onstrated the exceptional stability of the images (both short-term and long-term) over a period of 4–8 hr, and the relatively high quality of the images at magnifications of 50–100 ×. The results obtained with this experimental approach and some interesting observations bearing on imaging phenomena with superconducting solenoids have been described here.

I gratefully acknowledge the expert technical assistance of Mr. Louis P. Ouwerkerk, Mr. R. J. Szara, and Dr. K. Yada in the course of these experiments. I also wish to thank Prof. L. Meyer and his colleagues of the Low-Temperature Laboratory, and Prof. W. Bloom of the University of Chicago for valuable suggestions. I am greatly indebted to Prof. W. H. Sweet, Massachusetts General Hospital, Dr. S. Autler, Prof. S. C. Collins, and Prof. F. O. Schmitt of Massachusetts Institute of Technology for their early interest and encouragement in this project since 1961. Sincere thanks are also due to Mr. C. L. Hough, Mr. R. Luftig, Miss L. LaDeur, Miss J. Richardson, Miss A. Hollinger, and other associates from our laboratory. I am also indebted to Mr. J. Hanáček, Mr. J. Costa, and other members of the workshop for their valuable assistance. Sincere appreciation is due Mr. F. Lins, Mr. C. L. Berrington, and other members of the Westinghouse Cryogenics Systems Department for their kind help.

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[1] Ruska, E., in *Proceedings Fifth International Congress for Electron Microscopy*, ed. S. Breese (New York: Academic Press 1962), vol. 1, A-1.

[2] Leisegang, S., in *Encyclopedia of Physics* (Berlin: Springer, 1955), vol. 33, p. 396.

[3] Haine, M. E., and V. E. Cosslett, *The Electron Microscope* (New York: Interscience Publishers, Inc., 1961).

[4] Autler, S. H., in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 324.

[5] Kunzler, J. E., *Rev. Mod. Phys.*, **33**, 1 (1961).

[6] Hulm, J. K., M. J. Fraser, H. Riemersma, A. J. Venturiano, and R. E. Wien, in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 332.

[7] Marshall, H. L., and H. E. Weaver, *J. Appl. Phys.*, **34**, 3175 (1963).

[8] Fernández-Morán, H., *Ann. N. Y. Acad. Sci.*, **85**, 689 (1960).

[9] Fernández-Morán, H., *J. Roy. Microscop. Soc.*, **83**, 183 (1964).

[10] Fernández-Morán, H., *Circulation*, **26**, 1039 (1962).

[11] Henry, W. E., in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 324.

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*ISOLATION AND CHARACTERIZATION OF RECOMBINATION-DEFICIENT MUTANTS OF ESCHERICHIA COLI K12**

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Certain features of the process of genetic recombination at the molecular level have recently become evident: (1) Recombination in bacteria and viruses involves the physical interaction of and subsequent inheritance by recombinant progeny of double-stranded elements of DNA derived from two parents.[1–5] (2) The unreplicated recombinant DNA may contain a double-stranded region in which

the two complementary strands are derived from different parents.[6] (3) Recombination may involve the removal and resynthesis of small amounts of DNA.[5]

Since it did not seem unlikely that enzymes participate in the events leading to the formation of the completed recombinant DNA structure, one of the authors (A. D. M.) undertook the isolation of mutants in which one or more of the hypothetical recombination enzymes would be defective.

After two mutants had been isolated, the authors learned that each of the above features had been incorporated into a model of recombination devised by Howard-Flanders.[7] His purpose was to show the similarity between the steps in recombination and the steps thought to be involved in the *in vivo* removal from DNA of photoproducts formed by exposure of cells to ultraviolet light and their replacement by undamaged nucleotides.[8, 9] The first step in this model of recombination is the breakage of the parental DNA's and synaptic pairing of complementary single-stranded ends of two parental fragments by the formation of hydrogen bonds between complementary sequences of bases. This step has no counterpart in the excision from DNA of photoproducts, the step which initiates the repair of irradiation damage. The subsequent steps of the two processes are formally similar, however, and can be described as follows: (1) In recombination there is degradation of the single strands of terminal regions of the parental fragments which are not involved in the double-stranded region holding the two fragments together. This step may be similar to the step in repair of photodamaged DNA which leads to removal from the DNA of 30 nucleotides for every thymine dimer excised.[9, 10] (2) Following the degradation step, a polymerization step occurs during which the gaps in the DNA are filled by newly synthesized single-stranded regions. (3) The final step in both recombination and repair of photodamage is the restoration of the integrity of the phospho-sugar backbone of the DNA by joining the newly synthesized single strands to the extant single strands at the side of the gap opposite the side from which the polymerization began.

The similarity of the models for recombination and the repair of photodamaged DNA has led Howard-Flanders[7] to predict that mutants would occur in which one of the enzymes common to the two processes was defective. Such mutants are expected to possess two phenotypic properties if the mutations are obtained in an F^- strain of *E. coli* K12: (1) inability to form recombinants by conjugation, hence appearing infertile in crosses with Hfr strains, and (2) inability to repair photodamaged DNA, hence appearing very sensitive to the lethal effects of UV irradiation. In this report we wish to describe the isolation and preliminary characterization of two such mutants.

Materials and Methods.—Strains used: The bacterial strains used in this study are characterized below according to the alleles of relevant nutritional and fermentative genes they carry,[11] their mating type,[11] and their phenotypic response to phages and other lethal agents:[11]

JC-182: *thr*+, *leu*+, *his*+, *pur*−1, *arg*+, *met*+, Thi−, *lac*+, *mal*+, λ^S, $P1^S$, $T6^S$, Sm^S, UV^R, D, double male strain. See reference 10.

JC-1020: *thr*+, *leu*+, *his*+, *pur*+, *arg*+, *met*+, Thi−, F-*lac*$^+$/*lac*$^-$, *mal*+, λ^S, $P1^S$, $T6^S$, Sm^S, $UV^?$, D, F-*lac* donor derived from strain 200P.

JC-1164: *thr*+, *leu*+, *his*+, *pur*+, *arg*+, *met*+, Thi−, *lac*+, *mal*+, (λ), $P1^S$, $T6^S$, Sm^S, $UV^?$, D, a lysogenic derivative of a Hayes Hfr.

JC-411: *thr*+, *leu*−2, *his*−1, *pur*+, *arg*−6, *met*−1, *thi*+, *lac*−1, 4, *mal*−1, λ^R, $P1^S$, $T6^S$, Sm^R, UV^R, ND.

JC-1553 and JC-1554: *thr*+, *leu*−2, *his*−1, *pur*+, *arg*−6, *met*−1, *thi*+, lac−1, 4, mal−1, λ^R, $P1^S$, $T6^S$, Sm^R, UV^S, ND.

Those strains which carry mutant alleles are either dependent for growth at 37°C on the presence of a nutritional supplement in minimal medium or are unable to utilize particular carbon sources for growth. Ordinarily, the nutritional supplement required can be inferred from the pathway affected by the mutation; however, this is not true of strains carrying *pur*-1. Such mutant strains will utilize adenine as the sole purine source.

Media and mating conditions: All of the media and most of the mating conditions used have been previously described fully by Clark[12] and by Adelberg and Burns.[13]

Mating on plates was accomplished by inoculating strains of one parent onto a "lawn" of the other parent spread on medium selective for recombinants. The inoculum was obtained from growth of nondonor strains on a complex or minimal agar medium either in the form of colonies or heavy confluent growth in patches. The Hfr strain was prepared by washing cells obtained from overnight growth in complex medium with *M*/20 phosphate buffer at pH 7.0. Approximately 2×10^9 washed cells of the Hfr strain were spread onto the surface of a minimal medium selective for recombinants. This plate was inoculated by replica plating as was a control plate of selective medium containing no Hfr cells. After the plates had been incubated for 24 and 48 hr they were examined for the presence of recombinant colonies within the areas inoculated by the nondonor strains. Generally, for comparison purposes, strains known to be capable of forming conjugational recombinants were present on every master plate which contained strains suspected of being incapable of forming such recombinants.

Technique of ultraviolet irradiation: Strains were tested for their sensitivity to ultraviolet irradiation by first inoculating a complex medium with cells obtained from confluent growth within patches on a master plate, and then subjecting the inoculated plate to 20 or 30 sec exposure to ultraviolet light. An inoculum was first transferred to a complex medium agar plate by replica plating. Then without prior incubation the freshly inoculated plate was used as a master plate to inoculate two other complex medium agar plates. In this fashion the inoculum was reduced to the point where exposure of one of the latter plates to 20–40 sec of ultraviolet light was sufficient to distinguish UV^S from UV^R strains after incubation of both plates at 37°C for 18 hr had permitted the growth of all surviving cells. All irradiations were carried out at a distance of 25 cm from a Mineralight lamp with an output measured at that distance to be 2.27 ergs/sec/mm^2 in the ultraviolet.

Selection of revertants: Independently isolated revertants were obtained from single colony isolates of mutant strains. Cultures from different colonies were kept on complex medium slants at 4°C after overnight incubation at 37°C. A flask containing fresh liquid complex medium was inoculated with cells from one slant and incubated at 37°C overnight. An aliquot was used to inoculate fresh medium and the remaining cells were harvested by centrifugation. Approximately $1–5 \times 10^9$ cells were spread on the surface of each of four complex medium agar plates. The plates were then exposed to 20, 30, 40, and 50 sec of ultraviolet irradiation and finally incubated in the dark at 37°C for 18–20 hr. The colonies which appeared were tested for the presence of UV^R cells and cells able to form conjugational recombinants. Usually several serial transfers were required before UV^R revertants were discovered among the survivors.

Mutagenic treatment: Stationary phase cells of JC-411, obtained after overnight growth in complex medium, were collected by centrifugation, washed with 0.1 *M* citrate buffer at pH 5.5, and resuspended in 0.1 *M* citrate buffer supplemented with 50 μg/ml of 1-methyl-3-nitro-1-nitrosoguanidine. They were incubated for 1 hr at 37°C to about 0.1% survival. Appropriate dilutions were then made in phosphate buffer and the cells plated onto minimal glucose medium. The plates were incubated for two days at 37°C and the resulting colonies were screened for mutants.

Results.—A multiply marked F- strain of *E. coli* K12, JC-411, was treated with the mutagen 1-methyl-3-nitro-1-nitrosoguanidine and the surviving cells were allowed to grow into colonies on minimal medium. The colonies were then screened for the ability of the cells they contained to produce recombinants when exposed to a population of Hfr cells. The colonies of Leu^- Ade^+ F- cells were replicated onto a lawn of Leu^+ Ade^- Hfr, JC-182, which had been spread onto a minimal

medium selective for Leu+ (Ade+) recombinants. After suitable incubation the plates were examined for the existence of areas which did not contain recombinant colonies although they had been inoculated from an F- colony. Part of the corresponding colony was taken from the master plate, suspended in a small amount of liquid medium, and streaked onto nutrient medium. Two successive single colony isolations were performed testing several colonies at each isolation for their phenotypic characteristics including their infertility when crossed with Hfr JC-182.

From approximately 2000 survivors of exposure to nitrosoguanidine two strains infertile with JC-182 were isolated: JC-1553 and JC-1554. Since their infertility with an *E. coli* Hfr is a trait which would characterize most bacteria of other genera, care was taken to establish the relationship of the isolates with JC-411. Both were examined microscopically under various conditions of growth and were found to be morphologically similar to JC-411. Both were also found to be phenotypically similar to JC-411 with respect to their growth factor requirements, their inability to ferment certain sugars, and their response to streptomycin and certain phages.

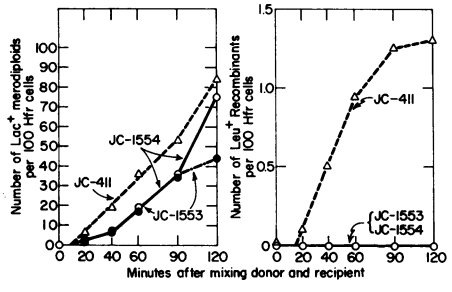

FIG. 1.—Results of crossing an F-*lac* donor, JC-1020, with JC-411 and two recombination-deficient mutants JC-1553 and JC-1554. Cells of each of the four strains were grown into log phase in complex medium at 37°C. Five ml of mating mixture were constituted at approximately 1×10^7 cells per ml of JC-1020 and 2×10^8 cells per ml of one of the F^- strains. The 3 mating mixtures were incubated in a 125-ml Erlenmeyer flask without shaking at 37°C. Periodically an aliquot was withdrawn, diluted 1:10 in buffer, and subjected to the shearing action of a Waring Blendor. Appropriate dilutions were then made and aliquots plated on medium selective for Lac^+ (Sm^R) merodiploids and Leu^+ (Sm^R) recombinants.

Four explanations of the infertility of the mutant strains with Hfr JC-182 may be advanced. (1) Transfer of genetic material from the Hfr to the mutant F- may be impossible because cells of the F- may be incapable of forming effective contacts with cells of the Hfr. (2) Transfer of chromosomal material from Hfr cells to the mutant cells may be prevented even though effective contacts are established between the cells. (3) Genetic material may be destroyed upon its entry into the mutant F-. (4) The mutant F- may be unable to catalyze recombination between the endogenote and exogenote. In order to test these possibilities two experiments were performed. In the first, JC-1020, a streptomycin-sensitive donor strain carrying the F-merogenote F-*lac*, was crossed to the streptomycin-resistant strains JC-411, JC-1553, and JC-1554. Samples of the three mating mixtures were removed after suitable intervals had elapsed from the mixing of the parent strains, and the effective pairs present were disrupted mechanically. In each sample the number of Lac+(Sm^R) merodiploids and Leu+(Sm^R) recombinants were determined. The results are plotted as a function of time in Figure 1. As can be seen from this figure, the kinetics of formation of Lac+(Sm^R) merodiploids is similar when JC-411 and the two mutant strains are used as recipients. The frequency of merodiploids formed is also similar in the three crosses. These facts indicate that the mutant cells participate in the formation of effective contacts and effective pairs and that transferred genetic material is not completely destroyed upon entry into the mutant cells. On the other hand, Figure 1 shows that after two

hours of mating the mixtures of JC-1553 and JC-1554 with the donor contain fewer than $^1/_{1000}$ the number of recombinants present in the mating mixture containing JC-411. This substantiates the infertility of the mutant strains detected first with JC-182 and indicates either that chromosomal transfer to the mutants is prevented or else that the mutants suffer from an inability to catalyze recombination.

The second experiment demonstrates that chromosomal markers are transferred to the mutant strains although they are not inherited by recombinants. A cross was performed with a lambda lysogenic donor JC-1164 and the T6-resistant mutants of JC-411 and JC-1553, JC-1166, and JC-1167, respectively. In Figure 2

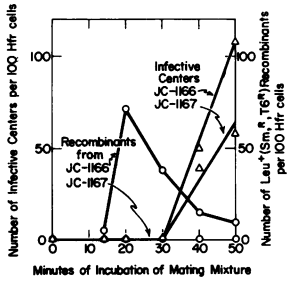

FIG. 2.—Cross of a lambda lysogenic Hfr, JC-1164, with non-lysogenic F^- strains JC-1166 and the recombination-deficient JC-1167. Cells of JC-1164 were harvested in log phase of growth by centrifugation at 4°C. They were washed once in complex medium. 0.5 ml of resuspended, washed cells were added to 4.5 ml of a log culture of each F^- strain. The final cell concentration was approximately 1×10^7 Hfr cells per ml and approximately 2×10^8 F^- cells per ml. The mixture was incubated in a 125-ml Erlenmeyer flask without shaking at 37°C for 10 min, and then 0.1 ml was withdrawn and was added to 19.9 ml of fresh complex medium prewarmed to 37°C in a 250-ml flask. At intervals, 0.5 ml of the diluted mating mixture was added to 0.5 ml of a lysate of bacteriophage T6 having a titer of 3×10^9 pfu per ml. The resulting suspension was incubated at 37°C with gentle shaking for 10 min and then was sampled to determine the titer of recombinants and infective centers. A parallel culture in which 0.5 ml of washed, resuspended Hfr cells were added to 4.5 ml of fresh complex broth was treated in the same fashion in order to ascertain the titer of phage produced by spontaneous lysis. The number so obtained (from 2×10^3 to 2×10^5 infective centers per ml) was subtracted from the number of infective centers present per ml of mating mixture in order to calculate the titer of zygoticallyinduced infective centers.

are shown the results obtained when mating is interrupted at intervals by the addition of T6 phage to samples of the mating mixtures. Leu^+ (Sm^R, $T6^R$) recombinants were formed from JC-1166 beginning about 14 min after mixing the parent strains. They increased in number until about the time the lambda prophage was transferred to the zygotes, and then they decreased in number as zygotic induction took place producing an increase in the number of infectious centers. This behavior has been observed by other authors[14] in a similar cross. In the cross of JC-1164 with JC-1167 zygotic induction occurred as the lambda prophage was transferred to zygotes beginning about 30 min after the parents were mixed. $Leu^+(Sm^RT6^R)$ recombinants were not formed in this cross, however, although it is clear from the cross with JC-1166 that *leu*$^+$ preceded the lambda prophage into the zygotes formed. Therefore it seems clear that the mutant JC-1553 and its derivatives are unable to catalyze recombination between endogenote and exogenote. A similar zygotic induction experiment performed with $T6^R$ mutant of JC-1554 shows substantially the same results, thereby demonstrating that the defect in both mutant strains is similar.

Having obtained mutants in which recombination was blocked we were able to determine whether or not their ability to repair photodamage to DNA was also impaired. Aliquots of cultures of JC-411, JC-1553, and JC-1554 were irradiated for different periods of time and the number of survivors determined by plating samples onto a complex medium. All operations were performed in dim light and

the plates were incubated in the dark. The results are shown in Figure 3. The fact that after 10 sec of irradiation 40 per cent of the cells of JC-411 but only 0.003 per cent of the cells of JC-1553 and JC-1554 are viable provides clear evidence that the mutant strains are more sensitive to ultraviolet light than is the parent strain.

The mutants JC-1553 and JC-1554 therefore differ from the parent strain in two characteristics, and it then becomes necessary to demonstrate whether one or two mutations are responsible for the mutant phenotype. Two methods of determining this information are available: (1) Back-mutants selected for their reversion to one of the parental phenotypic characteristics may be examined for reversion to the other parental characteristic. (2) Conjugational or transductional recombinants formed by crossing a wild-type donor with the mutant strains may be selected for their inheritance of one of the wild-type traits and then examined for inheritance of the other wild-type trait. The second of these methods depends upon recombination between two mutant genes and their wild-type alleles to produce recombinants usually inheriting only one of the wild-type

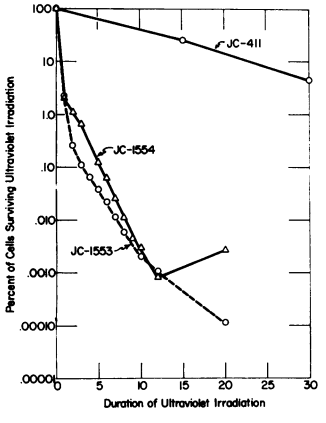

FIG. 3.—Sensitivity to ultraviolet irradiation of two recombination-deficient strains JC-1553 and JC-1554 as compared to the relative resistance of JC-411. Cells were prepared for irradiation by inoculating a liquid minimal medium with approximately 10^7 cells per ml obtained from stationary phase cultures in complex medium. The cells were harvested when they reached 5×10^8 per ml and were washed once with $M/20$ phosphate buffer at pH 7. Five-ml aliquots of the cells, resuspended in buffer at 10^8 cells per ml, were placed in glass Petri dishes and were irradiated at a distance of 25 cm from a Mineralight lamp. The samples were kept in the dark until they were diluted and plated on complex medium. All operations were carried out in dim light and the plates were incubated in the dark.

alleles. In general, this result would distinguish a double mutant from a single mutant; in this case, however, the distinction is not possible. If the mutant strains carried two mutations, only the locus conferring the trait of sensitivity to ultraviolet light would be of selective disadvantage permitting selection for UV^R recombinants. The other mutant locus would presumably prevent recombination, since it is assumed to determine that trait in the mutant strains. Recombination would then be possible only if both wild-type alleles had been transferred to the recipient. This would increase the probability of inheritance by recombinants of both wild-type alleles; in fact the wild-type allele controlling UV^R may not be integrated unless prior integration of the wild-type allele permitting recombination occurs. Thus, recombinational separation of two mutant alleles, one of which prevents recombination, may be impossible.

Because of these considerations the method of reversion was used to determine the number of mutations present in JC-1553 and JC-1554. Two revertants were obtained from among the cells of JC-1553 surviving ultraviolet irradiation in the experiment shown in Figure 3. In similar but independent experiments,

another UV^R revertant of JC-1553 and one of JC-1554 were obtained. The properties of these revertants are listed in Table 1; all four have regained either fully or partially the wild-type ability to form conjugational recombinants, as well as resistance to ultraviolet light. In all the experiments performed to obtain the four revertant strains, several thousand survivors of ultraviolet irradiation were tested. Most of these survivors were UV^S and in no case had they recovered wild-type proficiency in conjugational recombinant formation.

TABLE 1

CHARACTERISTICS OF REVERTANTS OF RECOMBINATION-DEFICIENT MUTANTS JC-1553 AND JC-1554

Strain	Source of revertant	Survival after 30 sec of UV (%)	Results of Crosses with F-*lac* Donor JC-1020: Freq. of $Lac^+(Sm^R)$ Merodiploid formation	Freq. of $Leu^+(Sm^R)$ Recombinant formation
JC-411	—	4.1	84*	1.3*
JC-1553	—	3.5×10^{-5}	44	1.1×10^{-4}
JC-1554	—	5.6×10^{-5}	75	1.4×10^{-4}
JC-679	JC-1553	39	108	3.4
JC-680	JC-1553	11	146	2.0
JC-678	JC-1553	8.6	95	1.7
JC-677	JC-1554	0.48	88	0.09

* Number of merodiploids or recombinants per 100 Hfr cells.
Sensitivity to ultraviolet irradiation was measured as described in the legend to Fig. 3. Proficiency in recombination was measured as described in the legend to Fig. 2.

Discussion.—The term "recombination" when used in the context of bacterial genetics connotes to many either the process of DNA transmission known as conjugation or the formation by conjugation of any progeny which inherit phenotypic traits derived from both parents. It can, however, be used more strictly to denote the series of physical and chemical events which serve to link genes derived from one parental DNA with those derived from another parental DNA.[15] It is in this sense that the word is used to describe the recombination-deficient (Rec-) mutants, JC-1553 and JC-1554, whose isolation is described in this report. These two strains were isolated after mutagenic treatment of their parent culture because they appeared to be unable to form recombinants when crossed with an Hfr strain. They were tested for their ability to engage in the process of zygote formation and were found to form F-*lac* merodiploids in a cross with an F-*lac* donor and to form infective centers by zygotic induction when crossed with an Hfr carrying lambda prophage. These results served to rule out the possibility that the mutants' infertility with an Hfr was a reflection of their inability to engage the donor cells in mating or their acquisition of a new ability to destroy exogenous DNA. It was therefore concluded that the mutants were unable to catalyze at least one of the steps involved in recombination. Upon further examination the mutant strains were found to be much more sensitive than their parent strain to the lethal effects of ultraviolet light. The inference is made that the mutation has affected the ability of irradiated cells to repair photodamage to DNA. Experiments carried out by one of the authors (A. J. C.) in collaboration with Drs. P. Howard-Flanders and R. Boyce at the Yale University Medical School have strengthened this inference. These experiments will be described elsewhere.

Since JC-1553 and JC-1554 were both isolated from the same culture treated with mutagen and show marked similarity in phenotype, they may be siblings. However, the fact that the cells were plated immediately after treatment and

that the mutants occurred on different plates renders a sibling relationship unlikely. Consequently they are considered to be independently isolated mutants.

An examination of a number of independently isolated revertants to ultraviolet resistance has lent support to the hypothesis that a single gene mutation is responsible for the UV^S and Rec- traits of the mutants. All revertants isolated had regained not only resistance to ultraviolet irradiation but proficiency in the formation of conjugational recombinants as well. This fact is consistent with the hypothesis that one gene determines an enzyme which catalyzes one of the steps in recombination and one of the steps in the replacement of photoproducts in ultraviolet-damaged DNA. There are, however, other hypotheses which could also account for all the facts reported here: (1) A single suppressor mutation could cause a phenotypic reversion of two independent mutations to Rec^- and UV^S. This possibility is supported by the observation that the revertants show a greater or lower resistance to ultraviolet irradiation than the wild-type strain, JC-411. (2) A mutant protein may cause a modification in the DNA of the mutant cell so that it can neither participate in the process of recombination, nor be repaired after being damaged by ultraviolet light. (3) A single mutation may affect the expression of more than one gene: for example, a mutation in a gene concerned with regulating the operation of genes, some of which participate in recombination and some of which participate in repair. A polarity mutation in an operon containing some genes participating in repair and some participating in recombination would also have the same effect. Tests of each of these hypotheses can be devised and are presently being conducted.

The authors appreciate the assistance of Miss Ann Templin and Miss Haruko Nagaishi in performing some of the experiments described. They also acknowledge the collaboration of Dr. Katherine Brooks in performing the zygotic induction experiments.

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[1] Kellenberger, G., M. L. Zichichi, and J. Weigle, these PROCEEDINGS, **47**, 869 (1961).

[2] Meselson, M., and J. J. Weigle, these PROCEEDINGS, **47**, 857 (1961).

[3] Ihler, G., and M. Meselson, *Virology*, **21**, 7 (1963).

[4] Siddiqi, O. H., these PROCEEDINGS, **49**, 589 (1962).

[5] Meselson, M., personal communication.

[6] Luria, S. E., *Ann. Rev. Microbiol.*, **16**, 205 (1962).

[7] Howard-Flanders, P., personal communications.

[8] Boyce, R. P., and P. Howard-Flanders, these PROCEEDINGS, **51**, 293 (1964).

[9] Setlow, R. B., and W. L. Carrier, these PROCEEDINGS, **51**, 226 (1964).

[10] Pettijohn, David E., *Biophysics Laboratory Report*, No. 103 (Stanford University, 1964).

[11] Genotypic symbols stand for the genes concerned with the biosynthesis of threonine, *thr;* leucine, *leu;* histidine, *his;* purines, *pur;* arginine, *arg;* methionine, *met;* thiamin, *thi;* and the fermentation of lactose, *lac;* and maltose, *mal.* When used with gene symbols, "+" refers to the wild-type state of the gene. Numbers indicate arbitrary site designations assigned to mutant alleles by Adelberg (personal communication).

Phenotypic characteristics are indicated by the following abbreviations: Thr, threonine; Leu, leucine; His, histidine; Ade, adenine; Arg, arginine; Met, methionine; Thi, thiamin; Lac, lactose; Mal, maltose; Sm, streptomycin; UV, ultraviolet light; D, donor in conjugation; ND, nondonor in conjugation; "−", "requiring," when used with the abbreviation of an amino acid, and "nonfermenting," when used with the abbreviation of a sugar; "+", nonrequiring and

fermenting, when used with the abbreviation of an amino acid and sugar, respectively: *S*, sensitivity to a lethal agent; *R*, resistance to a lethal agent.

[12] Clark, A. J., *Genetics*, **48**, 105 (1963).

[13] Adelberg, E. A., and S. N. Burns, *J. Bacteriol.*, **29**, 321 (1960).

[14] Jacob, F., and E. L., Wollman, *Sexuality and the Genetics of Bacteria* (Academic Press, 1961).

[15] Schaeffer, P., in *The Bacteria*, ed. I. C. Gunsalus and R. Y. Stanier (New York: Academic Press, 1964), vol. 5, p. 115.

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*THE SELECTIVITY OF BIOSYNTHESIS OF GLUCOSYL COMPOUNDS AS ILLUSTRATED BY AN E. COLI MUTANT DEFECTIVE IN UDPG SYNTHETASE**

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It has recently been reported that mutants of *Escherichia coli* K12 which are defective in the ability to synthetize UDPG synthetase (UDPG pyrophosphorylase) show also a great decrease in the amount of glucose incorporated into their cell-wall polysaccharides.[1, 2]

In their analysis of cell walls isolated by Salton's technique,[3] Fukasawa *et al.*[4] reported that the UDPG synthetase-defective mutants of *E. coli* K12, W4597, and W3142, contained only 15–20 per cent of the amount of glucose present in the wild-type strain W3110.

A study has been made in this laboratory of the cell-wall lipopolysaccharides (LPS) of various *E. coli* K12 mutants.[2] LPS was isolated by the hot phenol procedure of Westphal *et al.*,[5] and a preliminary investigation of its sugar composition was made by paper chromatography and by various colorimetric reactions. It was reported in this study that in the case of the UDPG synthetase-defective mutant Gal 23 (identical with W4597), glucose could not be detected in the LPS;[2] this was also the case of a galactokinaseless derivative of this strain, Gal 23 K^-.[2]

In the present study we wish to report some observations which seem to be of interest from the point of view of cellular physiology. By using the highly sensitive hexokinase-Zwischenferment assay for glucose, we have found that the LPS of Gal 23 contains only minute amounts of glucose (less than 0.1% of the lyophilized LPS), whereas in the corresponding parental strain (W3100), glucose is a major constituent, amounting to 8–12 per cent of the LPS.

We have found that in various strains of *E. coli* K12 there are, in addition to LPS, glucose-containing phosphorylated polysaccharides. We would like to refer to these acidic polysaccharides in the present article as "soluble polysaccharide fraction" or sPF. A subsequent publication will deal with the fractionation and the chemical makeup of this sPF.[6]

It seems noteworthy that sPF is present both in the original strain (W3100) as well as in the UDPG synthetase-defective mutant (Gal 23 K^-) and that in the latter the sPF contains 5–10 per cent of the amount of glucose found in wild-type sPF. (W3100 ferments both glucose and galactose and is considered here as "wild