COMPARATIVE STUDIES OF MONOEPOXIDES AS INDUCERS OF REVERSE MUTATIONS IN NEUROSPORA[1]

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IN two previous papers (KØLMARK and WESTERGAARD 1953; KØLMARK 1953) experiments have been reported dealing with studies of epoxides as mutagens. In these experiments the induction of reverse mutations, principally in a purple adenineless mutant of *Neurospora crassa*, served as the criterion of mutagenic activity for the various compounds tested. Certain of these compounds proved to be very potent mutagens. The general results suggested that additional comparative studies of related substances should prove fruitful in elucidating certain aspects of the mechanism of action of these chemical mutagens. In the present paper the results of comparative studies of six different monoepoxides as inducers of reverse mutation in a purple adenineless mutant will be presented.

MATERIALS AND METHODS

The monoepoxides used in these experiments were obtained from the following commercial sources: from Eastman Kodak Chemical Corporation—glycidol, propylenoxide, 1,2-monoepoxybutane, 2,3-monoepoxybutane; from Farchan Research Laboratories—epichlorohydrin and epibromohydrin. All these compounds are liquids at room temperature.

The general method employed in testing various chemicals for mutagenic activity by the back-mutation technique has been described previously (KØLMARK and WESTERGAARD 1949; JENSEN, *et al.* 1951). In the present experiments the test culture, W. 40 "distinctus" A (an extreme colonial strain of purple adenineless mutant 38701), and the methods of chemical treatment and reversion detection have been the same as those utilized in the initial experiments with epoxides (KØLMARK and WESTERGAARD 1953). The various chemicals are added directly to suspensions of macroconidia in sterile distilled water, utilizing a micropipette attached to a screw syringe. The conidial suspensions are kept at 25°C and gently agitated during the period of treatment. The treatment is stopped by centrifuging the conidia and washing several times with sterile water. The conidia are then plated at appropriate concentrations into a minimal agar medium. The numbers of viable and of surviving conidia in the control series and in the treated series respectively are determined by plating diluted samples on minimal medium supplemented with adenine.

When starting to test a chemical whose toxicity for Neurospora conidia is unknown, the usual initial procedure is to perform an experiment in which the concentration of the chemical is varied over a wide range, the time of treatment being kept constant.

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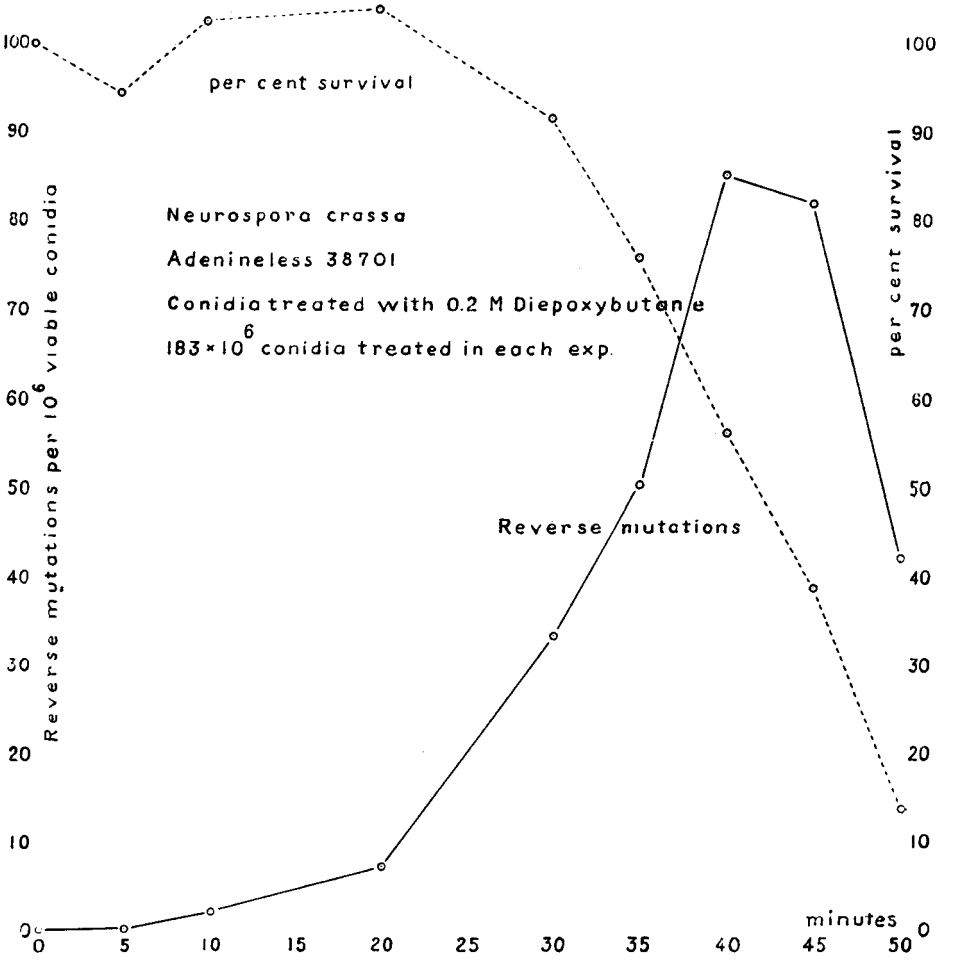


FIGURE 1.—The effect of exposures of macroconidia of purple adenineless mutant 38701 (strain W 40 "distinctus" A) to 0.2 M diepoxybutane for varying lengths of time. The lower curve gives the frequency of reverse mutations, the upper, percent conidial survival, based on the value of 100 percent in the control.

From such an experiment a concentration will usually be found which produces a considerable conidial killing within a time convenient for the experiment, for example, 50 percent killing in 45 minutes. This concentration can then be used in a second experiment in which the time of exposure is varied. Figure 1 illustrates the type of curve which is obtained in an experiment in which a constant concentration of a chemical has been utilized and the time of treatment varied. The data on which this figure is based are those obtained by KØLMARK and WESTERGAARD (1953) with diepoxybutane, the chemical mutagen having the greatest activity of any so far tested in producing reversions in purple adenineless mutant 38701. This curve may be used in comparing the activity of this diepoxide with the various monoepoxides tested in the present experiments. It will also prove useful in discussing the concept of

"optimal conditions" which is used in making comparisons of the mutagenic activities of the various compounds tested.

In the experiment plotted in figure 1, diepoxybutane was added to the conidia at time zero. Aliquots were removed at intervals, the reaction stopped by washing the conidia with distilled water, and the conidia plated to determine the number of reversions and the percent conidial survival. Each point represents a treatment of 183×10^6 conidia. In the control there were a few spontaneous reversions (the number was 5). With increasing time of exposure the number of induced reversions increased as shown in the lower curve until it reached a maximum at 40 minutes treatment, at which point more than 15,000 reversions were scored. In the curve the derived numbers of mutations per 10^6 treated initially viable spores have been plotted. The slow rise in the number of reversions during the first 20 minutes of treatment is probably due to the time required for the chemical to penetrate into the cell and into the nucleus. The upper curve gives the percent conidial survival at various times. A pronounced mutagenic effect is observed before the toxic effect becomes significant. However, this time difference in toxic and mutagenic activity is presumably only an apparent one. Since macroconidia have been treated, more than one nucleus is present in the majority of the conidia. Hence it is expected that in most instances each conidium containing a nucleus which has undergone reversion to the non-requiring state will give rise to a colony on minimal medium, whereas in most instances the occurrence of a single lethal mutation in a conidium will not result in the death of the conidium. For example, if a conidium contains three nuclei, a reversion in only one nucleus is required to give rise to a colony on minimal medium, whereas at least three lethal mutations are presumably needed to prevent this conidium from forming a colony on minimal plus adenine medium where the viability count is made.

The maximal yield of mutations is obtained after an exposure which gives approximately 56 percent survival. Beyond this point, the total yield of reversions begins to decrease because the toxic effect increases so rapidly that conidial killing occurs at a faster rate than reversion production. When a particular mutant, such as this purple adenineless is treated with a chemical, such as diepoxybutane, the experimental conditions giving the highest yield of mutations may be designated the "optimal conditions." The relative mutagenic activities of the various monoepoxides used in the present experiments have been compared on the basis of their effectiveness under "optimal conditions" for each mutagen. It is realized that this basis of comparison is not a perfect one. In some respects it would appear desirable to compare the various chemicals on an equimolar basis. Unfortunately, since toxic effects vary markedly from one chemical to another, such comparisons are often difficult if not impossible. It appears, however, that even if the molar concentrations are rather different, the percent survival under "optimal conditions" is usually rather similar. Hence, to a considerable degree the present comparisons are being made at equal or similar survival levels.

Clearly, there are many variables involved in determining how effective a particular chemical will be as a mutagen. Some of these variables have been discussed by KØLMARK and WESTERGAARD (1953) and it is apparent that numerous factors other

than relative chemical affinity for a particular gene may influence the mutagenic effectiveness of structurally similar compounds. Although we are aware of these difficulties, it has seemed desirable in these initial comparative experiments to make a first attempt at establishing the order of mutagenic activity of the monoepoxides being studied. Because of the great differences in activity found even between closely similar compounds, it appears likely that these indicate basic differences in the mutagenic effectiveness of these chemicals with respect to this adenineless mutant. The relative effectiveness of these compounds as determined by the experimental procedures outlined will be used as the basis for a discussion later in this paper of a possible mechanism of mutation induction by monoepoxides.

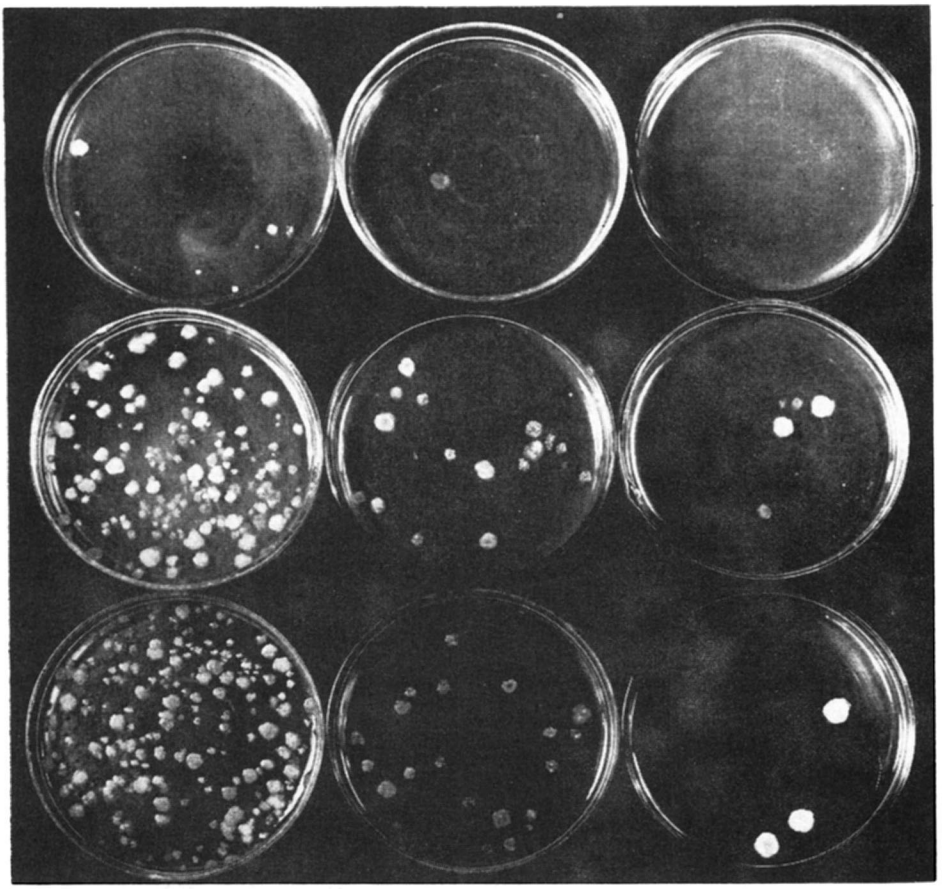
In connection with the experiments performed to determine the mutagenic activity of the various compounds, control platings were carried out to ascertain that no competition effects were present resulting in the selective suppression of reversions under particular conditions (cf. GRIGG 1952; KØLMARK and WESTERGAARD 1952). Representative results of one such series of platings are given in table 1 and illustrated in figure 2. It is clear that there is no disproportionate increase in reversion frequencies with either a 10- or 100-fold conidial dilution.

EXPERIMENTAL RESULTS

The quantitative data obtained in experiments with the various monoepoxides are given in tables 2–5. A detailed consideration of these results will be deferred until the discussion.

Because of the marked differences in the mutagenic effectiveness of the various compounds, it seemed especially desirable to make genetic tests of a number of reversions to determine whether suppressor rather than reverse mutations might have occurred. In most instances, reversions appearing on minimal are not only adenine-independent, but no longer form purple pigment. In some experiments with monoepoxides, however, a number of colonies appeared on minimal which still produced some purple pigment. It seemed possible that these might be the result of suppressor mutation. Tests of a number of these purple revertants revealed, however, that they were heterocaryons. In the majority of cases both revertant (adenine-independent, non-purple) and non-revertant (adenineless, purple) components could be separated by conidial platings. In a few cases no non-requiring colonies were obtained by diluted conidial platings on minimal medium. Only the very slight type of growth—called "shadows"—was observed. This type of growth is typical for this adenineless strain when only a few spores germinate on a plate with minimal medium. These latter cases apparently are due to heterocaryons with a great preponderance of requiring nuclei, so that occasionally no non-requiring nuclei have been transferred by the isolation. Since almost all reversions induced in macroconidia are heterocaryotic, pigment expression is not always evident and apparently occurs when the nuclear ratio favors adenineless nuclei.

Crossing analyses were carried out with a total of 38 independent reversions arising spontaneously or induced by four of the monoepoxides tested. The results of these genetic tests are summarized in table 6. Most of the reversions were induced in the macroconidiating strain W. 40 "distinctus" A. In all such instances, the revertants



Spores undiluted diluted 10× diluted 100×

Reverse mutations in *Neurospora crassa*, purple adenineless, 38701, macroconidiating, "distinctus"
Upper row: control, untreated.
Middle row: 0.5 M. propylenoxide, 15 min.
Lower row: 0.5 M. glycidol, 15 min.

FIGURE 2.—Photographs of representative plates from a dilution series containing reverse mutations induced in purple adenineless mutant 38701 by two epoxides. See table 1 and text for further discussion.

were first outcrossed to a strain of purple adenineless 38701a and homocaryotic isolates obtained for crossing with wild type (strains 74A or 73a). In some instances reversions induced in a microconidiating strain (8743-CL20-12.1a) were tested and these were crossed directly to wild type. In all cases the progeny from crosses of homocaryotic reversions with wild type were scored for the presence of typical purple pigment. The number of such isolates tested ranged between 24 and 196 for 38 crosses, the average number being 90. In no instance was a culture with typical purple pigment found. In addition, in a number of crosses, all the progeny were also tested on minimal medium, and no instances of adenineless segregants were obtained.

TABLE 1

Evidence for the absence of a suppression effect of high conidial concentrations on the recovery of reversions as demonstrated by the effect of conidial dilution on the frequencies of reversions after treatment with two epoxides. 132 × 10^6 viable macroconidia treated in each experiment. See text for further discussion

Treatment	No. of colonies counted on five plates each at the conidial dilutions indicated		
	Undiluted	10^{-1}	10^{-2}
Control	8	3	1
0.5 M Propylenoxide			
15 min.	780	77	8
30 "	1647	156	17
45 "	ca. 2500	271	—
60 "	ca. 2750	282	—
0.5 M Glycidol			
15 min.	1025	101	9
30 "	ca. 2000	195	17
45 "	ca. 3500	349	—
60 "	ca. 4250	445	—

TABLE 2

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments with 0.5 M propylenoxide or 0.5 M glycidol for varying lengths of time. 132 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	8	0.0	0.0
	0.5 M Propylenoxide				
II	15 min.	94.0	780	5.9	6.2
III	30 "	83.5	1647	12.4	14.9
IV	45 "	63.1	2710	20.5	32.5
V	60 "	26.7	2820	21.4	80.0
	0.5 M Glycidol				
VI	15 min.	92.0	1025	7.7	8.4
VII	30 "	85.7	1950	14.7	17.4
VIII	45 "	62.3	3490	26.4	42.5
IX	60 "	26.3	4450	33.8	128.5

* The frequencies have been corrected for the spontaneous background.

These results indicate that the majority of reversions induced by these chemicals do not involve suppressor mutations, unless the latter are quite closely linked with the purple adenineless locus. Hence it appears reasonable to compare the various epoxides on the basis of their relative effectiveness as inducers of reverse mutation in the purple adenineless mutant.

TABLE 3

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments for 30 minutes with varying concentrations of either 1,2-monoepoxybutane or 2,3-monoepoxybutane. 234 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	29	0.0	0.0
	1,2-Monoepoxybutane, 30 min.				
II	0.005 M	100	32	0.013	0.013
III	0.025 M	56.9	36	0.09	0.16
IV	0.050 M	57.3	91	0.32	0.55
V	0.10 M	52.2	239	0.96	1.8
VI	0.20 M	34.5	506	2.1	6.1
	2,3-Monoepoxybutane, 30 min.				
VII	0.05 M	67.5	25	0.021	0.032
VIII	0.10 M	ca. 50	32	0.07	ca. 0.15
IX	0.20 M	ca. 40	35	0.10	ca. 0.25
X	0.40 M	28.8	10	0.009	0.03

* The frequencies have been corrected for the spontaneous background.

TABLE 4

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments with 0.15 M epichlorohydrin for varying lengths of time. 73.6 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	39	0.0	0.0
	0.15 M Epichlorohydrin				
II	15 min.	94.7	626	8.0	8.5
III	30 "	87.8	2480	32.6	37.0
IV	45 "	41.5	4140	56.1	135.2
V	60 "	0.72	218	3.0	411.0

* The frequencies have been corrected for the spontaneous background.

TABLE 5

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments for 30 minutes with varying concentrations of epibromohydrin. 71.3 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	10	0.0	0.0
	Epibromohydrin, 30 min.				
II	0.005 M	97.2	13	0.042	0.043
III	0.01 M	99.1	17	0.098	0.099
IV	0.02 M	85.5	30	0.29	0.34
V	0.04 M	77.5	65	0.80	1.0
VI	0.08 M	39.3	206	2.8	7.2
VII	0.16 M	0.0005	0	—	—

* The frequencies have been corrected for the spontaneous background.

DISCUSSION

On the basis of the data already presented, an attempt can be made to compare the relative mutagenic effectiveness of the six monoepoxides tested. To this end, figures on reverse mutation rates under "optimal conditions" for the various chemicals, derived from tables 2–5, have been collected in table 7, together with the structural formulae

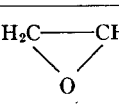
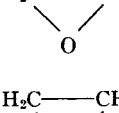
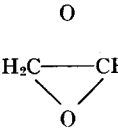
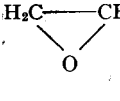
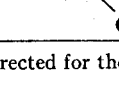
TABLE 6

Genetic analyses of reversions induced by various chemical mutagens in purple adenineless strain 38701. See text for further details

Mutagen	No. homocaryotic reversions tested	No. reversions giving typical purple segregants	No. reversions giving adenineless segregants
None (control)	7	0 in 7	0 in 3
Propylenoxide	12	0 in 12	0 in 6
Glycidol	4	0 in 4	0 in 4
1,2-Monoepoxybutane	4	0 in 4	0 in 3
Epichlorohydrin	11	0 in 11	no tests

TABLE 7

Relative mutagenic activity of six different monoepoxides. Comparisons have been made of reverse mutation rates under "optimal conditions" (see text). Figures obtained from the foregoing tables 2–5

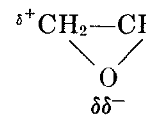
Chemical	Formula	Molar conc.	Percent survival	Reverse mutations induced*	
				Per 10^6 viable	Per 10^6 surviving
Epichlorohydrin		0.15	41.5	56.1	135.2
Epibromohydrin		0.08	39.3	2.8	7.2
Glycidol		0.5	26.3	33.8	128.5
Propylenoxide		0.5	26.7	21.4	80.0
1,2-Monoepoxybutane		0.2	34.5	2.1	6.1
2,3-Monoepoxybutane		0.2	ca. 40	0.10	0.25

* The frequencies have been corrected for the spontaneous background.

of the six monoepoxides. It is clear that there are marked differences in the comparative mutagenic effectiveness of the six compounds. Epichlorohydrin appears to be the most active mutagen, but both glycidol and propylenoxide are also quite active. Both the monoepoxybutanes are much less active, the 2,3 compound being especially ineffective.

As judged by the structural formulae included in the table these six compounds are all quite similar. Consequently, their marked differences in mutagenic effectiveness are of particular interest, since it seems possible that these differences may give some clue as the mechanism of action of these compounds in mutation production. Following a suggestion made by W. C. J. Ross of the Chester Beatty Institute, consideration of certain physico-chemical properties of monoepoxides has lead to the formulation of an hypothesis to explain the marked differences in mutagenic effectiveness already noted.

This hypothesis is based on the assumption that the reaction producing a mutation takes place between electrically charged entities. In the specific case as regards monoepoxides this reaction is assumed to occur between the positively charged terminal carbon atom of the epoxide ring and a negative center in the biological material, in this instance in the adenineless locus. Chemical evidence (Ross 1950) indicates that, although the epoxide ring is always polarized to some extent even when there are no substitutes, the presence of electron attracting (i.e., electronegative groups) in the side chain increases the positive charge on the terminal carbon atom and decreases the negative charge on the oxygen atom. Thus a monoepoxide molecule can be written as a dipole with a distribution of charges as follows:



Side chains which have stronger electronegative (electron attracting) properties will result in a relative increase in the positive charge on the terminal carbon atom of the epoxide ring. The side chains of the compounds used here are chemically rather inactive. The epoxyring, therefore, can be considered as the only active group in the molecule. Thus, if the mutation reaction involves a negative center in the genetic material, a monoepoxide with a more positively charged terminal carbon atom in the ring should be a more effective mutagen. The first two compounds tested with this idea in mind were propylenoxide and glycidol. The CH_2OH group in glycidol is more electronegative than the CH_3 group in propylenoxide. Thus, if the hypothesis outlined above is correct, glycidol should be the more effective mutagen. As indicated in table 7, glycidol does in fact produce about one and a half times as many mutations as propylenoxide. The results with the other monoepoxides generally support the hypothesis. In table 8 the groups in the side chains of the six monoepoxides have been arranged in order of decreasing electronegativity (reading from top of the table) and the mutagenic effectiveness of the compounds indicated.

Pairwise comparisons between the compounds most like each other give the following results: (1) The Cl-compound should be more effective than the Br-com-

TABLE 8

Six monoepoxides arranged in the order of decreasing electronegativity of their side chains (reading from top of table) and compared for mutagenic effectiveness

Monoepoxide	Side chain	Mutations per 10^6 viable conidia under "optimal conditions"
Epichlorohydrin	CH_2Cl	56.1
Epibromohydrin	CH_2Br	2.8
Glycidol	CH_2OH	33.8
Propylenoxide	CH_2H	21.4
1,2-Monoepoxybutane	CH_2CH_3	2.1
2,3-Monoepoxybutane	$2(CH_2H)$	0.1

pound, which accords with the results obtained; (2) The OH-compound should be more active than the H-compound, which is also the case; (3) A CH_3 side chain should be more active than a CH_2CH_3 side chain, which agrees with the results for propylenoxide vs 1,2-epoxybutane; (4) One CH_3 side chain should be more active than two CH_3 side chains, which also is the case in the comparison of propylenoxide and 2,3-monoepoxybutane.

If the order of the entire series is considered, the mutagenic activity of epibromohydrin is too low. On the basis of its position in the sequence it would be expected to yield more mutations than glycidol, which is contrary to the observations. This result, which is in disagreement with the working hypothesis, can perhaps be explained in part, if not entirely, by the greater toxicity of epibromohydrin, which makes difficult a direct comparison between it and the other substances. If a comparison is desired between two compounds with widely different toxicities, such as glycidol and epibromohydrin, used in equimolar concentrations, it is necessary to use a concentration of the more toxic substance which is low enough to be tolerated by the test organism. Consequently an attempt has been made to compare the activity of glycidol and epibromohydrin at lower equimolar concentrations, both compounds being used in 0.1 molar concentration. The results are shown in figure 3. Under these conditions the mutagenic and toxic effects of epibromohydrin occur very much faster than do those for glycidol.

On the basis of this experiment it would appear that epibromohydrin is the stronger mutagen of the two, which is in accord with our working hypothesis. However, it is also clear from this curve that the total number of mutations with epibromohydrin fails to reach a high value because of the toxic effect. Thus, the figure "mutations per 10^6 treated conidia" can never reach as high a value (33.8) as was found for glycidol when this compound was used at 0.5 molar concentration. Glycidol at 0.1 molar concentration acts very slowly as a mutagen and is far from its maximal effect after 120 minutes, at which time there is also little evidence of a toxic effect. If, in a comparison of this type, the same percent survival is desired, there will obviously have to be a very large time differential. If, by contrast, equal times of treatment are utilized, the percent survival will be very different for the two compounds. In view of

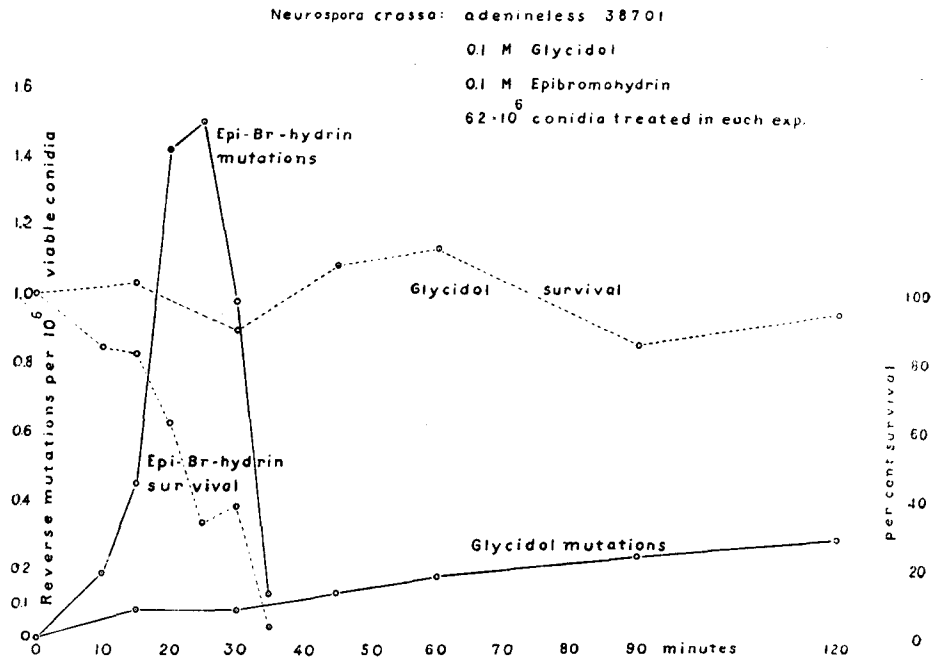
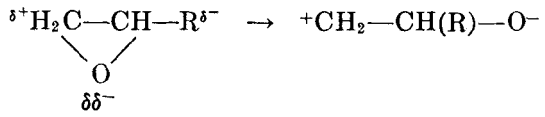


FIGURE 3.—The effect of exposures of macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) to 0.1 M glycidol and 0.1 M epibromohydrin for varying lengths of time. Mutation rates corrected for the spontaneous background. Percent conidial survival based on the value of 100 percent in the control.

these considerations it appears difficult to achieve a good basis for a comparison of the mutagenic activities of two compounds which differ considerably in toxicity. It is clear that comparisons between mutagenic activities based on the mutation rate under "optimal conditions" must be evaluated with caution if the compounds differ markedly in their toxicities. With these reservations in mind, it appears reasonable to conclude from these experiments that the possibility still remains open that epibromohydrin fits into the appropriate position in the sequence of activities.

The possibility has been mentioned by KØLMARK and WESTERGAARD (1953) that the mutagenic effect of epoxides might be due to a release or transfer of energy when the three-membered ring is opened. There is also the possibility that the product of a general chemical reaction gives rise to a mutation. Of course, it need not be that an initial chemical product between mutagen and gene be reduplicated in the induced mutant. Such a reaction might simply give rise to a steric disturbance in the normal gene during the first mitosis, the modified gene then being reduplicated without any alteration in its molecular size. The epoxides react with a variety of other groups to give alkylation compounds. Both these mechanisms still remain as possibilities if the epoxides are considered as dipoles—the electric charge might act merely to direct the mutagenic chemical to the location where a reaction will produce the particular mutation studied. Thus the actual mutagenic event might still be due to a chemical reaction or to a transfer of energy.

The different theories for biological reaction of radiomimetic agents have been reviewed recently by HOMER (1954). Our experimental results fall closely in line with one of the viewpoints cited in this paper which maintains that "all that is required for radiomimetic activity is that the drug shall give rise to a reactive positive ion, sometimes so unstable as to be formed only at the moment of reaction. . . ." Different types of radiomimetic compounds, including the epoxides, have the ability to form carbonium ions. The ionic form of an epoxide, created by opening the ring may be written:



which then can react further and, for example, form esters with carboxyl or phosphoryl groups. If such a preformed ion is involved in the mutagenic reaction, this will presumably be a chemical reaction. If, on the other hand, the ring is opened at the moment of mutation production, the mutation inducing mechanism might well be dependent on a simultaneous transfer of energy. It is not possible to decide which of these two possibilities is realized. The fact that a fractional positive charge is carried on the terminal carbon atom before the ring has been opened might favor the view that the compound is reacting directly from the ring structure at the critical time and place. Electrostatic forces would direct the positive terminal carbon atom to the place of the anion. This directing force would increase with the magnitude of the positive charge. Such a situation would lead to series of compounds with different activities as outlined in the working hypothesis and found in our experiments. We cannot provide any experimental evidence to identify the anion in the gene with which the epoxides presumably react, but it is tempting to think of phosphoryl groups in the backbone of DNA or carboxyl groups in nucleoproteins as the site of reaction.

The general conclusion of this investigation is that there exists a quite striking correlation between two properties of the epoxides used here: a high positive charge on the terminal carbon atom in the epoxyring and a high mutagenic activity. So far we know only that this correlation exists for reverse mutations in this particular adenineless strain (38701). We may in the future expect to find other relations with regard to other genes. Experiments with gene specificity (KØLMARK and WESTERGAARD 1953; KØLMARK 1953) already suggest that the correlations between mutagens and mutation rates will be different for different genes treated with the same mutagen.

For a given reverse mutation reaction, as in the work presented here, it appears that we can predict, by examining the formulae of the chemicals and taking into consideration their properties as dipoles, whether they will behave as strong or weak mutagens. On this basis it would appear desirable to study in future experiments epoxides having side chains with nitro (NO_2) and cyano (CN) groups. Such compounds would be expected to be more potent mutagens than any of the compounds yet tested. Also the sulphonic acid group (SO_3H) can be expected to increase mutagenic activity, but this effect will presumably be dependent on the pH of the solution as well (W. C. J. Ross personal communication).

SUMMARY

1. Six different monoepoxides have been shown to induce reversions in the purple adenineless mutant (38701) of *Neurospora crassa*.

2. Genetic tests of reversions induced by four of these monoepoxides have been carried out. Only true reverse mutations and no suppressor mutations were found.

3. A comparison of the mutagenic effectiveness of the six epoxides has been made. This comparison is based on the relative number of reversions recovered per 10^6 viable treated spores when treatments have been performed under "optimal conditions." "Optimal conditions" are defined as the experimental conditions under which the highest yield of reversions is obtained.

4. The data indicate that in general epoxyrings carrying side chains with strong electronegative properties are stronger mutagens than compounds carrying weaker electronegative side chains.

5. On the basis of these experimental observations, a working hypothesis to explain the mutagenic effect of these compounds is developed and discussed. This hypothesis proposes that the mutation reaction involves negatively charged centers at the site of mutation which react relatively more readily with those epoxides carrying an increased positive charge on the terminal carbon atom of the epoxyring.

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