The Mutagenic Action of Various Chemicals on *Micrococcus aureus*

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Reports of a large number of experiments on the mutagenic action of various chemicals have been published in recent years. The ultimate goal of such work has been to find some new tool of research that would aid in the determination of the exact chemical nature of the gene or an elucida-

tion of its action. However, it has been found that mutagenicity is not a specific property of any one group of chemicals. Work by Witkin (4), Demerec, Wallace, and Witkin (2), and Demerec, Bertani, and Flint (1) has demonstrated that a wide variety of compounds with different properties may be mutagenic for microorganisms. Herskowitz (3) has summarized the mutagenic effects of a large number of chemicals on *Drosophila*.

In the past six years the author has had occasion to test several chemicals for mutagenic action. This work was done in the Laboratory of Bacteriology and Genetics at the University of Texas and in the Department of Plant Sciences at the University of Oklahoma.

Materials and Methods

The mutations tested for in these experiments were penicillin and/or streptomycin resistance in *Micrococcus pyogenes* var, *aureus* strain FDA209. The organism was maintained in stock on nutrient agar slants, and a log phase culture in nutrient broth was used as inoculum.

In each case the chemical used was added to 50 ml. aliquots of nutrient broth prior to autoclaving. The resulting broth containing the chemical and possibly its degradation products was inoculated with approximately 1000 cells of the log phase culture per ml. of medium and allowed to incubate for six hours. The culture was then plated in appropriate dilutions in nutrient agar for a total viable count, in nutrient agar containing 0.055 units of penicillin per ml. for penicillin resistance count, and in nutrient agar containing 3.0 units of streptomycin per ml. for streptomycin resistance counts.

Penicillin resistant and streptomycin resistant cultures were inoculated into aliquots of the chemically treated broth and the growth rate compared with that of the parent culture. In no case was any selective depression of the growth rate of the parent culture found; so any apparent increase in mutation could not be attributed to this factor. No other tests were performed to determine factors of selection, and it is apparent that several other selective factors could create population pressures which could give misleading results. However, the purpose of this work was to determine any apparent mutagenic activity of the chemicals and not to fully investigate each chemical tested.

The frequency of mutation is expressed in terms of the number of mutants per 10^6 living cells in the culture at the time of plating. No statistical analysis was made of the results, but each experiment was repeated several times and the results were always within the limits of experimental error inherent in a plate count.

Results

For convenience, the results are presented in two tables. These contain those chemicals which increased the frequncy of mutation, and those which had no appreciable effect. Each chemical was tested in several concentrations, but only the concentration used which gave the minimum killing action was chosen for the results. The wide discrepancies in the spontaneous frequency of mutation between the various experiments is due to the inexactitude of maintaining an accurate antibiotic concentration in different flasks of nutrient agar. Plates for each chemical tested and its control were poured from the same flask of antibiotic agar to minimize this error in the individual experiments.

Those chemicals which increase the frequency of mutation under the conditions used are listed in Table I. A few of these are confirmations of previously published reports. In several cases it appeared that the apparent increase in mutation might be due to the destruction of the

TABLE I

Chemicals Which Induce Antibiotic Resistant Mutants in Micrococcus Aureus Populations.

CHEMICAL	CONCENTRATION	TOTAL COUNT x 10^6	PENICILLIN RESISTANT MUTANTS/10^6 CELLS	STREPTOMYCIN RESISTANT MUTANTS/10^6 CELLS
none	——	36	3	13
ascorbic acid	0.1%	3	880	160
none	——	13	39	27
catalase	5%	7	457	400
none	——	36	7	2
crystal violet	0.0001%	0.5	12	60
none	——	33	——	10
formaldehyde	0.001%	39	——	25
none	——	47	5	19
hydrogen peroxide	0.0006%	33	151	696
none	——	80	100	475
hydroxyaspartic acid	0.02%	18	234	7770
none	——	47	5	19
menadione	0.0006%	12	97	156
none	——	37	37	2
methylene blue	0.002%	15	47	31
none	——	37	37	2
nile blue	0.0001%	11	123	8
none	——	50	4	49
sodium azide	0.06%	6	500	1165
none	——	64	7	246
sodium bisulfite	0.1%	40	79	1029
none	——	14	40	179
sodium nitrite (acid pH, then neutralized)	0.1%	12	125	333
none	——	32	——	10
sodium sulfite	0.06%	5	——	236
none	——	47	1	——
sulfanilic acid	1.0%	0.5	20	——

antibiotic by the test chemical as it was carried to the antibiotic agar plate in the dilution process. However, the concentration of chemical carried to the plate was negligible, and colonies picked from the antibiotic plates were tested and found to be actually resistant to the concentrations of antibiotics used.

Under the conditions used in these experiments, several chemicals were found to exert no significant alteration of the frequency of mutation (Table II). Since only two genetic markers were used, it cannot be assumed that these chemicals are totally non-mutagenic. It is possible that some of them might induce other genetic changes which were not detected, or they might be mutagenic under different cultural conditions.

DISCUSSION

The mutagenicity of a relatively large percentage of chemicals which have been tested on bacterial systems is a factor which must be considered when planning experimental approaches to the mechanism of gene activity, composition, and specificity. It is possible that some of these chemicals react directly with the gene complex, *per se*. If such were known to be the case, a great deal of information could be obtained about the exact

TABLE II

Chemicals Which Exert No Apparent Mutagenic Effect on Formation of Antibiotic Resistant Mutants in Micrococcus Aureus Populations.

CHEMICAL	CONCENTRATION	TOTAL COUNT x 10^6	PENICILLIN RESISTANT MUTANTS/10^6 CELLS	STREPTOMYCIN RESISTANT MUTANTS/10^6 CELLS
none	——	46	——	19
acenaphthene	sat.	36	——	17
none	——	54	18	40
acetone	1.5%	16	2	30
none	——	80	10	47
benzoyl peroxide	sat.	16	7	14
none	——	39	13	20
chlorine	0.0006%	30	20	17
none	——	30	44	10
colchicine	0.1%	2	40	11
none	——	14	40	179
copper sulfate	0.000001%	28	18	111
none	——	30	44	10
1,2,5,6, dibenzanthracene	sat.	58	4	1
none	——	20	10	13
hydroquinone	0.0006%	24	8	10
none	——	46	——	19
indole-3 acetic acid	0.00002%	64	——	21
none	——	20	10	13
inosital	5%	9	1	12
none	——	39	13	20
iodine	0.0006%	20	4	8
none	——	14	40	179
mercuric chloride	0.000001%	10	44	181
none	——	14	40	179
merthiolate	0.00000005%	24	19	110
none	——	30	44	10
methylcholanthrene	sat.	16	30	6
none	——	39	13	20
potassium permanganate	0.001%	37	17	23
none	——	14	40	179
silver nitrate	0.000001%	20	9	90
none	——	63	7	25
sodium formate	0.01%	27	8	22
none	——	63	7	25
sodium hydrosulfite	0.01%	64	7	23
none	——	32	——	10
sodium nitrite (neutral *p*H)	0.1%	20	——	9
none	——	32	——	10
sodium sulfide	0.05%	11	——	11
none	——	30	8	27
sodium thioglycollate	0.05%	30	10	28
none	——	26	13	4
tertiary butanol	1%	48	11	4

chemical nature of the gene. However, as pointed out by Demerec, *et al.* (1), since so many compounds have been shown to exhibit some mutagenic activity, it is plausible that any chemical which can enter the cell and interfere with some metabolic pathway could cause a mutation to occur. Since it would be virtually impossible in most cases to determine if a chemical is acting directly with the gene complex or interfering in some

metabolic pathway leading to gene synthesis, it appears fruitless to try to gain any insight into gene composition or action by determining which chemicals may exert a mutagenic effect under a certain set of experimental conditions. A more profitable approach to the general problem would be to study the metabolic pathways which might be involved in gene synthesis and thus learn something of the composition of the gene by determining how the gene could be formed as the result of many chains of metabolic events.

If interference in a metabolic pathway can result in mutation, it might be expected that mutuation would occur predominantly at cell division due to an inexact replication of the gene. It is, however, plausible that mutation could occur without cell division as a result of the dynamic state of the proteinaceous components of the genetic mechanisms. For example, the amino acid pool could furnish homologs of the naturally occurring protein components, which could then cause alteration of the gene composition and result in a new genotype.

The non-specificity of the type of chemical which is mutagenic suggests an explanation for the failure to find a specific mutagen which will induce the formation of only one new genotype. If a mutagen acted directly with the gene and always reacted in the same manner, it might be expected that a specific mutation would occur consistently. However, if the mutagen acted by interfering with some metabolic pathway leading to gene synthesis, it would not be expected that only one specific gene change would occur. If this metabolic interference were many steps away from the termination of the metabolic sequence, a large number of probable alterations could be expected. If the metabolic interference occurred close to the final product, one might expect to approach a specific mutation.

SUMMARY

Thirty-six chemicals were tested for mutagenic activity against *Micrococcus aureus* using penicillin and streptomycin resistance as genetic markers. Of these, 14 were mutagenic while 22 exerted no apparent activity. The wide diversity of chemicals which are mutagenic suggests the possibility that a metabolic approach to the problems of gene composition and action would be of more value than a systematic testing of chemicals for mutagenic action.

LITERATURE CITED

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