that most methods for estimating the rate of mutations tend to underestimate their frequency due to many reasons. Firstly, lethal mutations which leave no progeny may be missed. Secondly, mutations which leave only a slight change in the phenotype may remain undetected.

Mutations occur much more frequently in certain regions of the gene than in others. The favoured regions are called 'hot spots'. Mutations involving single nucleotides can revert to normal gene structure. Most single nucleotide mutations are reversible. In many cases the rate of reverse mutations is similar to the rate of forward mutations. In rare locations the rate of forward mutation is much greater than the rate of backward mutation.

Effects of mutations on the phenotype. According to their effects on the phenotype mutations may be classified as *lethals*, *subvitals* and *supervitals*. *Lethal mutations* result in the death of the cells or organisms in which they occur. *Subvital mutations* reduce the chances of survival of the organism in which they are found. *Supervital mutations* on the other hand may result in the improvement of biological fitness under certain conditions. There may also be mutations which are neither harmful nor beneficial to the organism in which they occur.

How does a mutation act? As mentioned previously, genes act by controlling the rate of production of specific proteins (enzymes). The scheme of protein synthesis in most organisms is as follows. (1) The DNA (gene) produces a complementary mRNA strand which has *codons* consisting of nucleotide triplets. (2) tRNA molecules, each forming a a complex with a specific amino acid, have three free nucleotides which form the *anticodon*. (3) The alignment of tRNA molecules on mRNA depends upon complementary *codon–anticodon pairing*. (4) Thus the sequence of amino acid molecules in an enzyme (and hence the structure and functions of the enzyme) depends upon the nucleotide sequence of mRNA. This in turn depends upon the nucleotide sequence in DNA.

It will be seen that any change in the sequence of nucleotides of DNA will result in a corresponding change in the nucleotide sequence of mRNA. This may result in alignment of different tRNA molecules on mRNA. Thus the amino acid sequence, and hence the structure and properties of the enzme formed,will be changed. This may effect the traits controlled by the enzyme.

Base pair substitutions

Gene mutations are of two main types, *base pair substitutions or* ***transitions*** and *frameshift mutations*.

Base pair substitutions are the most common mutations. They result in the incorporation of wrong bases during replication or repair of

DNA. Examples of base pair switches are from A-T to G-C, C-G or T-A. In base pair switches one base of a triplet condon is substituted by another, resulting in a changed codon.

(1) Original (wild type) message of reading frame.
CAT GAT CAT GAT CAT GAT CAT.....

(2) Substitution or replacement.
A replaced by G
CAT GAT CGT GAT CAT GAT CAT.....
Message out of frame

Fig. 10.1. Mutation by substitution.

If the mutated codon specifies another amino acid it will result in amino acid substitution in the polypeptide chain during translation.

Substitutions usually result in a simple change of one amino acid in the polypeptide chain synthesized. Such changes are called *missense mutations*. If a single base substitution modifies a codon to a *termination codon*, the chain may terminate at the point of mutation (e.g. CAG→UAG). Such mutations are called *nonsense mutations*. They result in incomplete polypeptide chains which may not be biologically active.

Base pair substitutions are of two main types, *transitions* and *transversions* (Fig. 10.2).

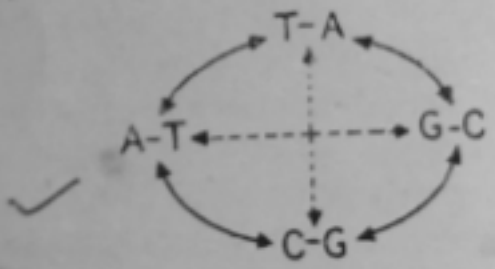


Fig. 10.2. Transversions (continuous lines) and transitions (dashed lines).

Transitions. If a *purine* base is replaced by another *purine* base (A by G or G by A) or a *pyrimidine* by another *pyrimidine* (T by C or C by T) the substitution is called a *transition*. Transitions are by far the most common types of mutations.

Transversions. If a *purine* base is substituted by a *pyrimidine*, or vice *versa*, the substitution is called a *transversion*.

It will be seen that each base pair can undergo one kind of *transition* and two kinds of *transversions*. In general, *transition* mutations code for chemically similar amino acids while *transversions* show a greater possibility of inserting amino acids with different charges. Although transitions and transversions can cause *nonsense mutations*, the chances of *missense* mutations are greater.

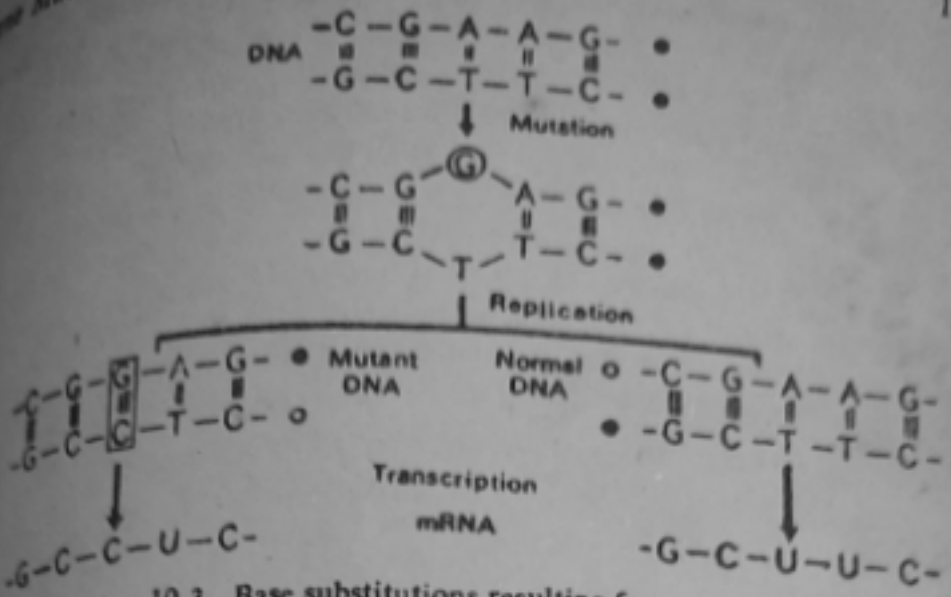


Fig. 10.3. Base substitutions resulting from mutation.

Mutations leading to base pair substitutions presumably take place in two steps. Let us consider a mutation in a DNA double strand in which the purine base A is substituted by another purine base G (transition).

When the DNA replicates it will give rise to two chains, one *normal* like the parent chain and the other *mutant*. Since the mutated base G pairs with C, the mutant DNA will have G-C at the point of mutation. Thus both chains have altered bases at the point of mutation.

Inversion. If a segment of DNA is removed and reinserted in a reverse direction it results in an inversion. As in substitution, the message is out of phase only in the triplets involved in the inversion.

CAT GAT TAC GAT CAT GAT CAT.....

Massage out of frame

Frameshift mutations

A mutation in which there is deletion or insertion of one or a few nucleotides is called a *frameshift mutation*. The name is derived from the fact that there is a shift in the reading frame backward or forward by one or two nucleotides. Addition or deletion of one or two bases results in a new sequence of codons which may code for entirely different amino acids. This results in a drastic change in the protein synthesized. The protein is usually nonfunctional. It should be noted that if the reading frame shifts by three nucleotides, the resulting protein is normal, except that it may lack one amino acid or may contain an extra amino

The site of the mutation has an important bearing on whether the protein formed will be slightly or drastically altered. Since translation takes place in the 5′→3′ direction, a frameshift mutation near the 3′ end of the gene results in only the terminal part of the polypeptide chain being altered. This may result in a functional protein. The several variants of haemoglobin are belived to have arisen in this manner.

Deletion. Removal of one or a few bases from a nucleotide chain is called a *deletion*. It will be seen that the removal of even one base will throw the genetic message out of frame beyond the point of deletion. A new sequence will be established. This will happen on deletion of any number of bases not divisible by three.

Original (wild type) message or reading frame.

CAT GAT CAT GAT CAT GAT CAT

Deletion.

$-C$

CAT GAT ATG ATC ATG ATC AT

Message out of frame

Insertion. The genetic message will be similarly disturbed if one or a few bases are added (*insertion*), provided that the number of such bases is not divisible by three.

$+G$

CAT GAT GCA TGA TCA TGA TCA T

Message out of frame

If there is simultaneous *deletion* and *addition* of a base, then the message will be out of frame only in the triplets between the deletion and addition.

Deletion and insertion

$-C$ $+C$

CAT GAT ATG ATC ATC GAT CAT

Message out of frame

Mutations can be explained by the following analogy. Suppose that the genetic message is contained in the sentence : THE MAN WHO HAS ONE EYE CAN SEE YOU. In this sentence each word consisting of three letters represents a codon.

If the letter W of WHO is removed (*deletion*) the sentence beomes THE MAN HOH ASO NEE YEC ANS EEY OU—. The sentence is meaningless after the word MAN.

If the letter A is added after MAN (*insertion*), then also the sentence becomes meaningless after MAN. THE MAN AWH OHA SON EEY ECA NSE EYO U—.

If the letter H of WHO is replaced by U (*substitution*) the sentence becomes : THE MAN **WUO** HAS ONE EYE CAN SEE YOU. In this case only one word, WUO, becomes meaningless.

If the letters of WHO are reversed (*inversion*) the sentence becomes : THE MAN **OHW** HAS ONE EYE CAN SFE YOU. In this case also only one word is meaningless (OHW).

If the letter W of WHO is removed and the letter Z is added after E (*deletion* and *insertion*) the sentence becomes : THE MAN **HOH** AZE EYE CAN SEE YOU. Only the words involving the deletion and the insertion are meaningless.

A change in only one amino acid can have a drastic effect on the phenotype. Haemoglobin, which is found in the R. B. C., is a protein molecule consisting if four chains, two *alpha* chains and two *beta* chains. These chains consist of amino acids arranged in a definite sequence. Normal R.B.C. are disc shaped. Changes in haemoglobin structure results in certain types of anaemia called *sickle cell anaemia* and *haemoglobin C anaemia*. In sickle cell anaemia the R. B. C. become sickle shaped when oxygen tension is reduced, and are much less effective in the transportation of oxygen. Death may occur in severe cases of sickle cell anaemia. Normal haemoglobin has the amino acid *glutamic* acid in the sixth position. In sickle cell haemoglobin C it is substituted by lysine.

	1	2	3	4	5	6	7	8
A.	Valine	histidine	leucine	threonine	proline	**glutamic acid**	glutamic acid	lysine
B.	Valine	histidine	leucine	threonine	proline	**valine**	glutamic acid	lysine
C.	Valine	histidine	leucine	threonine	proline	**lysine**	glutamic acid	lysine

Fig. 13.4. Parts of haemoglobin molecules showing differences in amino acids in the sixth position.
A. Normal haemoglobin.
B. Sickle cell haemoglobin.
C. Haemoglobin C.

Missense mutations

A missense mutation is one which results in the replacement of one amino acid in a polypeptide chain by another. As a result of mutation a base of a codon may be substituted by another base. The changed codon may then code for another amino acid. A missense mutation can be caused by substitution, deletion or insertion.

Missense mutations arising by substitution result in proteins which differ from their normal counterparts only in a single amino acid. Such

proteins therefore frequently have normal biological activty. One of the codons for *phenylalanine* is UUU. A single base substitution (U→G) changes it to UGU, the codon for *cysteine*. Thus the protein formed after mutation is identical to the normal protein except that phenylalanine is substituted by cysteine. About half the known human haemoglobins have amino acid substitutions involving single base transversions.

Table 10.1. Some codon changes and amino acid replacements in haemoglobins.

Amino acid in normal haemoglobin		*Base Substitution*	*Amino acid in mutant haemoglobin*	
Lysine	(AAA)	A → G	Glutamic acid	(GAA)
Glycine	(GGU)	G → A	Aspartic acid	(GAU)
Asparagine	(AAU)	U → A	Lysine	(AAA)
Glutamic acid	(GAA)	A → U	Valine	(GUA)

Nonsense mutations

Of the 64 codons 61 code for amino acids, while three are *termination codons* which do not specify any amino acid. The three termination codons are UAA, UAG, and UGA. Any mutation resulting in the alteration of of a codon specifying an amino acid to a termination codon is called a *nonsense mutation*. Thus if the codon UAC (for *tyrosine*) undergoes a one-base substitution (C→G) it becomes UAG, a termination condon.

A nonsense mutation brings about termination of polypeptide synthesis at that point (unless there is *genetic suppression*: see later part of this chapter). As a result the polypeptide chain synthesized is incomplete. Such chains are likely to be biologically inactive. Since a nonsense mutation brings about a relatively drastic change in the enzyme synthesized it is more likely to have a deleterious effect on the phenotype than a missense mutation.

Polypeptide chain synthesis takes place in the 5′ → 3′ direction. Therefore a nonsense mutation near the 5′ end results in a very short chain with probably very little or no biological activity. Conversely, a nonsense mutation near the 3′ end results in a chain which is nearly complete, and which may have some or normal biological activity.

Mutations which are the reverse of nonsense mutations also occur. Thus a mutation can convert a *termination codon* to a *sense codon* specifying some amino acid. The α chain of human haelogmobin is normally 141 amino acid residues long. A mutation (U→C) converts the *termination codon* UAA to CAA, the codon for *glutamine*. Chain synthesis therefore proceeds beyond the normal termination point, producing a polypeptide chain containing 172 amino acids.

Silent mutations

Any gene mutation which does not result in phenotypic expression is called a *silent mutation*. Silent mutations are of several types.

1. The genetic code is *degenerate*, i.e. more than one codon may specify an amino acid. For example both AAG and AAA specify *lysine*. If the codon AAG undergoes a mutation to AAA the latter codon will still specify lysine. When a mutated triplet codes for the same amino acid as the original there is no change in the amino acid. This mutation is of the *silent* type, because although there is a change in the base sequence of DNA there is no alteration in the amino acid sequence of the protein synthesized.

2. The codon change may result in an amino acid substitution, but this is not sufficient to modify the function of the protein appreciably.

3. The mutation may occur in a gene that is no longer functional or whose protein is not essential at the particular stage of testing.

4. Simultaneous presence of *suppressor mutations* may cause a mutation to become silent. In genetic suppression a second mutation at a different site neutralizes the effects of the first mutation (See 'Genetic Suppression').

Genetic suppression

The effect of a mutation on the phenotype can be reversed, so that the original wildtype phenotype is brought back. This reversal may be due to *true reversion* or *suppression*.

In a *true reversion* there is a reversal of the original genetic change. A C→A mutation would change the codon GCU (*alanine*) to GAU (*aspartate*). This may result in the enzyme formed becoming inactive. In a true reversion a reverse mutation from A→C would restore the codon for alanine (GAU→GCU). Such a mutation is called a *back mutation*.

In a *suppression* a change at a *different site* brings about phenotypic correction of the mutation. True reversions can be distinguished from

If a wrong amino acid is inserted a mutant may be produced which does not give rise to a functional protein. The mutated tRNA gene producing such a change cannot be considered a suppressor gene.

Another factor which must be considered is the effect of suppressors on normal chain termination. When the termination codon is suppressed, polypeptide chain synthesis will continue beyond the normal termination point. This will result in the production of abnormally long chains, causing cell growth to stop. Suppressor strains protect themselves against this possibility by having *double termination codons*, e.g. UAA and UAG in MS2 coat protein mRNA.

5′ UCC GGC AUC UAC **UAA UAG** ACG CCG...

This acts as a safety device if one of the termination codons is suppressed. Since the suppression of UAA is relatively poor (1-5% as compared to about 50% for UAG and UGA) it is possible that it is the normal codon for chain termination, and many genes terminate in UAA alone.

The Molecular Basis of Mutation

Gene mutations at the molecular level involve *modification* of one base by another, or *addition* or *deletion* of one or more bases. Mutations may be *spontaneous* or *induced*.

I. Spontaneous mutations

Mutations which occur under natural conditions are called *spontaneous mutations*. It should be noted that some spontaneous mutations arise by the action of mutagens present in the environment. These mutagens include cosmic radiation, radioactive compounds, heat, and such naturally occuring base analogues like caffeine. These will be considered under 'induced mutations' as they are external agents bringing about mutations. Truly spontaneous mutations that will be dealt with here are those arising from *tautomerism*.

Tautomerism. The ability of a molecule to exist in more than one chemical form is called *tautomerism* (Fig. 10.7). All the four common bases of DNA (adenine, guanine, cytosine and thymine) have unusual tautomeric forms, which are, however, rare. The normal bases of DNA are usually present in the *keto* form. As a result of tautomeric rearrangement they can be momentarily transformed into the rare *enol* form in which the distribution of electrons is slightly different.

Normal base pairing in DNA is A—T and G—C. The tautomeric forms are, however, capable of unusual ('forbidden') base pairing (Figs. 10.8 and 10.9) like T—G, G—T, C—A and A—C.

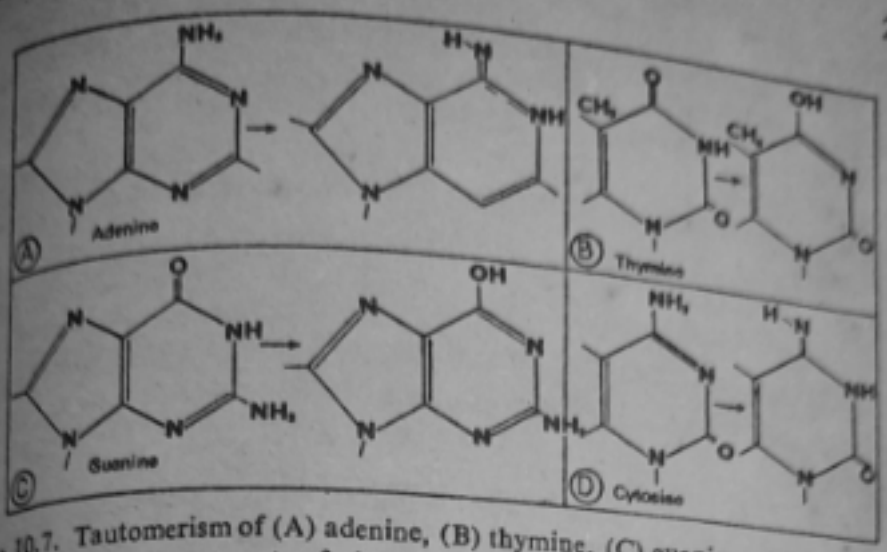


Fig. 10.7. Tautomerism of (A) adenine, (B) thymine, (C) guanine and (D) cytosine. The common state of each base is shown on the left and the rare state on the right.

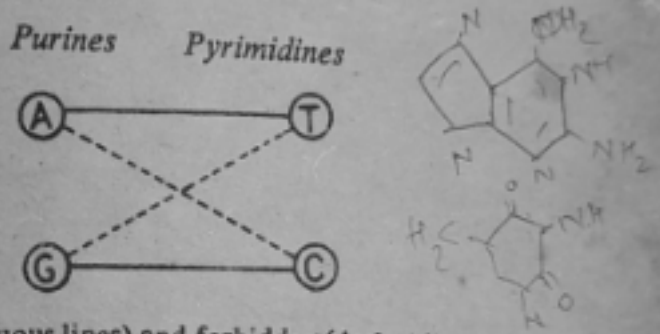


Fig. 10.8. Normal (continuous lines) and forbidden (dashed lines) base pairing.

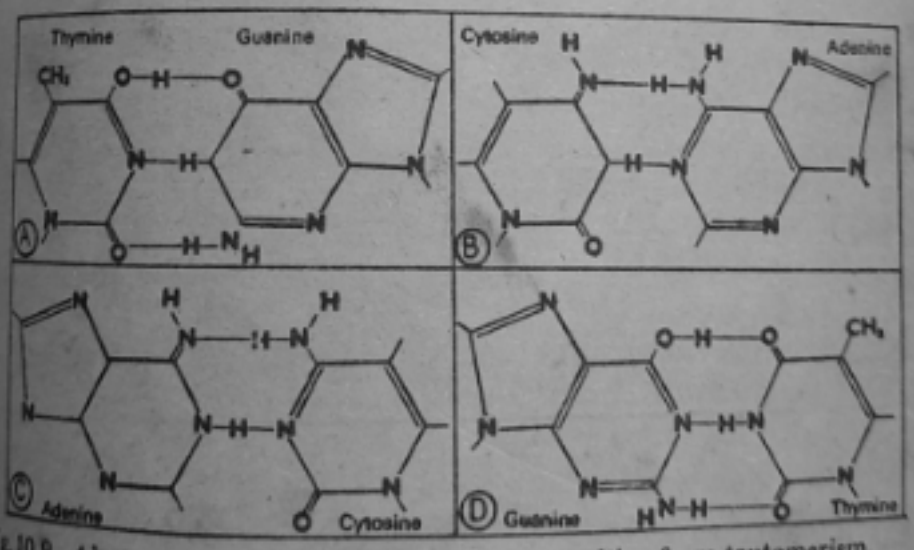
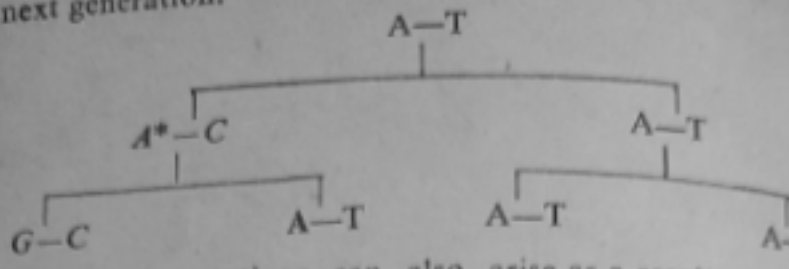


Fig. 10.9. Abnormal or forbidden base pairing resulting from tautomerism.
(A) Thymine–guanine. (B) Cytosine–adenine.
(C) Adenine–cytosine. (D) Guanine–thymine.

This unusual base pairing results in misreplication of the DNA strand, giving rise to mutants in some of the progeny. Thus A*, a rare

tautomer of adenine (A) pairs with cytosine. This leads to G—C pairing in the next generation.



Spontaneous mutations can also arise as a result of ambiguity of base pairing during replication because of 'wobble' (see : 'The Genetic Code').

II. Induced mutations

A variety of agents increase the frequency of mutation. Such agents are called *mutagens*. They include *chemical mutagens*, and *radiations* like X-rays, γ-rays and UV-light.

A. Chemical mutagens

The first chemical mutagen discovered was *mustard 'gas'*. In the 1950s chemical mutagens with more or less specific action were developed. Chemical mutagens can be classified according to the way in which they bring about mutations : (1) *base analogues* which are incorporated into DNA instead of normal bases, (2) agents *modifying purines and pyridines* and agents *labilizing bases*, and (3) agents producing *distortions in DNA*.

The agents in categories (1) and (3) require replication for their action, while agents in category (2) can modify even non-replicating DNA.

(1) **Base analogues.** A chemical substance resembling a base is called a *base analogue*. A base analogue may be incorporated into newly synthesized DNA instead of a normal base.

The pyrimidine analogue *5-bromouracil* (5-BU) is structurally very much similar to *thymine*. If bacteriophages are grown in the presence of 5-BU they incorporate the substance as if it were thymine. 5-BU does not have a lethal action because it is incorporated in place of T and functions almost normally. 5-BU can, however, undergo internal rearrangement (*tautomerization*) from the usual *keto* state to the rare *enol* state. 5-BU now pairs with *guanine* instead of *adenine*, the natural partner of thymine (Fig. 10.10). Thus there is 5-BU-G pairing instead of T-A pairing. Because of this property 5-BU is used in the chemotherapy of virus infections and cancer. By pairing with guanine it disturbs the normal replication mechanism of micro-organisms.

5-Bromodeoxyuridine (5-BDU) can replace thymidine in DNA. *2-Aminopurine* (2-AP) and *2,6-Diaminopurine* (2,6-DAP) are purine analogues.

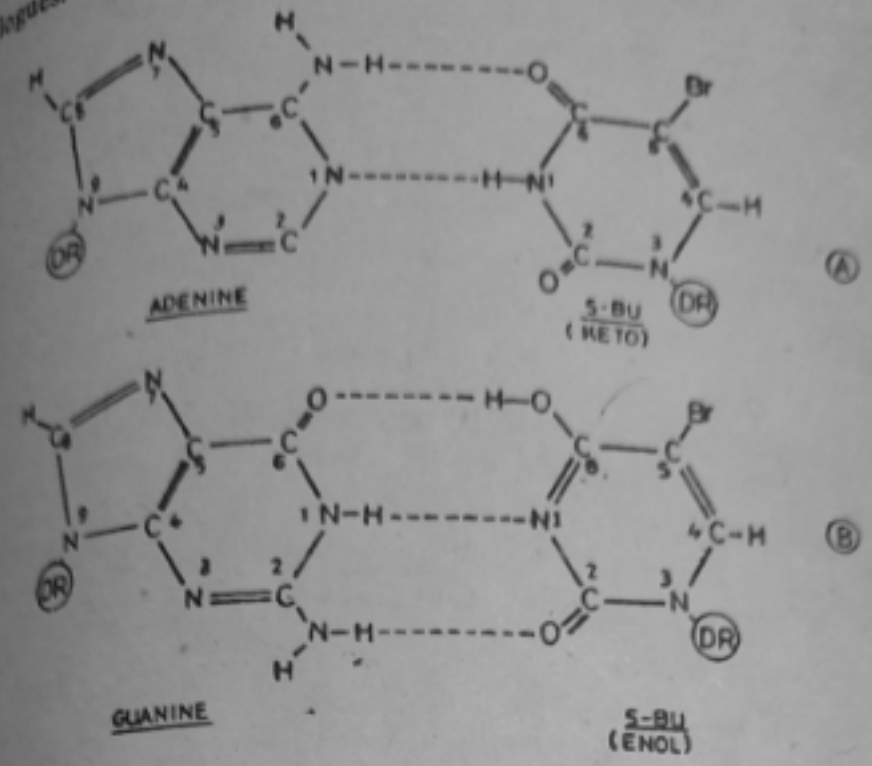


Fig. 10.10. Tautomerism of 5–bromouracil (5–BU). DR = Deoxyribose.
A. Regular base pairing of adenine with 5–bromouracil in the normal keto form.
B. Forbidden base pairing of 5–BU (in the rare enol form) with guanine.

2-aminopurine can be read as either *adenine* or *guanine*. It normally pairs with *thymine* but can also form a single hydrogen bond with *guanine*. It can therefore produce A-T → G-C transitions. 2-AP and 2,6-DAP are less effective as mutagens than 2-BU and 5BDU.

DNA from any sources contains *methylated bases*. Methylation of the bases takes place after the synthesis of the polynucleotide. Thus cytosine on methylation becomes *5-methylcytosine* (Fig. 10.12). In many organisms DNA contains both cytosine and 5-methyl cytosine. The amount of guanine is equal to the sum of these two bases. Methylation appears to protect DNA from enzymes formed under the direction of invading viruses. *5-hydroxymethyl cytosine* is formed when there is a hydroxymethyl ($-CH_2OH$) group at the fifth position of cytosine. The bacteriophage T2 contains 5-hydroxymethyl cytosine instead of cytosine. Similarly in a bacteriophage of *Bacillus subtilis* there is *hydroxymethyl uracil* instead of *uracil* and *5-dihydroxypentyl uracil* instead of

thymine. It should be noted that the methylated bases mentioned above are normal constituents of DNA and are not mutagens.

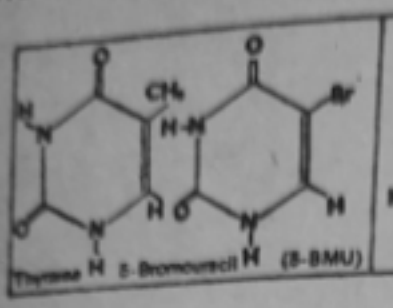


Fig. 10.11.
Structure of thymine (5-methyl uracil) and 5-bromouracil (5-BU).

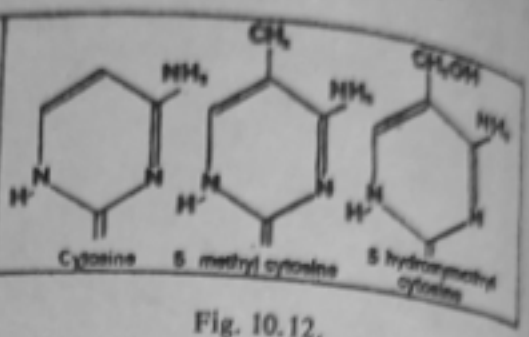


Fig. 10.12.
Methylation of cytosine resulting in formation of 5-methyl cytosine and 5-hydroxymethyl cytosine.

(2) **Agents modifying purines and pyrimidines or agents which labilize the bases** include *nitrous oxide*, *hydroxylamine* and *alkylating agents*.

(i) **Nitrous oxide** (HNO_2) reacts with bases containing amino groups. It can change the structure of such bases by deamination (removal of the amino group). When purines or pyrimidines containing the amino group are treated with nitrous oxide, the *amino group* ($-NH_2$) is replaced by the *hydroxyl group* (-OH). The order of frequency of deamination is adenine, cytosine and guanine.

Deamination of *adenine* results in the formation of *hypoxanthine* (Fig. 10.13). The pairing behaviour of hypoxanthine is like that of *guanine*. Therefore hypoxanthine pairs with *cytosine* rather than with *thymine*. Thus A-T pairing is replaced by G-C pairing.

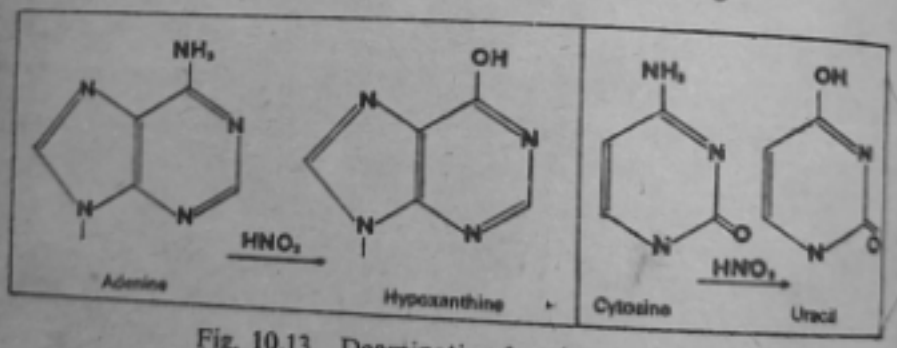


Fig. 10.13. Deamination by nitrous acid.

The deamination of *cytosine* (at the 6-position) results in the formation of *uracil*. The hydrogen bonding properties of uracil are similar to those of *thymine*. Therefore, instead of C-G pairing there is U-A pairing.

Guanine is deaminated to *xanthine*. There is no change in pairing behaviour in this case, because xanthine behaves like guanine and pairs

with cytosine. Instead of G-C pairing there is X-C pairing. Thus deamination of guanine is not mutagenic.

Table 10.2. Changes in structure and pairing behaviour of DNA bases as a result of deamination by *nitrous oxide*.

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

The bases formed after deamination of *adenine* and *cytosine* have a different pairing behaviour. As a result changes in DNA take place in 50% of the progeny. Deamination of *guanine*, however, does not result in a heritable mutation, since there is no change in the pairing behaviour of the deaminated base (*xanthine*).

(ii) **Hydroxylamine** (NH_2OH) is very specific in its action. It reacts mainly with *cytosine* and *guanine* residues and brings about *transitions* and *mispairing*. It deaminates cytosine to a base which pairs with *adenine* instead of *guanine*. Thus C-G pairing is changed to A-T pairing.

(iii) **Alkylating agents** are the most widely used mutagenic reagents. They include :

dimethyl sulphate (DMS)
ethyl methane sulphonate (EMS) $-CH_3CH_2SO_3CH_3$
ethyl ethane sulphonate (EES) $-CH_3CH_2SO_3CH_2CH_3$

The main chemical reaction of these agents is alkylation at the N-7 position of *guanine* residues or at the N-3 position of *adenine* residues. Alkylation increases the probability of ionization and introduces pairing error. The base-sugar linkage undergoes hydrolysis and releases the base from the DNA molecule. This creates a gap in one chain.

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 (A, 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. Where the correct base is inserted, the DNA is normal. Insertion of an incorrect base may result in a *transversion* (purine replaced by a pyrimidine and *vice versa*) or a *transition* (purine replaced by a purine and a pyrimidine by a pyrimidine).

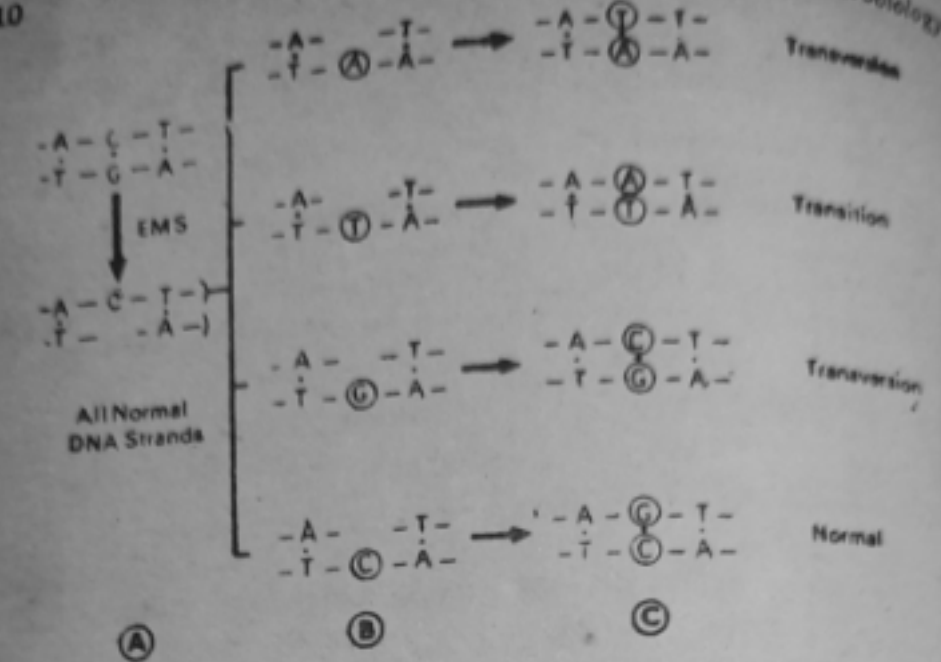


Fig. 10.14. Effect of the alkylating agent ethyl methane sulphonate (EMS) on DNA.
A. EMS removes G and creates a gap in DNA.
B. 1st replication. A/T/G/C inserted across the gap.
C. 2nd replication. Complementary base fills the gap.

(3) Agents producing distortions in DNA. Certain flourescent acridine dyes such as *proflavine* and *acridine orange* (Fig. 10.15) cause muta-

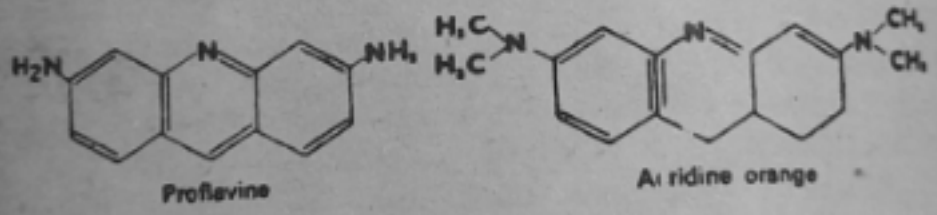


Fig. 10.15. The acrine dyes proflavine and acridine orange.

tions by *insertion* or *deletion* of bases. Crick's work on acridine mutants has provided strong evidence for the genetic code. The acridines are planer (flat) molecules, like the purine bases, and can be intercalated between the bases of the DNA helix (Fig. 10·16). This distorts the

Fig. 10.16. Insertion of acridine dye molecule (black) between bases of DNA.

structure of DNA and can result in deletion or insertion of bases during recombination (Fig. 10.17).

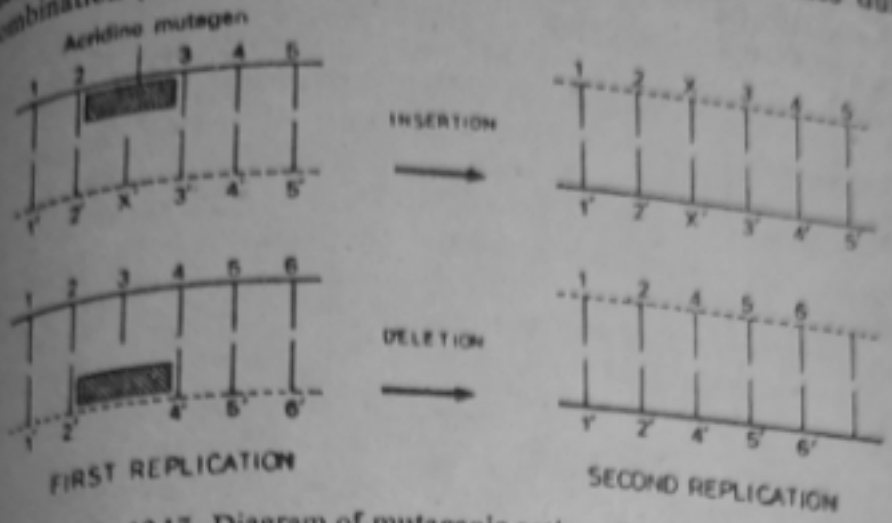


Fig. 10.17. Diagram of mutagenic action of acridine dyes.

(i) *Intercalation resulting in insertion of base.* Intercalation of the acridine molecule between two bases of the template strand results in the lengthning of the DNA molecule. During replication a base (X') is inserted at random opposite the acridine molecule in the new chain. In the next replication a complementary base (X) will pair with the newly inserted base. Thus the new DNA has an *additional* base pair.

(ii) *Intercalation resulting in deletion of base.* The acridine molecule may be inserted in the new chain *during synthesis.* This blocks the base in the template strand and does not permit any base to pair with it. The chain produced is thus deficient in one base, and in the next replication produces DNA with a deficient base pair.

B. RADIATION

Among the physical mutagens radiation is the most important. The energy content of a radiation depends upon its wavelength. In general, the shorter the wavelength the greater the energy value of the radiation. High-energy radiations can change the atomic structure of a substance by causing the *loss of an electron* and the formation of an *ion*. Sometimes an electron pair may be moved from an inner to an outer orbital shell. This brings about *excitation* of the atom. In this excited state the atom is highly reactive and is called a *free radical*. Radiation which brings about such a state is called *ionizing radiation*.

Alterations in nucleic acids caused by radiation are of great genetic importance. High-energy ionizing radiation and ultraviolet (UV) light are important mutagenic agents. Ionizing radiation has greater penetra-

tion power than UV-radiation and produces free radicals which tend to labilize molecules. This type of radiation causes single-strand breaks in DNA and produces deletions.

Both DNA and RNA preferentially absorb UV-light, causing their nitrogen-containing bases to become highly reactive free radicals. The resulting unstability causes the conversion of one base to another (a purine to another purine or a pyrimidine to another pyrimidine). If this change occurs in mRNA only a few inactive proteins will be formed, because mRNA is soon broken down. Substitutions in DNA, however, may have a lasting effect. All the proteins coded by the DNA may be defective. Moreover, if the mutation happens to take place in germ cells the mutated DNA strands could be passed on to succeeding generations.

The primary mutagenic effect of UV-light appears to be due to the production of *thymine dimers* (Fig. 10·18). The 5,6 unsaturated bonds of adjacent pyrimidines become covalently linked to form a cyclobutane ring. Irradiation of a bacterial culture and subsequent extraction of DNA yields three possible types of pyrimidine dimers in DNA :

Thymine-thymine	—	50%
Thymine-cytosine	—	40%
Cytosine-cytosine	—	10%

Pyrimidine dimers can also be formed between adjacent strands. In RNA, pyrimidine dimers are formed between adjacent *uracil* and *cytosine* rings. Pyrimidine dimers cannot fit into the DNA double helix and cause distortion of the molecule. If the damage is not repaired, replication is blocked, leading to lethal effects.

Distortions in DNA caused by thymine dimers can be corrected by a repair mechanism. An *exonuclease* recognizes the distorted region and excises it. A second enzyme, *DNA polymerase* inserts the correct bases in the gap. A third enzyme, *ligase*, joins the inserted bases. The DNA is thus restored to its original condition.

UV-radiation also causes addition of water molecules to pyrimidines in both DNA and RNA resulting in the formation of *photohydrates* (Fig. 10·19). The water molecule is added across the C5–C6 double bond.

X-rays bring about mutations by breaking the phosphate ester linkages in DNA. The breakage may take place at one or more points. As a result, a large number of bases are lost (*deletion*) or *rearranged*. In double-stranded DNA breaks may occur in one or both strands. Only the latter type are lethal. Sometimes two double-stranded breaks may occur in the same molecule and the two broken ends may rejoin. The part of the DNA between the two breaks is eliminated, resulting in a

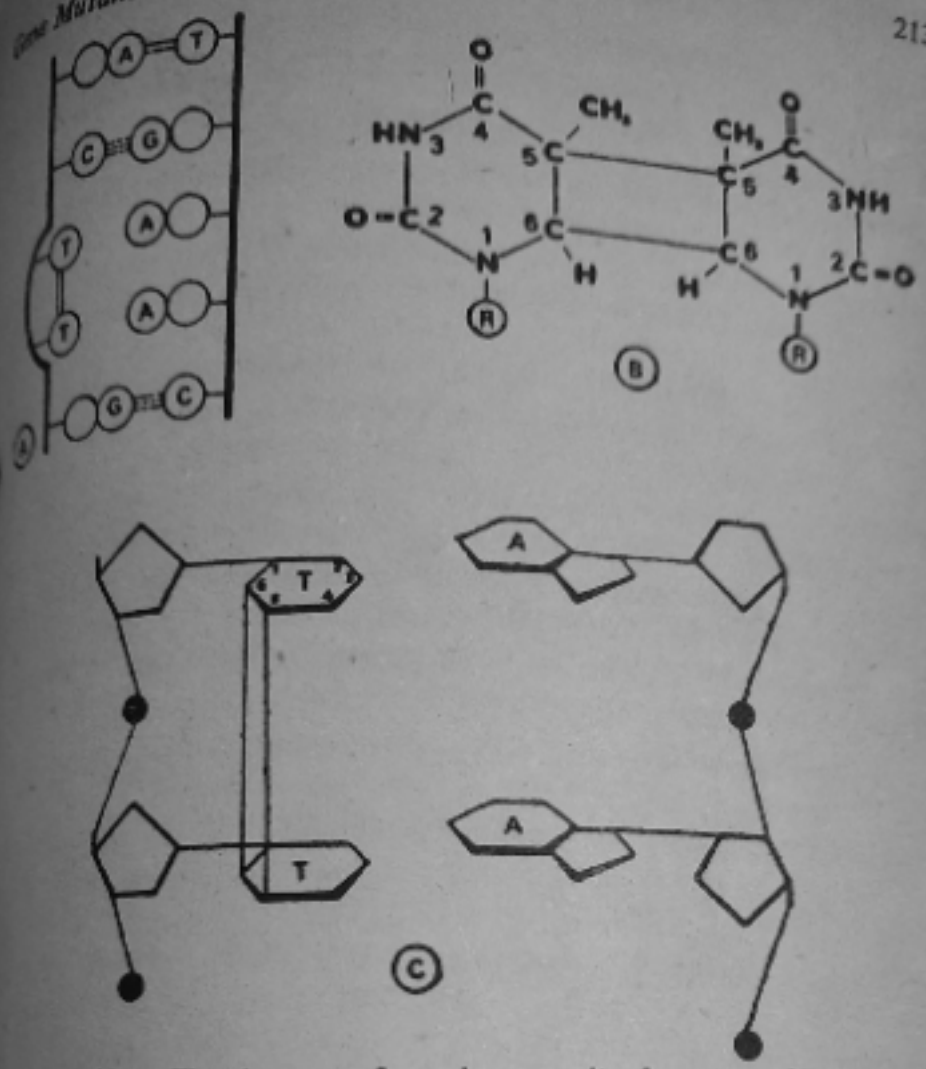


Fig. 10.18. Thymine dimer formed as a result of exposure of DNA to ultraviolet radiation.
A. Distortion of DNA by thymine dimer.
B. Molecular structure of a thymine dimer.
C. Linking of two adjacent thymine residues to form a dimer.

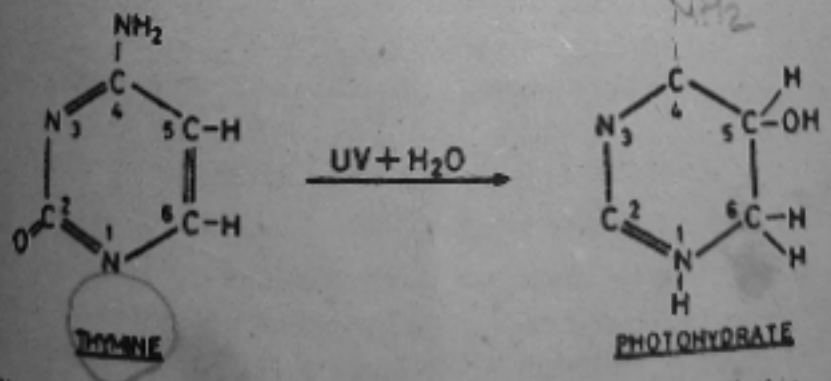


Fig. 10.19. Formation of photohydrate by ultravoilet radiation.

...diation. The damage caused to nucleic acids by UV-light and X-ray ...utilized to sterilize bacteria and viruses.

Evidences for Occurrence of Spontaneous Mutation :

There are many experiments to prove the spontaneous mutation in bacteria. Some of them have been discussed here :

The Fluctuation Test : (Fig. 5–3)

The fluctuation test was developed by S. Luria and M. Delbrück in 1943. This test is administered to prove the spontaneous nature of bacterial mutation.

Procedure : (1) A young suspension of a bacterium (500 *E. coli* cells/ml) is divided into two large cultures, A and B, of 10 ml. each.

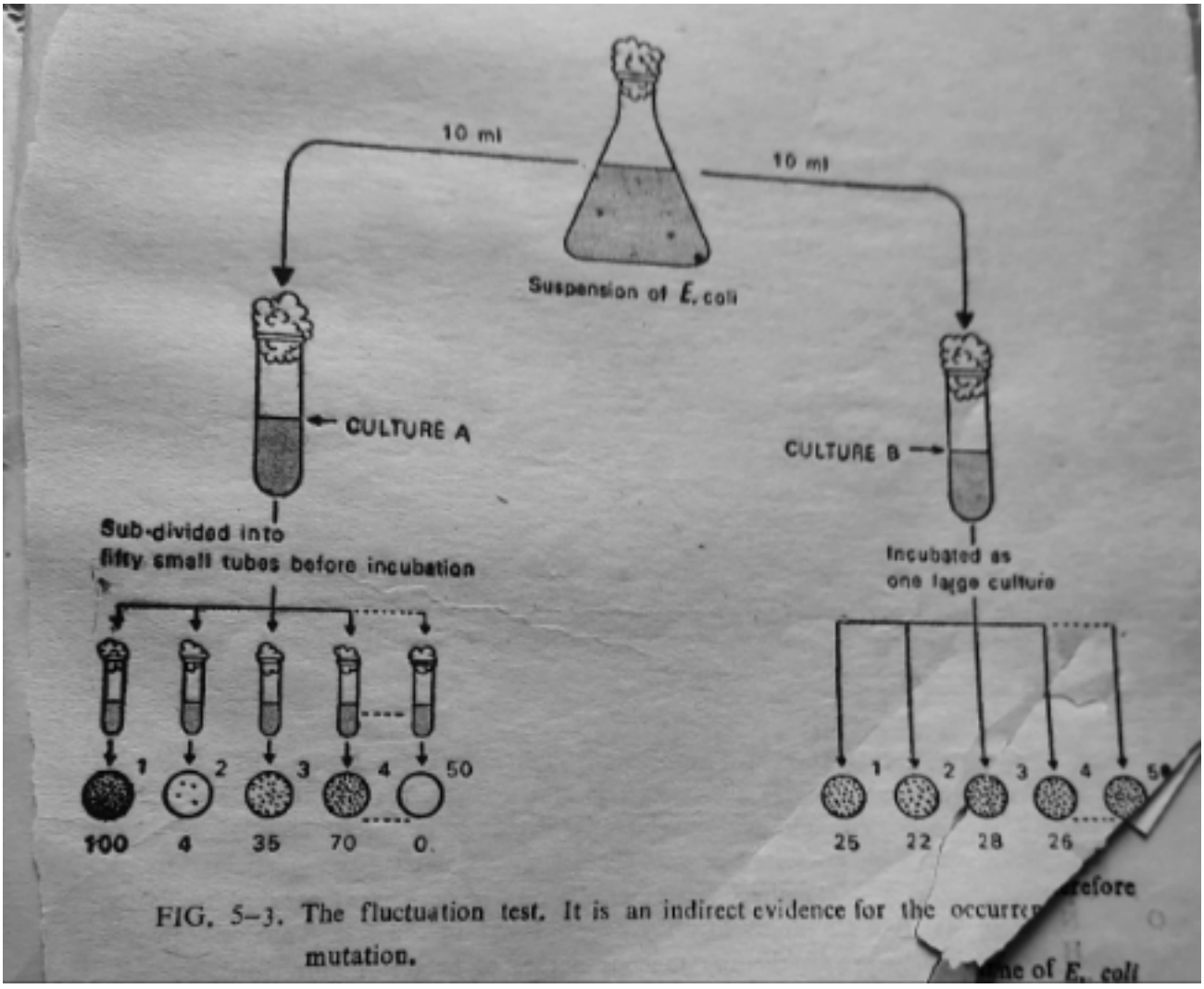


FIG. 5–3. The fluctuation test. It is an indirect evidence for the occurre[illegible]efore mutation.

(2) Large culture A is further subdivided into 50 small cultures (Each small culture contains 0.2 ml.).

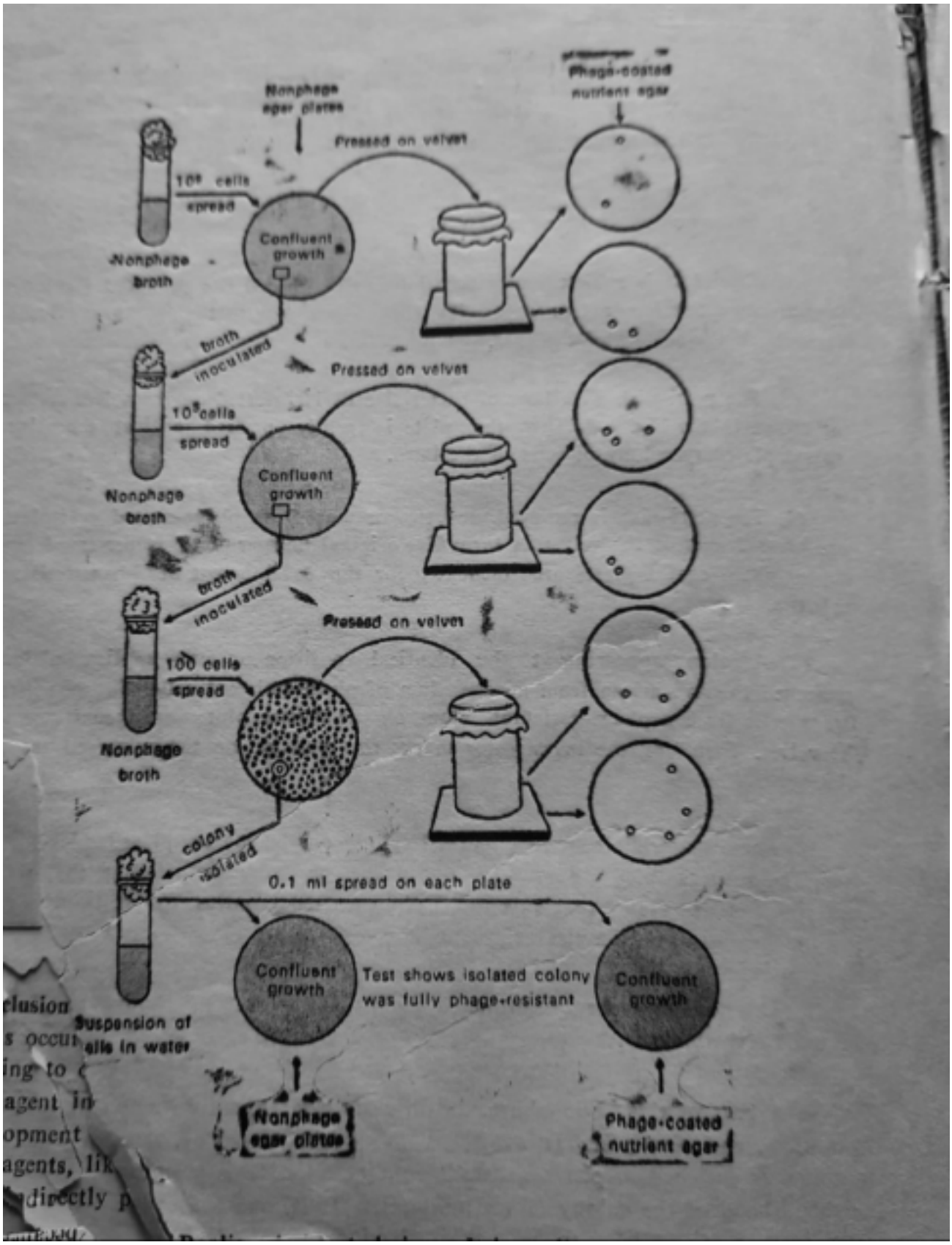
(3) All the 50 small cultures as well as one B large culture are incubated until a suitable population density is reached.

(4) The contents of each of the 50 small cultures are plated separately on phage-coated agar plates. Also, fifty equal samples (0.2 ml.) from B large culture are likewise plated separately.

(5) All the plates are incubated until the development of phage-resistant colonies.

(6) Number of phage--resistant colonies on each plate is recorded.

If, on the other hand, phage resistance resulted from spontaneous mutations during growth in phage-free medium, there should be a large fluctuation between the numbers of phage-resistant colonies which have developed from each of the 50 small cultures. This fluctuation in the numbers of resistant colonies is a consequence of the randomness in the time of the occurrence of spontaneous mutations. If the mutation to resistance occurred early during incubation period, there would be many resistant bacteria present at the time of plating. This happens because the resistant mutant cell would pass through a large number of cell divisions. If the mutation occurred just before the cells were plated on phage-coated agar, only one resistant colony should develop on the plate. In other words, early development of a phage-resistant cell in small culture produces more number of phage-resistant colonies on subsequent plating. Late development of a phage-resistant cell, on the other hand, produces less number of phage-resistant colonies. Between these two extremes, a phage-resistant mutant cell develops randomly and the number of phage-resistant colonies is found depending upon the quickness of the phage-resistant cell development in phage-free medium.


Nonphage agar plates
Pressed on velvet
Phage-coated nutrient agar
10⁸ cells spread
Confluent growth
Nonphage broth
broth inoculated
Pressed on velvet
10⁶ cells spread
Confluent growth
Nonphage broth
broth inoculated
Pressed on velvet
100 cells spread
Nonphage broth
colony isolated
0.1 ml spread on each plate
Confluent growth
Test shows isolated colony was fully phage-resistant
Confluent growth
Suspension of cells in water
Nonphage agar plates
Phage-coated nutrient agar

Conclusion : This experiment supports the spontaneous mutation theory. Bacterial mutations occur as rare chance events in the phage-free cultures. The phage has nothing to do with the development of resistant cells, but it simply acts as a selective agent in phage-coated plates. The selective agent (phage particles) permits the development of only phage-resistant cells on phage-coated agar plates. Other selective agents, like streptomycin, may be employed in the test. Thus, the fluctuation test indirectly proves the spontaneous nature of bacterial mutation.

(9) The spread plate is subjected to incubation.

(10) The lawn of cells developed after incubation is replicated to phage-coated agar. The phage-resistant colonies developed on phage-coated agar may be located on the master plate.

(11) An inoculum is picked from the suspected site on the master-plate. It contains more number of phage-resistant cells.

(12) Repetition of one more cycle of replica plating gives well-isolated colonies of phage-resistant mutants.

Result : The final non-phage agar plate (master-plate) after three rounds of replica plating has well-isolated colonies. The phage-coated nutrient agar plate prepared from the final master plate has confluent growth of phage-resistant colonies.

Interpretation : In this experiment, the phage-resistant colonies on the master plates (non-phage agar plates) may be located by superimposing them on the replica plates (phage-coated nutrient agar plates). Here, phage-resistant colonies on the master plates are never brought into direct contact with phage particles.

Conclusion : This experiment proves that spontaneous mutation is possible in bacteria. The phage particles are used as a selective agent and not as a mutagenic agent.

The other experiments, proving spontaneous nature of bacterial mutation, are the Newcombe's spreading experiment, the Sib-selection test and others.

(5) A colony appearing at the identical position on every replica plate is presumed to have arisen from an inoculum of phage-resistant cells. The phage-resistant cells from a phage-resistant clone on the master plate are transferred via the velvet. Therefore, the investigator marks this position on the preserved master plate.

(6) Some material from that marked colony on the master plate is picked with care. The suspension is prepared in non-phage broth by suspending the picked material. Let us assume that this inoculating needle removes 10^3 cells, of which two or three are phage resistant.

(7) The non-phage broth culture is incubated for several hours to [illegible] test-tube the total cell number.

(8) A part of this non-phage broth culture is spread on a fresh [illegible] Therefore agar plate, and the plate is subjected to incubation. This time, [illegible] inoculum is enriched in phage-resistant mutant cells. This happens [illegible] time of *E. coli* neighbourhood of the colony of resistant cells. Thus, one has only [illegible] not [illegible] wait cells, instead of 10^8 cells, to be sure of having several phage-r[illegible] to spread the plate. [illegible]esistant clone

Replica Plating : (Fig. 5–4)

'Replica plating' technique was introduced by J. Lederberg and E. Lederberg in 1952. This technique was devised to prove the spontaneous origin of bacterial resistance. Now-a-days it has many applications in the field of molecular engineering (e. g., in the isolation of auxotrophic mutants).

Procedure : (1) About 10^8 cells of a phage-sensitive strain of *E. coli* are spread on a non-phage nutrient agar plate.

(2) The plate is subjected to incubation until each cell has produced a colony containing a few hundred progeny cells. This will appear to the eye as confluent growth on the surface of the agar.

(3) The petri-dish with lawn of bacterial cells is inverted on the velvet surface as shown in the Fig. 5-4. Then the plate is gently pressed against the velvet to sample every colony of cells in the lawn.

(4) The petri-dish is removed, and two or more fresh phage-coated agar plates are pressed against the velvet in turn. The original 'master plate' is preserved, and copied phage-coated plates are incubated for the development of phage-resistant colonies.

Result : A large fluctuation is noticed between the numbers of phage-resistant colonies developed from each of the 50 small cultures. But, roughly the same number of phage-resistant colonies is found on each of the 50 plates prepared from B large culture.

Interpretation : Luria and Delbrück reasoned that if bacterial resistance is developed by direct adaptation to the phage, there should be no significant differences in the numbers of phage-resistant colonies on any of the plates made from both large cultures. By this theory it is made evident that it does not matter whether the cultures were incubated in a series of separate tubes, or were all grown together in one large tube, since all the bacterial cells are presumed to be sensitive until brought into contact with phage.