[4] D. W. Woolley and R. B. Merrifield, *J. Am. Chem. Soc.*, **76**, 316, 1954.

[5] J. K. McAnelly and M. L. Speck, *J. Bacteriol.*, **73**, 676, 1957.

[6] M. L. Scott, L. C. Norris, and G. F. Heuser, *J. Biol. Chem.*, **166**, 481, 1946.

[7] J. C. Rabinowitz, N. I. Mondy, and E. E. Snell, *J. Biol. Chem.*, **175**, 147, 1948.

[8] E. E. Snell, *Physiol. Revs.*, **28**, 255, 1948.

[9] The tryptic casein digest (TDC) was prepared from vitamin-free casein with twice-recrystallized trypsin by the procedure of H. Sprince and D. W. Woolley, *J. Am. Chem. Soc.*, **67**, 1734, 1945. The preparation designated "EDC" is that sold by Nutritional Biochemicals Corporation as "Casein hydrolysate-enzymatic."

[10] The nature of the growth stimulation by the nucleic acid derivatives has not been further investigated, nor have impurities been ruled out as a contributing factor to their activity. The favorable effects of ascorbic acid on growth of *L. casei* in media of this type were observed by E. L. Rickes, P. J. Koch, and T. R. Wood, *J. Biol. Chem.*, **178**, 103, 1949. These supplements are not those supplied by either TDC or EDC, for they do not eliminate the effect of these preparations on growth.

[11] We are indebted to Dr. H. Tabor for a sample of spermidine, and to Dr. E. J. Herbst for 1,3-propanediamine and N-(3-aminopropyl)-1,3-propanediamine.

[12] E. J. Herbst and E. E. Snell, *J. Biol. Chem.*, **176**, 989, 1948; **181**, 47, 1949.

[13] W. H. Martin, Jr., M. J. Pelczar, Jr., and P. A. Hansen, *Science*, **116**, 483, 1952.

[14] P. H. A. Sneath, *Nature*, **175**, 818, 1955.

[14a] A. Traub, J. Meyer, and N. Grossowicz, *J. Bacteriol.*, **70**, 60, 1955.

[15] J. G. Hirsch and R. J. Dubos, *J. Exptl. Med.*, **95**, 191, 1952.

[16] J. G. Hirsch, *J. Exptl. Med.*, **97**, 323, 1953.

[17] R. Rozansky, U. Bachrach, and N. Grossowicz, *J. Gen. Microbiol.*, **10**, 11, 1954.

[18] C. W. Tabor and S. M. Rosenthal, *J. Pharmacol. Exptl. Therap.*, **116**, 139, 1956.

[19] M. Silverman and E. A. Evans, Jr., *J. Biol. Chem.*, **154**, 521, 1944.

[20] E. E. Snell, *J. Biol. Chem.*, **152**, 475, 1944. The culture of *Streptococcus lactis* used there is now called *Streptococcus faecalis* R.

[21] H. W. Dudley, M. C. Rosenheim, and O. Rosenheim, *Biochem. J.*, **18**, 1263, 1924.

[22] S. M. Rosenthal and C. W Tabor, *J. Pharmacol. Exptl. Therap.*, **116**, 131, 1956.

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*A RELATION OF NUCLEIC ACID SYNTHESIS TO RADIATION-INDUCED MUTATION FREQUENCY IN BACTERIA**

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During the past few years it has become increasingly evident that an appreciable portion of the mutations induced by radiant energy in various organisms are due to chemical reactions of the radiation with certain intracellular substances. The shape of the induced mutation frequency curves found by many investigators[1] suggests that radiation-sensitive material present in the cell is activated or altered by radiation in the process of mutagenesis. The mutation frequency reaches a plateau at high doses of radiation and then may decrease at still higher doses. Newcombe[2] and Newcombe and McGregor[3] have recently made an extensive investigation of the nonlinearity of the mutation frequency–X-ray dose curve with *Streptomyces griseus* and find evidence that nonlinearity in the curve is the result of a saturation effect which causes a lowering of the capacity of *Streptomyces* spores to

respond when the radiation dose is high. Based on accumulated evidence, a hypothesis may be advanced that the radiation-activated mutagen is limited in amount and is perhaps destroyed by the radiation.

Witkin[4] in a recent publication states that "the time interval between absorption of radiant energy and production of stable genetic changes can no longer be regarded as infinitesimal." She points out that a variety of postirradiation treatments (visible light, temperature changes, metabolites, and metabolic inhibitors) can drastically alter the mutation frequency produced by radiation. Her recent investigations establish that with certain *Escherichia coli* and *Salmonella typhimurium* auxotrophs, mutation to the prototrophic state induced by ultraviolet light requires the immediate postirradiation synthesis of protein.

We have studied the mechanism of ultraviolet mutation induction in bacteria by attempting to establish the nature of the intracellular material reacting with the radiation. This has been accomplished by preirradiation growth-factor supplementation techniques. We have also sought to establish by these studies, and by postirradiation studies similar to those of Witkin, the nature of the metabolic processes involved in establishing mutations in the genetic structure. Experiments reported here support a hypothesis that at least one induced mutation process in *E. coli* is dependent on two distinct but related events: (1) the action of radiation on the biosynthetic products of certain purine and pyrimidine bases found in nucleic acids; (2) the immediate postirradiation incorporation of the radiation-activated or radiation-modified products into the nucleic acid macromolecule. This latter process appears to be dependent on *concurrent* protein synthesis.

Methods.—The mutations of *E. coli* strain B studied were those giving aberrant colonial color response after 2 days of incubation on Difco eosin–methylene blue agar (EMB). The basic phenotype produced by mutation and responsible for this effect is due to modification of glucose or lactose fermentation. Some of the experiments described in the present report have been repeated in essential detail with *E. coli* B/r, using a similar color response on mannitol-tetrazolium agar.[5]

The basal growth medium used in these experiments was a salts-glucose medium, hereafter called "M medium."[6] This medium was supplemented with the various growth factors under test, as indicated in the experiments.

The radiation source was a Model 30600 Hanovia mercury-vapor lamp. Two-milliliter aliquots of the cells in small Petri dishes were placed 30 cm. from this source and were agitated during radiation. The output of the lamp at the position of the cells was 92.5 ergs/mm^2/sec at wave lengths below 2800 A (determined by Hanovia Model AV-971 ultraviolet meter). Operations during and subsequent to irradiation were carried out under yellow light to prevent photoreactivation.

The basic procedures used in these experiments were as follows: Fifty-milliliter portions of M medium were inoculated from a 24-hour nutrient agar slant of *E. coli* strain B. This culture was grown at 37° C. with aeration for 16 hours. Following incubation, the cells were held for 1 hour at 6° C., to obtain synchronization of cell growth and division.[7] The cells were removed from suspension by centrifuging in 10-ml. quantities and were resuspended in 10-ml. portions of M medium supplemented with various factors as indicated. These cultures were then incubated at 37° C. on a reciprocal shaker for the indicated time. Following incubation, the cells were immediately chilled and held at 2° C. for 10 minutes. They were

then centrifuged out in a refrigerated centrifuge, washed twice, and resuspended in cold 0.9 per cent saline. The turbidities of various cell suspensions were then adjusted to an optical density of 0.10–0.12 at 700 mμ with cold saline. The suspensions were distributed in 2-ml. aliquots to small cold Petri dishes and given various doses of ultraviolet. In experiments not concerned with postirradiation effects, the suspensions were plated immediately after irradiation onto EMB agar. One-tenth-milliliter quantities of the proper dilution were plated by the glass-rod-spreader technique, using 0.9 per cent saline for making serial dilutions. In experiments concerned with postirradiation effects, a 1:100 dilution of the suspension was made into M media containing various supplements. These cultures were incubated for the indicated time on the shaker at 37° C., and the cells were then plated on EMB agar as described above.

Effect of Various Supplements on Induced Mutation Frequency.—In the initial experiments the effect of cell incubation for a 4-hour period in M medium was compared with that of M medium plus yeast extract (20 mg/ml). Results indicated that cells incubated in yeast extract gave a materially higher subsequent induced mutation frequency than those incubated in unsupplemented M medium. The radiation-survival curves of the two cultures were almost identical. Experiments were next carried out to determine the factors in yeast extract responsible for the observed increase in induced mutation frequency. It was found that vitamin supplementation[8] or RNA purine and pyrimidine supplementation (0.01 mg/ml of adenine, guanine, uracil, cytosine) produced increases in the induced mutation frequency of the same order of magnitude as those produced by yeast extract. Casein hydrolyzate (2 mg/ml) produced little or no increase in induced mutation frequency, and the DNA purines and pyrimidines (0.01 mg/ml of adenine, guanine, thymine, cytosine) showed only a slight effect.

In further experiments of the same type in which supplementation was with single vitamins, it was established that *p*-aminobenzoic acid and riboflavin were responsible for the observed increase in mutation frequency. Either growth factor, when present in concentrations of 1 μg/ml, increased the mutation frequency to a rate comparable with that observed with yeast extract. The combined effects of these factors were not additive. Experiments on the mechanism involved in increasing induced mutation frequency by these vitamins will be reported elsewhere. The present report deals with the effect of purines and pyrimidines.

Relation of Ultraviolet Dose to Mutation Frequency.—Figure 1 presents the mutation frequencies and surviving fractions at various ultraviolet doses for cells incubated in M medium and in M medium supplemented with adenine, guanine, uracil, and cytosine for 1 hour before exposure to the radiation. The results demonstrate that incubation in purines and pyrimidines contained in RNA produces a marked increase in both the frequency of mutation with unit dose and the level of maximum mutation frequency occurring at the same radiation dose (20–25 seconds) in suspensions incubated in either M medium or supplemented medium. When the dose is increased above 25 seconds, there occurs a marked decline in mutation frequency in both cases.

A consideration of the survival curves demonstrates, on the other hand, that survival following irradiation is affected little if any by incubation in the presence of purines and pyrimidines. This argues against the possibility that preincubation

in purines and pyrimidines renders mutants more radiation-resistant, or wild type more radiation-sensitive. The break in the survival curves from the initial exponential rate of very high killing to a second exponential rate of greater radiation resistance occurs at an ultraviolet dose of 7.5–10 seconds—a dose considerably lower than that at which the maximum mutational frequency is obtained. Survival curves for a number of mutant and wild-type isolates established from colonies plated at various dose levels have been obtained. No consistent difference in the survival characteristics of the mutant and wild-type strains could be demonstrated from these curves. An identical experiment was conducted, using *E. coli* strain B/r, which has markedly different ultraviolet survival characteristics. The ultraviolet dose–mutation frequency curves obtained under the two conditions of preirradiation incubation were almost identical with those demonstrated with *E. coli* strain B, regardless of the fact that strain B/r was much more resistant than strain B at all doses of ultraviolet. All these studies lead to the conclusion that selection through differential killing is not involved in the results obtained.

Purine and Pyrimidine Combinations.—Preirradiation incubation experiments were carried out in M medium supplemented with purines and pyrimidines singly and in various combinations. These experiments demonstrated that maximum increase in mutation frequency was not observed unless the supplementation included uracil, cytosine, and either adenine or guanine. That either adenine or guanine will satisfy the purine requirement is to be expected in *E. coli*. Figure 2 gives the maximum mutation frequencies attained in a typical experiment of this type. The results demonstrate the requirement for the presence of either adenine or guanine in order for an increase in the induced mutation frequency to occur. Of the bases added individually, uracil has the greatest effect; however, it was only when guanine or adenine and cytosine were also added that a maximum frequency comparable to that of yeast extract was obtained. Substitution of thymine for uracil resulted in a drastic reduction in the maximum mutation frequency obtained.

It is not possible on the basis of this evidence to determine whether RNA or DNA synthesis (or possibly both) is involved in the observed increase in induced mutation frequency. The fact that uracil is effective, and not thymine, suggests that RNA synthesis is directly involved. However, recent studies demonstrate that labeled thymine supplied in the medium to *E. coli* is not incorporated into the RNA or DNA of the bacterium. The labeling of uracil, on the other hand, is incorporated into both RNA and DNA, presumably being transformed to thymine by methylation of some intermediate.[9,10] The singularity of uracil in the purine and pyrimidine effect therefore does not serve as a reliable indication that RNA synthesis, as opposed to DNA synthesis, is involved. Further work on both the genetic and the biochemical nature of the particular hereditary systems under study will be necessary to determine the nucleic acid macromolecule(s) involved.

Incubation Interval Studies.—Experiments were next conducted to determine the period of incubation necessary to attain a maximum increase in mutation frequency. In the first experiments, periods of preincubation for 1, 2, 3, and 4 hours were compared. The results indicated that the maximum increase in induced mutation frequency is obtained with cells incubated 1 hour or less in the purine-pyrimidine-supplemented medium. The maximum induced mutation frequencies were progressively less after 2, 3, and 4 hours of incubation.

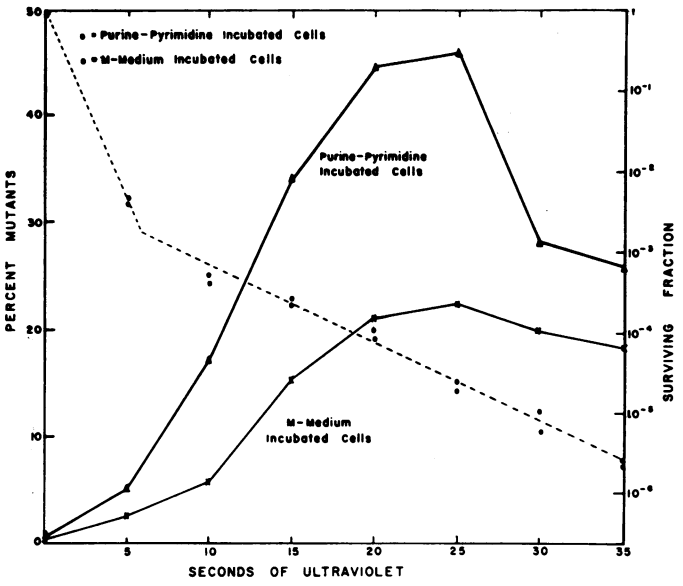

FIG. 1.—Ultraviolet survival and induction of "EMB color" mutants in *E. coli* strain B grown for 1 hour in the indicated medium prior to irradiation. The broken line represents the survival curve of both cultures; the solid lines, the mutation frequency–radiation dose curves.

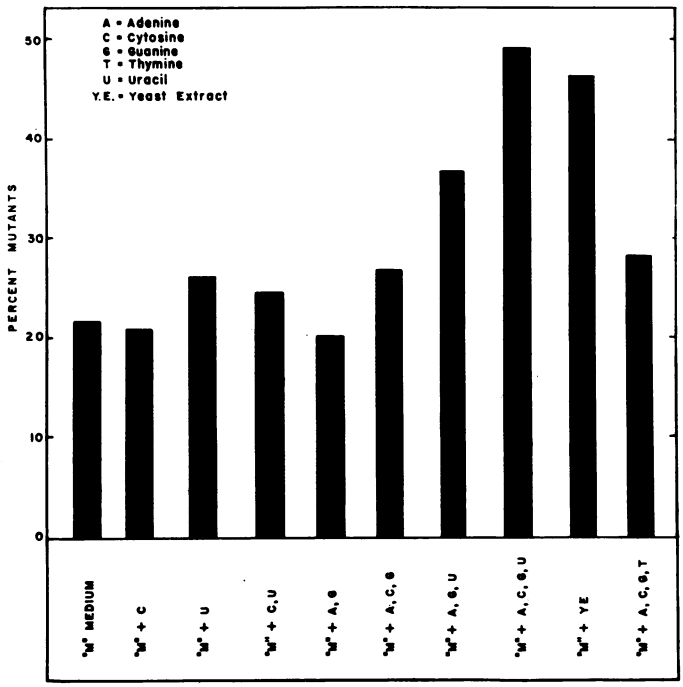

FIG. 2.—Per cent of "EMB color" mutations induced by 25 seconds of ultraviolet in *E. coli* strain B grown for 1 hour prior to irradiation in M medium supplemented with the indicated purines and pyrimidines.

The experiments were then repeated, using short incubation intervals from 0 to 60 minutes. The results of such an experiment, in which yeast-extract supplementation was used in one medium and adenine, guanine, cytosine, and uracil in another, are presented in Figure 3. The results show that an initial incubation interval of approximately 20 minutes in purine and pyrimidine medium is necessary before an appreciable increase in radiation-induced mutation frequency takes place. Following this initial lag (which varies somewhat in duration from experiment to experiment), a rapid increase is observed, until maximum frequency is attained after 30–35 minutes of incubation. With yeast extract, the increase in susceptibility to subsequent induced mutation starts immediately with incubation. Further experiments have shown that if the bacteria are incubated in M medium supplemented with the ribosides (adenosine, guanosine, uridine, and cytidine) rather than with the purines and pyrimidines, the lag is reduced to less than 5 minutes.

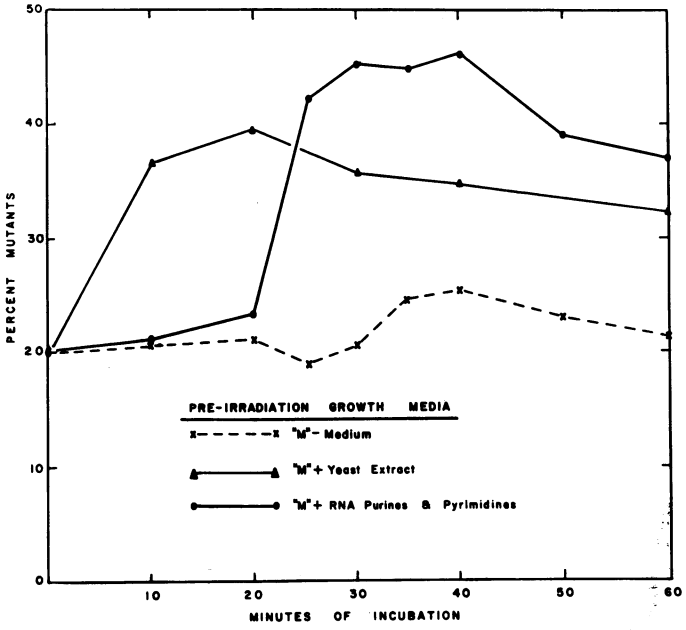

FIG. 3.—Effect of preirradiation growth medium and incubation time on the frequency of "EMB color" mutants induced in *E. coli* strain B by 25 seconds of ultraviolet.

The Relation of the Physiological State of the Cell to Induced Mutation Frequency.—Synchronized culture experiments were repeated, and the physiological state of the culture was studied by determining at each increment of incubation the average nuclear number (Robinow's stain[11]), increase in colony-forming organisms, increase in RNA synthesis,[12] increase in DNA synthesis (by the Burton-Dische method[13]), and the mutation frequency induced by 25 seconds of ultraviolet. The results of these experiments are given in Figure 4 and Table 1. Doubling in colony-forming organisms during the 40–50-minute incubation period shows that the cultures are in a high state of synchrony. The maximum frequency of mutation induction is attained at about 20 minutes of incubation in purine-pyrimidine-supplemented

TABLE 1*

Incubation Time (Minutes)	DNA Net Increase		RNA Net Increase		Relative Turbidity		Cell Counts after Incubation ($\times 10^8$)†		Per Cent Induced Mutants†	
	M Medium	M Medium + Pur/Pyrim	M Medium	M Medium + Pur/Pyrim	M Medium	M Medium + Pur/Pyrim	M Medium	M Medium + Pur/Pyrim	M Medium	M Medium + Pur/Pyrim
0	1.00	1.00	1.0	1.0	1.00	1.00	1.0	1.6	22.0	19.0
10	1.09	1.00	1.3	1.3	1.11	1.11	1.4	1.7	21.2	22.0
20	1.18	1.08	1.6	1.6	1.33	1.44	1.4	1.5	20.2	47.0
30	1.36	1.15	1.9	2.0	1.44	1.67	1.2	1.5	10.5	46.0
40	1.45	1.15	2.5	2.2	1.67	1.89	1.8	1.3	22.9	50.4
50	1.45	1.46	2.6	2.2	2.11	2.22	3.5	3.0	24.0	40.2
60	1.82	1.46	2.9	2.8	2.33	2.44	3.8	4.0	18.1	61.7
70	1.82	1.69	3.7	3.2	2.67	2.78	4.1	4.3	20.2	61.0
80	2.00	1.69	3.9	..	2.78	2.78	4.1	..	21.0	49.0

* Two identical cultures of *E. coli* strain B in synchronized growth were tested. One culture was incubated in M medium, and the second in M medium supplemented with guanine, adenine, cytosine, and uracil. Aliquots of each culture were withdrawn at the indicated incubation times, and in each case the following factors were determined: (1) DNA content, (2) RNA content, (3) cell protein (relative turbidity), (4) time of cellular division, (5) time of first nuclear division, (6) frequency of "EMB color" mutations induced by 25 seconds of ultraviolet.

† The cell counts and induced mutation frequencies for each culture are plotted against incubation time in Fig. 4. The approximate time of the first nuclear division in each culture is also indicated in Fig. 4.

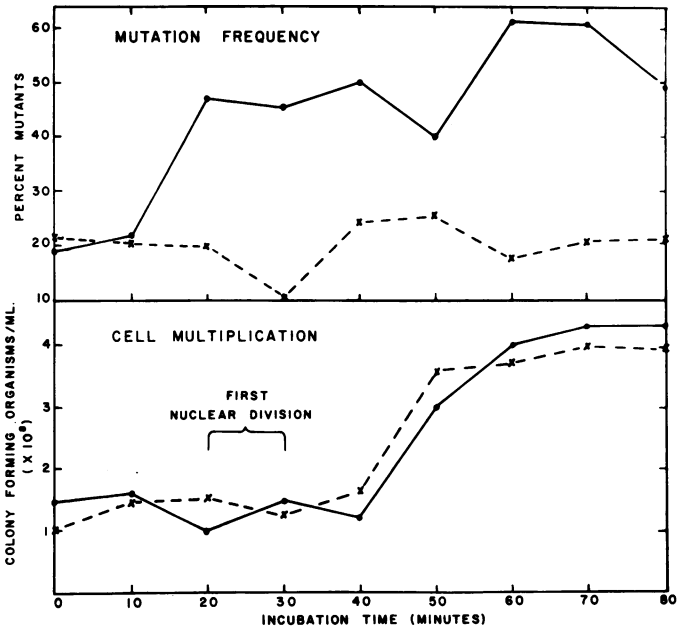

FIG. 4.—Correlation of cellular physiology with per cent of "EMB color" mutants induced in synchronized cultures of *E. coli* strain B by 25 seconds of ultraviolet. The broken line represents induced mutation frequency and colony-forming cells immediately prior to irradiation in a culture grown for the indicated time in M medium prior to irradiation. The solid line represents the same factors found in identical cultures grown for the indicated time in M medium supplemented with adenine, guanine, cytosine, and uracil. The first nuclear division takes place in both cultures after 20–30 minutes of incubation. RNA, DNA, and turbidity values for each incubation period and both cultures are given in Table 1.

medium. On further incubation, the frequency appears to level off or fall slightly during the period of 20–30 minutes. It is during this time that the first nuclear division is observed to take place. The decrease in induced mutation frequency which occurs after 40–50 minutes of incubation appears to be correlated with cell division. When cell division is completed, a second increase in frequency takes place which is comparable to the initial increase and additive to it.

RNA synthesis occurs at a fairly constant rate in both media until the initiation of cell division (Table 1). At this time, RNA synthesis ceases for a period of about 10 minutes—presumably until division is completed. It is during this period that a decline in maximum mutation frequency is observed. At the time of cell division the total quantities of RNA in cells grown in M medium or in supplemented medium are the same, while the maximum mutation frequency is considerably higher in cells incubated in purines and pyrimidines. Therefore, if RNA is involved at all, it would appear that the quantity of RNA precursors immediately preceding nuclear division, and not the actual quantity of RNA at a given time, is the important factor in increasing the mutation frequency. Similar considerations apply to DNA. While the ratio of cellular DNA of cells incubated in the two media remains practically at unity throughout the course of incubation, different mutation frequencies are observed; and DNA synthesis after the first nuclear division has no effect in increasing maximum mutation frequency with purine-pyrimidine-incubated bacteria. This strongly suggests that the marked increase in ultraviolet-induced mutation frequency following incubation in purines and pyrimidines is ascribable to the presence in the cell of increased quantities of nucleic acid precursors at the time of irradiation Quantitatively different protein synthesis is probably not involved, since the turbidity increase occurs at regular and similar rates in both media.

Cellular synthetic processes dependent on inorganic nitrogen, and an energy source must occur prior to irradiation in order for any increase in mutation frequency to take place at subsequent irradiation. This was established in similar experiments using purine-pyrimidine-supplemented M medium from which the nitrogen source or the carbon source has been omitted. When either of these were omitted from supplemented medium, the usual increase in mutation frequency did not occur.

Postirradiation Treatment.—Witkin[4] has demonstrated that postirradiation synthesis of protein is necessary for expression of induced prototrophs with certain auxotrophs of *E. coli* and *S. typhimurium*. To be effective, protein synthesis must occur within the hour immediately following irradiation.

Figure 5 demonstrates that increases in the maximum frequency of radiation-induced mutations brought about by preirradiation incubation in purine-pyrimidine-supplemented medium are similarly dependent on postirradiation protein synthesis for expression. Experimentation with this mutational system confirms Witkin's observation that protein synthesis within the hour following radiation exposure is necessary for mutation expression. The suspensions, regardless of their preirradiation incubation medium, have a greatly reduced mutagenic response if incubated for 1 hour immediately following irradiation in M medium minus a nitrogen source. Similar results were obtained by inhibition of protein synthesis with chloramphenicol. Postirradiation incubation of the organisms in M medium also limits the mutagenic response of cells incubated in purines and pyrimidines

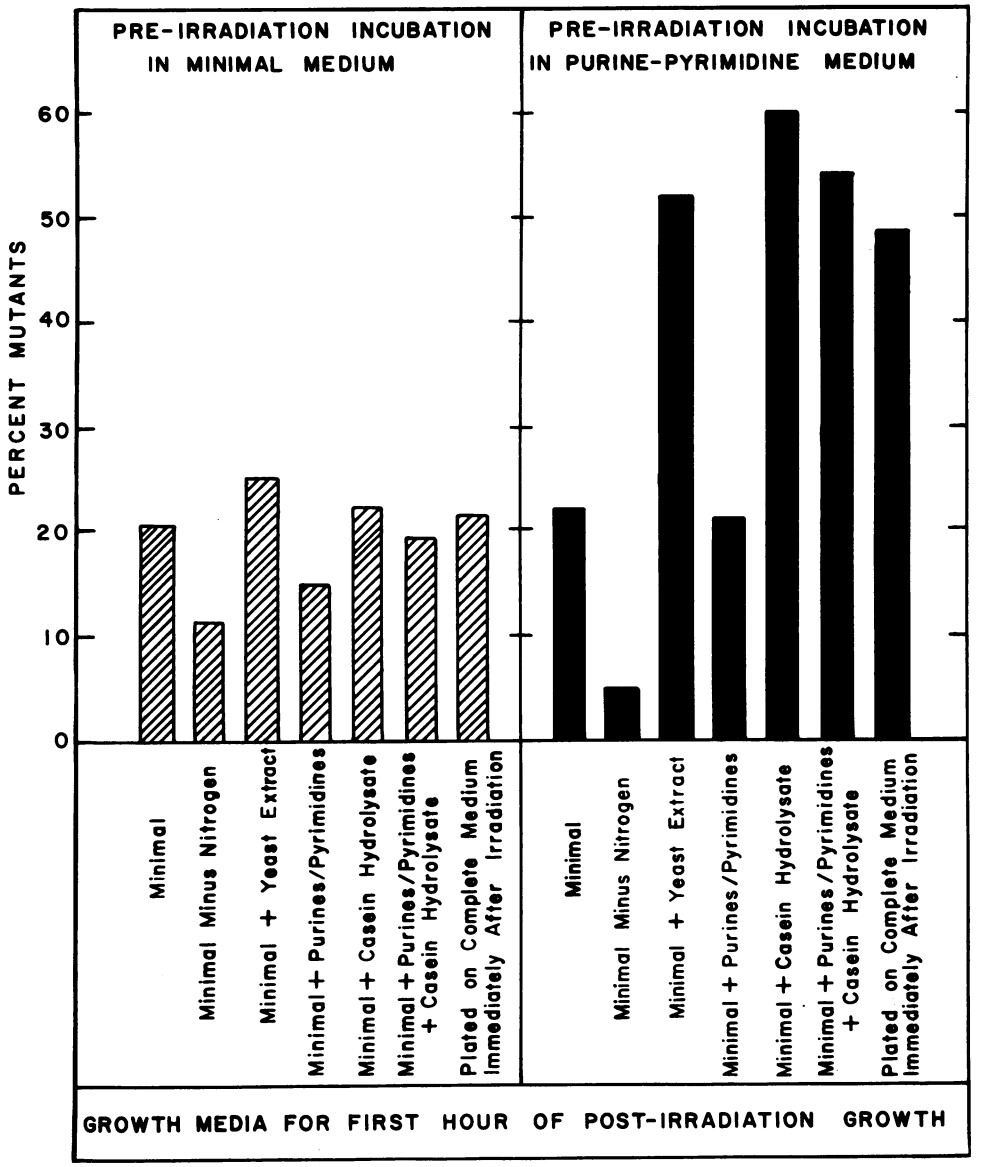

FIG. 5.—Effect of postirradiation growth medium on the frequency of "EMB color" mutations induced by 25 seconds of ultraviolet in *E. coli* strain B. One culture was grown in M medium, and a second identical culture in M medium supplemented with adenine, guanine, cytosine, and uracil, for 1 hour prior to irradiation. Immediately following irradiation, aliquots of the two irradiated suspensions were incubated in each of the indicated media for 1 hour and then plated on EMB agar.

prior to irradiation. Presumably, this is related to the fact that protein synthesis is somewhat limited in M medium, since cells have to synthesize the amino acid components of protein *de novo*. On the other hand, this intermediate limitation of protein synthesis does not seem to affect the lesser mutagenic response of cells incubated in an unsupplemented medium prior to radiation exposure. It is only when a full complement of amino acids (casein hydrolyzate) or a complex natural extract (yeast extract) is supplied to the suspension immediately following irradiation that the greatly increased mutagenic response produced by preincubation in purines and pyrimidines is observed. These experiments also demonstrate the ineffectiveness of the purines and pyrimidines in increasing the induced mutation frequency when they are added immediately following radiation exposure.

Discussion.—Most of the findings in this preliminary survey warrant more extensive study. However, sufficient evidence has been accumulated to support the following hypothesis concerning the underlying mechanisms of mutation induction by ultraviolet light, at least for certain types of mutation in *E. coli:*

1. Intermediates in nucleic acid synthesis arising from purines and pyrimidines must be present during ultraviolet exposure in order for mutagenesis to occur.

2. Incorporation of these intermediates, which presumably have been activated or modified by the radiation, into the hereditary macromolecule is dependent on the synthesis of protein immediately following radiation exposure.

By utilizing cultures synchronized in cellular development and division, it has been possible to compare the physiological characteristics of the culture at various points during cellular development with the observed increase in mutation frequency dependent on the presence of purines and pyrimidines in the medium. These studies have led to two significant observations. In the first place, it has been demonstrated by cytological investigations that the maximum increase in mutagenic response of the purine-pyrimidine-incubated cells occurs prior to and during the first nuclear division. Following nuclear division, no further increase is observed until cell division has been completed. This relation of mutagenic frequency to nuclear division could not be due to an exhaustion of purines and pyrimidines in the medium, since increased purine-pyrimidine concentration does not stimulate any increase in the mutagenic response after the first nuclear division. In addition, further increase in mutation frequency dependent on purines and pyrimidines is observed following cell division. Whether this relation of maximum mutation frequency increase to nuclear division is direct or only coincidental cannot be ascertained on the basis of evidence presently available. A comparable relation of mutation response to nuclear division has been demonstrated in *Streptomyces griseus* spores by Newcombe[2] and by Newcombe and McGregor.[3] These investigators demonstrated that the maximum mutational frequency induced by X-rays is observed in the binucleate stage following germination. Further nuclear division results in a marked decline in mutation frequency in these organisms. Newcombe emphasizes a close association between nuclear number and radiation-induced mutation.

The synchronized growth experiments point up a second significant fact. There appears to be no correlation between nucleic acid content of the cell and the mutagenic response observed. Cultures incubated in either M medium or the purines and pyrimidines show comparable nucleic acid contents (both RNA and DNA)

but widely different induced mutation frequencies. If nucleic acid formed prior to irradiation is involved at all in increasing mutagenic frequencies, then that which is synthesized later than the first nuclear division has no effect (at least until after cellular division). Our data suggest that increased amount of nucleic acid precursors present in the cell during radiation exposure are the important factors involved in increasing induced mutation frequency.

Since a combination of purines and pyrimidines in the medium is required for maximum mutation frequency, it is suggested that these basic components of nucleic acid are involved in the final process responsible for occurrence of mutation in the genetic factors concerned here. Since the nucleic acid precursors must be supplied prior to irradiation, it is probable that induced mutation expression is related to the rate of postirradiation nucleic acid formation. Maximum nucleic acid synthesis would be realized when all component precursors or their proper facsimiles were present in the cell in nonlimiting quantities following radiation.

It is quite evident that the precursors must be exposed to radiation for increases in mutation frequency, since purines and pyrimidines added immediately following exposure do not cause such an increase. There is some evidence that under certain conditions purines and pyrimidines added immediately following radiation exposure act to decrease the mutation frequency.[14]

On the basis of present evidence it is not possible to determine the biosynthetic intermediates to nucleic acid which are affected by ultraviolet light. It is improbable that radiation-modified purines and pyrimidines are the effective compounds, since a considerable lag (15–25 minutes) is noted in the incubation-time experiments. This could be due to an adaptive lag in uptake of purines and pyrimidines, but, since the corresponding ribosides show an almost immediate effect similar to that of yeast extract, it is most likely that the radiation-precursor interaction is at the least at the stage of ribosides. It is entirely possible that the ribotides are involved, and the ribotides may actually have to be associated with the nucleic acid "template" in the final stage of nucleic acid synthesis for maximum mutagenic effect.

That increase in mutation frequency by incubation with purines and pyrimidines is dependent on postirradiation protein synthesis has been demonstrated in the present studies. It seems probable in view of the marked similarity of these results to those of Witkin[4] that both investigations deal with the same mechanism of induced mutagenesis in bacteria. If a relation of nucleic acid synthesis to mutation induction can be demonstrated in the protein-dependent mutational response described by Witkin, the mechanism of mutation induction described in the present report may have widespread application. We have obtained preliminary evidence that this mechanism possibly has general application to mutations in *E. coli*. In three out of four experiments we have been able to increase significantly the maximum frequency of mutation to bacteriophage T1 resistance in *E. coli* strain B by incubation in purines and pyrimidines prior to ultraviolet irradiation. Also, studies using X-rays instead of ultraviolet show that similar results are obtained with this radiation, although increases in the induced frequencies are of much smaller magnitude.[14]

Spiegelman[15] has found it possible to derive from osmotically ruptured protoplasts of *Bacillus megaterium* a fraction which retains a considerable capacity to

synthesize DNA, RNA, and protein. In a study of RNA synthesis of this fraction, an absolute requirement for the presence of a complex mixture of amino acids was noted.[16] These data suggest that RNA synthesis is accompanied by the fabrication of a particular kind of protein molecule. Ezekiel and Spiegelman[17] have found that a complex mixture of amino acids is an absolute requirement for extensive synthesis of DNA in these preparations. This can be considered strong evidence that formation of the macromolecules of primary hereditary significance—DNA and RNA—is accompanied by, and dependent on, synthesis of specific protein. The possibility that the process of induced mutagenesis involves concurrent synthesis of protein and the nucleic acid macromolecule responsible for a particular hereditary characteristic must be considered in the light of these findings.

It is quite possible that the preformed nucleic acid molecule is relatively resistant to mutation and that most (or even all) mutagenic agents exert a major portion of their effects through the postexposure synthesis of nucleic acid. If this is the case, and if it holds true in higher organisms, then its significance in problems of radiation exposure and protection is quite apparent. In case abnormal purines and pyrimidines, or nucleic acid presursors containing them, are the mutagens involved, this would be quite pertinent to the study of malignant growth. It may be that certain abnormal purines and pyrimidines produced as products of cell degeneration or disintegration, and from normal purines and pyrimidines by other body processes, are built into the genetic mechanism of newly synthesized cells, to produce mutations which result in neoplastic growth.

Summary.—Both the frequency of mutation per unit dose and the maximum mutation frequency obtained with minimal-medium-grown *E. coli* strain B exposed to ultraviolet light may be increased considerably for certain mutations (aberrant color response on EMB agar). This increase is obtained by short-period incubation prior to irradiation in minimal medium supplemented with yeast extract, a combination of purines and pyrimidines (adenine or guanine, uracil and cytosine), riboflavin, or *p*-aminobenzoic acid.

By using cultures synchronized in growth and division, the following has been established in regard to the purine-pyrimidine influence: (1) maximum increase in frequency is attained during periods of maximum nucleic acid synthesis; (2) little or no increase is observed following the first nuclear division; (3) the maximum mutation frequency is reduced by cellular division; (4) the increased mutation frequency obtained by incubation in the presence of purines and pyrimidines bears no relation to nucleic acid content of the cell—rather, an increase in nucleic acid precursors in the cell prior to radiation exposure may be the important factor; (5) the increase in mutation frequency promoted by preirradiation incubation with purines and pyrimidines is dependent on postirradiation protein synthesis.

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[1] For a review of the literature see M. R. Zelle, in *Radiation Biology*, ed. A. Hollaender (1955), Book II, chap. x, pp. 404–414.

[2] H. B. Newcombe, *Atomic Energy of Canada Limited Publ. No. 144*, pp. 326–338, 1955.

[3] H. B. Newcombe and J. F. McGregor, *Genetics*, **39**, 619–627, 1954.

[4] E. M. Witkin, *Cold Spring Harbor Symposia Quant. Biol.*, **21**, 123–140, 1956.

[5] H. B. Newcombe and H. A. Whitehead, *J. Bacteriol.*, 61, 243–251, 1951.

[6] M medium is a glucose-salts basal medium of the following composition: KH_2PO_4, 3.9 gm.; K_2HPO_4, 7.9 gm.; sodium citrate; and $2H_2O$, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.1 gm.; $(NH_4)_2SO_4$, 1.0 gm.; glucose, 2.0 gm.; H_2O, 1 liter. The glucose is sterilized separately and added after cooling.

[7] D. B. McNair Scott and Eileen Chu, *Bacteriol. Proc.*, p. 114, 1957.

[8] Vitamin supplementation was with the following, so that the final concentration was 1 μg/ml: niacin, riboflavin, thiamine, biotin, *p*-aminobenzoic acid, folic acid, calcium pantothenate, pyridoxine, pyridodoxal, pyridoxamine, cobalamine.

[9] L. Siminovitch and A. F. Graham, *Can. J. Microbiol.*, 1, 721–732, 1955.

[10] M. Green and S. S. Cohen, *J. Biol. Chem.*, 225, 387–396, 1957.

[11] C. F. Robinow, *Proc. Roy. Soc. London, B*, Vol. 130, 1942.

[12] P. C. Cadwell and Cyril Hinschelwood, *J. Chem. Soc.*, pp. 1415–1418, 1950.

[13] E. Volkin and W. E. Cohn, "Estimation of Nucleic Acids," in *Methods of Biochemical Analyses*, ed. David Glick (New York: Interscience Publishers, Inc., 1955), pp. 287–303.

[14] F. L. Haas and C. O. Doudney, unpublished data.

[15] S. Spiegelman, in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (Baltimore, Md.: Johns Hopkins Press, 1956), pp. 232–267.

[16] A. I. Aronson and S. Spiegelman, *Bacteriol Proc.*, p. 138, 1957.

[17] D. H. Ezekiel and S. Spiegelman, *Bacteriol Proc.*, p. 137, 1957.

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*THE FORMATION OF ANTIBODIES IN MAN AFTER INJECTION OF PNEUMOCOCCAL POLYSACCHARIDES**

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The studies summarized in the present paper began with the injection of one to six of the type-specific pneumococcal polysaccharides, first into volunteers and then into trainees for the air force, followed by quantitative measurement[1] of the amounts of circulating antibody nitrogen in bleedings taken at varying intervals after the injections. The results, which played their part in the speedy termination of an epidemic of pneumonia,[2] have been published elsewhere.[3, 4] Additional data obtained later[5–9] increased the body of information regarding the human response to the injection of pneumococcal polysaccharides.[10] Analysis and evaluation of the material as a whole are as yet lacking and are accordingly presented herewith.

Data from the papers mentioned above are summarized in Tables 1 and 2 and in Figures 1 and 2. In these, the first two series of subjects in an earlier study[3] are omitted, since the dose given, 20–30 μg., was considerably less than the optimal 50–70 μg. of each of the type-specific polysaccharides. No attempt has been made to separate the groups which received only one of the polysaccharides or received two to six simultaneously, since an increase in the number of type-specific polysaccharides administered did not seem to diminish the response to any one type. Moreover, only those analyses are included which were made after prior removal of antibodies to pneumococcal C-substance, the common somatic polysaccharide of all pneumococci, since some, at least, of the type-specific polysaccha-