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MOLECULAR MECHANISMS OF ALKYLATION OF A BIOLOGICAL SYSTEM

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Among chemical mutagens the group of alkylating compounds occupies a singular position both in intensity of action on the mutagenesis of various organisms, as well as in diversity of structure of functional centers. Investigation of the molecular mechanism of action of mutagens has a direct bearing on a most crucial problem in genetics -- directed hereditary variability. Therefore, we must first look at the fundamentals of the process underlying formation of mutations from the chemical vantage point.

The substitution of a hydrogen atom by an alkyl group, and also the direct addition of this radical to the molecule is called alkylation. In this reaction, either a simple alkyl group ($-CH_3$, $-CH_2CH_3$, etc.) or a complex group (CH_2CH_2OH, $-CH_2CH_2NH_2$, etc.) is introduced into the molecule. Cyclic and acyclic alkylating agents can have one or several radicals in a reactive form. In the case of polyfunctional compounds, they react individually as well as concomitantly in several centers, forming overlapping cross-linkings between molecules, and this bond can be induced with various atoms (C-, O-, N-, S-) of the nucleophilic groups. It is believed that in the cleavage of the alkyl radical from the rest of the alkylating agent molecule both electrons that participate in forming a covalent bond remain with the latter. Therefore, a positively charged carbon in the alkyl radical strives to fill the outer valency orbital through addition to centers that are electron-rich. In this case, the positively charged Alk^+ group is the carbonium ion, which enters into reaction at the site of the highest electron density of the alkylating compound. $:X^- + Alk:Y \longrightarrow Alk:X + :Y^-$

Hypothetically, this process can occur by two mechanisms. First of all, asynchronously under the monomolecular type of reaction (S_N1),

when complete separation of the Alk^+ alkyl radical from Y^- takes place slowly in the presence of a polar solvent. This stage, determinative of the rate of the overall alkylating reaction, is reversible and will be slowed down with increase in concentration of the anion (Y^-) in the medium. The energy of solvation of the ions compensates for the energy needed for heterolytic cleavage. Then the resulting cation reacts rapidly with the nucleophilic center. In the second place, synchronously under the bimolecular mechanism (S_N2), when complete separation into two ions does not occur, but an unstable transitional complex is formed in which the Alk is united simultaneously with two groups (Y and X). In this case, the carbonium ion is not in a free state, since processes of bond formation and bond breaking occur simultaneously. The reaction rate depends on the concentration of the substituent group X^- and on its affinity with the electrophilic Alk^+ group.

Achievement of the transitional state is promoted by solvation of the polar complex, then processes of desolvation, charge transfer, and chemical bond formation take place. The cause responsible for cleavage of the bond in the complex is the attack of the nucleophilic X^- group on the positively charged carbon atom in the group of the alkylating compound, and the outcome of the reaction depends on the extent of nucleophilicity of the X^- and Y^- groups, and also on the polarity and solvent capacity of the solvent.

Most alkylating compounds are mutagens to some extent in physiological conditions of temperature and pH in aqueous solutions. Lethal effect, mutational activity, and specificity of action depend on the structure of the attacking group and of the entire alkylating agent. Many alkylation reactions used in organic chemistry are inapplicable under in vivo conditions for various reasons or because of overhigh reactivity (chloromethyl esters) that preclude selectivity of action, or, in contrast, because of extreme inertness in conditions of an intact biological system (alcohols, certain halogenalkyls, phosphoric acid esters). The chemical structure of the most typical classes of biologically active alkylating compounds is given in the table.

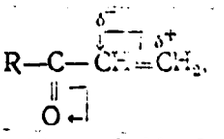
Halogenalkyls. Primary and secondary halogen-substituted saturated hydrocarbons react with the nucleophilic center mainly according to the bimolecular mechanism. For example, methyl iodide reacting with the aceto-group forms an ester

$$RCOO^- + CH_3I \rightarrow CH_3COOCH_3 + I^-.$$

In contrast, tertiary compounds are first subjected to ionization in aqueous solutions and the S_N1 mechanism is more characteristic of them. The resulting carbonium ion reacts more with the solvent molecules (water) than with the functional groups, which substantially reduces the role of tertiary derivatives as biological alkylating agents. The activity level of Alk-R in mild physiological conditions varies depending

on the halogen used, in the following order: R = F $\ll$ Cl $<$ Br $<$ I. 1,2-Dichloroethane ($ClCH_2-CH_2Cl$) exhibits a strong mutagenic effect, inducing visible and lethal mutations in Drosophila (Rapoport, 1960b). In contrast to most chemical and physical mutagens, dichloroethane acts more strongly on immature spermatogonia in larvae than on mature sperm in males. Females prove to be ten times more sensitive to this mutagen than males. This selectivity of action is explained by the author as owing to the ability of dichloroethane to overcome the lipoidal barriers of the ovum freely. 1,2-Dibromoethane and 1,3-dichloropropane act somewhat more weakly (Rapoport, 1965). I. A. Rapoport believes the mutational reaction of the halogenalkyl to be interaction with amino- oxy- and sulfhydryl radicals.

Activated ethylene compounds. Activation of the pi-bond occurs through introduction of an electron-acceptor group, for example, the sulfone or carbonyl radical, etc.



where the arrow points to the direction of displacement of the pi-electrons, that is, the direction of bond polarization. As a result of displacement of unpaired electrons, and also in part as a result of delta-bond polarization, the peripheral carbon atom acquires a certain (incomplete) positive charge, and the other atom -- an incomplete negative charge, therefore this molecule exhibits a certain spontaneous dipole moment. In this case, the compound reacts with the nucleophilic center according to the bimolecular mechanism through the polarized ethylene bond.

Acryloylethylenimine ($CH_2=CH-C(=O)-N<^{CH_2}_{CH_2}$) is a strong bifunctional mutagen, containing an ethylenimine ring and an ethylene radical, which are bonded through an acyl group (Bartoshevich, 1965b). Through mutational analysis of several compounds, including derivatives of ethylenimine with acyl radicals and pi-electron substituents, it was found that the activated ethylene bond is mainly responsible for high mutagenic activity. Also highly revealing in this respect is a comparison of the action of propionic aldehyde (CH_3-CH_2-CHO) with acrolein ($CH_2=CH-CHO$). It is probable that owing to the activated double bond, the unsaturated aldehyde causes ten times more mutations than the saturated aliphatic aldehyde (Rapoport, 1948a).

Aldehydes. The simplest representative -- formaldehyde (CH_2O) -- has proven to be the most active mutagen in this series (Rapoport, 1946; Auerbach, 1951; Jensen et al., 1951; Iyer, Szybalski, 1958). Spectrophotometric and isotopic methods have established that formaldehyde reacts with free amino-groups of nucleic bases (RNA and single-chain DNA) and aminoacids (Staehelin, 1958). At low concentrations, it reacts with

Classification of Alkylating Agents

1. Alk – R
   R = F, Cl, Br, I

Halogenide alkyls

2. CH_2=CH–R

R = NO_2, SO_2R_1, $COOR_1$, COR_1 etc.

3. R – C(H)=O

R = alk, – CH = CH_2

Aldehydes

4. HN(R) – C(=O) – O – C_2H_5

R = H, Alk, phenyl, etc.

Urethanes (ethyl ester of carbamic acid)

5. Alk = N^+ = N^-

Diazoalkanes

6. ON – N(–R_1) – R_2

R_1 = H, Alk

R_2-group, urethane, urea, guanidine, alk

N – nitrosubstituted compounds

7. Alk – O – S(=O)(=O) – O – Alk

Dialkylsulfates (esters of sulfuric acid)

8. Alk – O – S(=O)(=O) – Alk

Alkylalkanesulfonates (esters of sulfonic acids)

9. (Alk – O)(Alk – O)(Alk – O) P = O

Esters of phosphoric and phosphorous acids

10. Cl – Alk – S – (Cl – Alk) or R

R = Alk

Yprite (β-chloroethylsulfides)

11. Cl – Alk – N(R) – (Cl – Alk) or R

R = H, Alk, Alk–Cl, etc.

Nitrogenous analogs of yprite (β-chloralkylamines)

12. H_2C – CH – R (epoxide ring)

R–H, Alk, CH_2Cl, CH_2OH, second epoxide group

Epoxides

13. $(CH_2)_n$ – C = O (lactone ring)

Lactones

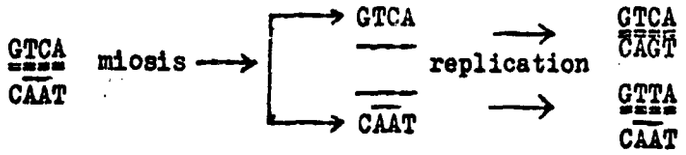
14. H_2C – CH – R (ethylenimine ring, NR)

R = H, Alk, phenyl, etc.

Ethylenimines

the attendant formation of a monomethyl derivative ($-NH-CH_2OH$), but at high concentrations the dimethylol derivative is formed, $-N=(CH_2OH)_2$. Similar methylene bridges are formed between two adenine molecules, which leads to formation of purine dimers. The length of the methylene bridge is 3.7 AU, but the distance between adjoining nucleotides is 3.4 AU. If thymine and cytosine are neighbors in the original DNA chain, in the course of replication erroneous incorporation of the adenine dimer is possible, which leads to the transitional substitution of base pairs

Original DNA Chain



The interphasal nucleus, that is, directly during the period of intensive DNA synthesis, is the nucleus most sensitive to the action of this mutagen.

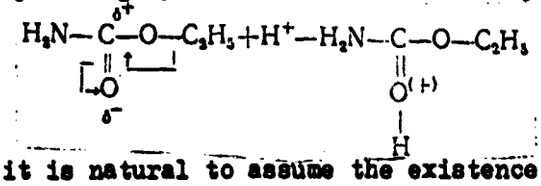
Formation of other dimers ($\overline{AC}$, $\overline{CC}$, $\overline{GG}$) is theoretically possible, but mutational changes are probably related to the adenine dimer, since the strong mutagenic action of formaldehyde is manifested only in the presence of adenine (Alderson, 1960a, b, 1961). In spite of the fact that formaldehyde is conventionally in the class of alkylating compounds (Friz, 1964), at the basis of dimeric complex formation lies the condensation reaction, as has been noted earlier by I. A. Rapoport (1948b). Several authors suggest that formaldehyde reacts with genic material via formation of an organic peroxide or free radical (Jensen et al., 1951; Auerbach, 1963; Friz, 1964). This reaction is catalyzed by ultraviolet rays and hydrogen peroxide.

In contrast to dichloroethane, formaldehyde acts only on male gametes in an early stage (in the meiosis period) and has no effect on the incidence of mutations in ova when Drosophila females were treated. On the same genetic model, the unsaturated aldehyde $CH_2=CH-C(=O)-H$, acrolein, exhibits high mutagenic index, substantially exceeding the effect of the nearest representatives of this homologous series (Rapoport, 1948a).

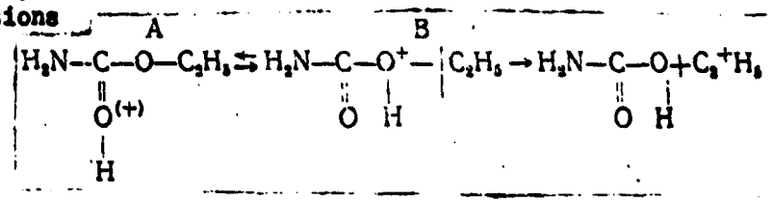
Urethanes. Among monofunctional compounds of this type, ethylurethane ($H_2N-C(=O)-OC_2H_5$) exhibits the highest mutagenic effect, the action of which was almost simultaneous and was found independently in plants and in Drosophila (Oehlkers, 1943; Rapoport, 1947a). If the ethyl analog increased the incidence of sex-linked lethals by 25 times compared

to controls, the remaining seven derivatives of carbamic acid did not show a marked effect. Just like other alkylating mutagens, urethane induces retarded mutations, which show up in the second generation in the form of gonadal mosaics. Mutational research on plant material using several urethane derivatives with substituents at the amine group and at the ester bond led the author to the conclusion that the degree of activity depends more on the size of the molecule than on the chemical structure of urethanes (Roebbelen, 1962). Ethylurethane manifested no mutagenic action on the model of reversible mutations of Neurospora and other microorganisms (Westergaard, 1957). This gave grounds to assume that urethanes act indirectly through formation of mediator complexes and therefore activity to a large extent depends on metabolic processes in the cell.

In spite of the lack of clear concepts of the mutational mechanism of urethanes, the following sequence of reactions in outline form can be proposed. It is known that urethanes are weakly basic esters and at acidic pH probably undergo protonation of the carbonyl oxygen atom



Further, it is natural to assume the existence of tautomerism between two cations



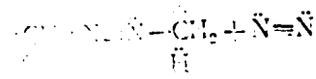
Cation B can be readily hydrolyzed with breaking of the alkyl-oxygen bond and the consequent formation of an ethyl cation, which enters into reaction with the nucleophilic center of the genetic molecule according to the mono- or bimolecular mechanism.

Less acceptable is the assumption that mutations under the effect of urethane occur due to inhibition of the synthesis of DNA precursors, and not due to direct alkylation.

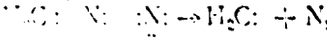
Diazoalkanes. This unusual group of mutagens includes diazomethane ($\bar{N}=\overset{+}{N}-CH_2$ or $N\equiv N-CH_2$), diazoacetic ester ($N_2CHCOOC_2H_5$), exhibiting the same activity and which were first studied on Drosophila (Rapoport, 1948b), and then on microbiological material (Jensen et al., 1951; Marquardt et al., 1963), an antibiotic product in the form of an aminoacid — azoserine

(Steinman et al., 1958; Iyer, Szybalski, 1958), and the unusually active compound 1,4-bis-diazoacetylbutane ($N_2CH-\overset{\overset{O}{\|}}{C}-(CH_2)_4-\overset{\overset{O}{\|}}{C}-CHN_2$) increasing by several orders of magnitude the incidence of mutation of Drosophila genes (Rapoport, 1960c), plant genes (Zoz, 1961), and actinomycetes (Bartoshevich, 1964; Bartoshevich, Lyubinskaya, 1965).

In acidic media diazo-compounds readily unite a proton to carbon, but the pair of valency electrons is attracted to nitrogen, as a outcome of which a methyl radical and a nitrogen molecule are formed



It is less probable that the effective principle of diazoalkanes in a biological system will be carbenes, that is, biradicals which have a bivalent carbon atom. The simplest carbene is free methylene, formed in the degradation of diazomethane



In the final analysis, the reaction amounts to methylation, but in contradistinction to most alkylating compounds diazoalkanes react not with nucleophilic centers (anions), but with carboxyl and amine groups of nonionized compounds, in which there is a labile hydrogen atom

This reaction occurs according to the bimolecular mechanism both in the presence, as well as in the absence of the ionizing solvent.

N-nitrososubstituted compounds. Products of the substitution of a hydrogen atom in secondary amines for a nitroso-group exhibit high reactivity and weakly basic character. A triad of concomitant properties is clearly pronounced for compounds in this group: carcinogenicity, carcinolyticity, and mutagenicity.

Study of the mutagenic effect of nitroso-compounds was commenced in the 1940's with the action of nitrosomethylurethane ($O=N-\underset{CH_3}{N}-C-OC_2H_5$, O) and led to discovery of extremely powerful mutagens -- nitrosoalkylurea ($O=N-\underset{Alk}{N}-\overset{\overset{O}{\|}}{C}-NH_2$), among which the most active proved to be the methyl- and ethyl-derivatives (Rapoport, 1948b, 1962b). Allowing for the emergence of several simultaneous mutational transformations in a single sex chromosome, nitrosoethylurea in the gaseous phase causes more than 100% lethal mutations. Not one of the known chemical and all the more so, physical

factors brought about such a high hereditary variability in Drosophila. As a consequence, on the model of reversible biochemical mutations in yeast and actinomycetes (Marquardt et al., 1963; Bartoshevich, 1965a), direct mutations in microorganisms (Khropova, 1965; Gumanov et al., 1965), and in plants (Zoz et al., 1964; Zoz, Makarova, 1965), the uniquely high effect of these mutagens was confirmed. Thus, in the plants Arabidopsis treated with nitrosomethylurea, up to 99.1% mutations were induced. For the same degree of plant growth suppression, X-rays induced 1/30 the number of mutations (Muller, 1964).

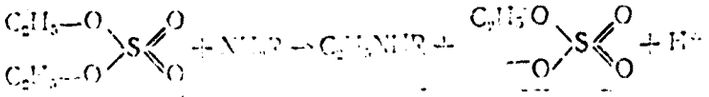
Other nitrosoamines show a more temperate genetic effect: dimethyl- and diethylnitrosoamines ($O{=}N{-}N\begin{smallmatrix}\nearrow Alk\\ \searrow Alk\end{smallmatrix}$) (Pasternak, 1962, 1963; Geissler, 1962, 1963), nitrosoalkylated ethylurethanes ($O{=}N{-}\overset{\displaystyle Alk}{\overset{|}{N}}{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}O{-}C_2H_5$) (Zetterberg, 1960, 1961, 1962; Kihlmann, 1960; Marquardt et al., 1963; Loprieno et al., 1964; Grant, Heslot, 1965), nitrosoaminophenyl derivatives ($\diagdown N{-}N{=}O$; R = alk, aryl) (Kihlmann, 1961), and nitrosomethylguanidine ($H_2N{-}\overset{\displaystyle R}{\overset{|}{C}}{-}N{-}NO$) (Mandell, Greenberg, 1960; Gichner et al., 1963; Muller, Gichner, 1964). Some nitrosoamides show the same strong mutagenic action (Zimmermann, Schwaier, 1963). Thus, the bifunctional derivative N,N-dinitroso-N,N-dimethyloxamide ($ON{-}\overset{\displaystyle CH_3}{\overset{|}{N}}{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}\overset{\displaystyle CH_3}{\overset{|}{N}}{-}NO$) increases the incidence of reversible mutations in Saccharomyces by almost three orders of magnitude (Marquardt et al., 1963).

The mechanism of the mutagenic action of nitrosoamines has not been decisively elucidated. In an acidic medium at elevated temperatures nitrosoamines cleave to the original amine and nitrous acid. Under alkaline conditions nitrosomethylurethane forms diazomethane and other products of degradation

$$\begin{smallmatrix}ON\\ \\ CH_3\end{smallmatrix}\!\!\diagdown N{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}O{-}C_2H_5 + 2KOH \rightarrow CH_2N_2 + K_2CO_3 + C_2H_5OH + H_2O.$$

Therefore, some authors believe that secondary products are responsible for mutagenesis and carcinogenesis: nitrous acid and diazoalkanes (Zetterberg, 1961, 1962; Kihlmann, 1960; Pasternak, 1962, 1963, 1963; Cichner et al., 1963). In model experiments the chromatography method was used to discover both products of the deamination of guanine, adenine, and cytosine, as well as the alkylation of guanine (Bednyak, 1965). In contrast, other authors propose that nitrosoamines induce mutations per se, since not one of the degradation products induces such a high mutation incidence as the original compounds (Rapoport, 1962b; Marquardt et al., 1963; Muller, Gichner, 1964; Loprieno et al., 1964).

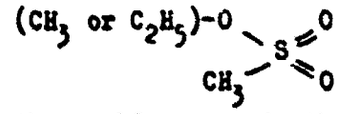
Esters of sulfuric acid. Dimethylsulfate $(CH_3O)_2SO_2$ and diethylsulfate $(C_2H_5O)_2SO_2$ have been studied most closely as to their chemical action on DNA, as well as their mutagenic effect on many materials (Rapoport, 1947b; Loveless, 1951; Westergaard, 1957; Heslot, 1962; Monti, Scorascia, 1964; Eisenstark, Rosner, 1964). They act as monofunctional agents, in spite of the presence of two alkylating groups, since only one of the two can be used for alkylation in biological systems



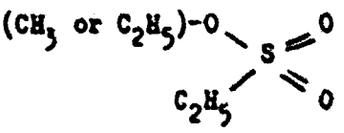
It is difficult to conceive that a negatively charged monoalkylsulfate ion, formed as a result of cleavage of the first alkyl-oxygen bond, can approach a nucleophilic center and enter into reaction with it. It is possible that this molecular reaction mechanism explains why dialkylsulfates induce predominantly point mutations in plants and animals (Rapoport, 1961; Heiner et al., 1962). If for the most effective physical and molecular mutagens the limit of induced mutations for a sex chromosome approaches 20-30%, treatment of Drosophila in diethylsulfate vapor raises this barrier to 75% lethals, and in so doing normal fecundity and viability of the treated material are preserved (Rapoport, 1965). Mature spermatozoids are the most sensitive to the action of diethylsulfate, but cells undergoing reductive division undergo less mutation (Alderson, Pelecanos, 1964). In the same way diethylsulfate-induced mutations appear in nondividing populations of bacteria and in extracellular phages (Ronin, 1963). The methyl homolog is more toxic than the ethyl and reveals a variable preference to mutagenic activity in certain materials and character

Esters of sulfonic acids. In genetics research, the esters of methyl- and ethylsulfurous (sulfonic) acids are most often used

methylmethanesulfonate and ethylmethanesulfonate



methylethanesulfonate and ethylethanesulfonate



Just like esters of sulfuric acid, alkylsulfonates -- monofunctional agents -- react in mild physiological conditions with cleavage of the bond between the alkyl radical and oxygen. Methyl esters reacts under the S_N2 mechanism, ethyl homologs -- under a mixed mechanism, that is, in the latter case ionization of the ester bond occurs even before

approach to a nucleophilic center, but complete cleavage occurs after formation of the complex with this center. Marked by good solubility, slow hydrolysis, low toxicity, and negligible aberrational effect, ethylmethanesulfonate invariably on all genetic materials and characters, beginning with viruses and ending in multicellular plant and animal organisms, manifests high mutagenic activity (Fahmy, O. G., Fahmy, M. J., 1957; Westergaard, 1957; Loveless, 1958; Heslot, 1960; Fress, 1961; Minocha, Arnason, 1962; Lobbecke, Boestel, 1962; Robbelen, 1962a; Bohme, 1962; Gaul, 1962; Strauss, 1962; Swaminathan et al., 1962; Neuffer, Ficsor, 1963; Kaplan et al., 1963, Ivanovics et al., 1963; Blixt et al., 1964; Lyubinskaya et al., 1965).

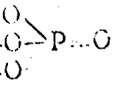
The halogenide derivative $-ClCH_2CH_2-O-SO_2-CH_3$, chloroethylmethanesulfonate, and the cyclic ester of propanesultone $(CH_2-CH_2-CH_2)$ with SO_2 and O have

shown no less mutageric action (Westergaard, 1957; Heslot, 1962; Auerbach, 1962; Watson, 1962; Mathew, 1964).

The mutagenic and toxic effect of alkylsulfonates drops proportionally to the increase in alkyl branch forming the ester bond.

Esters of phosphoric acid. Under different conditions, the cleavage of the ester bond occurs differently: in an alkaline solution the phosphorus-oxygen bond is cleaved, and only at lower pH is the alkyl-oxygen bond broken

acidic medium

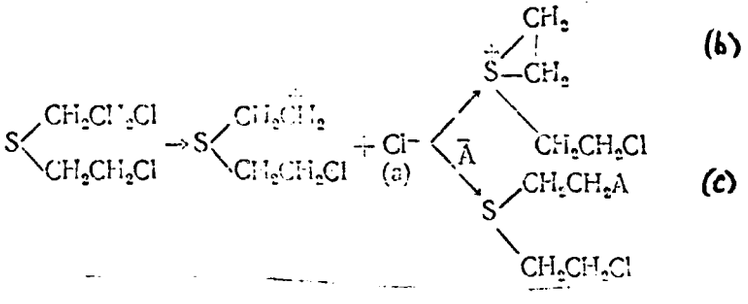


alkaline medium

Therefore, alkylation with these agents in mild physiological conditions is limited either to intramolecular regroupings in the ester itself, or to a reaction which takes place with a very strong nucleophilic center. Thus, for example, triethylphosphate in vivo alkylates cysteine at the mercapto-group (Ross, 1962). No strong mutagens have been found among the alkylphosphates. Dimethylphosphate $(CH_3)_2HPO_4$, inducing modifications in Drosophila, does not induce mutations (Rapoport, 1962a), but trimethylphosphate on Neurospora (reversible mutations) has demonstrated a very low mutagenic effect (Westergaard, 1957). When alkyl radicals are substituted by two chloroethyl groups -- di-β, β'-chloroethylphosphorous acid -- $HPO(OCH_2CH_2Cl)_2$ -- mutagenic activity rises precipitously (Rapoport, 1960a). Probably, the hydrogen-phosphorus bond in phosphorous acid acts on the lability of the alkylating group, since upon substitution of hydrogen by a third chloroethyl radical the mutagenic effect drops to 1/10th the value. Moreover, the high reactivity can be explained on the basis of ionization with formation of a carbonium ion. A sharp rise in mutational effect has also been observed for the

ester of methylphosphinic acid (Kapoport, Kostyanovskiy, 1960).

β-Chloralkylsulfides. The presence in the molecule of these compounds of an electron-rich central sulfur atom raises the reactivity of the chloralkyl radical in aqueous solution. Chloralkylsulfides are active in aqueous solution and are capable of alkylating the most diverse functional groups. The reaction takes place at a high rate; thus, the half-period of yprite hydrolysis in water at 37° C is 3 minutes, therefore it is a strong mutagen only in biological systems in which the reaction is not restricted by numerous cytoplasmic barriers, and conditions promote a rapid reaction. And ionization of the chlorine atom occurs readily owing to the electron-repelling properties of the sulfonium ion (a)



Via the internal bimolecular reaction, the resulting carbonium cation either cyclizes with formation of a stable ethylenesulfonium ion (b), or immediately enters into reaction with the nucleophilic center (c). The concentration of the sulfonium ion in aqueous solution is insignificant, therefore, probably, the direct reaction of the carbonium cation with the nucleophilic group must be regarded as the main form of the reaction. As to the alkylating molecule, this reaction follows the monomolecular mechanism with a rapid stage of ionization and slow interaction with the electron-rich center. The extremely high toxic effect of this compound type narrows the chance of their laboratory use in mutation experiments (Auerbach, Robson, 1944; Stevens, Mylroie, 1950; Loveless, 1951).

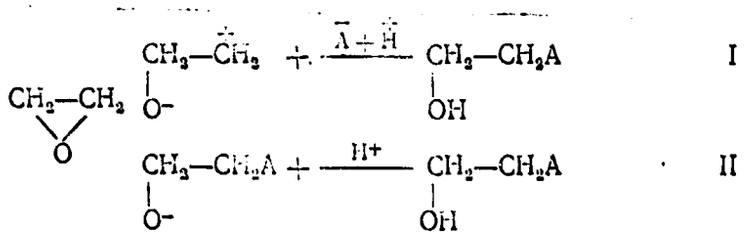
β-Chloralkylamines. This extensive group of compounds under the name of nitrogenous analogs of yprite is used with success as carcinolytic preparations. Depending on the content in the molecule of the β-chloralkyl groupings, mono-, bi-, and trifunctional compounds are distinguished (they are denoted by HN1, HN2, and HN3, respectively). In contrast to the true yprites, in which the sulfur atom exhibits a negligible basicity, the cyclic ethylenimmonium ion formed via ionization of β-chlorethylamines is more stable in aqueous media than is the sulfonium ion. In addition, the cyclic ethylenimmonium ion exhibits increased reactivity through stretching of valency bonds in the three-membered cycle. Therefore, it is assumed that the main reaction stages are rapid ionization with cyclization under the S_N1 mechanism in a period of a few minutes and then

a bimolecular reaction with the nucleophilic center

Yet it was noted that nitrogenous analogs of yprite, which have the attacking group in the form of chloropropyl radicals, are stronger mutagens than chloroethyl derivatives (Alikhanyan, Oganesyan, 1964). In this case, as a result cyclization a four-membered immonium ion should have been formed, the straining of its bonds being considerably less than in a three-membered cycle and, consequently, reactivity also being reduced. Hence, the possibility of alkylation of functional genetic molecules via formation of a carbonium ion with cyclization is not excluded.

In spite of the fact that at present studies have been made of mutagenic action of more than 100 yprite analogs on different materials, no success has been won in revealing a direct correlation between chemical and mutagenic activity (Westergaard, 1957; Dulaney, 1959; Fahmy, O. G., Fahmy, M. J., 1960; Loebbecke, 1963; Degen, 1963; Oganesyan, Alikhanyan, 1964).

Epoxides. Ethylene oxide, or ethyleneoxide (CH_2-CH_2, O) is the first member and is the attacking mutagenic center of all subsequent representatives of the epoxide group (Rapoport, 1948a; Heslot, 1962; Zacharias, Ehrenberg, 1962). Two principal pathways are possible for the alkylation of the nucleophilic center by ethylene oxide

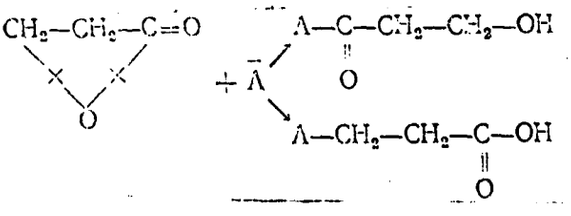


In the first, rupture of the cycle, ionization with formation of a carbonium cation, and subsequent monomolecular reaction take place. This type of alkylation is activated in compounds with acceptor substituents at the terminal carbon atom, for example, epichlorohydride ($CH_2-CH-CH_2Cl$, O) and glycidol ($CH_2-CH-CH_2-OH$, O), which are more powerful mutagens than ethylene oxide (Rapoport, 1948c; Westergaard, 1957; Heslot, 1962). The second pathway is the bimolecular process (S_N2) with protonation of the

of the resulting complex. Epoxides react slowly and the reaction rate in the latter case can be raised through increase in concentration of the reacting functional groups. Diepoxybutane shows especially increased mutagenic activity ($CH_2-CH-CH-CH_2$, O, O) (Kolmark, Westergaard, 1953; Emery, 1960; Kreizinger, 1960; Cohn, 1961; Kolmark, Kilbey, 1962; Heslot, 1962; Northrop, King, 1963). And of the two stereoisomers, the levo-form of diepoxybutane as a mutagen is several times more active than the dextro-form (Bianchi, Contin, 1962; Moutschen-Dahmen et al., 1963).

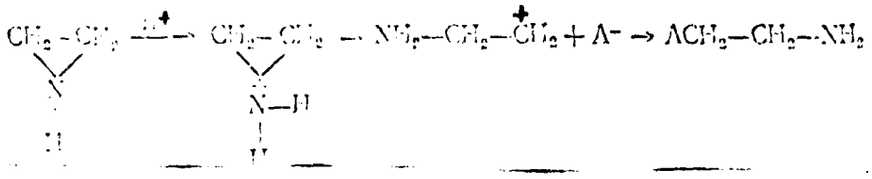
In bifunctional epoxides substitution at the peripheral carbon atom and increase in carbon chain length reduces the chemical and mutagenic activity (Heslot, 1962). Upon substitution of the oxygen in the cycle with a sulfur atom (ethylenesulfide CH_2-CH_2, S), the mutagenic index is sharply reduced (Rapoport, 1962).

Lactones. The greatest mutagenic effect is exhibited by the cyclic ester β-propiolactone ($CH_2-CH_2-C=O$, O) (Smith, Srb, 1951; Iyer, Szybalski, 1958; Eisenstark, Rosner, 1964; Withers, 1965). Esters of carbonic acids are hydrolyzed at the carbonyl-oxygen bond and therefore cannot be electrophilic agents in alkylation reactions. Via cyclization, lactones exhibit internal strain, rising with decrease in the number of members in the heterocycle, and as an outcome cleavage can occur in aqueous solutions at both oxygen bonds, forming ions that enter into the following reaction



with different nucleophilic centers. As a consequence, it is more correct to call lactones not alkylating, but acylating agents. This group of compounds can include with full warrant ketene ($CH_2=C=O$) and also several genetically active ketones (Rapoport, 1946).

Ethylenimines. In the reaction period ethylenimine undergoes the following energy changes: activation of the strained three-member ring via protonation of the tertiary nitrogen atom, which leads to formation of an ethylenimmonium ion. The instability of this cation is caused by repulsion of the electron pair from the nitrogen atom, therefore weakened valency bonds in the cycle are ruptured and a reactive carbonium ion is the result, entering into reaction with a nucleophilic center



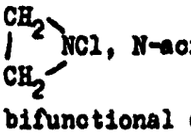
Displacement and interaction of the carbonium ion with the functional group of a gene in the terminal reaction stage occurs under the bimolecular mechanism S_N2.

Examining the mutational process on the basis of a unified field of electrical asymmetry, I. A. Rapoport proposed that affinity with genic material is the function of the cyclic molecule ethylenimine, and not the cleaved radical (Rapoport, 1965). Here, in contrast to genic changes, where reactions proceed at covalent bonds, but ethylenimine enters a homeopolar state, chromosomal rearrangements are manifest at electrovalent bonds, but the mutagen reacts in the ionic form.

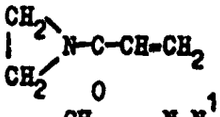
Ethylenimines are one of the most active groups of chemical mutagens persistently manifesting a strong genetic effect on organisms of diverse levels of organization: on animals (Rapoport, 1962c; Alexander, Glanges, 1964); on plants (Shkvarnikov, 1948; Gustafsson, Ehrenberg, 1959; Heslot, 1960; Zoz, Dubinin, 1961); on algae (Khropova, 1965); fungi (Kolmark, Westergaard, 1953; Alikhanyan, Mindlin, 1956; Heslot, 1962); actinomycetes (Zhdanova, 1961; Klepikova, Alikhanyan, 1963; Bartoshevich, Kostyanovskiy, 1965); on bacteria (Szybalski, 1958; Steinman et al., 1958); and on extra- and intracellular bacteriophages (Drake, 1963; Degen, 1963).

As a rule, increase in functional ethylenimine centers in a complex mutagen molecule reduces the point mechanism of action and intensifies the abberational effect (Westergaard, 1957; Rieger, Michaelis, 1962).

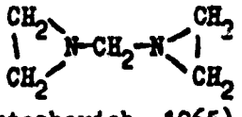
On models of reversible mutations of ausotrophic strains of yeasts and actinomycetes, an extremely high activity was found, surpassing by many times the effect of ethylenimine, N-chloroethylenimine



, N-acrylcylethylenimine



and a bifunctional derivative



, N,N^1-methylene-bis-ethylenimine (Heslot, 1962; Bartoshevich, 1965).

In studying the mechanism of the induction of mutations, it is necessary to take into account in part the possibility of direct action of mutagens through intermediate formation of nucleotide analogs. Therefore, control of directed variability of organisms is attainable through

an understanding of the complex mutational reaction occurring not as a single-act interaction of mutagen with DNA, but as a process proceeding through a number of stages.

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