INDUCED MUTATIONS

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➢ Chemical mutagenesis

• Harmful chemicals in the environment are of many different classes organic and inorganic, natural and synthetic.
• We are exposed to toxic chemicals through air pollution, contaminated food or water and medicines.
• It should be noted that exposure to environmental agents may result in increase in human cancers and mutations even after several decades or generations.

• There are many different structural classes of mutagenic chemicals or their metabolites.

❖ Mechanism of chemical mutagenesis

• Genetic changes in DNA can either be the result of small alterations involving very few base residues or large alterations within the gene or chromosome.
• Examples of small alterations are point mutations including frameshift (microlesions).
• They are direct results of the interaction of the chemical mutagen with DNA.

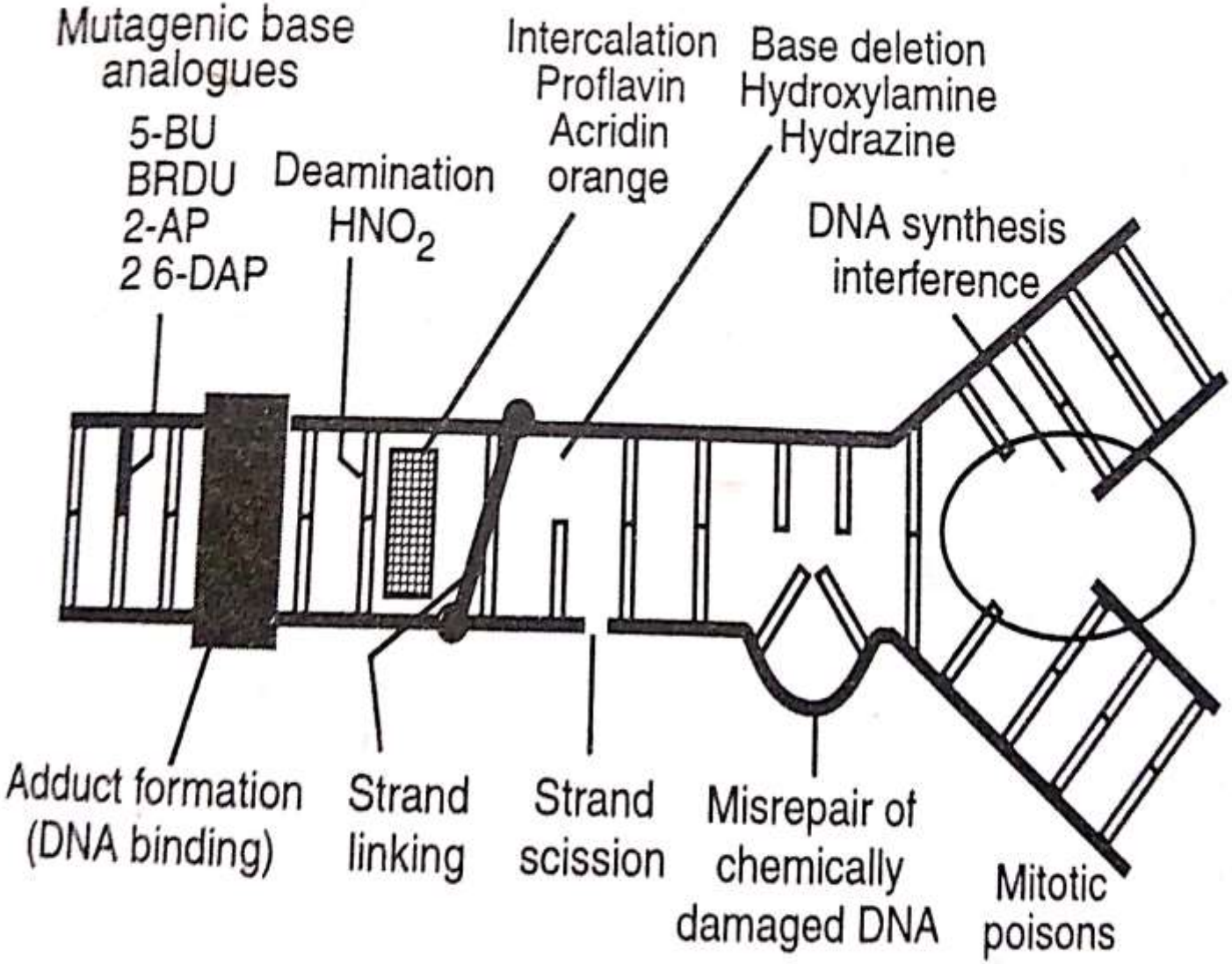


Fig. 18.14. Mechanisms of chemical mutagenesis.

• Large alterations (macrolesions) involve changes such as gaps, deletions or rearrangements of the genetic material.

• They may result secondarily from chemical damage in response to recombination and repair of damaged DNA.

• Extensive chromosomal damage frequently may lead to nonviable cells.

• Mutagens may bring about their effects through more than one of these mechanisms.

- Mutagenic base analogues:

A chemical substance resembling a base is called a base analogue. A base analogue may be incorporated into a replicating DNA strand instead of a normal base.

1) 5-halouracil: The pyrimidine analogues, 5-halouracils are structurally very similar to thymine. They can be incorporated into DNA in place of thymine residues.

Thymine

5 - Bromouracil
(5-BU)

Fig. 18.15. Structure of thymine (5-methyl uracil) and 5-bromouracil (5-BU).

Keto state

Enol state

Fig. 18.16. The keto and enol states of 5-bromouracil (5-BU).

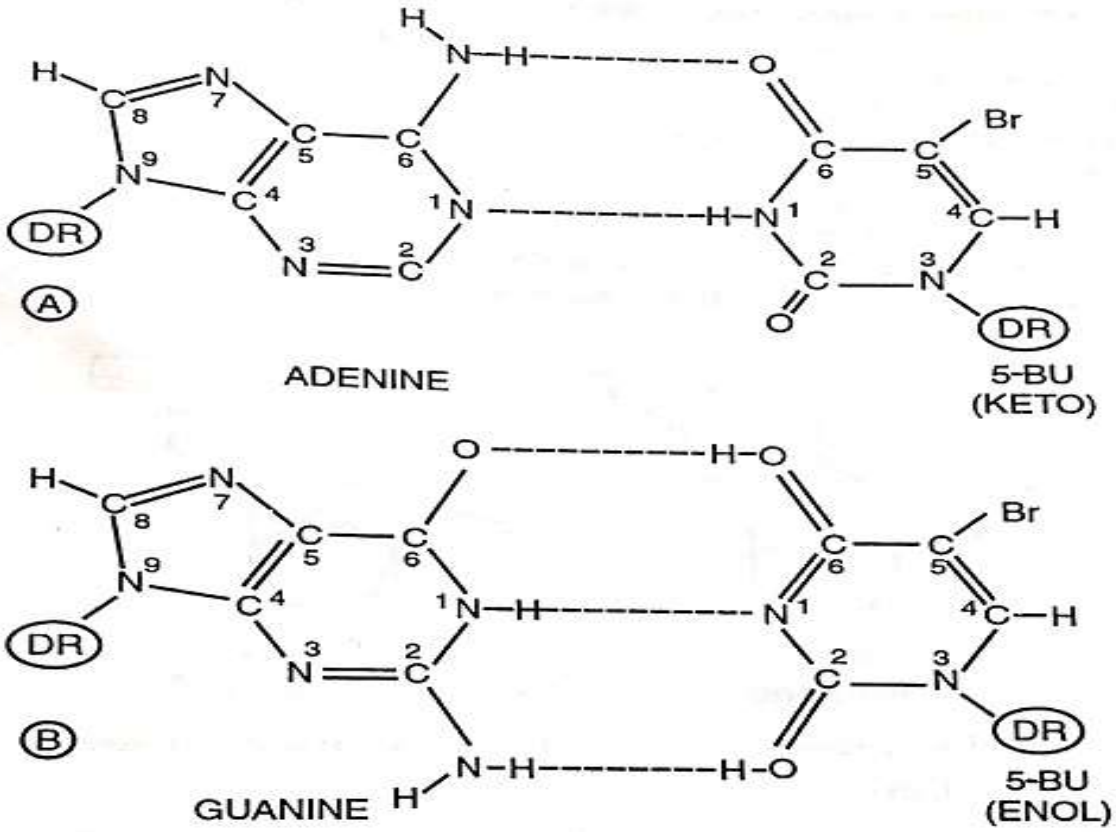


Fig. 18.17. Tautomerism of 5-bromouracil (5-BU).

(A) Regular base pairing of adenine with 5-bromouracil in the normal *keto* from.

(B) Forbidden base pairing of 5-BU (in the rare *enol* form) with guanine.

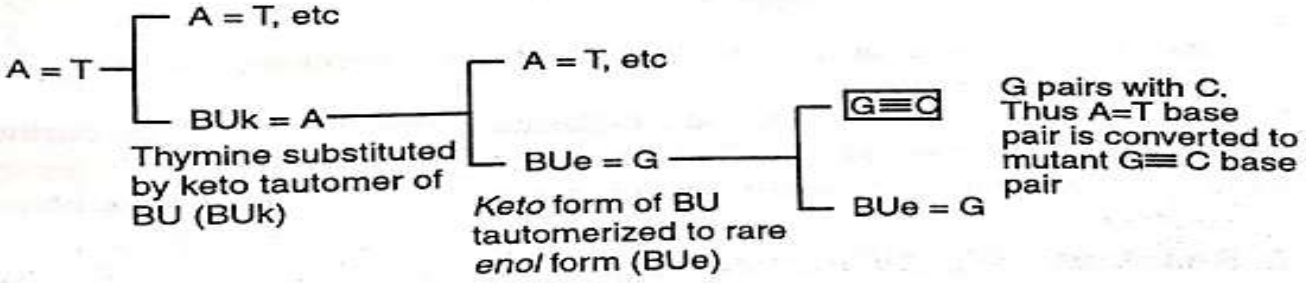


Fig. 18.18. Incorporation of the base analogue 5-bromouracil and its effect.

2) 5-bromodeoxyuridine (BRDU) :
It can replace thymidine in DNA.

3) 2-aminopurine (2-AP) and 2,6-diaminopurine (2,6-DAP) :

They are purine analogues. On incorporation into DNA they act in place of adenine residues by pairing with thymine. They sometimes pair erroneously with cytosine.

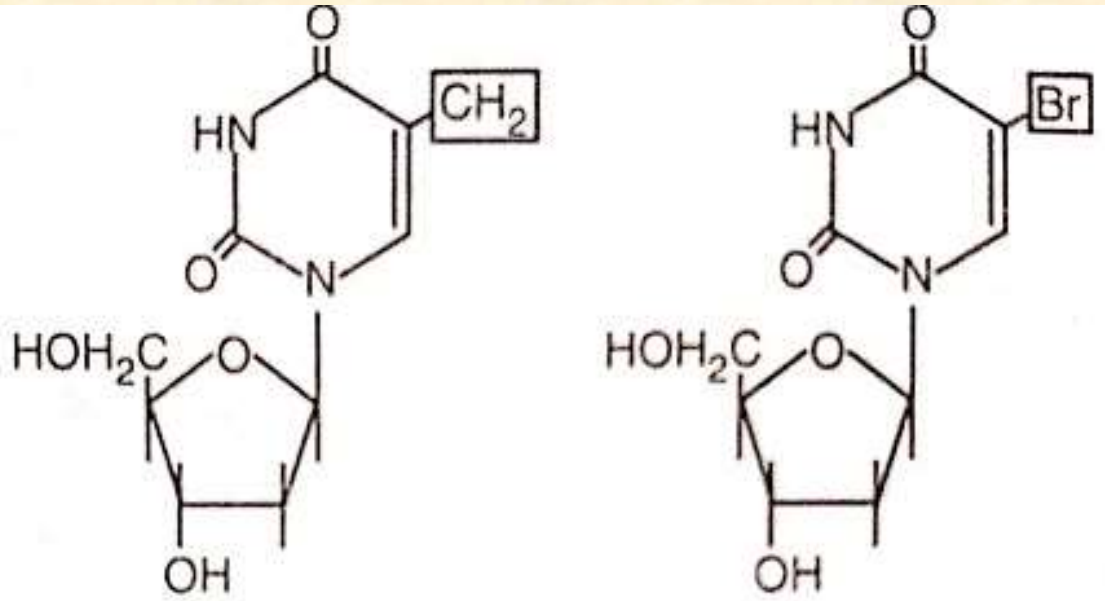


Fig. 18.19. Structure of thymidine and 5-bromodeoxyuridine (BRDU).

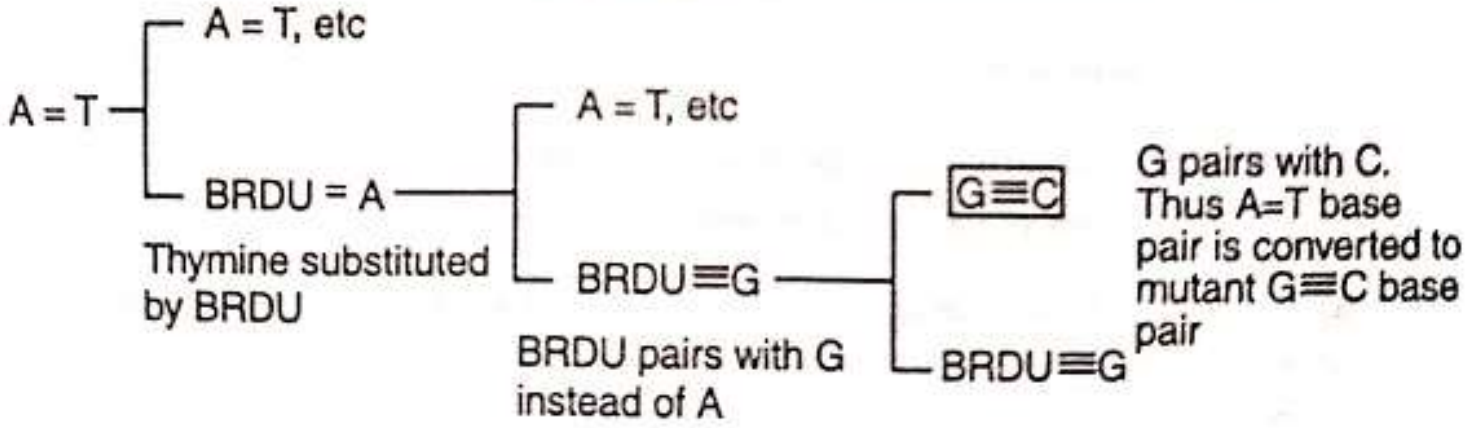


Fig. 18.20. Incorporation of the base analogue 5-bromodeoxyuridine (BRDU) and its effect.

▪ Reactants with purines and pyrimidines:

Nitrous acid (HNO_2)

• At low pH sodium or potassium nitrites are readily converted into nitrous acid.

• This mutagen reacts with bases containing the amino group and causes oxidative deamination (removal of amino group).

• When purines or pyrimidines containing the amino group are treated with nitrous acid, the amino group ($-NH_2$) is replaced by the hydroxyl group ($-OH$).

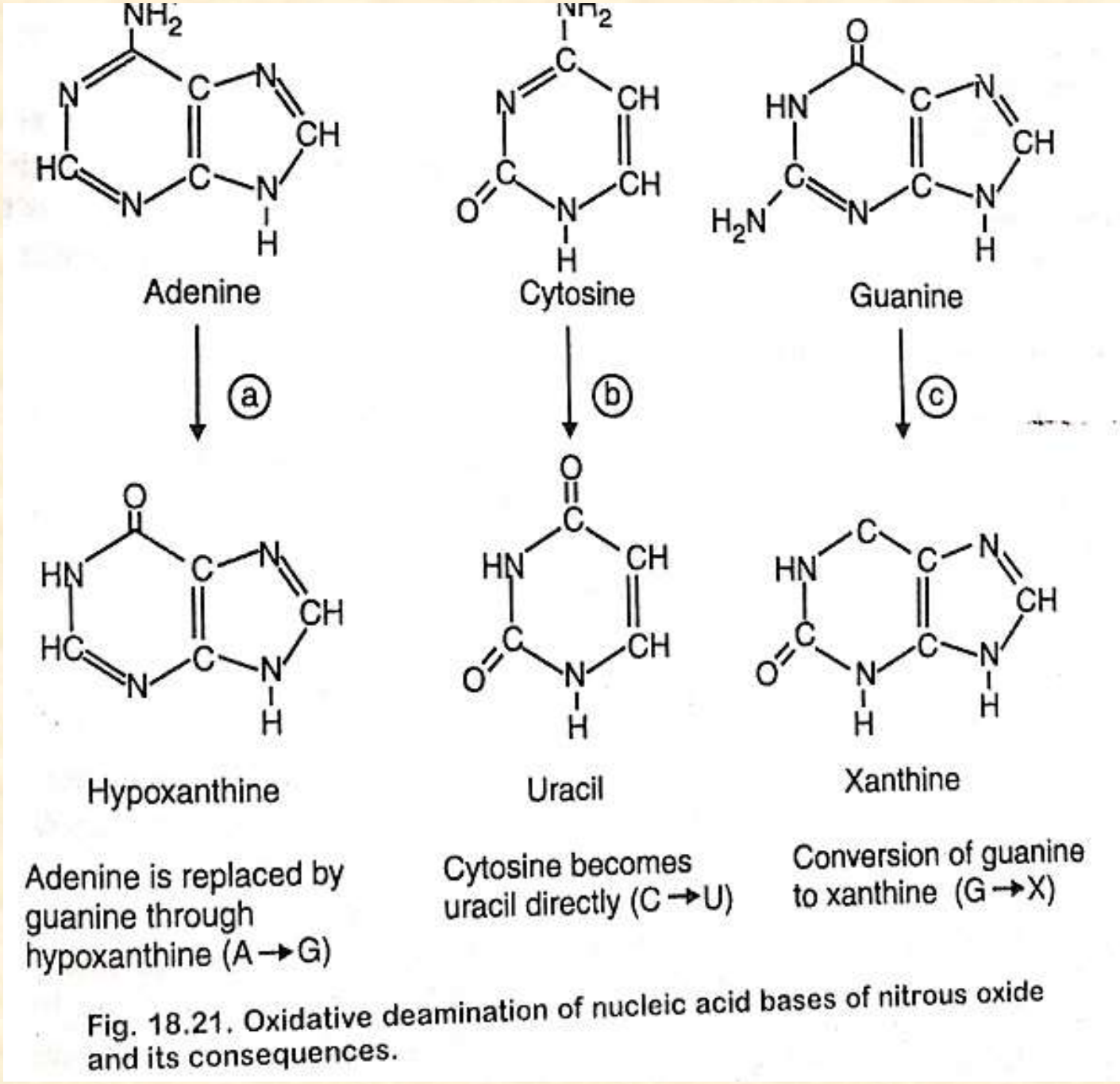


Fig. 18.21. Oxidative deamination of nucleic acid bases of nitrous oxide and its consequences.

i) Deamination of adenine : formation of hypoxanthine
ii) Deamination of cytosine : formation of uracil
iii) Deamination of guanine : formation of xanthine

Table: Changes in the structure and pairing behaviour of DNA bases as a result of deamination of nitrous acid.

Normal bases of DNA	**Normal pairing**	**Bases formed by deamination**	**New pairing**
Adenine	A-T	Hypoxanthine	G-C
Cytosine	C-G	Uracil	U-A
Guanine	G-C	Xanthine	X-C

▪ Intercalating agents:

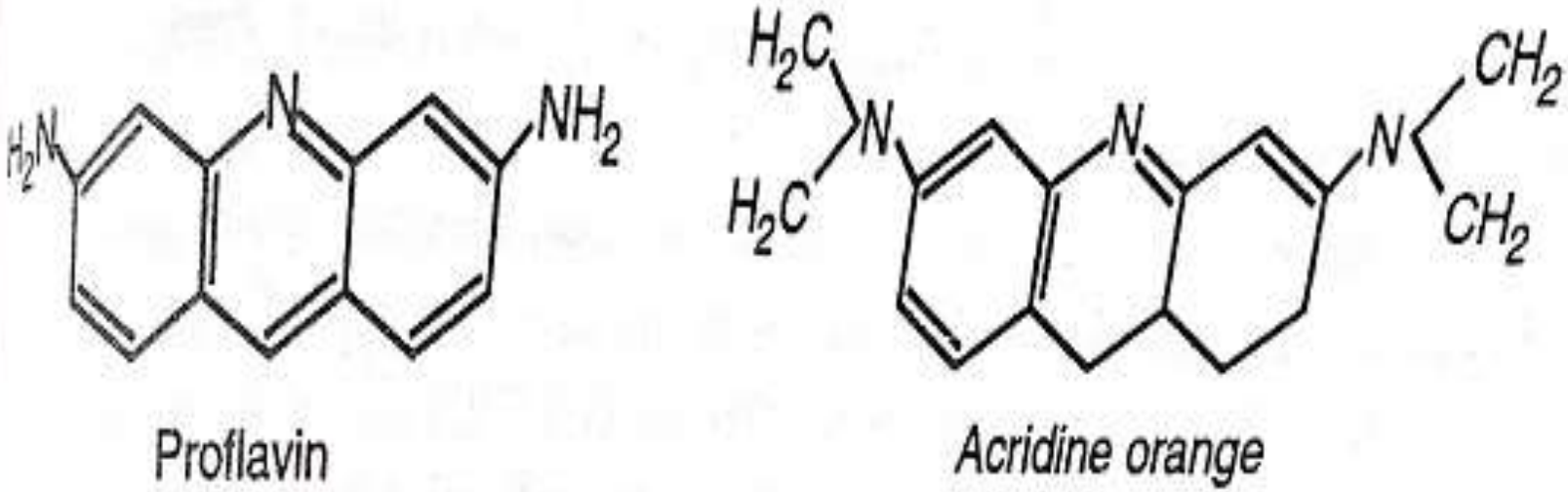
• Certain planar compounds consisting of multiple, fused rings cause certain mutations by insertions between stacked base pairs of DNA.

• Intercalation results in lengthening or unwinding of DNA, leading to mutations during replications or recombination.

• Such mutations are characterized by deletion or addition of base pairs during DNA replication or repair.

• This shifts the reading frame of messenger RNA molecule, with a correspondingly shifted reading frame (frameshift mutation).

• Intercalating mutagens are of two types, non-interactive agents (e.g. proflavin) and reactive intercalators (e.g. electrophilic derivatives of polycylic aromatic hydrocarbons).
• Acridine dyes: Certain flourescent acridine dyes such as proflavin and acridine orange cause mutations by insertion or deletion of bases.
• The acridines are planar molecules like the purine bases, and can be intercalated between the bases of the DNA helix.
• This distorts the structure of DNA, and can result in deletion or insertion of bases during recombination.



Proflavin

Acridine orange

Fig. 18.22. The acridine dyes proflavin and acridine orange.

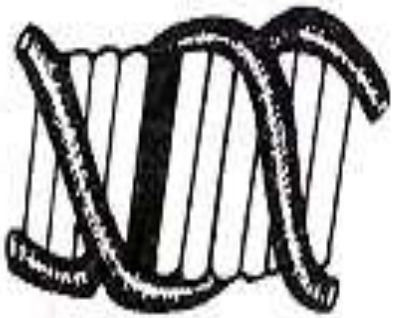


Fig. 18.23. Insertion of acridine dye molecule (black) between bases of DNA.

• The intercalation of an acridine and consequent stretching of the DNA molecule may occur during crossing over of DNA.
• This can result in unequal crossing over.
• One strand may have one more nucleotide than the other.
• This results in the production of one daughter helix with one more base than the other.
• The acridines are generally mutagenic in bacteria only during recombination.

i) Interaction resulting in insertion of base

ii) Interaction resulting in deletion of base

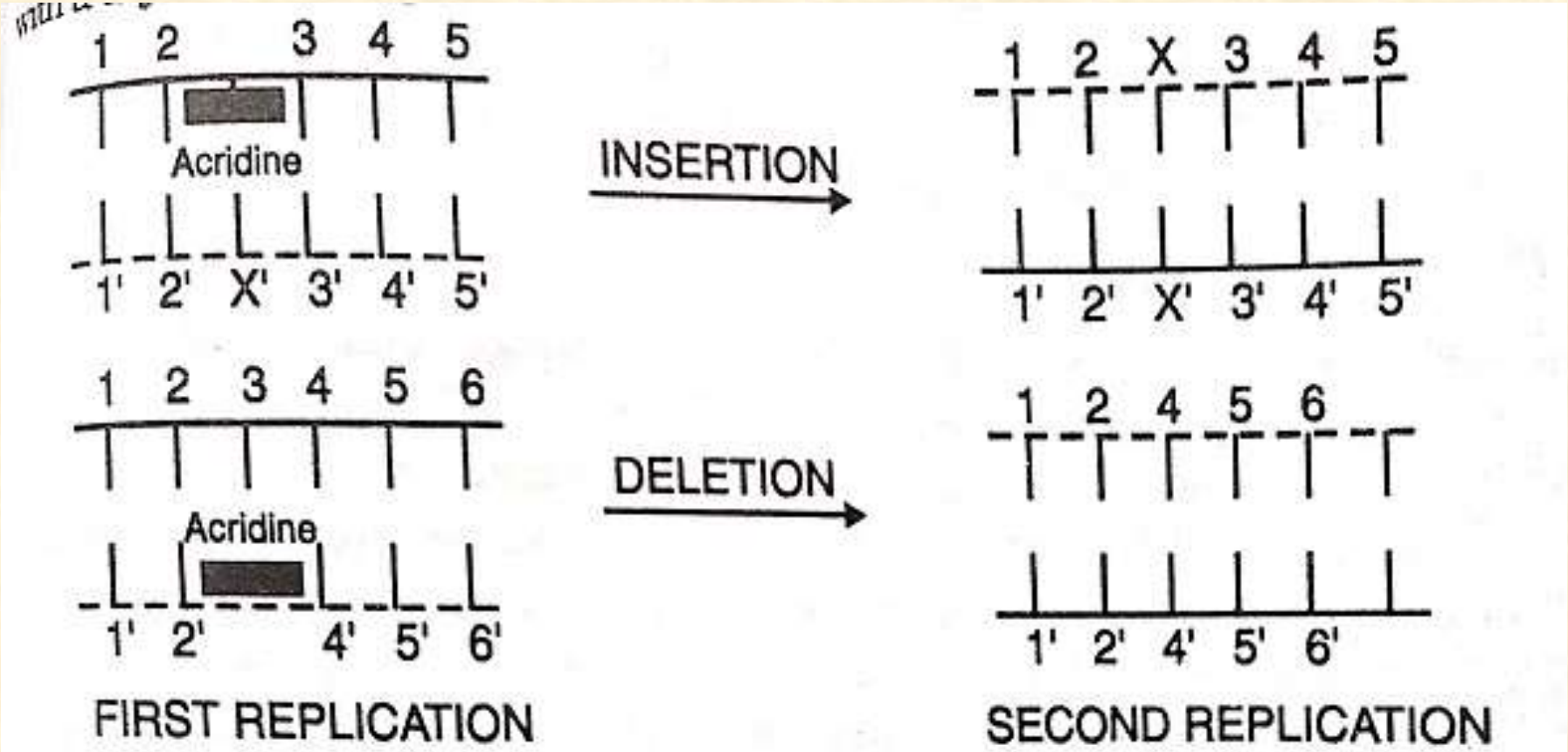


Fig. 18.24. Diagram of mutagenic action of acridine dyes.

- Chemically induced base deletions :

• Loss of base residues from DNA can result from the action of hydroxylamine or hydrazine on pyrimidines.
• The formation of certain base substitution products such as 3- or 7-alkylpurines, or O^6 –alkylpyrimidines can also result in loss of base residues.
• Hydroxylamine (NH_2OH) is one of the most specific agents known for inducing point mutations.
• It reacts with the amino group of cytosine. The modified C(C´) can base pair only with A (C ´= A).

• Thus at the next and subsequent replications, a transition mutation from G to A is produced in the complementary strand.

• Hydroxylamine can also produce inactivating DNA alterations in transforming DNA.

• These alterations can be suppressed by
i) removal of oxygen
ii) addition of compounds such as catalase peroxidase or EDTA .

from G to A is produced in the complementary

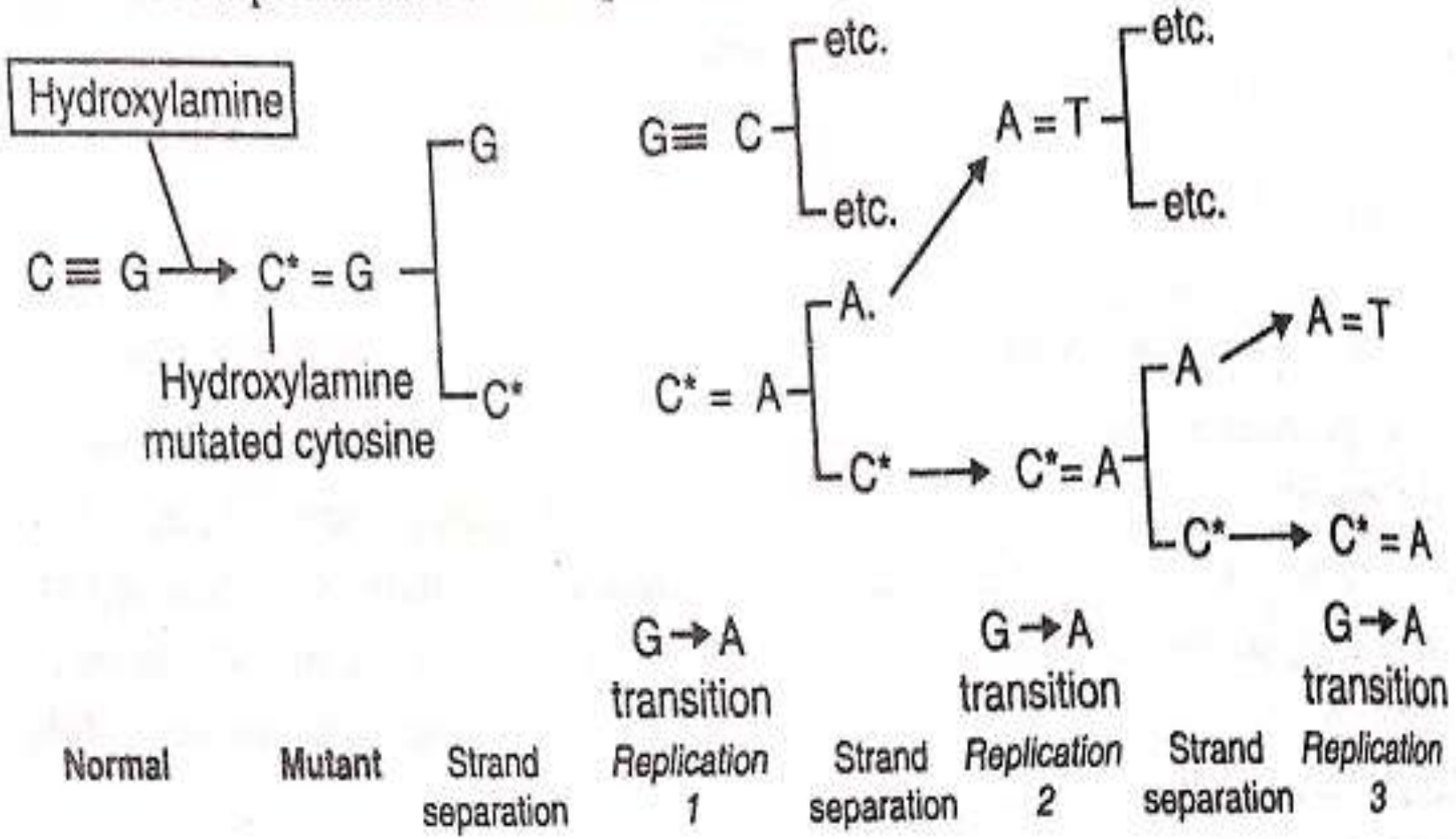


Fig. 18.25. Action of hydroxylamine. Hydroxylamine brings about mutation of cytosine (C) to C*. The mutated cytosine C* can base pair only with A, and not G. Thus G → A transition is produced at each replication.

▪ Adduct formation:

• The formation of a covalent bond between a mutagen and the DNA template can result in miscoding during replication.

• Three types of adduct formation with DNA have been found alkylation, arylation and acylation.

i) Alkylation:

• Electrophiles are substances that seek centres of negative charge in other molecules and bind them.

• One compound in the body which contains centres of negative charge is DNA.

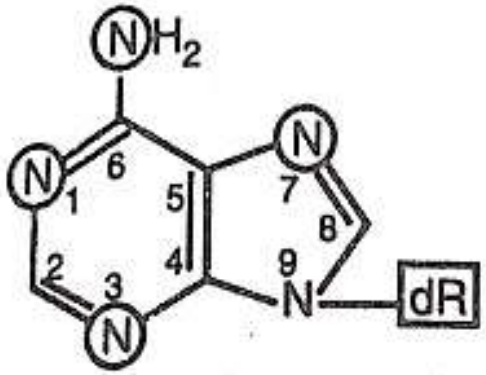
• Electrophiles on coming into contact with the negative centres attack them and usually add carbon containing alkyl groups.
• This process is called alkylation.
• In the alkylation reaction, alkanes or substituted alkanes react with macromolecules, especially nucleic acids.
• The alkyl group include methyl (CH_3), ethyl (CH_3CH_2) and propyl ($CH_3CH_3CH_2$), etc.
• Alkylating agents are used to determine the sites of modification in DNA.

• Commonly used alkylating mutagens are alkyl sulphonates, nitroso compounds and various nitrigen mustards.

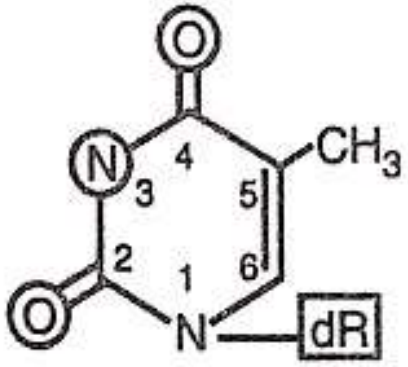
• Alkylating agents are electrophiles by themselves, and do not require metabolic activation.

• They are the most powerful mutagens for both prokaryotes and eukaryotes.

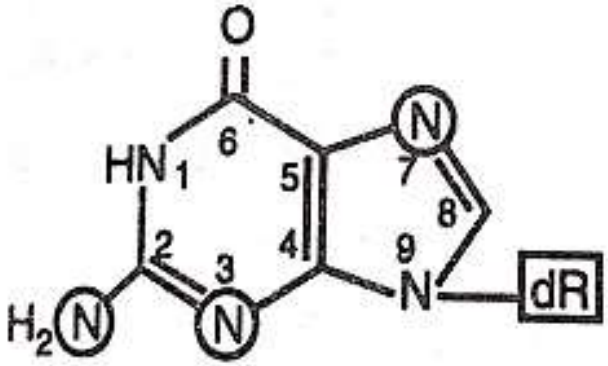
• The sites of DNA undergoing electrophilic substitution are as follows:



a. Deoxyadenosine

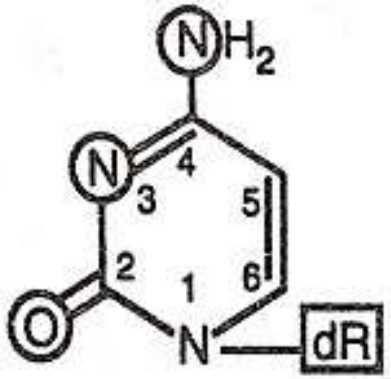


b. Thymidine



c. Deoxyguanosine

PURINES



d. Deoxycytidine

PYRIMIDINES

. 18.26. Substitution sites (circles) of purines and pyrimidines.

❖ Substitution sites of purines and pyrimidines :

Adenine at N^6, N-1, N-3 and N-7 positions.

Thymine at O^2, N-3 and O^4 positions.

Guanine at O^6, N^2 , N-3 and N-7 positions.

Cytosine at O^2, N-3 and N^4 positions.

Different alkylating agents show selectivity towards the different nucleophilic sites on DNA. This in turn may be the cause for the differing mutagenic activity of alkylating agents.

• In addition to the phosphodiester bonds, alkylating agents commonly attack the following sites:

i) N^3 of adenine which lies in the minor groove of DNA and N7 of guanine. Such alkylations do not immediately lead to mispairing since the positions are not involved in base pairing. However, these base modifications make the bond between the sugar and the base more labile, and a break may occur. When this happens, the sugar is left without its purine (apurinic site). This cannot undergo replication without repair, and the repair itself can produce mutations.

ii) O^6 of guanine lies in the major groove, and its alkylation results in mispairing.
iii) O^4 of thymine is situated in the major groove and O^2 of pyrimidines in the minor groove.

- Alkyl sulphonates : The alkyl sulphonates include

a) Ethyl methane sulphonate (EMS):
$CH_3CH_2SO_3CH_3$

b) Ehtyl ethane sulphonate (EES):
$CH_3CH_2SO_3CH_2CH_3$

Many sulphonate compounds are highly mutagenic to DNA bases. They may bring about moderate changes in bases resulting in transitions or drastic changes resulting in transversions.

- EMS places an alkyl group on guanine and thymine producing O^6-ethylgunine or O^6-ethylthymine.
- O^6-ethylgunine pairs with thymine and O^6-ethylthymine pairs with guanine.
- Alkylation increases the probability of ionization and introduces pairing error.
- The base-sugar linkage undergoes hydrolysis and releases the base from the DNA molecule.

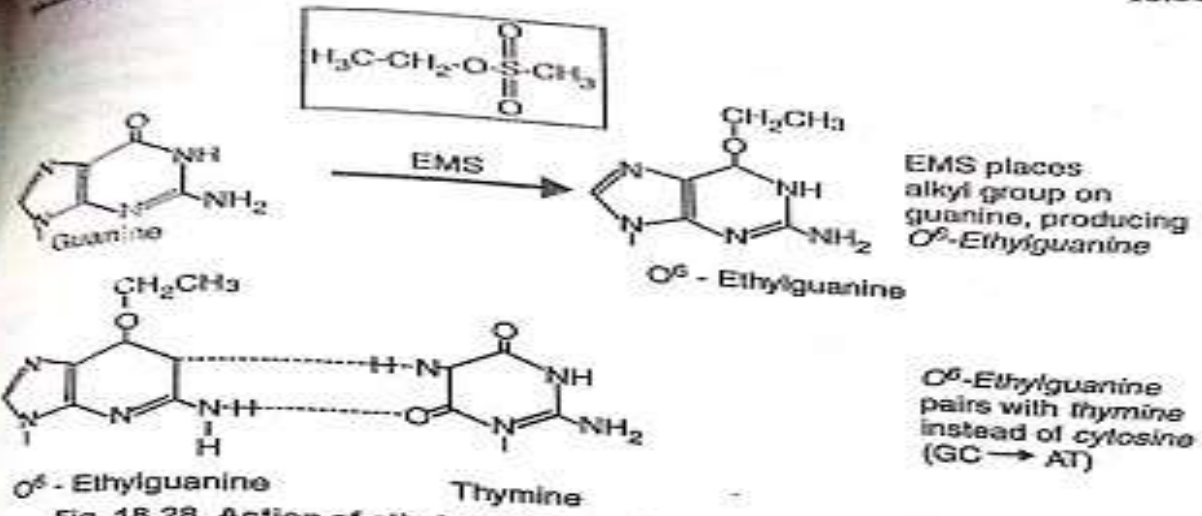


Fig. 18.28. Action of ethyl methane sulphonate (EMS).

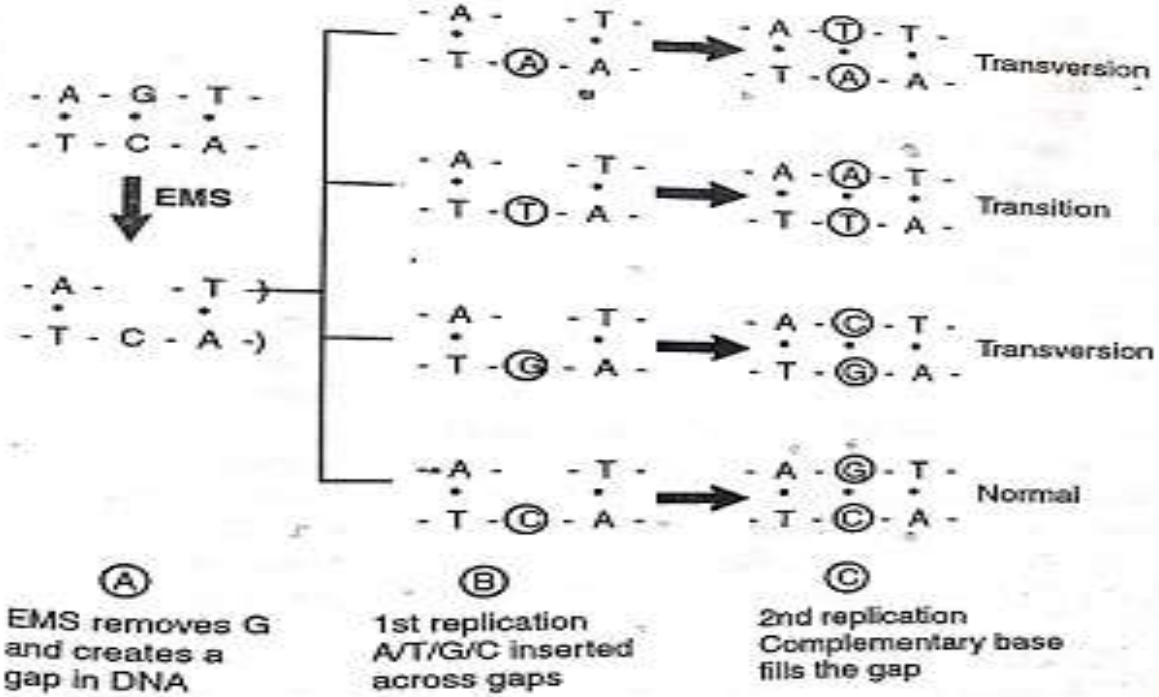


Fig. 18.29. Effect of the alkylating agent ethyl methane sulphonate (EMS) on DNA

• EMS specifically removes guanine from the chain.
• During replication the chain without gaps will give rise to normal DNA.
• In the chain with gaps, however, any base (Á,T,G or C) may be inserted across the gap.
• This may be a correct base or an incorrect one.
• In the next replication the gap is filled by a base which is complementary to the inserted base.
• When the correct base is inserted the DNA is normal.
• Insertion of an incorrect base may result in transition or a transversion.

• The substitution sites on proteins include the sulphur atoms of methionine and cysteine, the imidazole nitrogens of histidine, the phenolic O-4 and C-3 of tyrosine and the primary amino and free carboxyl groups of amino acids.

ii) Arylation: The aryl group is the phenyl group and corresponding groups of the more complex ring structures. Electrophiles such as arylamines or polycyclic aromatic hydrocarbon species have aromaticity in the structure. These compounds are highly reactive towards O6 of guanine . This may be

due to Van der Waal reactions between the mutagen and the base residues of DNA. Such reactions hold the electrophilic sites of the mutagen close to the nucleophilic sites on DNA. (mutagen-DNA adduct formation). Substitution at the C-8 position of the guanine residue does not take place under physiological conditions with simple alkylating agents.

iii) Acylation: In acylation, the H is replaced by an acetyl (RC=O) group, e.g. $CH_3C=O$
Adduct formation would be expected to result in base-substitution mutations only.

- Strand-linking (interstrand cross-links)
- Strand scission
- Misrepair of chemically damaged DNA
- Interference with DNA synthesis
- Mitotic poisons
- Comutagenesis