INDUCTION OF MUTATIONS IN THE *lac* REGION OF *ESCHERICHIA COLI* STRAIN S¹

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THERE is a group of chemicals, including nitrogen mustards, acridines, nitro-soguanidine, azaserine, nitrofurazone, mitomycin, nitrous acid, and 2-aminopurine, which, despite their diverse chemical or physical properties (see review by FREESE, 1963), share many biological properties among themselves and with ultraviolet radiation (UV) in affecting microorganisms. They are mutagenic; several of them induce temperate phage in the prophage state; in certain sensitive strains of bacteria (e.g., *Escherichia coli* B) they inhibit cell division mechanisms causing filaments to be formed; some specifically inhibit DNA synthesis and cross-link DNA. Most significant, however, is the observation that mutants of a sensitive strain such as *E. coli* S or B selected for resistance to any one of these agents are almost invariably found to be resistant to all the other agents and to UV (WOODY-KARRER and GREENBERG 1964; ZAMPIERI and GREENBERG 1964, 1965a). Furthermore, the amount of lethal damage induced by most of these chemicals and by UV in a sensitive strain is dependent on post-treatment conditions such as the composition of the plating medium and the temperature of incubation (ZAMPIERI and GREENBERG 1965b). These facts argue that all these chemicals have a common effect on a common target, presumably DNA, which is repaired in resistant strains by a common mechanism. The last idea is reinforced by the observation that resistance to these agents as well as to UV can be transferred as a single gene by conjugation from resistant donor strains of *E. coli* to recipient sensitive strains (GREENBERG 1964). The idea is further reinforced by the observations that the so-called dark reactivation process in bacteria (HCR) which is also under the control of each of several, presumably independent, genes (HOWARD-FLANDERS, BOYCE, SIMSON and THERIOT 1962; HOWARD-FLANDERS, BOYCE and THERIOT 1966) is able to excise not only thymine dimers induced in DNA by UV (BOYCE and HOWARD-FLANDERS 1964; SETLOW and CARRIER 1964) but also alkylated purines produced by mustards (LAWLEY and BROOKES 1965; PAPIRMEISTER and DAVISON 1964). One might expect that if, in a sensitive strain of bacteria such as *E. coli* strain S, all the radiomimetic agents as well as UV have a common effect, that this might be reflected in mutually revertible mutations induced by these agents. This report will show that mutations to Lac⁻ (inability to ferment lactose) induced in strain S by any of the chemicals and UV were revertible by all the other chemicals and UV.

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MATERIALS AND METHODS

Bacterial strain: *Escherichia coli* strain S, originally obtained from Dr. A. D. HERSHEY, was used throughout, except for controls for permease assay.

Media: M9 agar: dibasic sodium phosphate, 5.8 g; monobasic potassium phosphate, 3.0 g; ammonium chloride, 1.0 g; sodium chloride 0.5 g; glucose, 2.0 g; magnesium sulphate ($0.7 \text{ H}_2\text{O}$), 250 mg; calcium chloride 14 mg; 1% gelatin solution, 10 ml; agar, 8.0 g (Ionagar, Oxo Ltd., London). M9 lactose was made in the same way except that lactose was substituted for glucose. Penassay agar was a commercial preparation (Difco Bacto-Penassay Antibiotic Medium 2). Phosphate-buffered saline (PBS) consisted of 1% sodium chloride in 0.02 M phosphate buffer (pH 6.8).

Chemicals used: 1-methyl-3-nitro-1-nitrosoguanidine (Aldrich); nitrofurazone (5-nitro-2-furaldehyde semicarbazone) (a gift from Eaton Laboratories); nitrogen mustard (a gift from Merck, Sharp and Dohme); azaserine and mitomycin C (supplied by the Cancer Chemotherapy National Service Center, Bethesda); sodium nitrite (Mallinckrodt). (To produce nitrous acid 0.005 M sodium nitrite was dissolved in 0.1 M acetate buffer (pH 4.2). Proflavine (Nutritional Biochemicals); 2-aminopurine (Cyclo Chemical Corp.); o-nitrophenyl-D-galactopyranoside (Mann); methyl- β -thiogalactopyranoside (Calbiochem).

Induction of *Lac*⁻ mutants. An overnight culture of *E. coli* S in peptone broth washed twice in buffered saline was resuspended and adjusted to 2×10^8 cells per ml of buffer containing concentrations of mutagenic agent which permitted 1.0 to 0.1% of the treated cells to survive. All treatments were at 37°C except UV, which was at ambient temperature.

Treatment with azaserine, mitomycin C, 1-methyl-3-nitro-1-nitrosoguanidine, and nitrogen mustard was performed for 1 hour in phosphate (0.02 M) buffered 1% saline, pH 6.8 (PBS), containing respectively 2, 0.3, 5 and 15 μg of chemical per ml. Nitrofurazone was used at 50 μg per ml for 3 hours in PBS. The induction of mutants by UV was performed in PBS exposing bacterial cell suspension to 308 ergs/mm². The UV source was a single 15 w General Electric germicidal lamp with a maximum output at 2537 Å which delivered 15.4 ergs/mm²/sec at a distance of 51.5 cm from the source. Nitrous acid mutants were induced by suspending cells for 10 minutes in acetate buffer (0.1 M, pH 4.2) containing 340 μg of sodium nitrite per ml. Exposure to proflavine hydrochloride at 150 μg /ml in tris(hydroxymethyl)aminomethane buffer (Tris) (0.05 M, pH 7.8) was for 5 minutes in ambient light. Treatment with 2-aminopurine (15 mg/ml) was in tryptone broth at pH 7.0 for 3 hours.

Samples were withdrawn at the end of the treatment time, diluted in PBS to 1000 viable colonies per plate, spread on penassay agar containing lactose (1%) and triphenyltetrazolium chloride (0.01%) as indicator (TTC lactose agar), and incubated for 18 to 24 hours. *Lac*⁻ mutants, distinguishable from the background of lactose fermenting colonies by their red color, were reisolated once and inoculated into stabs. (Stab medium = nutrient broth 0.8%, BBL agar 0.8%.)

Permease test. The procedure described by COOK and LEDERBERG (1962) was followed. As permease negative control the strain *E. coli* K-12 W4133 F⁻ lac 73, T6^r, a gift of Dr. A. GANESAN, was used.

Induction of *Lac*⁺ revertants. An overnight culture in peptone broth of each lactose non-fermenting mutant, grown in a shaking water bath, was washed twice in buffered saline. Suspension of 10^9 cells per ml in appropriate buffer or broth were treated with the test mutagen. Reversion experiments with azaserine, mitomycin C, nitrofurazone, 1-methyl-3-nitro-1-nitrosoguanidine and nitrogen mustard were performed for 1 hour in PBS containing a concentration of each drug which permitted survival of 10% to 50% of the initial titer of the treated bacterial cells. UV revertants were induced by exposing bacterial suspensions in PBS to 616 ergs/mm². Proflavine reversion experiments were performed in Tris (0.05, M pH 7.8) for 5 minutes with illumination from a 500 w bulb at a distance of 23 cm. Nitrous acid revertants were induced in acetate buffer (0.1 M, pH 4.2) containing 340 μg /ml sodium nitrite for 1 hour. The concentrations of proflavine, nitrous acid and 2-aminopurine used gave survival frequencies of 30, 50 and 55% respectively. At the end of the exposure the treated bacterial cells were centrifuged, washed and resuspended to volume with PBS. 0.1 ml of this suspension was added

to 2.5 ml of soft agar (0.5%) and poured undiluted on M9 lactose agar and after suitable dilution on M9 glucose agar. The plates were incubated four to five days. The plating on M9 glucose medium permitted the total number of surviving cells to be determined.

In M9 lactose plates the colony morphology of the revertants was of two main kinds: large colonies surrounded by a halo of background cells (feeder type) and colonies of variable colony size without haloes (nonfeeder type). Feeder and nonfeeder colonies of different sizes were picked, resuspended in PBS, and after streaking on M9 lactose agar and incubation for three to four days, isolated into stabs, incubated overnight and maintained at room temperature.

β -galactosidase test. Overnight cultures of revertants in peptone broth containing methyl- β -thiogalactopyranoside (TMG) (10^{-3} M) were washed twice, resuspended in PBS and adjusted to 4×10^8 cells per ml. For determination of β -galactosidase activity the procedure described by REVEL, LURIA and ROTMAN (1961) was used. Optical density was measured at 420 m μ with a Coleman colorimeter. One unit of β -galactosidase was defined as the amount of enzyme which liberates 1 μ mole *o*-nitrophenyl- β -D-galactopyranoside (ONPG) per minute at pH 7.0.

Growth in minimum broth. Revertant cultures grown overnight in peptone broth were washed twice in buffered saline, adjusted to 2×10^8 cells per ml in minimum glucose or lactose broth, incubated with shaking. Samples were assayed periodically for number of viable cells to measure the rate of growth.

Extraction of β -galactosidase. The procedure for the extraction of β -galactosidase was as described in detail by KAMEYAMA and NOVELLI (1962). β -galactosidase from *E. coli* S wild type and Lac⁺ revertant mutants was purified 15 to 20 times.

Heat stability of β -galactosidase. Enzyme purified 15 to 20 times was incubated in a water bath at the desired temperature. At intervals samples were withdrawn and diluted in ice-cold Tris (0.0275 M, pH 7.8), and β -galactosidase activity was determined following the method of LEDERBERG (1950). The activity remaining after heat treatment is expressed as percent of the activity of an untreated sample.

RESULTS

Extensive preliminary experiments were done as follows: *E. coli* S was treated with each chemical under conditions which permitted 1 to 10% survival. Isolated Lac⁻ clones were tested for frequency of spontaneous mutation to Lac⁺. They were then treated in turn with each of the mutagenic agents to determine the frequency of induced reversions (Lac⁻ $\rightarrow$ Lac⁺). The frequency of reversions was expressed as the number of clones able to grow on minimal lactose medium as a function of survivors of treatment determined on complete medium.

The following observations were made. Unless otherwise stated, they apply to all agents, including UV.

1. About 15 to 20% of all Lac⁻ mutants did not revert spontaneously to Lac⁺ (frequency $\leq 10^{-9}$) and could not be induced to revert by any agent. About 15 to 20% of all Lac⁻ mutants reverted spontaneously at a very high frequency ($\geq 10^{-6}$). The remaining 60% of the forward mutants reverted spontaneously at a frequency of $\approx 10^{-8}$. Nothing further was done with nonreverting mutants.

2. Two kinds of clones were observed in about equal proportions among all the revertants, both spontaneous and induced, of the high and low frequency reverting mutants: clones approximately the size of the wild type, which were surrounded by a halo of minute colonies, presumably Lac⁻ colonies being fed by the revertant (feeder type); and clones smaller than wild type, without haloes (nonfeeder type).

3. All Lac⁻ mutants which reverted spontaneously were revertible by all the

agents. Few differences were observed in frequencies of reversions induced by any single agent tested against forward mutations induced by any other agent.

4. Significant differences in frequencies of induction of both forward and back mutations were observed among the nine mutagenic agents.

Preliminary observations suggested that under conditions producing equal lethality some agents were markedly less efficient as mutagens than others (e.g., nitrofurazone $\ll$ nitrosoguanidine). There was no specificity of mutagenesis as judged by (a) frequency of nonrevertible, or high or low frequency revertible forward mutations, (b) proportion of feeder and nonfeeder revertants or (c) revertibility of mutations induced by any agent.

More precise data were obtained on Lac⁻ mutants known to be β -galactosidase negative from among those whose spontaneous reversion frequency was about 10^{-8} . Lac⁻ mutants induced by each agent were examined sequentially for permease as described in methods until four β -galactosidase negative mutants were found. A total of 43 mutants were examined, of which three were permease negative, and these were not further examined. Of the three permease negative mutants, one was induced by nitrogen mustard and two (of ten examined) by UV. None were found among Lac⁻ mutants induced by other agents and tested in the sequence. The 40 β -galactosidase negative mutants examined had normal permease activity.

Table 1 shows the frequency of Lac⁺ revertants induced by each of four muta-

TABLE 1
Induction of Lac⁻ to Lac⁺ reversions by various mutagens

Lac ⁻ induced by	Lac ⁻ mutant No.	Lac ⁺ revertants* induced by				Spontaneous revertants $\times 10^{-8}$
		PF	2-AP	UV	NG	
PF	2	10.0	7.5	42	100	4.4
	4	9.6	8.1	29	87	3.8
	6	11.0	10.0	38	300	3.1
	7	9.6	7.5	33	96	5.5
2-AP	14	6.4	16.0	32	97	1.7
	34	3.9	13.0	15	75	4.1
	37	3.7	13.0	17	96	3.2
	45	5.4	18.0	20	99	2.7
UV	159	3.8	7.6	10	72	1.4
	163	4.9	4.4	14	54	1.3
	164	3.9	11.0	18	97	2.1
	1	2.9	11.0	21	100	3.0
NG	210	6.2	38.0	100	450	3.7
	226	5.4	12.0	22	98	1.0
	11	4.1	18.0	30	100	2.0
	26	3.9	11.0	32	100	1.3

Abbreviations: PF=proflavine; 2-AP=2-aminopurine; UV=ultraviolet light; NG=1-methyl-3-nitro-1-nitrosoguanidine; NM=nitrogen mustard. Lac⁻=inability to ferment lactose.

* Number of revertants induced $\times 10^{-1}$ /spontaneous revertants.

genic agents in each of the galactosidase negative forward mutations induced by each of the same four agents. It should be noted that all the mutants were revertible by all the agents, and that there were large differences in the frequencies of revertants induced by the various agents. Thus proflavine induced reversions at a frequency about 50 times above background (spontaneous), whereas the nitro-soguanidine induced reversions at a frequency about 1000 times above background. The frequency of back mutation was a function of the mutagen inducing the reversion and not of the mutagen inducing the forward mutation. As seen in Table 2 the proportion of feeders among the revertants was similar for all Lac⁻ mutants tested, regardless of the agent inducing the reversions. About half of the revertants were feeder-type.

Similar data were obtained with nitrofurazone, azaserine, mitomycin, nitrous acid and nitrogen mustard. Lac⁻ mutants induced by these agents were reverted by all of the agents. Furthermore, each of the agents reverted all of the Lac⁻ mutants at frequencies characteristic of each reverting agent. Thus, nitrofurazone reverted Lac⁻ mutants at a frequency 30 times, azaserine 60 times, mitomycin 90 times, nitrous acid 100 times and nitrogen mustard 350 times the spontaneous frequency. In addition, the frequency of feeder-type revertants in every instance was about 50%.

It was assumed that feeder-type revertants were reversions to wild-type β -galactosidase and nonfeeder-type revertants were the result of suppressors or of the substitution of an alternative nucleotide at the mutational site. To examine

TABLE 2

Proportion of feeder types among revertants from Lac⁻ to Lac⁺ induced by various mutagens

Lac ⁻ induced by	Lac ⁻ mutant No.	Percent feeder types among Lac ⁺ revertants induced by				
		PF	2-AP	UV	NG	Spontaneous
PF	2	49	51	48	50	39
	4	45	36	46	52	50
	6	43	38	40	50	46
	7	37	33	44	32	22
2-AP	14	51	46	46	49	47
	34	49	49	41	47	49
	37	44	53	49	46	54
	45	51	41	54	54	51
UV	159	49	51	51	47	51
	163	65	62	50	43	60
	164	57	54	51	47	53
	1	49	51	49	51	51
NG	210	47	47	38	41	44
	226	49	54	49	49	55
	11	52	50	51	54	54
	26	49	49	49	50	51

Abbreviations as in Table 1.

TABLE 3

β-galactosidase activity of wild-type *E. coli* strain S and of feeder- and nonfeeder-type revertants of *Lac*⁻ nitrogen mustard-induced #357

Strain	<i>β</i> -galactosidase activity (percent of <i>E. coli</i> S)
<i>E. coli</i> S	100
<i>E. coli</i> S <i>Lac</i> ⁻ NM induced #357	2.3
Feeder type <i>Lac</i> ⁺ revertant (reverted by UV #1)	100
Feeder type <i>Lac</i> ⁺ revertant (reverted by 2-AP #2)	104
Nonfeeder type <i>Lac</i> ⁺ revertant (reverted by UV #3)	13
Nonfeeder type <i>Lac</i> ⁺ revertant (reverted by 2-AP #4)	11

Abbreviations as in Table 1.

FIGURE 1.—Growth in M9 lactose broth of *E. coli* S wild-type (●), feeder-type revertants reverted by UV No. 1 (■) and reverted by 2-AP No. 2 (▲), nonfeeder 2-AP-induced revertant No. 4 and nonfeeder UV-induced revertant No. 3 (□). On the ordinate the logarithm of the ratio: number of viable cells at each interval of incubation time (N_t) divided by the number of viable cells at 0 time (N_0). *E. coli* S *Lac*⁻ NM-induced No. 357 is not represented. However, the logarithm of the ratio N_t/N_0 was constantly equal to 1 during incubation.

FIGURE 2.—Growth in M9 glucose broth of *E. coli* S *Lac*⁺ wild type, compared with the feeder (UV-induced No. 1) and nonfeeder revertants. Δ represents the growth of nonfeeder revertant No. 4 2-AP-induced; □ represents the growth of nonfeeder revertant No. 2 UV-induced. Ordinate as in Figure 1.

these assumptions two feeder and two nonfeeder-type revertants of Lac⁻ 357 (nitrogen mustard induced) were reisolated on minimal-salts lactose medium. One of each type of revertant had been induced by 2-aminopurine and one each by UV. As shown in Table 3, the β -galactosidase activity of the feeder-type revertants was as high as the wild-type; the nonfeeder type exhibited about one tenth the β -galactosidase activity of the wild one.

In Figure 1 is shown the rate of growth in minimal salts lactose broth of *E. coli* S Lac⁺ wild type and the two feeder and nonfeeder revertants of Lac⁻ 375. Viable counts were made at intervals over a period of 5 hours. *E. coli* S and the feeder-type revertants had a lag period of 2 hours, the rate of growth becoming exponential after about 3 hours. The growth curves of wild-type and feeder-type revertants were indistinguishable. The nonfeeder revertants exhibited an increased lag period and a markedly decreased rate of growth.

When examined for growth in minimal salts glucose broth (Figure 2), all strains had a lag period of one hour. Thereafter the rates of growth of the Lac⁺ wild-type (strain S) and the feeder-type revertants were indistinguishable. The nonfeeder types grew more slowly than wild type and achieved a lower plateau. Nonfeeder revertant No. 2 grew more slowly on glucose than No. 4; this was true also on minimal lactose broth (Figure 1).

Heat stability is a sensitive test of identity of enzymes and, therefore, this property of the extracted β -galactosidase of wild type and the four revertants was next examined. At 55°C (Figure 3) there was no detectable difference in rate of heat inactivation of wild-type enzyme and that from either feeder-type

FIGURE 3.—Inactivation by heat of β -galactosidase of *E. coli* strain S Lac⁺ wild type (●), feeder revertant 2-AP-induced No. 2 (▲) and UV-induced No. 1 (■).

FIGURE 4.—Inactivation by heat of β -galactosidase of *E. coli* strain S Lac⁺ wild type (●) and nonfeeder revertants 2-AP No. 4 (Δ) and UV No. 3 (□) induced.

revertant. The rate of inactivation of enzyme from the nonfeeder type was too rapid to record accurately. The experiment was, therefore, repeated at 50°C (Figure 4). Again there was no detectable difference between wild-type enzyme and that extracted from feeder-type revertants. The two nonfeeder-type revertants yielded enzymes markedly more sensitive than wild type, though not strikingly different from each other.

DISCUSSION

By several criteria such as the proportion of nonrevertible forward mutations, of highly revertible forward mutations, and of feeder- to nonfeeder-type back mutations, the types of mutations produced by all mutagens in the *lac* region of *E. coli* S appear to be similar. The main difference is their efficiency in producing mutations relative to their lethal action. The data suggest, furthermore, that about half the revertants are wild-type. They grow as rapidly in minimal medium, lactose and glucose, as the wild type. They excrete β -galactosidase into the medium. The heat stability of their β -galactosidase is identical with the wild-type enzyme. It is possible, as in revertants of mutants in the tryptophan A-protein (YANOFSKY, HORN and THORPE 1964; YANOFSKY 1963), that an amino acid substitution, coded by a nucleotide different from the wild type, might yield an enzyme only slightly different in phenotypic expression from the wild type. However, the heat inactivation curves suggest identity of the enzyme of the wild-type and feeder-type revertants.

The nonfeeder type of revertants do not feed Lac^- organisms presumably because they excrete little or no β -galactosidase; they synthesize less, or less efficient, enzyme, which is also less heat stable; and they grow more slowly than wild type on minimal medium containing either lactose or glucose. Nonfeeder revertants have been shown by SCHWARTZ (1963) to result from allele-specific, extragenic suppressors which produce a β -galactosidase of slightly different electrophoretic mobility than wild type. The β -galactosidase of one such revertant examined by SCHWARTZ was more stable at 55°C than wild type but less stable at pH 9.0. The enzymes of both nonfeeder-type revertants we examined were less heat stable than wild type. A heat labile enzyme in a Lac^+ revertant was previously described by JACOB and MONOD (1961). Without careful genetic studies, such as those performed by SCHWARTZ (1963) it is impossible to distinguish among our nonfeeder revertants those resulting from extragenic allele specific suppressors from those resulting from the mutation to a nucleotide other than that in the wild type at the point of the original mutation. It might be expected that cells containing β -galactosidase with a single amino acid different from wild type might be less efficient in growing on medium with lactose as sole source of energy. However, they would be expected to grow normally in medium containing glucose. The two mutants we examined grew less efficiently than wild-type cells on minimal glucose medium. Such a response might be expected of a suppressor mutation which misreads a mutant codon as though it were an appropriate amino acid for the synthesis of β -galactosidase. It would misread this codon, presumably,

wherever it occurred in the genome, thus producing other less efficient enzymes, leading in turn to a slower rate of growth.

Nonspecificity of mutagenesis among a variety of chemical and physical agents is contrary to most, if not all, previous reports of mutagen specificity in a large variety of organisms. Our results differ from those recently reported by SCHWARTZ (1963) who compared the properties of Lac⁻ mutants induced in *E. coli* by ethyl-methansulfonate (EMS) and UV. He found that EMS-induced mutants were induced to revert to wild-type phenotype by 2-aminopurine but not by UV or EMS, while UV-induced mutants were induced to revert by UV but not EMS and 2-aminopurine. EMS is a monofunctional alkylating agent and as such may be comparable in chemical activity to nitrosoguanidine and azaserine used in our experiments. An explanation for the lack of specificity we observed may be in the peculiar properties of *E. coli* S, which is a variant of strain B. *E. coli* S lacks what KNESER, METZGER and SAUERBIER (1965) have called K-type reactivation of UV lesions. Strains which lack this type of reactivation (and mutants of K-12 have been found in this class) (KNESER *et al.* 1965; HOWARD-FLANDERS, SIMSON and THERIOT 1964) have the following properties: (1) They form filaments when irradiated by UV or when treated by the chemicals listed in Table 1 (KILGORE and GREENBERG 1961). (2) They have a distinctive UV inactivation curve, characterized by a constantly changing slope, as though the organisms were becoming increasingly resistant with increasing exposure to the lethal agent. (3) Some of them are mucoid (HOWARD-FLANDERS, *et al.* 1964; MARKOVITZ and ROSENBAUM 1965) and, in fact, all may be mucoid, the ability to produce mucous being masked in some by mutations at other loci. (4) Damage by UV, and most, but not all (e.g., acridines), chemical agents is lessened if the organisms are incubated at an elevated temperature or plated on minimal sugar-salts medium rather than organically rich media, or if pantoyl lactone is added to the medium (VAN DE PUTTE, WESTENBROEK and RÖRSCH 1963; ADLER and HARDIGREE 1965). (5) They result from mutations at a locus or loci mapping close to *T6*. (6) They are much more mutable than their UV- or chemical-resistant derivatives (ZAMENHOF, HELDENMUTH and ZAMENHOF 1966).

This last item may provide a clue to the mechanism of resistance and mutagenesis. The high mutability of this class of organisms may explain why acridines can be shown to be mutagenic in them but not in radioresistant *E. coli*. A possible model for the mechanism of mutagenesis and cross-resistance is the following: all the agents produce a common but unidentified lesion in the DNA of all strains of *E. coli*. Most, but not all, of these lesions are repaired by the K-reactivation present in radiation resistant organisms. In the radiation sensitive organism in which K-reactivation does not take place, the unrepaired lesions result in a common type mutation. The abundance of such mutations obscures the background of such specific mutations as are observed in radiation resistant strains. This model does not describe the nature of the common lesion. It is relevant that the host cell or dark reactivating systems are also nonspecific (PAPIRMEISTER and DAVISON 1964; LAWLEY and BROOKES 1965).

SUMMARY

Lac⁻ mutations were induced by each of eight chemical mutagens (2-aminopurine, proflavine, 1-methyl-3-nitro-1-nitrosoguanidine, nitrogen mustard, nitrofurazone, azaserine, mitomycin C, nitrous acid) and ultraviolet light. Most of the mutants were deficient in β -galactosidase. About 15 to 20% did not revert spontaneously to Lac⁺ nor could they be induced to revert by any of the mutagens. About 15 to 20% reverted at a very high frequency ($\geq 10^{-6}$). The remainder reverted spontaneously at a frequency of $\approx 10^{-8}$. The last group was used for studies in which Lac⁻ mutants induced by each mutagen were induced to revert to Lac⁺ by each mutagen. Under conditions producing equivalent lethality, some agents were significantly more efficient mutagens than others. Beyond this there was no observed specificity of mutagenesis. The frequency of nonrevertible and highly revertible forward mutations, and the proportion of revertants which were feeders (excreting β -galactosidase into the medium), were the same for all agents. Mutations induced by all agents and which reverted spontaneously were revertible by all other agents tested. Two feeder-type revertants were found to have β -galactoside with exactly the same heat stability as the wild type. The two non-feeder-type revertants tested had β -galactosidase significantly more heat sensitive than the wild type, and are considered to be the result of suppressor mutations. This also argues against the specificity of mutagens with this strain at the *lac* locus.

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