	0.130	0.110	0.155	0.038
	60	0.190	0.144	0.235	0.047
	90	0.280	0.200	0.330	0.055
0.02	0	0.085	0.070	0.110	0.030
	30	0.120	0.105	0.165	0.035
	60	0.185	0.160	0.230	0.030
	90	0.265	0.220	0.320	0.028
0.1	0	0.090	0.065	0.110	0.030
	30	0.135	0.100	0.155	0.030
	60	0.185	0.140	0.230	0.030
	90	0.225	0.190	0.320	0.030
1.0	0	0.095	0.065	0.115	0.028
	30	0.110	0.085	0.150	0.022
	60	0.130	0.095	0.185	0.023
	90	0.110	0.090	0.160	0.015

were then reincubated with shaking at 35°C and the growth and RNA and DNA contents were measured at intervals during the incubation. As shown in Fig. 4, growth, RNA and DNA synthesis in the control medium increased at the same rate as that in un-washed culture. On the contrary, when cells were treated with $0.02 \mu\text{g.}/\text{ml.}$ of MC, the growth and RNA synthesis continued at a rate similar to that of the control for 60 minutes after the start of reincubation, but DNA synthesis did not proceed at all.

iii) Effect of MC on *E. coli* B in the non-growing state: To ascertain whether the selective inhibition of DNA synthesis by the antibiotic might occur only in dividing cells, the effect of MC on *E. coli* B suspended in Simmons' medium lacking glucose was observed.

The cells harvested from 2.5 hours culture were resuspended in the medium lacking glucose. The cells could be incubated for an hour without these being any reduction in the motility or viability, but with no multiplication or increase in the nucleic acid or protein content of the cells. Such a suspension of cells was mixed with MC and then incubated at 35°C for 15 minutes. The whole culture was then centrifuged, washed twice with a medium containing no glucose, resuspended in Glucose-Simmons' medium and finally incubated at 35°C with

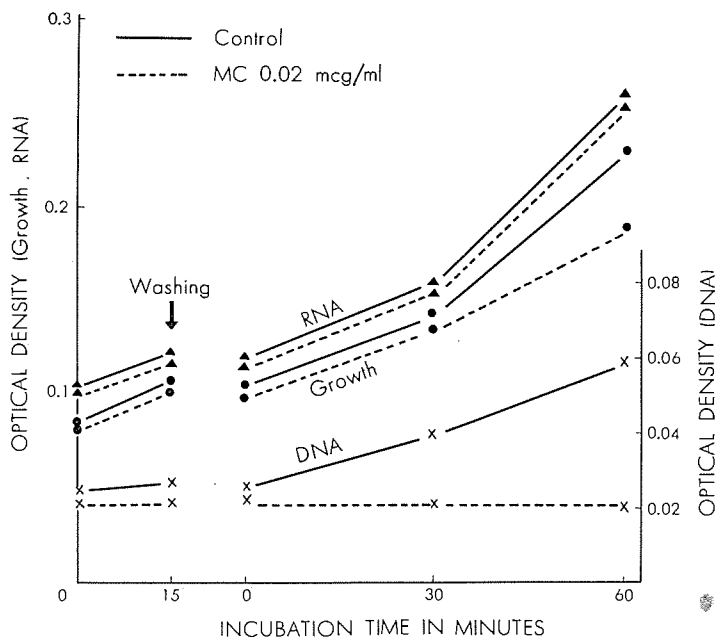


Fig. 4. Irreversibility of MC-induced inhibition of DNA synthesis in *E. coli* B.

Samples were removed from the MC-containing and MC-free cultures at the time indicated in the Fig., kept at 20°C, washed twice with physiological saline solution, resuspended in Glucose-Simmons' medium, re-incubated at 35°C and the growth, RNA and DNA assayed as before.

shaking. A control culture containing no MC was treated similarly. The results are shown in Fig. 5. In MC-treated cells growth occurred normally during the early stage of incubation but showed a little decrease after 90 minutes incubation, while DNA synthesis was inhibited completely from the beginning. The inhibition was not recovered even after 2 hours incubation.

III. Partial restoration of DNA synthesis blocked by MC in the medium containing yeast extract

From the preceding experiments, it was shown that MC inhibited selectively the DNA synthesis of *E. coli* B. Attempts were made to clarify the mechanisms of this inhibition.

Various compounds shown in Table 3, related to nucleic acid metabolism were added to the culture medium and the effect of MC on DNA synthesis of *E. coli* B was observed by similar methods to those described above. There was no reversal of inhibition by any compounds except yeast extract. As shown in Fig. 6, when the cells incubated in Glucose-Simmons' medium containing 20 mg./ml. of yeast extract are exposed to MC, the DNA synthesis is restored to degree depending to the concentration of MC added. That MC is not inactivated by yeast extract is obvious from the result illustrated in Fig. 7. The restoration of DNA synthesis by yeast extract was also observed when washed cells which

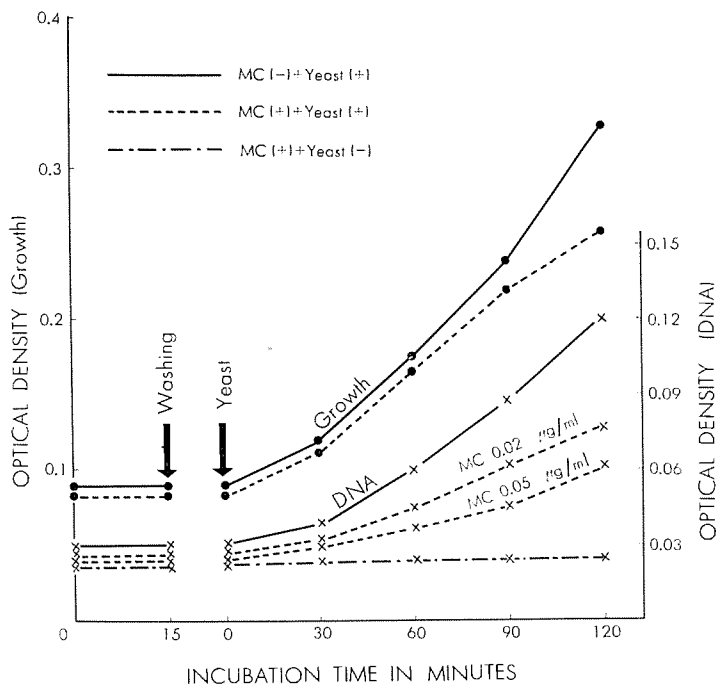


Fig. 5. Effect of MC on *E. coli* B in non-dividing state a medium lacking glucose.

E. coli in the log phase were harvested by centrifugation, suspended in the medium lacking glucose, and mixed with 0.02 $\mu\text{g./ml.}$ of MC.

The mixture was incubated for 15 min. and the cells were again collected by centrifugation, washed twice with the same medium, re-suspended in Glucose-Simmons' medium and incubated at 35°C. The control sample was treated similarly. Samples were withdrawn at 30 min. intervals for chemical analysis.

had been in contact with MC, were incubated in culture medium containing yeast extract.

IV. Effect of MC on the adaptive formation of β -galactosidase in *E. coli* B

By studying the relationship between synthesis of an enzyme protein and DNA synthesis during ultraviolet light irradiation, it was postulated that DNA synthesis might be associated with the induction stage of adaptive enzyme formation since ultraviolet rays strikingly inhibit enzyme formation at a dose sufficient to inhibit DNA synthesis (Torriani, 1956). In this respect, it may be of considerable interest to observe the effect of MC on adaptive enzyme formation.

As shown in Fig. 8, β -galactosidase formation of *E. coli* B was not inhibited by treating the organism with 0.1 $\mu\text{g./ml.}$ MC. Under the same conditions DNA synthesis was inhibited completely as mentioned above.

V. Effect of MC on protein and nucleic acid synthesis in *E. coli* 15 T⁻, a thymine requiring mutant

This strain is unable to synthesize methylpyrimidine, that is a specific component of DNA, but is able to synthesize DNA in the medium containing thymine. The effect of MC on the organism therefore, may provide us with

TABLE 3. REVERSAL BY VARIOUS COMPOUNDS OF MC INDUCED BLOCK IN DNA SYNTHESIS OF *E. COLI* B

addition to glucose Simmons' medium	amount of DNA after incubation for 120 min. at 37°C <i>E. 600m_μ</i>
control	0.125
control-Yeast extract 20mg./ml.	0.135
MC 0.05mg./ml.	0.032
MC-Yeast extract	0.060
MC-adenine 30μg/ml	0.034
MC-guanine "	" 0.030
MC-cytosine "	0.030
MC-uracil "	0.030
MC-thymine "	0.030
MC-thymidine "	0.035
MC-folic acid 5μg/ml	0.030
MC-casaminoacid 10mg/ml	0.030

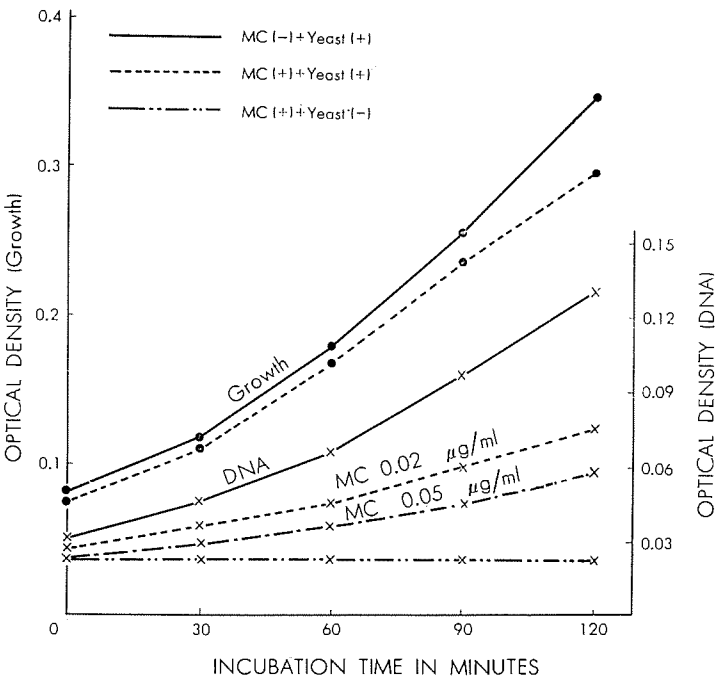


Fig. 6. Reversal of MC-induced block in DNA synthesis of *E. coli* B by yeast extract. Log phase *E. coli* B cells were resuspended in Glucose-Simmons' medium containing 20 mg./ml. of baker's yeast extract, mixed with MC, and reincubated at 35°C. The growth and DNA contents were then measured.

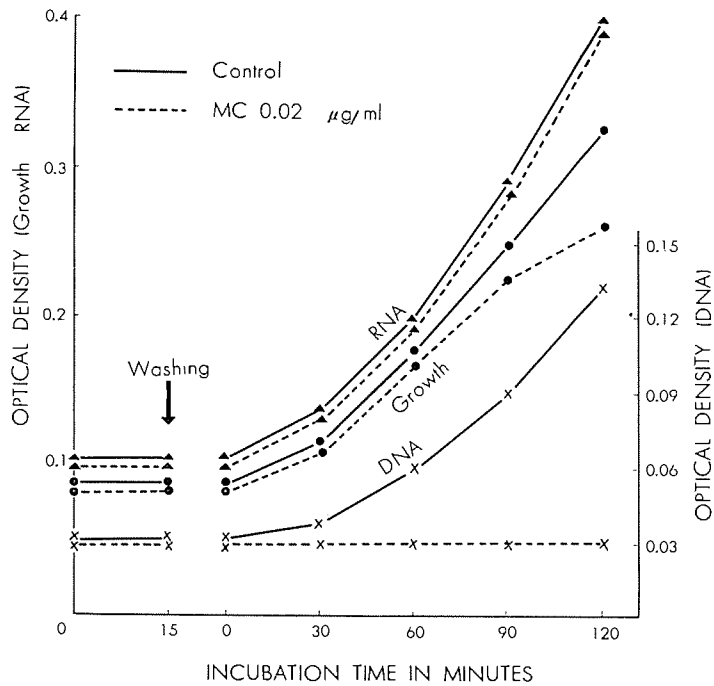


Fig. 7. Reversal of MC-induced block in DNA synthesis in *E. coli* B by yeast extract. Cells incubated with MC in the glucose-free medium at 35°C for 15 min. were washed twice with the same medium and resuspended in Glucose-Simmons' medium supplemented with 20 mg./ml. of baker's yeast extract. Growth and DNA contents were then measured.

TABLE 4. EFFECT OF MC ON THE GROWTH, PROTEIN AND NUCLEIC ACID OF *E. coli* 15T—

MC in suspension µg/ml	Time incubated (min)	Turbidity E. 660mµ	RNA orcinal reaction E. 660mµ	DNA diphenylamine reaction E. 660mµ
0	0	0.105	0.120	0.025
	30	0.150	0.180	0.035
	60	0.230	0.280	0.058
	90	0.350	0.375	0.085
0.1	0	0.100	0.115	0.025
	30	0.145	0.180	0.030
	60	0.220	0.280	0.055
	90	0.320	0.390	0.080
1	0	0.110	0.120	0.030
	30	0.150	0.185	0.038
	60	0.230	0.270	0.060
	90	0.330	0.360	0.080

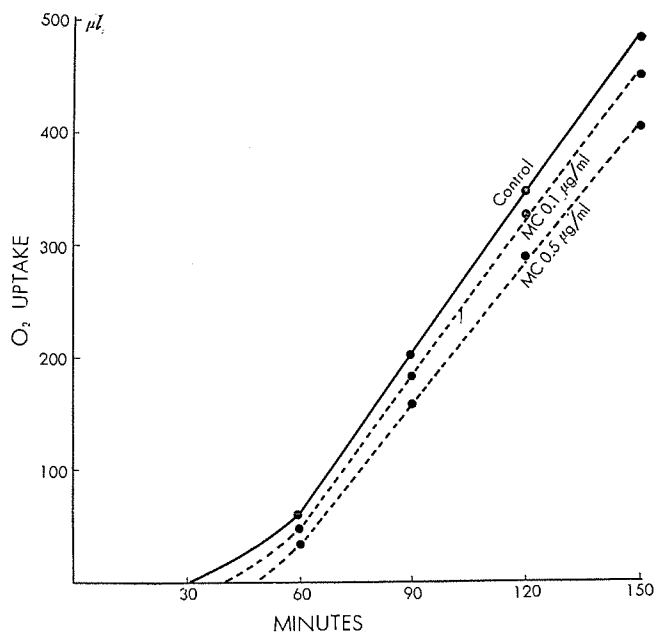


Fig. 8. Adaptive formation of β -galactosidase in MC-treated *E. coli* B. Log phase *E. coli* B was harvested by centrifugation, resuspended in a medium lacking glucose, mixed with MC, incubated for 15 min. at 35°C, washed twice with physiological saline and resuspended in the same solution. Adaptive formation of β -galactosidase in this suspension was measured as before. Each flask contained 3 mg. (dry weight) of *E. coli* B.

a clue for understanding the inhibition mechanism of DNA synthesis by MC in *E. coli* B. Thus the following experiments were carried out.

Experiments were made with *E. coli* 15 T- by the same method as with *E. coli* B, except that thymine (30 μ g./ml.) was added to the medium.

As indicated in Table 4, when this strain in the logarithmic phase of growth was used for the determination of nucleic acid and protein no inhibition was observable either in the growth or DNA synthesis in the presence of 0.1 μ g./ml. of MC.

The growth and the synthesis of DNA, RNA and protein of the organism was unaffected even by the presence of a much higher concentration of MC (1.0 μ g./ml.).

DISCUSSION

As described in the previous section it was observed that in the presence of MC at a concentration of 0.1-0.02 μ g./ml. the growth of *E. coli* B, measured by the optical density, began to be inhibited after 90 minutes incubation, whereas the numbers of viable cells markedly decreased before that time. This may indicate that the growth inhibitory action of MC is not bacteriostatic but bacteriocidal. It also suggests that the growth of the cell in the presence of MC may be abnormal and not accompanied by an increase of viable cells.

A characteristic finding in the experiments was that, in the presence of MC at a concentration sufficient to reduce the viability of cells, the DNA synthesis was found to be inhibited specifically. Thus the inhibition of DNA synthesis occurs immediately after the addition of 0.02 $\mu\text{g./ml.}$ of MC, the minimum dose for killing 95% of the viable cells. However protein and RNA synthesis and the adaptive formation of β -galactosidase of the cells are entirely unaffected even in the presence of 0.1 $\mu\text{g./ml.}$ of MC.

Protein synthesis was found to decrease at a concentration of the antibiotic of more than 1 $\mu\text{g./ml.}$

In the previous papers we reported that MC moderately inhibited the respiration and glycolysis of Ehrlich ascites tumor cells at a cell level while no inhibition was observed in homogenates of the tumor cells. A similar results were obtained with bacteria, but at the much higher concentration of 10 $\mu\text{g./ml.}$ MC markedly inhibited the substrate respiration of *Bacillus subtilis* at a cell level, whereas such an inhibition of respiration was not observable in an extract of the organisms (Shiba, 1957). This suggests that the inhibition of respiration may not represent the primary action of MC but a secondary phenomenon induced by certain other basic changes caused by the antibiotic.

Furthermore, the inhibitory action of the antibiotic seems to be irreversible since when the inhibition has once occurred, it is not reversed by washing MC-contacted cells with glucose free medium or with growth medium. Since in terms of the inhibition of the further DNA synthesis, MC was equally effective either on multiplying or non-multiplying cells the inhibition may not result from the cessation of cell division but from a primary action of the antibiotic on DNA synthesis.

Ultraviolet radiation and alkylating agents have been reported to be inhibitors of DNA synthesis in *E. coli* B (Kelner, 1953; Herriott, 1951). In 1953 Kelner reported that DNA synthesis in *E. coli* B was temporarily inhibited immediately after irradiation at 2600 Å for 15 minutes, while the growth rate and RNA and protein synthesis were not fundamentally altered. After 40 minutes' irradiation however, inhibition of both protein synthesis on a DNA synthesis had occurred. According to Harold and Ziporin (1957), sulfur-mustard exerts a selective inhibition on DNA synthesis in *E. coli* B at the concentration of 85 $\mu\text{g./ml.}$ and it also inhibits growth and RNA synthesis at a concentration of 110 $\mu\text{g./ml.}$ The inhibition of DNA synthesis caused by the drug however, was found to be reversed by washing with a drug-free medium indicating the reversibility of the inhibition.

MC, on the other hand, can completely inhibit DNA synthesis in *E. coli* B even at a concentration of below 0.1 $\mu\text{g./ml.}$ The difference between the concentration of MC necessary to inhibit DNA and that to inhibit RNA or protein synthesis is much greater than that in cases of ultraviolet radiation or of alkylating agents. Furthermore, unlike inhibition by mustard derivatives, the inhibitory action of MC is irreversible by washings. These finding clearly suggest that the action of MC may be much more selective for DNA synthesis than that of other agents such as ultraviolet radiation or alkylating agents.

Moreover, the apparent specific inhibition of DNA synthesis by MC may indicate that the antibiotic may serve as an important tool to clarify the metabolic pathway involved in the DNA synthesis of the particular organism.

Ultraviolet ray has so far been regarded as an important tool for the clarification of the physiological role of DNA in cells, yet there are still many unsolved questions. As stated above, ultraviolet radiation inhibits adaptive enzyme formation at a dose sufficient to inhibit DNA synthesis.

In the present work, however, it was shown that β -galactosidase formation was entirely unaffected by 0.1 $\mu\text{g./ml.}$ of MC, a concentration much higher than that sufficient to inhibit DNA synthesis (0.02 $\mu\text{g./ml.}$). It is possible, therefore, to predict that DNA synthesis may not be essential for β -galactosidase formation.

As to the relation between DNA synthesis and cell division, it has been proved by many investigators that DNA synthesis is essential for cell division, serving as an impartible component of the chromosome. In these experiments, it was observed that MC-treated cells are markedly elongated, which may perhaps be attributed to the cessation of cell division. The inhibition of DNA synthesis by MC could result in preventing division.

From the present results it can be deduced that the intrinsic mode of action of MC may be invariably associated with the specific inhibition of the synthesis of DNA, which being genetic material is one of the most important components of the cell. This may explain the bacteriocidal effect of MC as well as its broad spectrum. The mechanism involved in the inhibition of DNA synthesis by the antibiotic, however, must await further studies.

The effect of MC on the deoxyribose content in an acid-soluble fraction of *E. coli* B has also been studied, but no remarkable effect could be noticed.

It must be pointed out here that MC does not inhibit the growth and the nucleic acid synthesis of *E. coli* 15 T- even at a concentration of 1 $\mu\text{g./ml.}$. The reason for this is now being studied. Finally clarification of the effective principle for reducing the inhibitory action of MC in yeast extract might be of some help for understanding the mechanism of the inhibition.

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