THE RELATIONSHIP BETWEEN THE SYNTHESIS OF DNA AND PROTEIN IN *ESCHERICHIA COLI* TREATED WITH SULFUR MUSTARD*

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INTRODUCTION

In the preceding paper[1] we discussed the pattern of growth and nucleic acid synthesis in *Escherichia coli* treated with nitrogen- and sulfur mustards. It was shown that cells treated with these agents during the logarithmic phase of growth lost the ability to divide but continued to grow*** (*i.e.*, enlarge) for several hours, giving rise to filamentous forms. The synthesis of RNA and of protein (*i.e.*, growth) was not markedly affected by mustard treatment. The synthesis of DNA, however, was inhibited for a period of time, the duration of which increased with increasing concentration of mustard. Ultimately DNA synthesis resumed and its rate soon equaled that of growth.

Upon closer examination of this phenomenon it became clear that the resumption of DNA synthesis in mustard-treated *E. coli* may be regarded as recovery from a lesion or block in DNA metabolism. Results of a study of the physiological factors involved in the recovery process are presented here.

EXPERIMENTAL

Bacterial strains.

The following strains of *E. coli* were used in the present study: B, B_{U-} (requires uracil), B_{45} (requires methionine or vitamin B_{12}), 15_{T-} (requires thymine) and 15_{T-PA-} (requires both thymine and phenylalanine). All of the strains were obtained from Prof. S. S. COHEN of the University of Pennsylvania School of Medicine, to whom our sincere thanks are due. The bacteria were maintained on nutrient agar slants and transferred at intervals of four to six weeks.

Chemicals

Sulfur mustard, bis (β-chloroethyl) sulfide, abbreviated HD, was furnished by Dr. M. D. GAON of the Rocky Mountain Arsenal, U. S. Army Chemical Corps, Denver, Colorado. 5-Methyltryptophan was purchased from Sigma Chemical Co., and *p*-fluorophenylalanine and 5-hydroxyuridine from the California Foundation for Biochemical Research.

Growth experiments

The basic techniques employed in the growth experiments have been described[1]. Bacteria were grown with aeration at 37° in the glucose–salts medium of COHEN AND ARBOGAST[2], supple-

* The opinions expressed in this paper are those of the authors, and do not necessarily represent the official views of any governmental agency.

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*** As in the preceding paper, growth is defined as cell enlargement and is measured by means of the optical density at 600 mμ. Thus "growth" and "increase in turbidity" are synonymous.

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mented with factors as required for the various mutants. When the bacteria, growing logarithmically had undergone at least one division and had attained an optical density of 0.3 (measured at 600 mμ in a Beckman DU spectrophotometer), they were collected by centrifugation, washed, and resuspended in buffer. Sulfur mustard was added and the mixture was incubated for 15 min at 37°. The cells were again collected by centrifugation and suspended in growth medium (with supplements or deletions as described below); the cultures were aerated at 37° and samples were withdrawn at frequent intervals for chemical analysis. In plotting the results, the point 0 min refers to the time at which the mustard-treated bacteria were suspended in growth medium.

Analytical methods

DNA was determined by BURTON's method[3], and RNA by that of CERIOTTI[4]. The extraction of the nucleic acids from the cells was described in the preceding paper[1].

RESULTS

1. *Nutritional requirements for recovery from the mustard-induced block in DNA synthesis*

When a solution of sulfur mustard is adjusted to a physiological pH, the mustard is hydrolyzed and loses its toxicity[5]. Under the conditions employed in our experiments, no bactericidal activity remained after 30 min. This observation suggested that resumption of DNA synthesis in mustard-treated *E. coli* might require only the removal of mustard from the solution. In support of this hypothesis it was found that if the concentration of mustard was maintained by frequent addition of HD to a growing culture of *E. coli* B, DNA synthesis did not resume and the turbidity ceased to increase when the optical density of the culture had approximately doubled.

However, the following experiment indicates that removal of the mustard does not, by itself, permit the resumption of DNA synthesis. *E. coli* B were suspended in phosphate buffer at pH 7 and treated with 85 μg/ml HD. The cells were then collected and divided into two portions. One was resuspended in growth medium; the other was incubated for 1 h in phosphate buffer (to destroy any residual HD) after which time the cells were centrifuged and returned to growth medium. In both flasks, growth* began as soon as the cells were placed in the growth medium, and DNA synthesis lagged behind growth by about 20 min. Thus incubation in buffer did not overcome the block in DNA synthesis.

A more specific experiment of this kind, shown in Fig. 1, defines the general nutritional requirements for the restoration of the capacity for DNA synthesis. *E. coli* B, treated with 85 μg/ml HD, were divided into three portions. Portion A was suspended in growth medium; portion B was placed in medium lacking glucose, which was added after 30 min; finally, portion C was similarly incubated in medium lacking a nitrogen source, with addition of $(NH_4)Cl$ after 30 min. It will be seen from Fig. 1 that in all three flasks, growth and RNA synthesis began as soon as the medium was complete. Again, in all three flasks, DNA synthesis was inhibited for about 20 min. Thus, the inhibition of DNA synthesis could not be overcome in the absence of nitrogen or glucose.

From the three experiments described in this section it is clear that, while removal of the mustard is a necessary condition for the resumption of DNA synthesis, it is not a sufficient one. We may rather regard the inhibition of DNA synthesis in mustard-treated *E. coli* as the consequence of a lesion, or block, induced by the mustard. The expression of this lesion evidently does not require the continued presence of the mustard. Moreover, it appears that repair of the lesion in DNA

* See footnote page 1.

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metabolism requires the participation of both nitrogen and carbon-energy sources, *i.e.*, the conditions required for growth. In subsequent sections we will examine the role of DNA, RNA, and protein synthesis in the repair process.

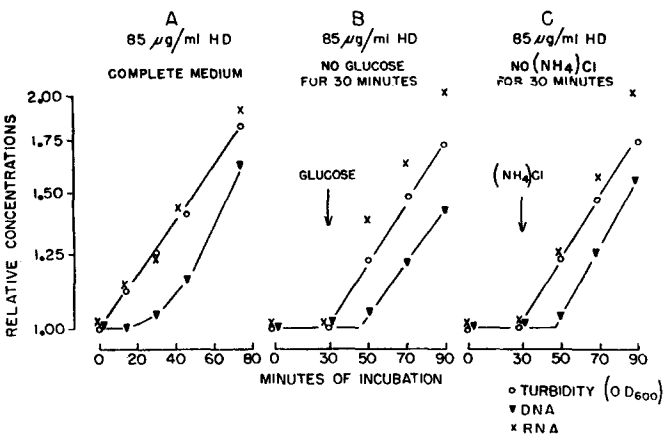

Fig. 1. Participation of carbon-energy and nitrogen source in repair of the lesion in DNA synthesis. *E. coli* B were treated with HD as described in the text and suspended in growth medium at o minutes. A – complete medium, B – medium lacking glucose, C – medium lacking nitrogen source.

Ordinates $= \frac{\text{concentration of cells and nucleic acid at time} = t}{\text{concentration of cells and nucleic acid at time} = o}$ plotted on a logarithmic scale.

2. *Non-participation of DNA synthesis in the repair of the lesion produced by HD*

Since the lesion produced by HD resulted in a transient inhibition of DNA synthesis, it seemed possible that repair of the lesion would not take place under conditions which specifically preclude DNA synthesis. Such conditions may be readily imposed upon a thymine-requiring mutant of *E. coli*, 15_{T-}, which is unable to synthesize DNA in absence of exogenous thymine. The physiology of this mutant has been studied in detail by COHEN AND BARNER[6-10] who have demonstrated that it is capable of performing many functions, including growth and the synthesis of RNA and protein, in absence of thymine; prolonged thymine starvation leads to the irreversible loss of the ability to multiply. If thymine is added to the thymine-starved bacteria, DNA synthesis resumes even though the bacteria may no longer be viable*. By use of this organism it was possible to inquire whether exogenous thymine—and hence, DNA synthesis— is required for repair of the lesion induced by HD.

E. coli 15_{T-} were grown in medium supplemented with thymine (2 μg/ml). The cells, washed free of thymine, were treated with 60 μg/ml HD in the usual manner (viable count: 5 % of untreated controls) and divided into two portions. One (A)

* In the course of these experiments we noted an unexplained discrepancy between the observations of BARNER AND COHEN[7] and our own, with respect to DNA synthesis in 15_{T-} inactivated by prolonged thymine starvation. Upon addition of thymine to such a culture, BARNER AND COHEN observed resumption of DNA synthesis which ceased when the DNA content of the bacteria had doubled. In our hands, this procedure led to much more extensive DNA synthesis, the DNA content increasing as much as four-fold. DNA synthesis under these conditions was not accompanied by RNA synthesis or increase in the turbidity.

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was suspended in growth medium supplemented with 2 μg/ml thymine; the other (B) was suspended in plain growth medium, thymine being added after 30 min. The results are shown in Fig. 2. In both flasks the turbidity increased rapidly at first, followed by a marked decline of the growth rate. In most experiments lysis was indicated by foaming in the aerated flasks, and by a decrease in the optical density. This behavior differs strikingly from that of *E. coli* B which continues to grow logarithmically for several hours. In flask A, with thymine present throughout, DNA synthesis was blocked for 20 min. By contrast in flask B, DNA synthesis began immediately upon addition of thymine, indicating that repair was complete after 30 min even in the absence of thymine. It is of great interest that DNA synthesis in mustard-treated 15_{T-}, once it had recovered, continued even after the turbidity began to decline.

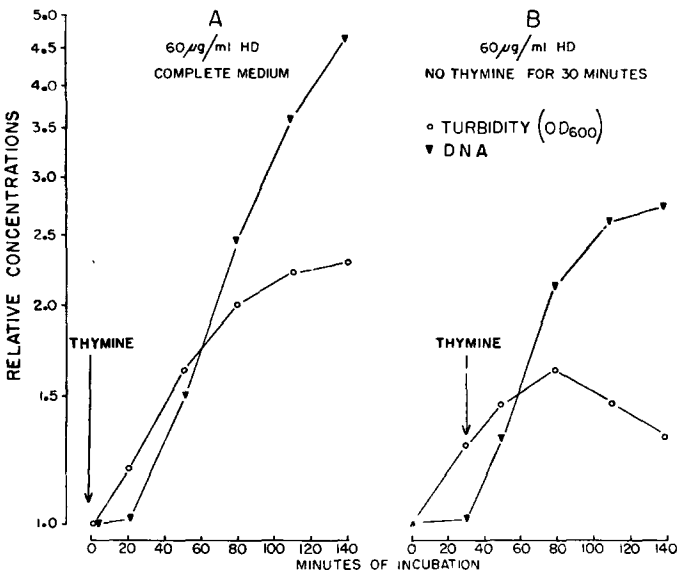

Fig. 2. Recovery from the HD-induced block in DNA synthesis in absence of thymine. *E. coli* 15_{T-}, treated with HD, were suspended in growth medium at 0 min, thymine being added at times indicated. Plot as in Fig. 1.

It is clear from this experiment that *E. coli* 15_{T-} can overcome the mustard-induced block in DNA synthesis in the absence of exogenous thymine. More generally, we interpret this result to indicate that DNA synthesis itself is not required for repair of the lesion. It may be argued that 15_{T-} has been shown to be capable of limited DNA synthesis during the early phase of thymine starvation[8] and that this amount of DNA synthesis might suffice to permit repair of the lesion. This objection is weakened by the observation that when the experiment described above was repeated, using 15_{T-} which had been subjected to 30 min of thymine starvation *before* mustard treatment, repair was nonetheless independent of exogenous thymine. Thus, at least in 15_{T-}, DNA synthesis apparently is not an obligatory part of the repair process.

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3. Participation of protein synthesis in the repair of the HD-induced lesion in DNA metabolism

The thesis that protein synthesis is required for recovery from the mustard-induced block is supported by experiments with amino acid auxotrophs, metabolic antagonists of the amino acids, and chloramphenicol. These will now be discussed in turn.

a) Amino acid auxotrophs. The mutants used in the present experiments require exogenous amino acids for growth and protein synthesis. It is therefore possible to determine whether amino acids (and hence presumably, protein synthesis) are required to overcome the block in DNA synthesis produced by HD. If HD-treated cells repair the lesion in absence of the required amino acid, DNA synthesis, as well as growth, should begin upon addition of the growth factor. Conversely, if the amino acid is required for the repair process, growth should begin as soon as the amino acid is supplied, but DNA synthesis would not resume until repair is complete and would

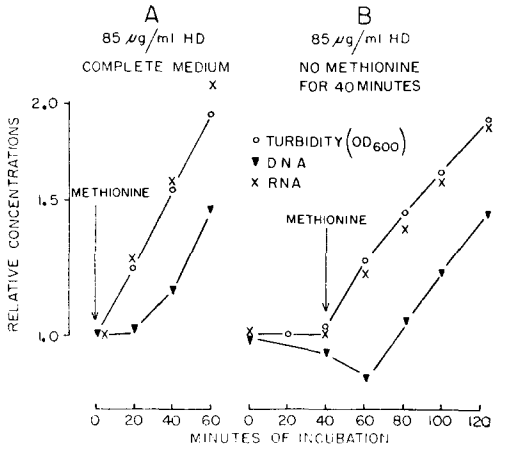

Fig. 3. Participation of methionine in the repair process. *E. coli* B_{45}, treated with HD, were suspended in growth medium at 0 min. Methionine was added at times indicated. Plot as in Fig. 1.

therefore lag behind growth. This approach is analogous to that used by MONOD *et al.*[11] and RICKENBERG *et al.*[12] to demonstrate that amino acids participate in induced enzyme biosynthesis.

E. coli B_{45}: This strain requires either methionine or vitamin B_{12} for growth. The bacteria were grown in the presence of methionine (45 μg/ml), washed, and treated with 85 μg/ml HD as described in the experimental section (viable count — 1 % of untreated controls). The cells were divided into two portions. One (A) was resuspended in growth medium supplemented with methionine; the other (B) was incubated in growth medium for 40 min before addition of methionine. The results are shown in Fig. 3. In flask A, which contained methionine from the start, the usual pattern was obtained, DNA synthesis being inhibited for 20 min. In flask B, cell enlargement and nucleic acid synthesis were blocked until methionine was added. Upon addition of this compound, growth and RNA synthesis began immediately, but DNA synthesis lagged behind growth by some 20 min. In appropriate control experiments it was shown that untreated B_{45}, suspended in a medium lacking methionine did not show a lag in DNA

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synthesis upon addition of methionine. It is thus clear that in this strain repair of the mustard-induced lesion in DNA synthesis required the presence of methionine.

A curious feature of the experiments with B_{45} was the loss of DNA from HD-treated cells under conditions of methionine starvation. Loss of DNA from untreated B_{45} under these conditions was not observed.

15T-PA-: Experiments with this mutant, which requires both thymine and phenylalanine[13], were particularly instructive, since the effects of thymine- and phenylalanine-starvation could be compared in a single experiment.

15T-PA- was grown in presence of thymine (5 μg/ml) and phenylalanine (25 μg/ml), washed and treated with 75 μg/ml HD. The bacteria were divided into three portions and were resuspended in growth medium supplemented as follows: (A) thymine and phenylalanine added at 0 min. (B) phenylalanine added at 0 min, thymine after 30 min (C) thymine added at 0 min, phenylalanine at 30 min.

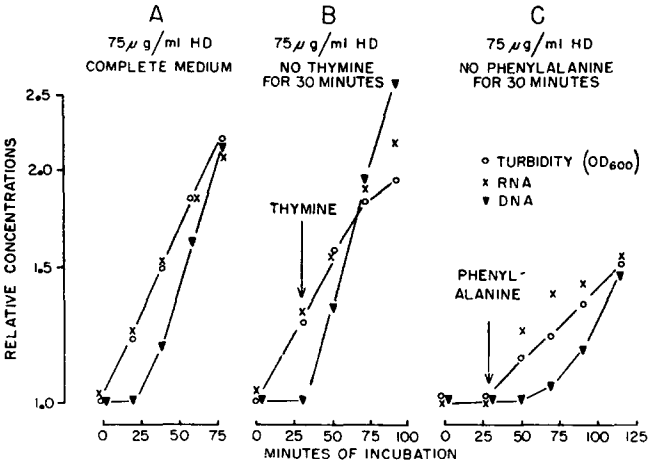

Fig. 4. Repair of DNA synthesis in 15T-PA-. *E. coli* 15T-PA-, treated with HD, were suspended in growth medium at 0 min, with supplementation as indicated. Plot as in Fig. 1.

The results, shown in Fig. 4, fully confirmed those of previous experiments. In presence of both supplements (A), growth and RNA synthesis began immediately but DNA synthesis was inhibited for 20 min. In flask B, DNA synthesis began immedi ately upon addition of thymine, in agreement with the previous conclusion that repair of the lesion is not dependent upon exogenous thymine. In flask C, however, addition of phenylalanine permitted the immediate resumption of growth and RNA synthesis only. The synthesis of DNA remained blocked for some 40 min, demonstrating the requirement for phenylalanine in the repair of the lesion induced by HD. It is of interest in this connection that untreated 15T-PA-, deprived of phenylalanine, were capable of limited DNA synthesis though unable to grow. Similar observations have been made by COHEN[10].

b) Amino acid antagonists. The use of amino acid analogs provides an alternative method of interfering with the metabolism of amino acids, and hence with protein synthesis. The experiments described below support the view that protein synthesis is requeird for recovery from the mustard-induced block in DNA synthesis.

5-methyl tryptophan, a tryptophan antagonist[14], is known to block protein

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synthesis in bacteria[15,16] and prevent growth until the inhibition is relieved by addition of tryptophan. In our hands, growth of cultures inhibited by 5-methyl tryptophan often resumed spontaneously after some 30–60 min, limiting the usefulness of this inhibitor.

E. coli B were treated with 85 μg/ml HD in the usual manner. The cells were divided into two portions. One (A) was suspended in growth medium, the other (B) in growth medium supplemented with 100 μg/ml 5-methyl tryptophan. In the latter flask, growth was virtually blocked until tryptophan (35 μg/ml) was added after 40 min.

The results are presented in Fig. 5, plotting DNA synthesis against growth. On a plot of this kind, DNA synthesis in untreated *E. coli* yields a straight line passing through the origin, since the rate of DNA synthesis equals that of growth (line C). The curve for HD-treated bacteria shows the usual transient inhibition of DNA

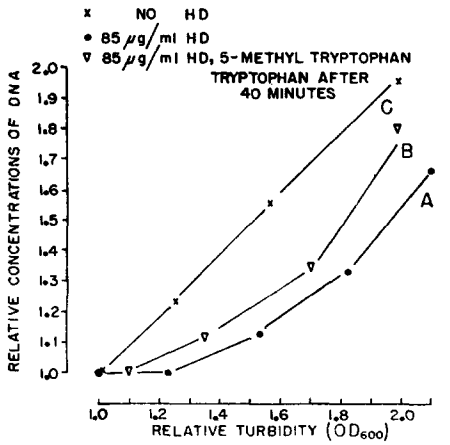

Fig. 5. Inhibition of repair by 5-methyltryptophan. DNA synthesis is plotted against growth as described in the text. Growth was blocked in presence of 5-methyltryptophan until tryptophan was added after 40 min.

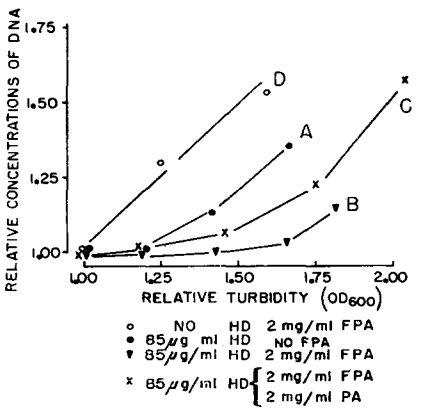

Fig. 6. Inhibition of repair of the lesion in DNA synthesis by *p*-fluorophenylalanine. *E. coli* B, treated with HD, were suspended in growth medium supplemented as described in the text. DNA synthesis is plotted against growth.

synthesis (line A). Finally, line B, representing HD-treated *E. coli* suspended in 5-methyl tryptophan, is similar to line A. This indicates that repair did not occur while growth was blocked with 5-methyl tryptophan since, had repair taken place in absence of growth, the plot of subsequent DNA synthesis against growth would have coincided with that of the untreated cells (line C). It may therefore be concluded that 5-methyl tryptophan blocks repair of the HD-induced lesion in DNA synthesis.

p-Fluorophenylalanine is a competitive antagonist of phenylalanine; it has been used as an inhibitor of microbial protein synthesis[17,18]. *E. coli* B were grown and treated with 85 μg/ml HD as usual. The bacteria were divided into three portions which were suspended respectively in plain growth medium (A), growth medium containing 2 mg/ml *p*-fluorophenylalanine (B), and medium containing 2 mg/ml each of *p*-fluorophenylalanine and phenylalanine (C). Under our conditions, *p*-fluorophenylalanine at this level did not reduce the growth rate for the first 40–60 min, after which it began to decline.

The results will be seen in Fig. 6, again plotting DNA synthesis against growth.

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Line D represents untreated *E. coli* B growing in presence of the inhibitor. It will be seen that *p*-fluorophenylalanine had no effect on DNA synthesis in normal bacteria. Line A, derived from HD-treated bacteria in plain growth medium, shows the usual transient block in DNA synthesis. Line B, corresponding to HD-treated *E. coli* growing in presence of *p*-fluorophenylalanine, shows that this substance inhibited repair more severely than growth. Finally line C shows that phenylalanine partly relieved the inhibition of repair.

From this experiment we may conclude, not only that phenylalanine participates in the repair of the lesion in DNA synthesis, but that it is possible to dissociate repair from cell enlargement. It appears then that repair is a process related to growth but distinct from it, presumably consisting of the synthesis of one or more specific proteins.

c) Chloramphenicol. Chloramphenicol has been shown to inhibit protein synthesis in microorganisms, including growth[19] and induced enzyme biosynthesis[20, 21]. Chloramphenicol does not inhibit the synthesis of nucleic acids; both RNA and DNA

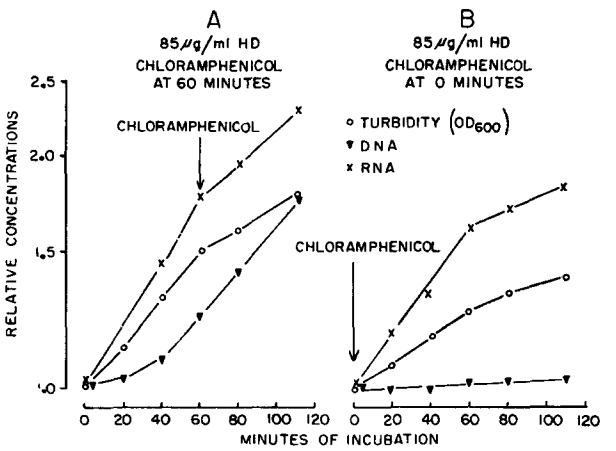

Fig. 7. Effect of chloramphenicol on nucleic acid synthesis in mustard-treated *E. coli* B. Bacteria, treated with HD, were suspended in growth medium at 0 min, chloramphenicol being added as indicated. Plot as in Fig. 1.

continue to be synthesized in bacteria whose growth is blocked by chloramphenicol[19, 22].

E. coli B were grown and treated with 85 μg/ml HD as usual. The cells were divided into two portions. One (A) was suspended in growth medium as described, chloramphenicol (75 μg/ml) being added after 60 min. The remaining cells (B) were suspended in growth medium containing 75 μg/ml chloramphenicol.

In Fig. 7 it will be noted that chloramphenicol severely inhibited the growth of the bacteria, but did not altogether stop it*. RNA synthesis was but little affected by chloramphenicol for the first 40 min. The effect of chloramphenicol on DNA synthesis in HD-treated *E. coli* was clear-cut. In absence of the inhibitor, DNA synthesis resumed after 20 min, and subsequent (60 min) addition of chloramphenicol

* In several experiments, *E. coli* suspended in presence of chloramphenicol resumed growth after 1–2 h. While this phenomenon was not further investigated, its existence does not invalidate our argument that protein synthesis is required for the repair process since resumption of growth (*i.e.*, protein synthesis) was accompanied by repair and ultimate resumption of DNA synthesis.

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had no inhibitory effect (Fig. 7, A). If, however, chloramphenicol was added at the start of the experiment, no resumption of DNA synthesis was observed (Fig. 7, B). This constitutes strong evidence for the thesis that DNA synthesis in HD-treated cells requires prior protein synthesis.

One may inquire whether the entire period, during which DNA synthesis is inhibited, is equally sensitive to chloramphenicol. The following experiment shows that the bulk of the required protein synthesis takes place during the first half of the repair period. *E. coli* B, treated with 85 μg/ml HD, were divided into three portions and suspended in growth medium. Chloramphenicol was added after 0, 10, and 20 min respectively. It will be seen, in Fig. 8, that while addition of the inhibitor at the start of the experiment blocked repair completely, it had but little effect when added after 10 min, and none when added after 20 min

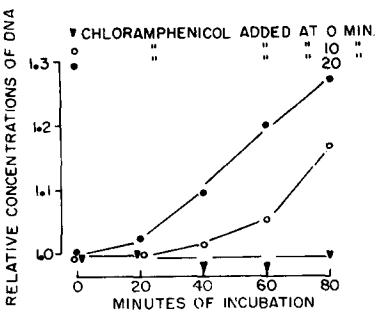

Fig. 8. Effect of chloramphenicol upon DNA synthesis at various times after mustard treatment. *E. coli* B, treated with chloramphenicol, were suspended in growth medium at 0 min. Chloramphenicol was added at 0, 10, and 20 min. Growth was severely inhibited after addition of chloramphenicol.

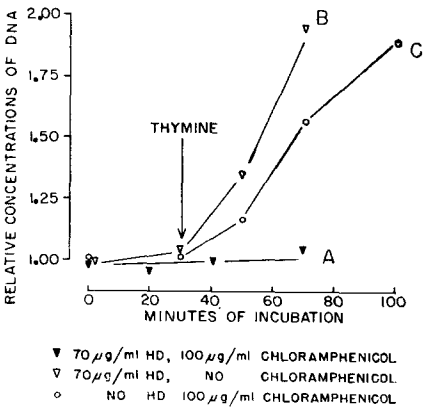

Fig. 9. Effect of chloramphenicol on DNA synthesis in *E. coli* 15_{T-}. *E. coli* 15_{T-} (mustard-treated and untreated) were suspended in growth medium with chloramphenicol as indicated. Thymine was added after 30 min of incubation.

The duration of the chloramphenicol-sensitive period also depends on the concentration of HD used. Thus, when *E. coli* B were treated with 125 μg/ml HD, DNA synthesis was inhibited for 45 min. Chloramphenicol inhibited repair of the lesion only if added within the first 25 min. Conversely, DNA synthesis in *E. coli* B treated with 25 μg/ml HD (which produces no detectable inhibition of DNA synthesis) was not affected at all by chloramphenicol.

From the above results it would appear that protein synthesis is a prerequisite for the resumption of DNA synthesis in HD-treated *E. coli*. The question now arises whether this requirement for protein synthesis is a consequence of mustard treatment, or is generally required for the initiation of DNA synthesis. The following experiment supports the former alternative: *E. coli* 15_{T-} were washed free of thymine and treated with 70 μg/ml HD in the usual manner; another portion of the bacteria was not treated. The bacteria were resuspended in growth medium as follows: (A) HD-treated cells in medium containing 100 μg/ml chloramphenicol; (B) HD-treated cells in absence of chloramphenicol; (C) untreated cells in presence of 100 μg/ml chloramphenicol. Thymine was added to all three flasks after 30 min of incubation.

Fig. 9 shows the results obtained. Upon addition of thymine, DNA synthesis

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began in flasks B and C, whereas there was no synthesis of DNA in flask A. Evidently chloramphenicol blocks DNA synthesis only in mustard-treated bacteria, suggesting that a specific effect of mustard is involved.

4. *Participation of RNA synthesis in the repair of the HD-induced block in DNA synthesis*

Considerable evidence is available indicating that protein synthesis in bacteria depends upon concomitant synthesis of RNA[23, 24]. Since we have found that resumption of DNA synthesis in mustard-treated *E. coli* requires prior protein synthesis, one may expect to find also a requirement for prior RNA synthesis. Experiments with a uracil-requiring *E. coli*, B_{U-}, and with the inhibitor 5-hydroxyuridine, bear out this prediction.

5-hydroxyuridine inhibits the formation of β-galactosidase in *E. coli*[21], presumably by blocking RNA synthesis. It was therefore hoped that this inhibitor would prove useful in analyzing the role of RNA synthesis in the repair of the HD-induced lesion.

Under our conditions, *E. coli* B treated with 85 μg/ml HD and suspended in growth medium containing 100 μg/ml 5-hydroxyuridine, grew at half the rate of HD-treated bacteria suspended in plain growth medium. The inhibition of DNA synthesis also lasted twice as long in presence of the inhibitor as in its absence. While this experiment supports a role for RNA synthesis in the repair process, it is open to alternative interpretations.

B_{U-}. Experiments with *E. coli* B_{U-}, which requires uracil for growth, parallel those previously described for B_{45} and 15_{T-PA-}.

B_{U-}, grown in presence of uracil, was washed and treated with 85 μg/ml HD. The cells were divided into two portions. One (A) was suspended in medium supplemented with uracil (25 μg/ml). The other (B) was incubated for 40 min in plain growth medium before addition of uracil. The results, shown in Fig. 10, followed the familiar pattern. It is clear that repair could not be accomplished in the absence of uracil. Appropriate control experiments showed that uracil starvation in untreated B_{U-} did not induce a lag in DNA synthesis. A small loss of RNA from HD-treated B_{U-} was generally noted during uracil starvation. It would thus appear that exogenous uracil is required for the repair of the HD-induced lesion in B_{U-}, suggesting the participation of RNA synthesis in this process.

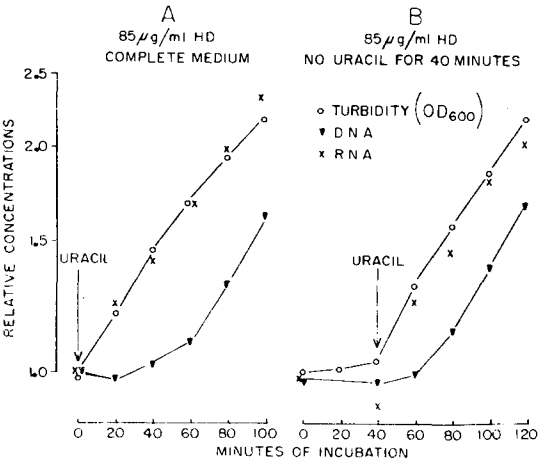

Fig. 10. Participation of uracil in the repair of the mustard-induced lesion in DNA synthesis. B_{U-}, treated with HD, were suspended in growth medium at 0 min. Uracil was added as indicated. Plot as in Fig. 1.

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DISCUSSION

The experiments discussed above constitute strong evidence that the resumption of DNA synthesis in mustard-treated *E. coli* depends upon prior synthesis of protein. In addition, limited evidence is presented supporting the view that RNA synthesis participates in the repair process. The latter observation finds an explanation in the experiments of PARDEE[23], indicating that in growing bacteria RNA synthesis must proceed concurrently with that of protein. DNA synthesis does not appear to be required for the repair of the lesion.

Dependence of DNA synthesis upon prior protein synthesis has been suggested for several other systems. Resumption of DNA synthesis in X-irradiated *E. coli* is facilitated by addition of amino acids and nucleic acid derivatives, suggesting participation of protein synthesis[25,26]. A similar situation may exist in bacteria subjected to ultraviolet irradiation since WITKIN[27] has demonstrated a relationship between protein synthesis and the expression of mutations. There also appears to be a relationship between protein synthesis and the expression of transformation in *Pneumococcus*[28]. However, the only comparable system which has been analyzed in detail is the multiplication of bacteriophage. Upon infection of *E. coli* with members of the T-even series of phages, DNA synthesis ceases. After a lag of several minutes, synthesis of phage DNA begins. If protein synthesis is inhibited during this lag period, the subsequent synthesis of phage DNA is blocked[15,16,29], establishing the dependence of DNA synthesis upon prior protein synthesis in this system.

We would like to consider briefly some possible hypotheses bearing upon the function of protein synthesis in the repair of the mustard-induced lesion in DNA synthesis. Alternative interpretations are by no means excluded.

a) Resynthesis of a cell constitutent. It may be proposed that mustard treatment causes the inactivation of a protein concerned with DNA synthesis (for example, an enzyme). Protein synthesis would then be required in order to replace the inactivated protein. Proteins, like nucleic acids, interact with mustards[30]; enzyme inactivation by treatment with mustard has been described[31]. Thus, though it would appear that DNA is particularly sensitive to inactivation by mustards[31,32], one should not dismiss the possibility that some of the biological effects of these substances, particularly at high concentrations, are to be attributed to their effect on cellular proteins.

b) Synthesis of a new protein. One may suppose that synthesis of proteins previously absent from the cell, or present in insufficient amounts, is required to overcome a block in DNA synthesis imposed by mustard. We might thus postulate that resumption of DNA synthesis requires the elaboration of an alternate pathway of DNA synthesis, by-passing the block. If this process involves enzyme synthesis, it might account for the observed participation of protein synthesis in repair. Another possibility is the synthesis of enzymes whose function is the destruction of a metabolic inhibitor derived from mustard.

c) The role of protein synthesis in the replication of DNA. Finally, we must consider the possibility that protein synthesis is an integral part of the replication of DNA. Studies on phage multiplication lend some support to the idea that, during the replication act, genetic information is transferred to newly synthesized protein which subsequently directs the synthesis of progeny DNA[29,33,34]. If the replication of bacterial DNA occurs by a similar mechanism, inactivation of the DNA by reaction

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with mustard may interrupt this process. Formation of new protein would then become a prerequisite for resumption of DNA synthesis.

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SUMMARY

1. Some of the physiological factors in the resumption of DNA synthesis in *E. coli* inactivated with sulfur mustard have been explored. Removal of the mustard was a necessary condition for the resumption of DNA synthesis, but not a sufficient one. Recovery from the block in DNA synthesis required the participation of both nitrogen- and carbon-energy sources.

2. Repair of the mustard-induced lesion in *E. coli* 15_{T-} could occur in absence of exogenous thymine, suggesting that DNA synthesis itself does not participate in the repair process.

3. Repair of the lesion was blocked under conditions which prevent protein synthesis. Thus, amino-acid requiring mutants of B and 15_{T-} were unable to effect repair when the required amino acids were withheld; repair was blocked by certain amino acid analogs and by chloramphenicol. It would appear that resumption of DNA synthesis after mustard treatment depends upon prior protein synthesis.

4. Evidence is presented suggesting that RNA synthesis is also required for repair of the lesion in DNA synthesis.

5. Possible explanations are considered.

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