SCIENCE EDUCATION SERIES

No. 10

MUTATIONS AND ENVIRONMENTAL MUTAGENS

by

WINSTON E. RATNAYAKE

NATURAL RESOURCES, ENERGY AND SCIENCE AUTHORITY
47/5 Maitland Place
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PREFACE

This booklet written in March 1979 is intended primarily for the teacher who is the most important member of society concerned with the future generation. As environmental mutagens will have the greatest effect on them it is the teacher who can warn them of the dangers facing them.

Since mutagens also act as carcinogens, or cancer inducing agents, this generation itself is exposed to the dangers of genetically toxic substances. Hence, this book is also addressed to the general reader to enable him to understand the problem. A well informed public is essential for any concerted action towards progress.

The subject has not been dealt with in detail here as the intention of the author is only to introduce the basic principles involved and to create an awareness of the problem, so that when specialists make frightening claims in the press, as they are apt to do sometimes, the public will not become panic stricken, but get together to assist the specialists in tackling the problem. The best assistance in this respect is to use those substances reckoned to be mutagenic with caution and care.

I wish to thank the Science Education Research Committee of the National Science Council of Sri Lanka for giving me the incentive to write this booklet, to Mrs. Devika Jayasinghe for helping in typing the manuscript and to Mr. G. F. de Alwis for preparing the diagrams.

Winston E. Ratnayake

175, Moratuwa Road,
Piliyandala,
16th February, 1983.

FOREWORD TO THE SERIES

The dissemination of scientific information is one of the main functions of the Natural Resources, Energy & Science Authority. The Journal provides a medium for the publication of scientific research papers, while "Vidurava," the quarterly science bulletin of this contains scientific articles of a general nature which is of interest to the public.

There is still a wide gap in the availability of reading material on scientific subjects of local interest. One result of this is that science students confine their reading only to their school notes and to the few available text books which are mostly published abroad. In an attempt to improve this situation, the working Committee on Science Education Research of the Natural Resources, Energy & Science Authority decided to publish a series of booklets on scientific topics of local interest as supplementary reading material for students and the general public. The authors who have been selected by the Committee to prepare these booklets are experts in their respective fields. The manuscripts that were submitted by the authors were examined by referees before being accepted for publication. The views expressed in these publications are those of authors and are not necessarily those of the Natural Resources, Energy & Science Authority.

In conclusion I must thank the working Committee on Science Education Research of the Natural Resources, Energy & Science Authority, and in particular Prof. K. Jayasena, who functioned as Hony. Director and now Prof. V. Basnayake who is the present Hony. Director for the work they have put into making this project a success.

R. P. Jayewardene
Director-General
Natural Resources, Energy & Science Authority
20th February, 1983

Chapter 1

INTRODUCTION

The hereditary material inside the cells of om bodies is the most valuable legacy we bequeath to prosterity. This material, now known to be the giant biopolymer deoxyribonucleic acid, or DNA, is passed onto the next generation through our generative cells, the sperm and ova. Just as much as our cultural legacy (the socio-economic legacy) is the cumulative activity of human beings leading to higher states of civilization, our unseen biochemical legacy, in the form of genes, collectively improve in populations under the action of natural selection thus producing evolutionary advances.

This improvement of the human gene pool is due to the random changes of the genes that go to produce better characters in us, and make us better adapted to our environment. This is a very slow process compared to the very rapid improvements in om socio-economic environment in recent times due to the concerted effects of our abilities to speak and write. We inherit harmful cultural legacies like, greed, passivity and belligerence which can lead to the destruction of entire populations. Such harmful cultural traits die with the death of those societies which exhibit them. Only those traits which will enable populations to survive will in turn be able to survive into future generations. So also with our genes. Harmful genes which cause disease and untimely death are ruthlessly eliminated from populations through the forces of Natural Selection.

Changes in the hereditary material are called mutations-most often they are harmful, seldom are they useful. The useful genes spread in a population due to the operation of Natural Selection while the harmful ones are eliminated. But as these changes occur recurrently, constantly, randomly and spontaneously, harmful mutations keep on affecting our health by persisting in populations. Some mutations by their very nature, can persist in populations for many generations before surfacing into phenotypes in a small fraction of individuals in a population. This is particularly true

of recessive genes which spread unseen in a population as heterozygotes. It is when closely related pei-sons, like cousins, marry and have children that these heterozygous genes become homozygous and show their mutant character in the phenotypes.

Most mutations are harmful because over the many generations of man's existence on this planet, only those genes which act correctly and beneficially have been selected. These are the best adjusted genes and that is why they survive – so that their action is best for a given situation, If such a well adjusted, balanced gene were to change or mutate then the gene product which is formed will be defective. If the gene product, which is a protein, is defective, it will not function properly and will produce a defective organ or an improper physiological function. This is genetic disease. If the malfunction is large enough so as to upset the orderly balance and sequence of the cellular processes, then death results. It is only very rarely that a mutation can produce a gene that is better than itself.

Over long periods of time genes in the human gene pools have had this incessant and dynamic struggle : namely, some genes mutate, most of the new genes thus produced are harmful and are eliminated, a very few are beneficial and they are fixed in the population. This process of Natural Selection helps an organism to adjust itself as efficiently as possible to the environment in which it lives. Its genes are therefore acting in the most harmonious manner. Any changes wrought in them will only lessen their efficiency and upset this harmony.

In human societies, better health and medical facilities are progressively mitigataing the severity of the processes of Natural Selection thereby preserving more a-id more defective genes in the population. For example, by the simple manufacture of spectacles for persons with weak eyesight we are preserving and spreading in our population the bad genes that go to produce the defective eyesight. Without spectacles such persons would perhaps get killed in childhood itself in some accident or other and the bad genes would be ruthlessly eliminated in this fashion. But due to the glasses they survive to old age and spread the genes that produce the defective eyes. There are numerous such examples. What we have done by medical means is to alter the environment for particular *genes* in such a way that these genes are no longer harmful.

However, as mentioned earlier, mutations occur spontaneously and at random all the time. The rate at which they occur is normally very low though at times the frequency may go up due to geological or cosmic reasons. The mutations that are thrown up are of various types, some of which may not respond to any known medical treatment.

If the mutation frequency increases due to changes in the environment brought about by the activities of humans, then, unlike in the case of the medical preservation and spread of a few particular defective genes that are already in a population the increased mutation frequency will produce large numbers of defective genes for which no known medical treatment is available. Human misery will concomitantly increase. The only way in which we can tackle such a situation is to prevent increases in the mutation frequency.

Herman J. Muller in 1927 showed, for the first time, that certain agents can increase mutation frequencies. X-rays, he showed, can increase mutation frequencies ten-fold or even more over the spontaneous level. Charlotte Auerbach in 1942 showed that large increases in mutation frequencies can be induced with the chemical mustard gas. Thus certain physical and chemical agents can mutate genes at much higher frequencies than that found in nature.

These mutation-inducing agents or mutagens, are produced by our own industrial activities. More and more irradiations and radioactive isotopes are accumulating in our environment due to the large number of atomic test explosions carried out by the super-powers. More and more mutagenic chemicals are produced and spread throughout the biosphere by human activity. For the first time in the earth's history, the environment is accumulating increasing quantities of such mutagens: and this is bound to increase the mutation frequencies in all organisms on this earth. As a result we can assume that in human populations more persons with incurable genetic defects will be born to live painful and miserable lives and to bring untold anguish and hardship to the normal parents of such patients. The sum total of human suffering is bound to increase.

Closely related to the induction of mutations in genes are two other processes, the induction of cancers and the production of birth defects (teratologies). They are induced by agents that are similar to the mutagens or by the mutagens themselves. These

diseases, carcinomas and teratologies, are largely confined to one generation and are aot inherited, unless they are caused by mutant genes in which case they can be inherited. These cancers and teratologies can also increase in incidence in populations and this will add to the suffering and misery of humans *at* each generation. The expression of mutations may take *a* few generations to take place but not so with cancers and teratologies. They appear in the very generation that is exposed to the mutagenic, carcinogenic or teratogenic agents.

Geneticists the world over are becoming increasingly concerned with this problem of environments1 mutagens. Already three International Conferences have been held to discuss the problem in depth. The first of these International Conferences was held in 1973 at Asilomar, California, and the second in 1977 in Edinburgh. The third conference **was** held in Tokyo in 1981. Many Environmental Mutagen Societies have been founded and active research into this problem is being pursued in many parts of the world. As a result of such research already **a** fair number of chemical substances have been shown to be mutagenic and/or carcinogenic and their production has been banned through legislation. The number of atomic bomb tests have been reduced and the public at large is becoming aware of the gravity of the problem.

The various aspects of this problem will be discussed in the following pages.

Chapter II

ABOUT CHROMOSOMAL MUTATIONS

2.8. Historical introduction

Hugo de Vries the Dutch Botanist and ooe of the discoverers in 1900 of Mendels work coined the word "mutation" to describe the sudden changes observed in the evening primrose, **Oenothera larmarkiana.**

With the discovery by Herman J Muller in 1927 that physical agents like X-rays can increase many-fold the frequency of mutations when compared to the spontaneous mutation frequency, and the discovery by Charlotte Auerbach in 1942 that chemical substances also can increase mutation frequency just as much as X-rays can, many other workers, like Altenburg, Stadler, Gershenson and others joined these two in studying the nature of mutations. Very soon a considerable amount of evidence began to accumulate from mutation research and clearer ideas about the mutation process emerged. Most of the work was carried out on the fruit fly **Drosophila melanogaster** and on the Maize or Corn plant **Zea mays.**

In 1953 the double helical structure of DNA was announced by James D. Watson and Francis H. C. Crick. Ernest Freeze in 1959 incorporated this knowledge in hypothysising about the nature of mutation at the molecular level based on the further knowledge of the genetic code which had been brilliantly worked out by Nirenberg, Mathaei, Khorana and others. We therefore, have a fairly clear idea as to what mutations are and as to how they arise, although obviously our knowledge of this, as of everything else, is not complete. The experiments on mutation in the latter period were carried out on the microbes and lower organisms like **Neurospora** crassa and **Saccharomyces cerevisiae** (yeast) (eukaryotic fungi) ; **Escherichia coli** and **Salmonella typhimurium** (prokaryotic bacteria) and the viruses of E. **coli.**

It is now known that in the higher organisms (eukaryotes) mutations are produced by three basic mechanisms. They are :-

I. Changes in numbers of chromosome sets.

2. Changes in the structure of individual chromosomes.

3. Changes in the molecular structure of DNA (or the genes).

Before these processes are described, however, the structure of chromosomes must be considered as this will help us to better understand the first two types of processes. It will certainly help us to understand the second.

2.2 Chromosome structure

Chromosomes are darkly staining structures inside the nucleus of a cell. They are composed of DNA molecules packed and held together by histone and non-histone proteins. These nucleoprotein structures have the genes arranged linearly in them, the genes being portions of the DNA molecule. The exact structure of the chromosome is not fully uaderstood, but on the basis of certain experiments initiated in 1974 by Olins and Olins it is becoming clear that the basic chromosome structure is made up of four pairs of histones (named H2A, H2B, H3 and H4) arranged into spherical bodies called nu-bodies or nucleosomes round the **outside** of which the DNA molecule is wound or wrapped with one turn for each nucleosome. (See Fig. 1). After a short gap of the continuing DNA molecule it wraps around another necleosome and so on. On these gaps rest another histone molecule (the H1 histone). This beaded nucleoprotein is supposed to be coiled on itself which will in turn get super-coiled again.

These chromosomes are constant in number in the nuclei of all the somatic cells of any individual organism, is constant (within certain limits) in the cells of the different individuals of the same species and may differ in numbers in the different species. **Drosophila** has 8 chromosomes, **Neurospora** has 14, **Zea mays** has **20,** cabbage has 36 and Man has 46. These are the diploid numbers – the numbers found in the somatic cells. The gametes (sperm and ova) have half this number or the haploid number.

The total number of chromosomes present in each cell of an organism is referred to as the **chromosome complement** of that organism. The sum total of the genes of an organism is known as the **genome,** but this is also sometimes taken to be the haploid number of chromosomes.

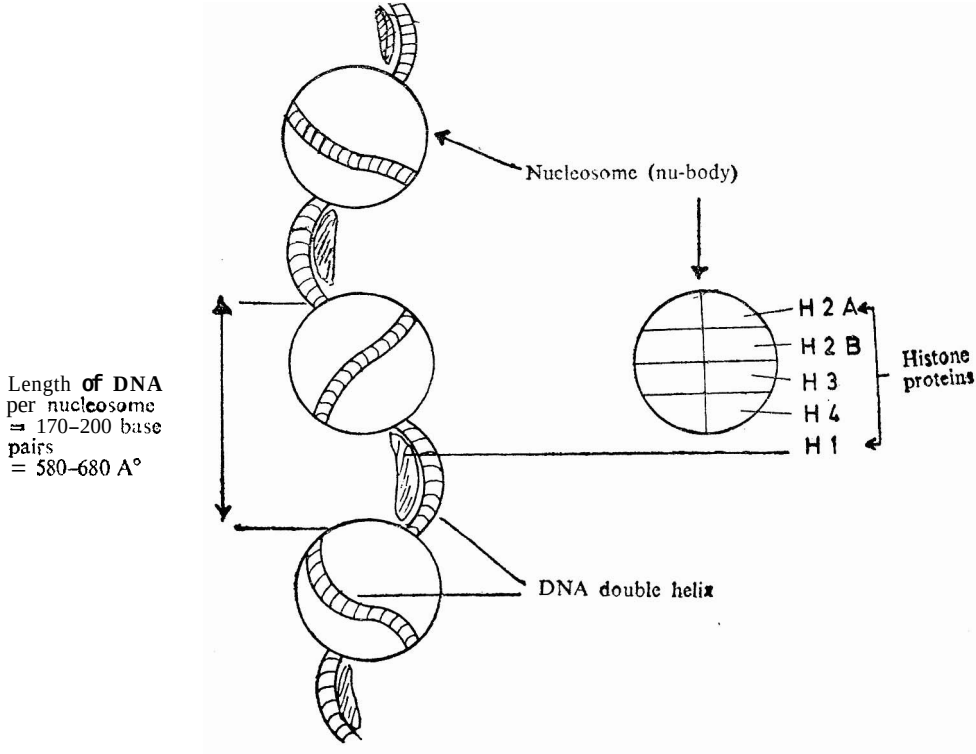


FIG. 1 — *Structure of a chromosome*

2.3 Changes in the chromosome complement (Euploidy)

Although the chromosome complement of a species is expected to be constant, variations of these numbers do occur. Variations may occur in the chromosome sets in different individuals of the same species and this affects the phenotypes of these organisms. Some of the changes originally referred to as mutations by de Vries were due to such changes in chromosome sets. (The other types of mutations were due to loss or gain of individual chromosomes; see 2.4).

Changes in the number of entire chromosome sets in organisms is called **Euploidy.** The chromosome complement in the gametes of eukaryotic organisms is the **haploid (n) number** (genome). The number of' chroinosome sets in the zygote and the somatic cells is the **diploid** (2n) number. Increases over this may be **triploid**

(3n) (as in theendosperm tissue of seeds of angiosperms), **tetraploid** (4n), **pentaploid** (5n), **hexaploid** (6n), etc. Increases over the diploid condition is collectively described as **polypoidy** (xn). These drastic increases in the number of chromosme sets occur mostly in plants (where vegetative propagation can occur) but is not entirely unknown in animals, particularly hermaphrodites. The best known examples of polypoids are afforded by the cultivated plants giving rise to well identifiable strains as in the varieties of bananas, paddys wheats, roses, grasses etc.

When diploids (2n) double the chromosome number in one individual of a species it is called an **autotetraploid** (4n) whereas if the doubling takes place after a cross between two individuals of two species, it is called an **allotetraploid** (4n). The common terms are autopolypoids and allopolypoids.

Polypoidy can be induced by treatment with the chemical extracted from plants called colchicine. This substance destroys the spindle fibres at cell division and the daughter chromosomes are not pulled apart. Doubling and quadrupling of chromosome sets can then occur in these cells.

Euploidy tends to produce larger individuals because of an increase in cell size (though not of cell number). However, most of the autopolypoids are generally sterile as their chromosome sets form multivalents. They are usually rare in nature. Allopolyploids, however, are fully fertile as they have homologous chromosome sets and can pair perfectly. Allopolypoids formed by a doubling of chromosome sets after the haploid gametes of two different species have formed semi-sterile hybrids, give rise to allotetraploids which **are** fully fertile. A good example of such an allopolyploid is obtained when a radish plant is crossed with a cabbage and the semi-sterile hybrid doubles its chromosome set giving rise to a Rhaphaaobrassicae strain.

2.4 Changes in the number of individual chromosomes (Aneuploidy)

When one or more of the homologous chromosome pairs do not segregate normally during meiosis then one gamete gets two chromosomes and the other gets none. When such gametes are fertilised by normal, gametes then the resultant zygotes arising from such abnormal gametes have either three of that chromosome or only one. The process which produces such abnormal gametes

is called primary **nondisjunction** and the zygotes formed by their fertilization with normal, gametes are called **trisomics** and **monosomics** respectively. The whole process is called **aneuploidy.**

If n in the haploid number of chromosomes then the diploid number is 2n and any deviation from this where an individual chromosome is lacking or more than two of a homologous pair are present are represented thus : 2n–1 (monosomy), 2n+1 (trisomy) and 2n–2 individuals where one chromosome pair is entirely lacking are called **nullosomics.**

There are many examples of aneuploidy in species of animals and plants. The best known examples are the ones obtained by de Vries for the evening primrose, the sudden appearence of which hc had termed mutations. The majority of 'sports' or mutations which he obtained in this plant were due to aneuploids. In humans, aneuploidy is well recognized now for the sex chromosomes. The monosomic condition for the sex-chromosoine in humans (2A+XO) where an X or the Y-chromosome is lacking gives rise to a distinct type of individual who is a male showing female characteristics and this genetic condition is called Turner's syndrome. The trisomic (2A + XXY) condition is called **Klinefelter** syndrome, where the female condition is mixed with male characteristics: 2A + XYY individuals are known. They are supermales. 2A + XXX are super-females. **A** monosomic for an autosome (21st chromosome) in man produces individuals with **Down's** Syndrome (Mongol idiots). These genetically abnormal individuals are born to mothers who are usually over 35 years of age. There seems to be a weakning of the meiotic process in their ova so that son-disjunction produces ova with either both or none of a pair of chromosomes.

Because these individuals have an odd number of chromosomes to begin with, if they are fertile, they produce aberrant gametes themselves through secondary non-disjunction. Once aneuploids arise, therefore, they pi-opagate themselves over the generations. In **Drosophils** no:?-disjunction can be induced with mutagens, but it also occurs spontaneously with the production of one aneuploid in 2000 gametes.

2.5 Changes in chromosome structure

Chromosomes may sometimes break and rejoin. Most breaks rejoin at the original break points and restore the original structure of the chromosome. They are then said to have **restituted.** Less

often they may rejoin in different ways leading to alterations in chromosome structure. Such alterations are called **chromosome aberrations.**

Breaks may occur spontaneously due to unknown environmental causes, or due to genetic internal causes or they could be induced by physical or chemical mutagenic agents. Breaks may be single, double or multiple. The broken pieces rejoin in many different ways. This rejoining, healing or repair process is supposed to be brought about by a host of repair enzymes called sealases, ligases, and so on. They bring about the Iinking up of the sugar phosphate backbone of the DNA. The chromosome aberrations can be detected cytologically. The giant (polytene) chromosomes in the salivary gland cells of Drosophila are the best studied in this respect. Each different type of aberration gives a characteristic look to these polytene chromosomes and can thus be identified.

According to whether the breaks are single or double, and according to how they rejoin, chromosomal aberrations are classified as follows :—

(a) Aberrations within chromosomes. There are three such aberrations which occur within chromosomes.

(i) Deletions or deficiencies: These defects arise either when a single chromosome breaks from an end and this small piece is lost, or when a piece is lost from the middle of a chromosome and the remaining two pieces rejoin. These can be described as follows :—

Terminal deletion, where one end of a chromosome breaks and the piece without the centromere is lost.

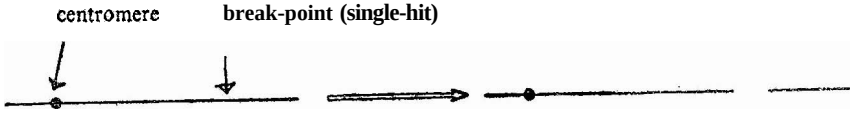


FIG. 2 *Origin of a terminal deletion*

Interstitial deletion, where two breaks occur in the middle region of a chromosome and this broken piece is lost and the two broken ends of the chromosome rejoin.

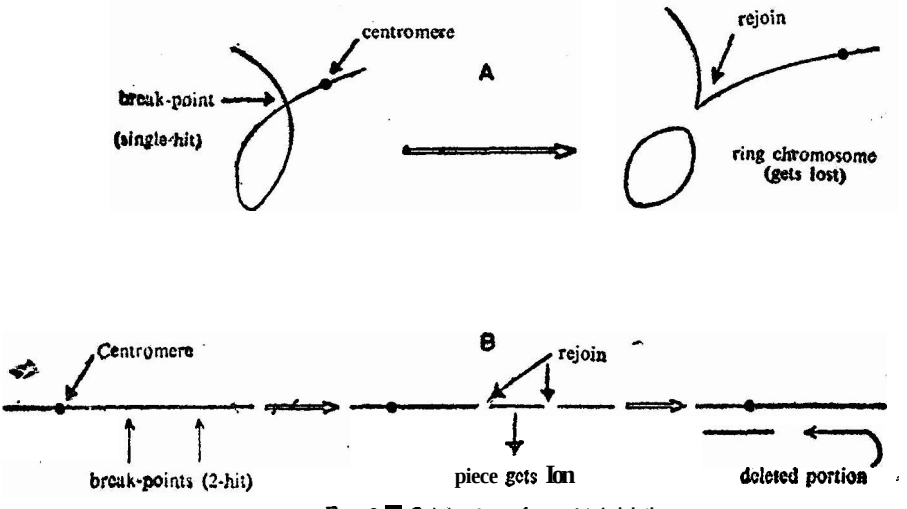


FIG. 3 — *Origin of an interstitial deletion*

Interstitial deletions can arise either from a single-hit or from a double-hit (2 hit) as shown in the above figure. Terminal deletions always arise from a single-hit.

Deletions are easily detected cytologically in the giant salivary gland chromosomes of Drosophila. by trained people One homologue appears shorter than the normal one, and if the deletion is terminal will appear as, ——————— , a detectable loss at the end of the chromosome pair. If interstitial, it will show up as a chromosomal loop, where the normal chromosome will form the loop as it has no corresponding (or homologous) portion on the deleted chromosome to pair or synapse with.

Depending on the size of the deletion it may be viable or lethal.

Deletions are detected genetically by a shortening of the map distance.

(ii) Inversion : These aberrations occur when a part of the chromosome has been broken and rejoins after turning around through 180". When thus rearranged, the inverted segment of

the chromosome will be in reverse order with respect to the rest of the chromosome. Inversions can be terminal (one-hit ; one break) or interstitial (two-hit ; two breaks), Most inversions, however, are interstitial. If an inversion occurs in one arm of *a* **metacentric** chromosome (where the centromere is in the middle of the chromosome) or anywhere on an **acrocentric** chromosome (where the centromere is at one end of the chromosome) it is referred to as a **paracentric** inversion. If an inversion involves both arms of **a** metacentric chromosome so that the centromere is also included then it is called a **pericentric inversion.**

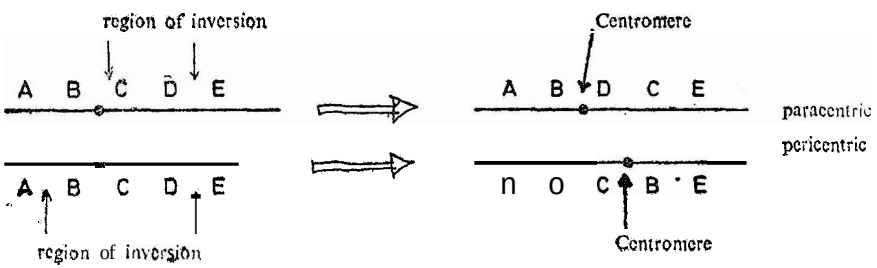


FIG. 4 — *Types of Inversions*

The two breaks required for the interstitial inversion may either arise from **a** single-hit or from two-hits as in the case of the crigin of an interstitial deletion (see Fig. **3).**

If crossing-over inside an inversion were to occur between two homologous chromosomes heterozygous for *a* paracenrtic inversion then at the segregation of the two chromosomes, deleted and duplicated (to be described latter) cross-over chromosomes arise. One crossover chromosome has two centromeres and is called. **dicentric,** the other has none and is called **acentric.** The dicentric chromosome will be pulled apart by the two centromeres which migrate to opposite poles at cell division which will make that chromosome to break in the middle. This process can occur over and over again at succeeding cell divisions, and is called the breakage-fusion-bridge cycle. The acentric fragment because it does not have a centromere cannot migrate to any one of the poles at cell divison and gets lost. (fig. 5)

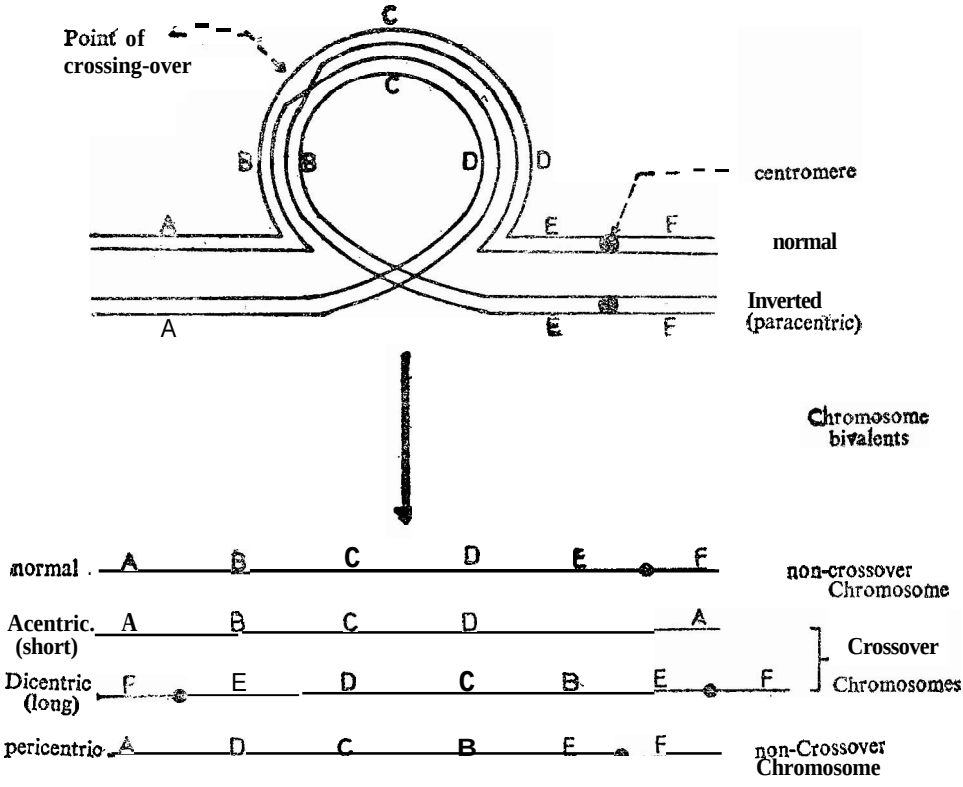


FIG. 5 — *Crossing-over in a paracentric inversion*

If crossing-over takes place inside the loop of a pericentric inversion similar products are formed. In this instance all the cross-over products have a centromere each but as in the case of the paracentric inversion the cross-over chromosomes are deleted and duplicated in certain regions of the chromosomes. (fig. *6)*

In both types of inversions, therefore, if a crossing - over were to take place in the region of the inversion under heterozygous conditions (double-chromosome loop) then the products of the crossing-over are aberrant and all the cells which receive such

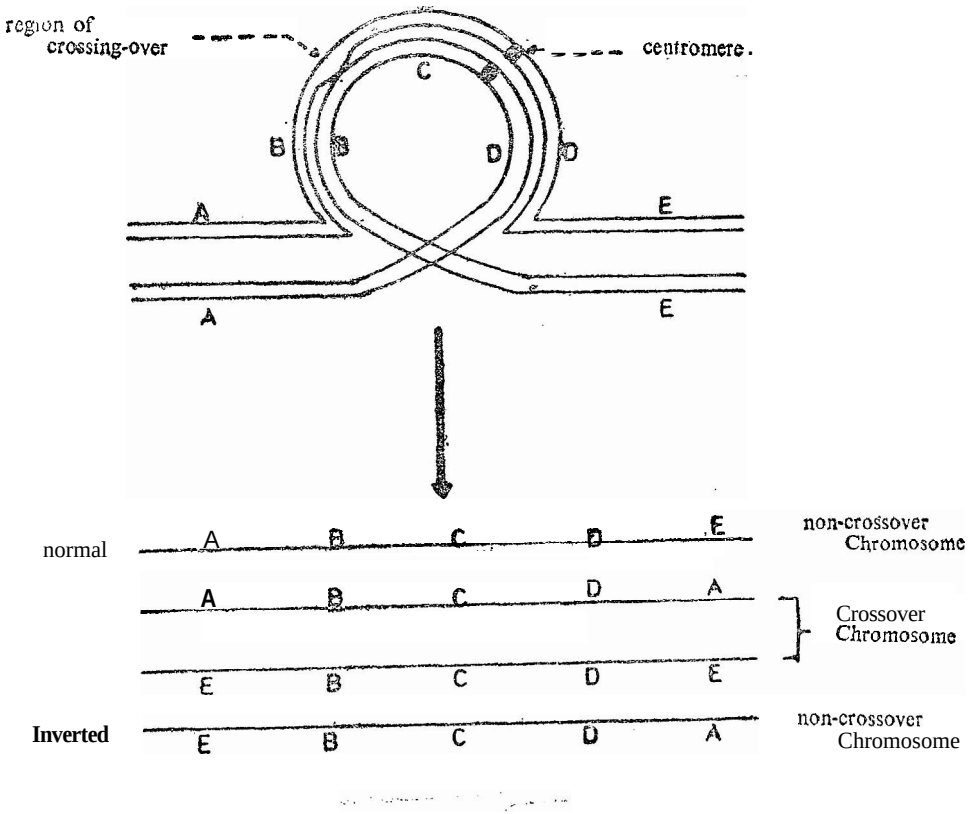


FIG. 6 — *Crossing-over in a pericentric inversion.*

aberrant chromosomes die. In practice, therefore, because the cross-over products cannot be recovered, it would appear that crossing-over does not take place in inversion heterozygotes and they are thus very good cross-over suppressors and are used to great advantage in situations where crossing-over is to be prevented. This will enable the preservation from generation to generation of a sequence of genes in a chromosome without disrupting that sequence.

In animal and plant breeding they can be used to keep a group of beneficial genes together without allowing them to break up and recombine. Such a cluster or group of genes held together by an inversion is called a **super-gene** where a syndrome of characters due to the action of many genes can be inherited from generation

to generation as a single gene. Similarly, inversions are used to carry mutant genes required for detecting genetically certain types of mutations in mutagenicity testing.

Cytologically, inversions are detected in salivary gland polytene chromosomes and even in other somatic (mitotic) cells by the presence of a characteristic two-chrornosorne loop as shown in figures 5 and 6. They are detected genetically by their suppression of crossing-over (cross-over suppression). Sometimes an inversion caa produce a phenotypic effect due to position effects. In Drosophila an inversion in the 2nd chromosome produces curly wings which acts as a dominant gene.

(iii) **Duplications :** When a region or segment of a chromosome repeats itself either on the same chromosome or on another chromosome, then it is called a duplication. Most often these duplications occur on the same chromosome and may be called tandem (adjacent), reversed-tandem or displaced. Displaced pieces may be normal or reversed. Duplications are also known as 'repeats.:

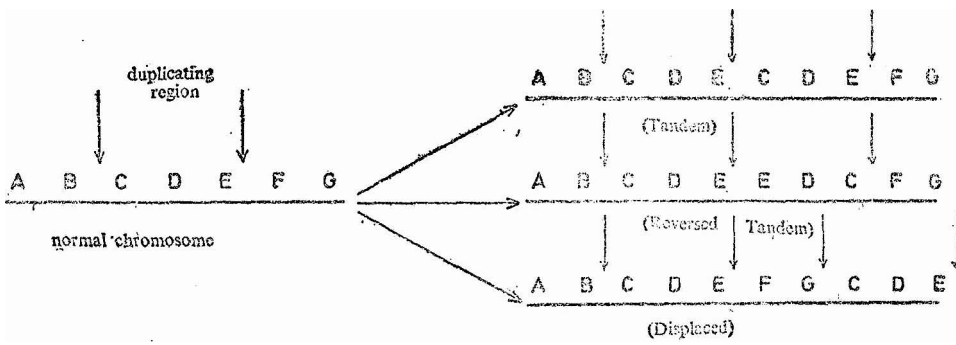


FIG. 7 — *Types of duplications*

Duplications are detected cytologically in the giant salivary gland chromosomes of Drosophila as single chromosome Ioops just as in the case of deletions, but the region of the loop will have an identical region elsewhere which can be recognized by the banding pattern. Genetically they are detected by the loss of recessive characters. The two tests have to be used in conjunction with one another to unambiguously defect duplications.

A good example of a duplication is found in Drosophila. Here a tandem duplication of 7 bands in the 16A region of the X-chromosome produces a distinct phenotype – Bar eyes. This acts as a semi-dominant gene. In the homozygous condition in the female and in the hemizygous (single chromosome-haploid) condition in the male it produces slitlike eyes. In the heterozygous condition in the female it produces kidmy-shaped eyes. Sometimes the condition called double-Bar occurs and this is due to additional repeats of the same region on the X-chromosome.

Duplications are important in evolution as they afford extra DNA or genetic material which can give rise to new genes. Individuals processing duplications can survive and these duplicated regions which have extra genes in them which are present in the required doses can support mutational changes in them while such mutations if found only in one of two alleles could be disadvantageous to the individual. Such mutations in the extra genes can be accumulated over the generations and can ultimately change them to entirely different genes which begin to perform entirely different functions than those of the genes they arose from.

Duplications arise spontaneously by unequal crossing-over or by the breakage-fusion-bridge cycle of dicentric chromosomes. They can be induced by mutagenic agents by the usual processes **of** breakage and fusion.

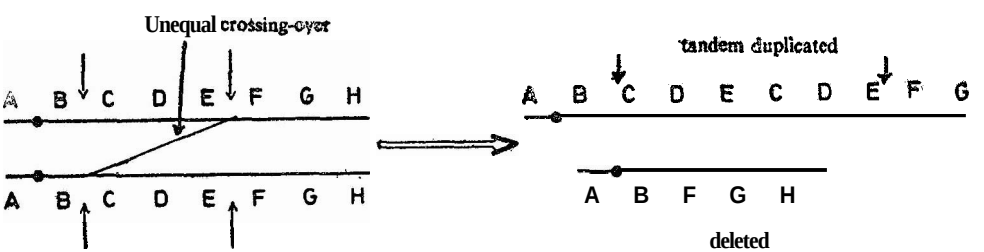


FIG. 8 – Origin of duplications through unequal crossing-over

(b) **Rearrangements between chromosomes :** These are exchanges of parts of chromosomes between non-homologous (heterologous) chromosomes and are called **translocations.** There are three types of translocations.

(i) **Simple translocations** which involve the transfer of one end of one chromosome to the end of another. These are very rare.

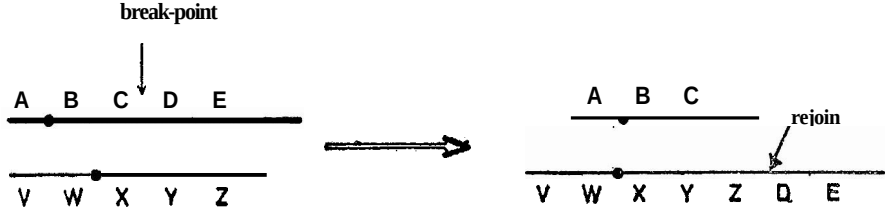


FIG. 9 – *Origin of simple translocation*

(ii) **Reciprocal translocations** arise when two heterologous chromosomes get fragmented, and exchange sich fragments between themselves. The two new chromosomes will function normally if they are both present in the same cell and if they each possess a single centromere.

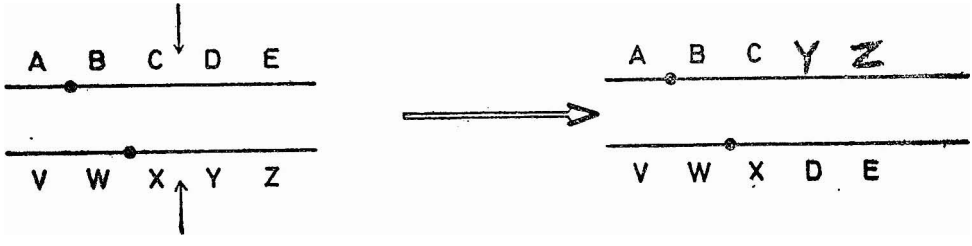


FIG. 10 – *Origin of Reciprocal translocation*.

(iii) **Shifts** occur if **an** interstitial piece is removed from one chromosome and is either re-inserted elsewhere in the same chromosome or is inserted interstitially in an heterologous chromosome.

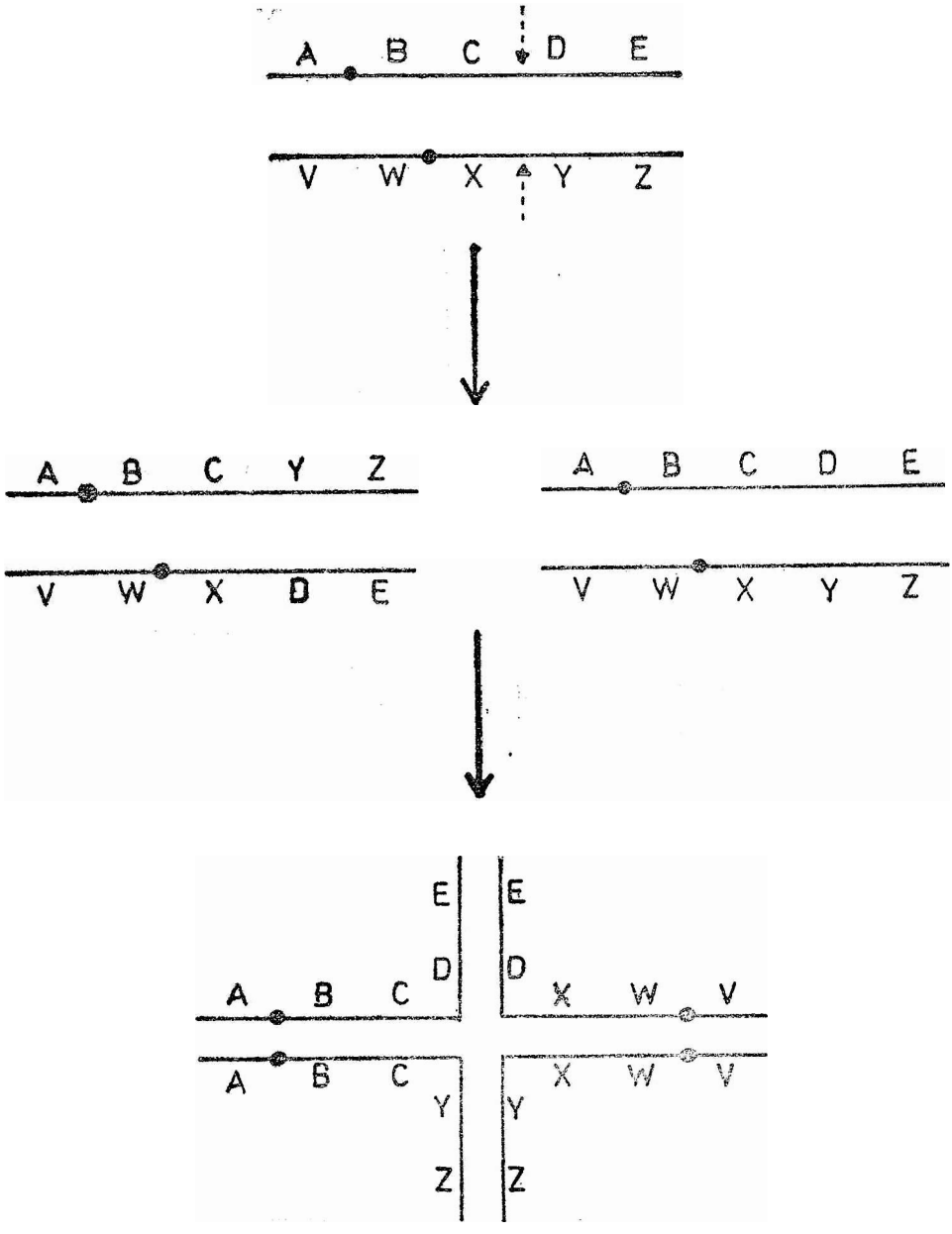


FIG. 11 — *Cross shaped configuration of chromosomes of translocation heterozygote*

Reciprocal Translocations can be present in a cell either homozygously when they are like normal chromosomes except that new linkage patterns of the genes are exhibited (spurious linkage) or heterozygously when due to the random assortment of chromosomes that take place at meiosis approximalely 50% of the gametes get chromosomes that are deleted and when these fertilize normal gametes, the resultant zygote is inviable and die in the first cleavage division itself. Fifty percent of the gametes are normal and when fertilized give rise to live offspring. Theoretically it is expected that only about one third should be viable but one type of segregation of the chromosome pairs does not occur frequently. Of these normal offspring half carry the reciprocal translocation heterozygously. In this fashion reciprocal translocations produce 50% sterility and transmit the reciprocal translocation through half the survivors from generation to generation. This fact is made use of in the control of insect pests.

The translocation heterozygote forms a characteristic cross-shaped figure of their chromosomes in cytological preparations and are thus easily recognised. This cross-shaped configuration is formed by the precise pairing or synapsis of the homologous regions of the two translocated chromosomes with their normal homologous chromosomes.

Due to the rearrangement of genes translocations may produce phenotypic modifications. This is due to the position effects of genes and is similar to that found in inversions. Of course, in the case of translocations genes get shifted from one chromosome to another and the position effect is more drastic.

(iv) Sister-chromatid exchanges

In addition to the types of aberrations mentioned above a more commonly occurring phenomenon is exchange of small fragments of chromosomes between homologous chromosomes themselves and is very much like crossing-over in that a number of such exchanges occur between the homologous chromosomes for small regions of the chromosomes.

These exchanges cannot be usually detected by normal cytological methods. However, by the use of isotopic labelling of the replicating chromatids exchanges between the mother and daughter chromatids can now be studied very effectively by cytological methods and it is being used extensively in such studies.

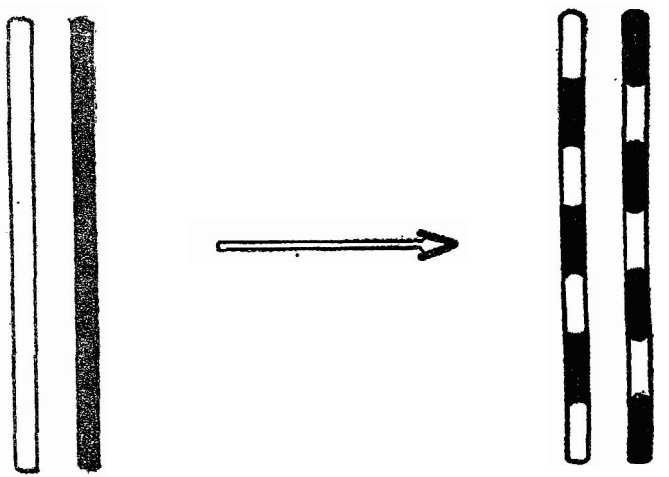


FIG. 12 — *Sister chromatid exchanges (SCEs)*

These sister chromatid exchanges or SCES are supposed to be involved in recombination repair of single-strand lesions of the DNA duplex. They are error correcting repair processes and most often hardly ever produce visible mutations or visible phenotypic effects.

(v) Crossing-Over

It is now known quite definitely that crossing over is due to a breakage-fusion event. The theories of Whitehouse and Holliday hypothyse that this breakage and fusion is of the single strands of the DNA double helix. These crossovers occur spontaneously in many organisms - but is totally absent in some as in the males of **Drosophila**.

This exchange of material between homologous chromosomes can be enhanced or depressed in the case of spontaneously occurring crossing-over or induced where crossingover does not take place by means of mutagenic agents. If induced crossing-over takes glace in organisms where crossing-over does not usually take place, then, as hypothysed by Ratnayake, it may also be due to breakage and fusion of single strands of the homologous DNA

molecules and is *a* measure of the chromosome breaking ability of the agent which induces the crossing-over. In that sense, agents that cause crossing-over between homologous chromosomes (recombinogens) can be to some extent be classed as mutagens. It has to be borne in mind, however, that crossing-over or recombination does not produce inheritable changes but only shows in some individuals that have been treated that single strands of DNA have been broken. Crossing-over can be used, therefore only to detect the ability of an agent to produce such breaks.

2.3 Another way of classifying these aberrations is according to the arrangement of genes on chromosomes.

(a) Alterations in the number of genes

(i) Deletions or deficiencies
(ii) Duplications

(b) Alterations in the position of genes

(i) Inversions
(ii) Translocations
(iii) Sister-strand exchanges

Chapter III

ABOUT GENE MUTATIONS

The word 'gene' as originally coined by Johannsen in 1909 referred to factors of inheritance as defined by Mendel in 1865 and was a unit of function that gave rise to a particular phenotypic character. With later advances in the knowledge of genetics, particularly the work of Seymour Benzer in 1955 on the T4 bacteriophage of E. coli it became clear that the 'gene' in its classical sense as giving rise to a particular phenotypic character was not strictly a unit of function. Most often two or more smaller units than the original 'gene' went to form that gene. These units could only be detected with the help of a very refined genetic test called the cis-trans test. These real functional units are called 'cistrons' and are now known to be the units that go to produce a single polypeptide chain. If this single polypeptide was by itself capable of acting as a protein then the cistron that produced it is equivalent to the older gene. But if two or more such polypeptide units by their cytoplasmic interaction are required to produce the definitive protein which gives rise to a single character then two or more cistrons together go to form a gene. The classical concept of the gene has, therefore, now been replaced by further sub-units. The gene has been split, like the atom !

Genes, or more correctly, cistrons, are linear portions of the giant biopolymer DNA whose triplet codons contain the message for sequencing the amino acids of a given polypeptide chain. It is the given length of the DNA duplex which goes to produce a stretch of polypeptide essential for the production of a protein which is referred to as a cistron.

Damage to the cistron or gene is referred to as gene mutation and is produced by structural changes of the DNA molecule DNA, as we all know, is a long, double-stranded helix of sugar (deoxyribose) and phosphate chains held together by H-bonds between the four bases, adenine (A), guanine (G), cytosine *(C)* and thymine (T) which are attached to the sugarphosphate backbone at right angles. The linear sequence of such bases read in threes (triplet codons) is the message for the production of *a* sequence

of amino-acids joined to each other by peptide bonds. This sequence of amino-acids is a polypeptide chain. These polypeptide chaios are the units of proteins. Either, one such chain acts directly as a protein or else two or more together by cross-linking with each other form a protein. Protein molecules are the building blocks of cells in which case they are called structural proteins or else they are the enzymes that drive the metabolic machinery of the cell.

The message from the DNA molecule is transcripted onto single stranded RNA molecules (the messenger or m—RNA) which are then **translated** into amino-acid sequences or polypeptide chains with the help of transfer RNA or t-RNAs, carrying the twenty different amino-acids, onto the m-RNA on cellular structures called the ribosomes. The triplet codes for the 20 amino acids and for the punctuation marks signalling start and stop for the genetic sentence have now been fully worked out at least for some lower organisms which seem to be universal. This code is non-overlapping and degenerate. The word, degenerate, refers to the fact that one amino-acid may have more than one codon reading for it.

Gene mutations are changes to the structure of DNA that disrupt the orderly sequence of the bases which codes for a particular polypeptide chain. These mutations are of various sorts and can be categorised according to their origins.

As in the case of chromosomes entire legions of **a** DNA molecule may get lost or deleted due to major breaks of the sugarphosphate backbofies of both strands. They may, therefore, be terminal, or interstitial and produce polypeptide chains lacking amino-acids ar various places in them. If in spite of a deletion, the polypeptide can function (say enzymatically) no visible phenotypic effects may be produced. Sometimes the protein so formed may malfunction and produce phenotypic effects. Some times the protein so formed may be altogether rendered ineffective in which case the character may not be formed at all – and if this character is of paramount importance for the survival of the organism then it will be lethal.

Similarly, due to 2-strand breaks of the DNA at two places, duplications and inversions may arise when the broken molecule rejoins. These changes will result in the changes in the sequence of bases of the genes thus producing entirely different polypeptide

chains - position effects. These gross aberrations in the DNA molecule is most likely to produce grossly different proteins to those required for the normal functioning of the organism.

Apart from these gross structural defects that could arise in DNA molecules changes in the base sequence can also arise in the following manner.

A. Base substitutions (B/S) : One base in the DNA could be replaced by another and this is called base substitution. For example, the base Adenine **(A)** may be substituted by Guanine (G). When this happens the base Thymine (T) on the complementary DNA strand which had paired with A originally now will change to Cytosine (C) in order to pair with the substituted base G. In this way the base-pair (bp) A–T has become converted into G-C.

There can be two types of such base substitutions: Transitions, when a purine is replaced by a purine or a pyrimidine by a pyrimidine and transversions when a purine can be replaced by a pyrimidine and vice versa.

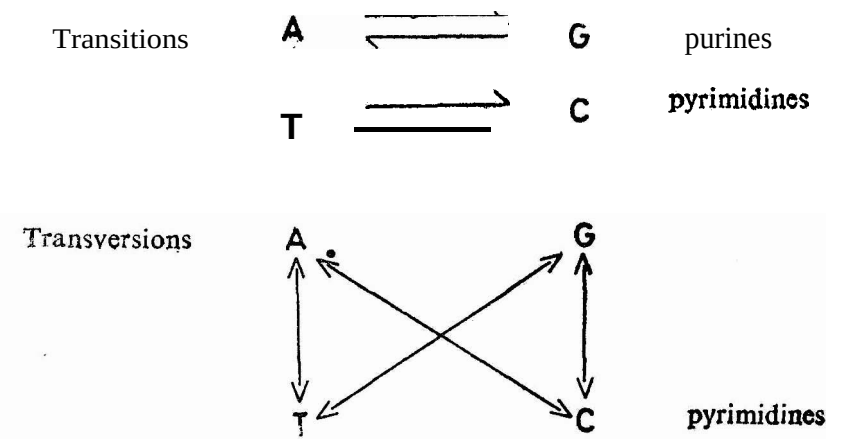


FIG. 13 – *Types of base-strbstitution (B/S) mutations*

This classification was given by Ernest Freese in 1959. Transition type B/S mutations are more frequent than transversions.

These B/S mutations have been extensively studied in the bacteria and their phages, where mutational changes can be mapped almost to the level of base pairs (muton) or recombinations between two base pairs (recon) and whose gene products (the proteins) can then be analysed for their amino-acid sequences. In this way very precise information is obtained with regard to such mutations.

A well known example of a base substitution is afforded by the sickle cell anaemia disease of man. This disease is found in certain parts of Africa and is now known to be due to the action of a single recessive autosomal gene. When the Beta-globin protein chains of the haemoglobins of both sickle cell anaemia patients and normal human were analysed by a two-way electrophoretic method it was found by Ingram in 1959 that the globin protein of the anaemic patient had a single different amino-acid at one position in the polypeptide chain when compared to the normal globin protein. In the 'finger-print' of the two blood samples it was very clearly shown that glutamic add had been replaced at position 6 of the Beta globin chain by valine. The codons for glutamic acid and valine (the mRNA codons) are AUG and UUG respectively. Hence it is seen that the A in the mRNA has been substituted by U which means that in the DNA molecule T has been replaced by A to produce the sickle cell disease. This is a transversion type of B/S mutation.

However, transition mutations are more common and these can arise by the tautomeric shifts of the steric structures, of the bases themselves or by the introduction of base analogues in the place of normal bases in the DNA. At subsequent replications different bases to those originally present will be inserted. Transition mutations, and in fact even transversions, are reversible mutations.

B. Frame-shift (FS) or Insertion-deletion (ID) Mutations

When even a single base is removed or inserted into a DNA molecule then from that point of insertion or deletion the base sequence, reading from left to right, gets completely altered. This would result in the triplet codons now ordering different amino-acids to form the polypeptide chain. Due to the degeneracy of the code a few amino-acids may remain unchanged from the original condition. The frame shift mutation would, therefore, produce polypeptide chains entirely different to the original chain and, therefore, with entirely different functions giving rise to an altered phenotype.

If the repeated sequence of the three bases **(C, A,** and **T)** in a gene were to produce a polypeptide chain it would appear thus :

	↓
DNA base sequence	**CAT CAT CAT CAT**
mRNA base sequence	**GUA GUA GUA GUA**
Polypeptide (amino-acid) sequence	**Val-Val-Val-**
	Val = valine

Now if another base (say, **G)** is added between the first **C** and A (at arrow in the above DNA base sequence) then the frameshift mutation thus produced and the final polypeptide chain resulting from it would be as follows :—

	Insertion
DNA	**C(G)A TCA TCA TCA TCA**
m–RNA	**GCU AGU AGU AGU AGU AGU**
Polypeptide	**Ala** – Ser – Ser – Ser – Ser
	Ala = Alanine
	Ser = Serine

The triplet codon starts reading now as **TCA, TCA** after the first **CGA** codon and this sequence produces an entirely different series of amino-acids in the polypeptide chain, **A** similar frame-shift occurs when a base is **deleted.**

If after an insertion at one point a deletion too were to occur in the same sequence of bases a few bases to the right of the insertion (or vice versa), then the base sequence between the insertion and the deletion only would get altered the rest of the sequence remaining unaltered. In the polypeptide that is formed only a few amino-acids (coded by the altered sequence of bases) will be different from the amino acid sequence of the original polypeptide chain the rest remaining unchanged, thus :

	(G) insert **(T)** remove
Original **DNA**	**CAT CAT CA CAT CAT CAT**
altered **DNA**	**CGA TCA TCA CAT CAT CAT CAT**
,, m RNA	**GCU** AGU **AGU GUA GUA GUA GUA**
,, polypeptide	**Ala** – Ser – Ser – Val – Val – Val – Val
	altered region

The same correction of the base sequence occurs if 3 bases are inserted at different places along a sequence of bases, or when 3 bases are deleted. From the point of first insertion (or deletion) to the point of the third insertion (or deletion) the base sequence gets changed but from the point of the third insertion (or deletion) onwards the base sequence reads as the original. These changes would be reflected in changes in the amino-acids for that region.

Unlike in the case of a BS mutation where only *one base* in the polynucleotide sequence gets altered with the consequent substitution of one amino-acid by another, in the case of the ID (or FS) mutations, a whole segment of the polynucleotide chain (and hence the polypeptide chain) gets altered producing a rather drastic modification of the protein.

Similarly, a rather large alteration takes place when an ***inversion*** within a polynucleotide chain is present, thus:

Original DNA	CAT CAT CAT CAT CAT CAT
Partially inverted DNA	CAT TAC TAC TAC CAT CAT
m RNA	GUA AUG AUG AUG GUA GUA
Polypeptide chain	Val - Met - Met - Met - Val - Val

Met = Methionine

The above DNA sequences are arbitrary ones and have no relation to actual genes. They are given to illustrate changes that take place within a gene by the different mutagenic processes that have been described. The messenger RNA (m–RNA) sequences are the correct complementary sequences of the arbitary DNA base sequences. The amino acids are the real ones coded by the mRNA triplet codons as have been worked out completely by Nirenberg, Matthaei and Khorana in the 1960s.

CHAPTER IV

Mutagens and Mutagen Specificity

4.1 Introduction

Any agent that induces mutations at frequencies above the spontaneous level is called a mutagen. They may be physical or chemical agents. That such agents can induce mutations was first shown for the physical agent X-rays, by Muller in 1927 and for the chemical agent, mustard gas, by Auerbach and Robson in 1942. Both workers demonstrated this mutagenic action in the fruitfly *Drosophila melanogaster*. To detect the mutations Muller had devised special strains of the fruit-fly. Later Stadler showed that mutations were induced by X-rays in the maize plant. An entire branch of study, called mutagenesis, was initiated by the work of these geneticists and with the further work of many others we now know much of the details of the processes of mutagenesis and have identified many new mutagens.

4.2 Physical Agents

With the discovery that X-rays are mutagenic other physical agents were tested soon after, and X-rays, gamma -rays, neutrons and ultraviolet light were found to be mutagenic. Many other test organisms have been developed for testing for mutagenicity and the various mutagens have been detected with the use of one such organism or another. Magnetism was tested in *Drosophila* which did not prove to be mutagenic for the particular test that was used but certain developmental delays brought about by the strong magnetic fields used in the experiment had been inherited over a few generations. Electric discharges, static electricity and similar physical events have not been tested but are supposed to be able to induce alterations to the DNA molecule from theoretical considerations. Heat can increase mutation frequencies to a very slight extent.

The basic finding with regard to the ionising radiations like X-rays, gamma-and Beta-rays (and even the non-ionising radiations like neutrons) which are the physical agents that produce drastic increases in mutation frequencies is that they all have a direct proportionality for induced mutation frequency with dose. That is, the mutation

frequency increases linearly with **dose.** This is true for low dose ranges and for single exposures to the irradiation - not for high doses nor for chronic or intermittent exposures where the mutation frequencies have different relationships with the dose.

As the **induced** mutation frequencies are dose-dependent a straight line graph is obtained for the low dose ranges and an upward bending curve at higher doses. The most alarming feature of this curve is that there is no threshold dose level for the induction of mutations by irradiations. Any increment of the dose can produce a certain increment of the mutation frequency. This means that any increases in the background irradiation can increase the mutation frequency of organisms in a population by a very definite amount. Furthermore, as for all mutagens, once mutations are fixed in the genes of a population any further exposures to the mutagen can add more mutant genes to the gene pool. Of course, if the mutations are deleterious, either producing death or reduced fertility and viability in the individuals possessing such mutant genes, then over many generations the forces of natural selection will tend to eliminate them (see next chapter.)

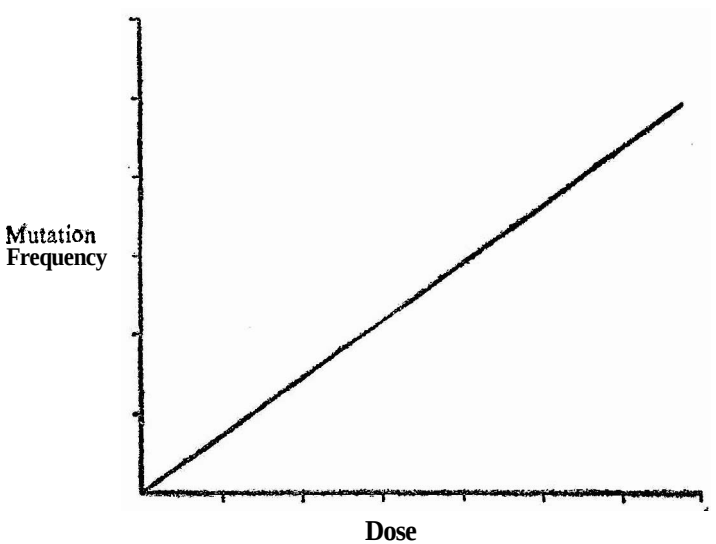


FIG. 14 — *Dose-response curve for irradiations*

The dose of irradiations are measured either as the amountt of energy incident on the treated organism in which case the unit employed is the *roetgen* or as the amount of ionization that takes place in the target tissues when the unit is the *rad*. The latter unit is more widely used now as it measures the actual amount of energy that goes ta damage the genetic material.

A consequence of the linear dose-frequency curve for low doses (upto about 1000 R) is the ***target theory*** which assumes that the irradiations bombard the genetic material and damage it by a single (one-hit) or double (2-hit) or even multiple hit events. When mutations produced by irradiations are expressed as recessive lethal mutations in ***Drosophila*** (or point mutations) then the linear dose effect is obtained and these mutations are reckoned to be produced by single hits. If the mutations are expressed as translocations then the curve takes a quadratic shape and the frequency of translocations increases as the square of the dose-when it is reckoned that it is a two-hit phenomenon.

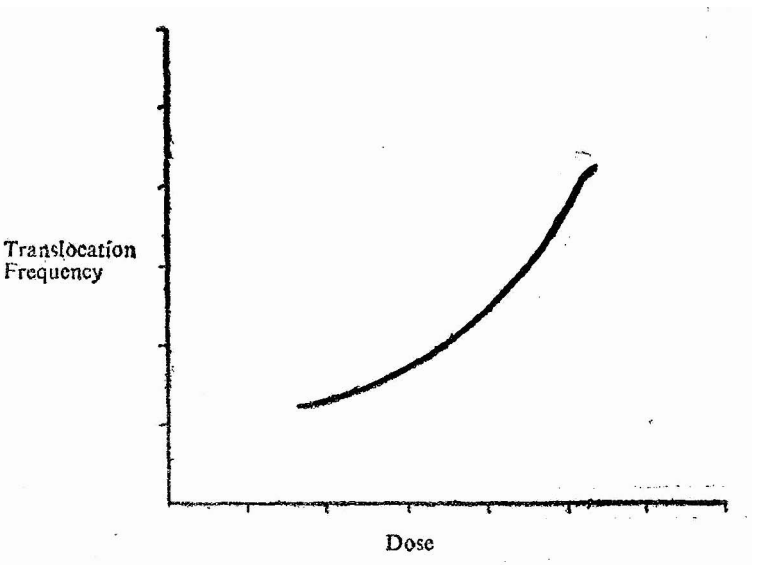


FIG. 15 — *Quadratic curve for increase of translocation frequency with dose*

Irradiations produce mostly chromosomal mutations (deletions, inversions, translocations etc) and only very few true gene (or point) mutations. Hence these are irreversible mutations and are very dangerous as they remain in the population, accumulating in the gene pool all the time, until natural selection eliminates them through the death and suffering (diseases) of the individuals who are unlucky to have inherited them from their apparently normal parents.

At the time of irradiation not all breaks form aberrations. Most actually rejoin correctly to restore the original condition, that is restitute while only a very few form the aberrations. The repair processes, which join breaks (either correctly or incorrectly) are mediated by a host of repair enzymes. They act differently under different conditions of temperature, mutation and oxygen tension. But once they are fixed they are stable as far as the molecule is concerned and are inherited in that form. If the mutation is a large deletion or a chromosome loss these most often act as dominant lethals . Such mutations(dominant lethals) are lost in one generation itself. In mammals they are observed as abortion of foetuses. Recessive lethals due to small deletions and point mutations can be carried in the heterozygous condition for many generations before they become homozygous and surface into the phenotypes causing death. If the mutation is a reciprocal translocation then their heterozygotes produce only 50% of viable gametes, the other 50% carrying deletions in them which when used to fertilize normal gametes produce zygotes which due to the large deletions in them die at the first cleavage division. Of the viable gametes half will be fully normal while the other half contain both translocated chromosomes and if fertilized by normal gametes will in turn become translocation heterozygotes. Thus translocations are semi-sterile from generation to generation. If *a* mutation is an inversion then no crossing-over in the inverted region will take place (or at least the cross-over products will act as super-genes preserving a group of linked genes closely associated to each other for many generations.) If the group of genes are for a syndrome of diseases they will be transmitted from generation to generation as a syndrome of characters for a disease.

Chromosomal damage produced by irradiations (or by any other mutagen for that matter) because of their irreversibility and because they keep on accumulating in the gene pool are therefore dangerous. They can keep on producing disease and death in a population at each generation with increasing frequency as that is the only way they get eliminated from the gene pool. Point or gene mutations can back mutate.

Ultra-violet light (UVL) is a mild mutagenic agent and produces mutations through a chemical reaction inside the DNA molecule. The reaction is the formation sf *thymine-dimers* either of thymine bases close to each other on the same polynucleotide strand or on opposite strands. These dimers upset the general functions of DNA (like replication and transcription) and are hence repaired as soon as they are formed-through the action of excision repair enzymes and rejoining enzymes. During these repair processes errors may arise and produce mutations. Unlike the more powerful irradiations however, UVL cannot penetrate deep into tissues and are hence less dangerous for higher organisms. Some humans, however, suffer from a genetic disease called *Xeroderma pigmentosum* which is a result of the malfunctioning of these excision repair enzymes. Such persons when exposed to UVL tend to break out into spots on their skins due to mutations arising in the ever-dividing malpighian layer of their skin. UVL however, cannot penetrate even a thin layer of glass and therefore, the gonads are protected from the harmful effects of UVL.

The damage to DNA of micro-organisms by UVL is lessened when exposed to suntight. This is reckoned to be due to photo-repair mediated by a group of other enzymes.

4.3 Chemical Agents

The first chemical that was shown to be mutagenic was mustard gas. This was the nerve poison used by the Germans in World War I. Auerbach and Robson using the *Drosophila melonogaster* stocks built by Muller showed quite conclusively in 1942 that mustard gas produced high mutation frequencies after treatment - but as World War II was on, this information was classified and they were allowed to publish their results only in 1947. They showed that mustard gas produced as much chromosomal damage as did X-rays.

After this break–through many other workers using the same test organism as well as other organisms have shown the mutagenic action of many other chemicals. Although the action of chemical mutagens is not so straight forward as the physical ones, yet, much is known about their action as well. A whole group of chemicals (mostly drugs used in cancer therapy) are found to act very much like mustard gas and X-rays. As they mimic the mutagenic action of irradiation. these.chemicals are called *radiomimetic* agents. They are technically known as the *alkylating agents* as their mutagenic action is due to the

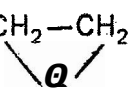
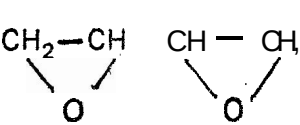
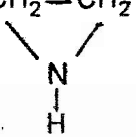
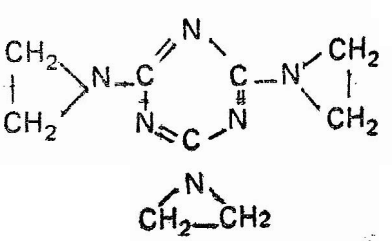
alkyl radicals they carry which react with the bases of DNA or the proteins of the chromosomes. The main reaction is now known to be the alkylation of the base guanine (G) at the N7 position.

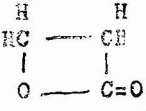
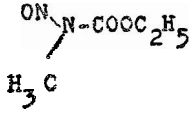
If an alkylating agent carries only one alkyl radical, then it is called a mono-functional alkylating agent, if two, then bifunctional, if three, trifunctional, and so on. If more than two alkyl radicals are present they are also collectively called polyfunctional alkylating agents. The poly-functional agents because they can react with two bases at the same time can cross-link DNA strands and produce greater damage than the monofunctional ones. It is also known that if sperms of Drosophila after treatment are stored before they are allowed to fertilize eggs then the frequency of translocations increase many fold with this storage whereas the frequency of small deletions do not increase so drastically. The monofunctional agents do not produce such a storage effect.

As in the case of thymine-dimer formation with UVL, bases with alkylating agents attached to them hioder the normal activities of the DNA and are hence excised and repaired. During this repair process errors arise and result in aberration formation and mutagenesis.

The following table gives a list of some of the well known alkylating agents.

TABLE I

	Name	Structure	Group Name
1.	Mustard gas	$S(CH_2CH_2Cl)_2$	Sulphur mustard
2.	Nitrogen mustard (HN_2)	$HN(CH_2CH_2CL)_2$	Nitrogen mustard
3.	Ethylene Oxide (EO) 		Epoxide
4.	Dipoxybutane (DEB) 		-do-
5.	Ethylineimine (EI) 		Ethylineimine
6.	Triethylenemelamine (TEM) 		-do-

7. Ethylmethane sulphonate (EMS)	CH_2H_5-OS O_2CH_3		Alkylsulphonate
8. Methylmethane sulphonate (MMS)	$CH_3OSO_2CH_3$		-do-
9. Diethyl sulphate (DES)	$SO_2(OC_2H_5)_2$		dialkylsulphate
10. Beta-propiolactone			-lactones
11. Diazomethane	CH_3 N=N		diazo compound
12, N-Nithroso-N-methyl urethane			Nitroso compound

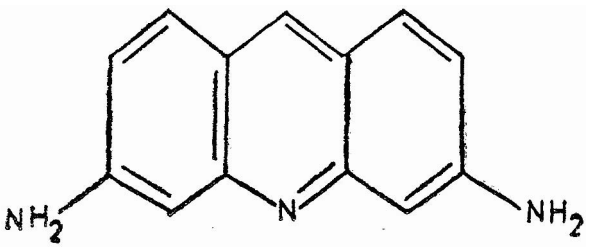
Apart from the alkylating agents various other groups of chemicals have been shown to be mutagenic and with the known structure of **DNA** their action on **DNA** is also well known. These are the purines, the base analogues, the acridines and other odd mutagens.

Various *purines* have been found to produce chromosome breaks in plants and produce mutations in fungi and bacteria. Adenine and Guanine in DNA are purines. The way that purines produce mutation is not fully understood. The best known of the mutagenic purines is *theophylline* which is better known as *caffeine*. This is used widely by man as **a** beverage. Caffeine, at 37^0 C, produces chromosome breaks independently of oxidative phosphorylation but dependent on DNA synthesis. Under these conditions chromosome fragmentation occurs. At 30^0 ***C,*** however, chromosome aberrations as well as fragmentation occurs which is dependent on oxidative phosphorylation and independent of **DNA** synthesis. There are thus two ways in which caffeine produces its mutagenic effects. In addition to this action of caffeine by itself, it is known that UVL and certain chemicals can enhance the mutagenic action of caffeine.

The ***base*** *analogues* ***are*** those substances which closely resemble the 2 purines and 2 pyrimidines found in **DNA.** The analogues are mutagenic by substituting for the normal bases in **DNA** which at replication (or even at time of incorporation) mis-pair through error. By this means a base-pair (bp) in the double-helix can change from

one to another, for example, A-T to G-C, T-A to C-G, G-C to A-T and C-G to T-A. 5-Bromouracil (5BU), the thymine analogue, and 2-Aminopurine (2AP) the purine analogue are well known mutagens. 5 Bromodeoxyuridine (5-BUdR) is the thymidine analogue and is more mutagenic than 5BU.

The acridines are dyes with 3 rings, the best known of which is proflavine:



The acridines are photodynamic, that is, act as photoreceptors and produce lesions in the DNA, but they also produce frame shift (FS) mutations in the dark by intercalating between the bases because of their flat structure. The bases get displaced and are then removed by repair enzymes with subsequent replacement of each base by any one of the four bases. thus producing frame-shift mutations.

In addition to the above chemicals a whole group of oddly assorted chemicals are also known to be mutagenic. They are quite different from each other and will be considered separately.

Hydroxylamine **(HA:** NH_2OH**).** This chemical reacts specifically with the base cytosine (and sometimes with uracil in the **RNA).** This is **a** weak mutagen.

Hydrazine **(HZ:** N_2H_4**)** is also ***a*** weak mutagen. It acts on the pyrimidine bases. Its derivative, maleic hydrazide, is a powerful mutagen.

Nitrous Acid (NA: HNO_2**)** deaminates guanine to Xanthine, adenine to Hypoxanthine and cytosine to Uracil. These base changes can lead to changes in base-pairs (bps) in the DNA.

Formaldehyde **(HCHO)** is ***a*** **very** powerful mutagen but only to a very specific stage of spermatogenesis in *Drosophila, melanogaster.* This is ***the*** early spermatocytic stage, and for these cells in the testes of the male its action is almost similar to that of X-rays and the radio-mimetic chemicals. Its mutagenicity was discovered by the Russian Gershenson also in the 1940s.

Urethane (ethyl carbomate: $NH_2COOC_2H_5$) was one of the original chemicals found to be mutagenic and was discovered by Oehlkers in Germany almost at the time that Auerbach in Scotland and Gershenson in Russia had discovered independently that chemicals are mutagenic. But Auerbach's discovery was slightly earlier, was very thorough and convincing and was published in English and hence became better known. Urethane, is, however, not such a clear mutagen as mustard gas.

Manganous chloride ($MnCl_2$) which is an inorganic salt is known to produce mutations in bacteria and is known also to enhance the mutagenic action of other chemical mutagens like EMS.

Some ***phenols*** and ***quinones*** (like pyrogallol) can produce chromosome fragmentation in the root-tip cells of the onion, *Allium cepa.* Some ***amino-acid*** *analogues* (like 2-amino-3-phenylbutanoic acid or 3-methy-phenylalamine, ethionine etc) are known to induce mutations.

Finally ***DNA*** itself has been shown to produce mutations, but by showing specific preference for certain regions of particular chromosomes (Second) in Drosophila. This mutation production is not like trans-formation found in bacteria.

An interesting feature of chemical mutagens that is not encountered with the physical agents is their ability to produce what are called ***delayed mutations.*** This delayed production of mutations arise by the formation of ***replicating instabilities.*** which are unstable pre-mutagenic states which are transmitted from generation to generation as instabilities and which throw off mutations at each generation.

Chemical, mutagens also differ somewhat from physical mutagens in yet another way. That is, they do not show a dose-response pattern as clearly as the physical agents. This may be due to the difficulties encountered by tbe chemical agents in penetrating to the germ cells through various organs and tissues. Physical agents have powers of penetrating directly to the gonads.

4.4 Mutagen specificity

Mutagens may induce mutations in all types of organisms or produce mutations only in some species but not in others. Then they are said to be species specific. They may induce mutations in one sex of an animal and not in the other sex, when they are said to be sex specific, or else they may be specific in action to a particular germ cell stage

only when they are referred to as being stage specific. Finally, some mutagens may induce mntations at one or more specific loci in the chromosome when they are said to be allele or site specific. Such sites are referred to as 'hot spots'.

These specificities only mean that more mutations are produced in different species, or in a particular sex. or for a given germ cell stage or at particular sites and lower mutation frequencies are cbtained at others.

The physical agents show the least amount of specificity of action and so do the radiomimetic chemicals. But even for these mutagens a slight specificity of action. is present, particularly for germ cell stages, the 'highest mutation frequencies being induced for the gametocytic stages as it is at these stages that meiotic reduction division takes place. There is also sterility induced in germ cells produced from gametocytes which have been exposed to such mutagens.

As referred to earlier UVL produces mutations in micro-organisms but not in higher organisms. This is purely as a result of lack of penetration by UVL to germinal tissue.

The chemical mutagens show most of the specificities described above. Some chemicals induce mutations only in some cases whilst others may show allele or site specificity. The single chemical which exhibits all these specificities is formaldehyde as referred to earlier.

Formaldehyde is without mutagenic action on Neurospora and other micro - organisms. It is without or only with slight action on females of ***Drosopkila melanogaster*** when treated as larva or adult by feeding or through injection. When adult males are treated no mutations occur. When larvae of males are fed formaldehyde treated food only are mutations induced - but only in the early spermatocytic stages, that is, in the cells undergoing meiotic division. In the mitotically dividing spermatogonial cells and in cells which had already undergone meiotic division like the spermatids and spermatozoa no mutations are induced, Other chemical mutagens may not show such extreme specificity though they do show some specificity of action.

These specificities of action are probably due to the powers of penetration of the chemical in the organism, so that not all chemicals are able to penetrate to the germinal tissues. They have also to go past metabolic barriers and may get transformed into other substances in the tissues, substances which are not mutagenic. This is certainly what happens to formaldehyde in female Drosophila whose catalase activity destroy the formaldehyde before it reaches the ovary.

Although mutations are not produced in the spermatogonial cells of *Drosophila* males, crossing over is induced in them, which goes to show that some chromosome breakage (single stranded breakage of DNA) takes place but that perhaps due to the high metabolic activity in these cells they are quickly repaired.

Mutagen specificity in the strict sense is the induction by a mutagen of higher mutation frequencies at certain loci and lower frequencies at other loci which may be preferentially mutated by another mutagen. These specific cases have been reported mostly for the lower organisms, but is not entirely unknown for higher organisms like Drosophila.

In the fission yeast, for example, *a* doubly auxotrophic strain for adenine and methionine (ad–met–) will show reversions at the ad locus at a frequency of 6×10^{-7} mutations while at the *met* locus it is 12.2×10^{-7} mutations. On the other hand with UVL the *ad* locus reverts only at a frequency of 0.3×10^{-7} mutations while the *met* locus reverts at a frequency of 99×10^{-7}. The ratio of met^-/ad^- reversions are only 2 for NA while it is 330 for UVL, which means that UVL induces over 160 times as many mutations at the methonine locus than at the adenine locus.

Such mutagen specificities have been shown for many gene pairs like the above and the way the specificity acts is very complex. It is not simply by the direct action of the mutagen on the DNA molecule.

CHAPTER V

Mutations in Populations

The nature of environmental mutagens is such that their effects are on whole populations of people. Therefore, it is important that we study the behaviour of mutations in populations, too, a least in a very elementary way.

It is only the genes that are inherited from generation to generation, not the genotypes. The genotypes that go to form the individuals at each generation, dissolve into the individual genes in the gonads - so that all the genes of the population that will be passed onto the next generation is held in the gonads of the individuals of that population. This is the gene pool.

The frequency of occurence of one gene as against its allele can be calculated from the frequency of the genotypes of these alleles. This is the gene frequency.

If A_1 and A_2 are co-dominant alleles, then the following are the genotypes and their frequencies, such that $P+H+Q=1$:

Genotypes	$A_1 A_1$	$A_1 A_2$	$A_2 A_2$
Frequencies	P	H	Q

The frequency of gene A_1 $= P + \frac{1}{2}H$ .....(1)

and ,, ,, ,, A_2 $= Q + \frac{1}{2}H$ .....(2)

If, as in the case of a recessive gene, A_1 is fully dominant over A_2 then only the frequency of A_2 A_2 can be determined, as both A_1 A_1 and A_1 A_2 will look alike and cannot be separated. In this case the frequency of the gene A_2 is obtained first.

Frequency of A_2 $= \sqrt{Q}$ .....(3)

because if frequency of gene A_1 $= p$

and ,, ,, ,, A_2 $= q$

then $p + q = 1$

when $(p+q)$ genes in one sex fertilize $(p+q)$ genes of the other sex (to put it rather crudely) the following frequencies of the genotypes are obtained by expanding $(p+q)\ (p+q)$:

$$p^2 + 2pq + q^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

In other words, the genotypic frequencies expressed in terms of the frequencies of their genes are:

Genotypes	A_1A_1	A_1A_2	A_2A_2
Genotype	P	H	Q
Frequencies	p^2	$2pq$	q^2

of course, if the population is a very large one, and the individuals in it are able to mate completely at random.

$$\therefore\ Q = q^2 \qquad \text{and} \quad q = \sqrt{Q}$$

Once q (the frequency of the allele A_2) is obtained then the frequency of A_1 (p) is obtained by subtracting q from 1 (as $p+q = 1$).

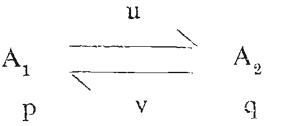
When the number of alleles at a single locus is more than two (as in the case of the ABO blood groups of man) then the calculation of gene frequencies get complicated - but can yet be calculated. The calculation of gene frequencies of two sex-linked alleles is also somewhat more complicated than that given above for two- co-dominant autosomal alleles.

Assuming that these calculations can be made, then it is possible to work out the gene frequencies for any given gene in a population provided of course, that the population is a large, panmictic (randomly mating) one.

When everything is constant in a large, panmictic population then the gene frequencies remain constant from generation to generation. This is called the Hardy-Weinberg Law of Equilibrium, and what is meant by "everything is constant" is that it is assumed no genes are added or taken away from the gene pool. These additions or subtractions to the gene pool occur through migration (emigration of individuals take genes away from the pool and immigration adds genes to the pool), mutation and selection.

In small, non - randomly mating populations other forces, namely, random genetic drift, can change gene frequencies. Mutation frequencies can also upset the Hardy-Weinberg Equilibrium.

Let the gene A_1 mutate to its allele A_2 at a forward mutation frequency of u and let A_2 back mutate to A_1 at a frequency of v, and let the original frequencies of the two alleles be p and q. This situation can be shown thus:



The change of gene frequency in one generation

$$\text{is} \quad \triangle q = up_o - vq_o \quad \ldots\ldots\ldots\ldots\ldots (5)$$

This will reach an equilibrium state because when less and less A_1 alleles are present, more and more A_2 alleles will backmutate to give A_1 s. Therefore, at equilibrium $\triangle q$ should be equal to zero.

$$\therefore \quad up - vq = O \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$\text{then.} \quad up = vq \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$\text{and} \quad p/q = v/u \ldots\ldots\ldots\ldots\ldots\ldots 8)$$

$$\text{also} \quad q = \frac{u}{u+v} \quad \ldots\ldots\ldots\ldots\ldots (9)$$

From these equations it becomes clear that gene frequencies are dependent entirely on mutation frequencies. However, the spontaneous mutation rates are very low normally (about 10^{-4} to 10^{-8} per gene per generation) and cannot by themselves change gene frequencies too drastically. But, because the back-mutation frequencies are about 10-fold less than the forward mutation frequency one would expect the mutant genes to come into equilibrium in such a way that the mutant genes are 9 times as frequent as the wild type normal alleles from which forward mutations produced the mutant form. In actual practice this is not so. The wild type genes are always more frequent than the mutant types. Therefore, something must be pushing this equilibrium in favour of the wild type allele. This force is Natural Selection.

Let us therefore, consider this force of selection on mutant genes. The individuals of one genotype who are less fit than those of a different genotype will produce less progeny by a factor, s, which is called the coefficient of selection. If the allele A_2 (recessive allele) when homozygous produces less fit individuals, then in the population the following holds:

Genotype	A A_1	A_1A_2	A_2A_2	Total
Initial frequencies	p^2	2pq	q^2	1
Fitness	1	1	1-s	
Subsequent frequencies	p^2	2pq	q^2 (1-s)	$1-sq^2$

Fitness can also be defined as that proportion of the genotypes which either survive or are fit to pass on their genes to the next generation. If a proportion of one genotype is unable to do so, such a proportion (coefficient of selection) is supposed to be selected against (or not selected) - and 1-s is the fitness of that genotype. The individuals produced in the next generation (after selection has operated) are the product of the fitness and the initial genotype frequency.

If q_1 is the gene frequency of the A_2 gene in the next generation, then

$$q_1 = \frac{Q + \frac{1}{2}H}{P+H+Q} = \frac{q^2(1-s) + pq}{1-sq^2} \quad \ldots\ldots(10)$$

and the change in gene frequency Δq, as a result of one generation of selection, is

$$\Delta q = q_1 - q$$

$$= \frac{q^2(1-s) + pq}{1-sq^2} - q$$

$$= -\frac{sq^2(1-q)}{1-sq^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots(11)$$

and is dependent only on the selection coefficient *s* and the frequency of the gene against which selection is operating.

If it is the dominant gene A_1 against which selection (s) is operating then

$$\Delta q = + \frac{sq^2 (1-q)}{1-s (1-q^2)} \quad \ldots\ldots\ldots\ldots\ldots\ldots (12)$$

and the recessive allele *increases* (+ sign) from generation to generation as shown in (12)

If s = 1 in equation (11) then

$$\Delta q = - \frac{q^2 (1 - q)}{1 - q^2}$$

$$= - \frac{q^2 (1 - q)}{(1-q)(1+q)}$$

$$= - \frac{q^2}{(1 + q)} \quad \ldots\ldots\ldots\ldots (13)$$

and the recessive allele will be removed in each generation by the amount

$$\frac{q^2}{(1 + q)}$$

If s = 1 in equation (12) then

$$\Delta q = + \frac{q^2 (1-q)}{1-(1-q^2)}$$

$$= + \frac{q^2 (1-q)}{1-1+q^2}$$

$$= + (1-q) \quad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

$$= + p$$

which means that only the dominant gene (frequency p) will survive after one generation of selection. s = 1 signifies the genetic death of the genotype against which selection is operating.

When both *mutation and selection* are considered at the same time the two sets of equations can be joined together. Thus in equation (6), $u(1-q)—vq = O$ and in equation (11) $\Delta q = -\frac{sq^2(1-q)}{1-sq^2}$ for the condition that selection is operating against the recessive gene. Therefore, when both mutation and selection are operating on the alleles A_1 and A_2 under these conditions, at equilibrium $u(1-q)-vq = -\frac{sq^2(1-q)}{1-sq^2}$.....(15)

Now this equation can be simplified if we make the assumption that q is small (as we are interested only in genes at low equilibrium frequencies). Then vq becomes very small and can be neglected. Furthermore $1-sq^2$ tends to 1, therefore, equation (15) can be written as follows:-

$$u(1-q) = -sq^2(1-q) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (16)$$

$$\text{and } \therefore \quad u = -sq^2 \ldots\ldots \quad \ldots\ldots\ldots\ldots\ldots (17)$$

sq^2 is the frequency of the recessive allele A_2 which is *lost* (negative sign) at each generation as a result of the action of selection. This is called the *mutation load* as it is also the proportion of new A_2 genes produced through mutation. As this frequency is lost at each generation it is also referred to as the number lost due to *genetic death.* This is the proportion of the recessive homozygotes that are unable to pass on their genes to the next generation either due to the death of those individuals before they become sexually mature or due to the reduction in viability and fertility of those that survive to sexual maturity. If the allele A_2 is a recessive lethal gene then equation (17) further reduces to: $u = -q^2$ .....(18) where all the recessive homozygotes are removed by the forces of natural selection and this is equal to the mutation frequency which produces these alleles.

Now if the mutation frequency were to double then the number of genetic deaths could also double. But this doubling of the genetic deaths would occur only when equilibrium is reached, which takes some time to occur. If the forward mutation rate (u) were to double it would be most likely also that the back mutation frequency would double or increase by the same amount. Hence the doubling of the forward mutation frequency need not directly produce a doubling of the frequency of genetic deaths. But this would be true only for gene mutations which can revert.

In the case of dominant mutations or those recessive mutations that do not revert (like small chromosomal aberrations) the mutation frequency has a direct bearing on the frequency of genetic death and in the case of human populations this would mean higher infant mortalities (or abortions) or higher incidence of genetic defects in the population if the frequency of mutations were to increase. This is where the danger of environmental mutagens lie. For by increasing the mutation load we are increasing the number of genetic deaths at each generation which in terms of money for medical care is very large and in terms of human misery is incalculable.

The above equations do not hold for small, non-random mating populations. For such cases stochastic equations of random genetic drift will have to be used and the effects of changes in mutation frequencies under these conditions will have to be worked out. Such equations have not yet been derived.

In all the above cases we have considered the production of mutations recurrently or repeatedly at each generation. It is the recurrent mutations that are important. If a single, once and for all mutation were to occur in a single germ cell of a single individual of a single population, the chances are that, that mutant gene will be lost and not transmitted in greater numbers into succeeding generations. Such single mutations will therefore, 'never' get fixed in a population - the odds against such an occurrence are really infinite.

CHAPTER VI

The Environment and Mutation

Environmental mutagens are those mutagenic agents which are so widespread in the environment that they are pervasive in their action on populations of living organisms, especially of humans.

In early times such agents were found only under natural conditions: cosmic radiations, natural products of other living organisms, heat, magnetism, electric thunder storms and naturally occuring inorganic chemicals. These agents appeared and disappeared in unpredictable ways according to the natural changes of the environment. Their presence was not widespread at any given time nor were they found in great excess.

From the time of the Industrial Revolution, however, this has changed drastically with the growth of industries and the rapid increase in human populations, more and more human intervention in the processes of nature has occurred. With the result that a great many substances are being produced on a vast scale and are being spread all over the globe. Naturally occuring radiation levels are being raised to dangerous heights by the various uses of nuclear power and isotopes either for peaceful purposes or for purposes of war. Of course, there is no threshold level for radiations but any increase in radiation levels is dangerous.

Various naturally occuring chemical mutagens are increasing in the enviroment due to the large quantities of food that is being produced and stored for future use. And entirely new synthetic chemicals are being manufactured for various purposes on a very large scale which are being systematically and thoroughly spread all over the globe in the name of commerce and progress. As more and more laboratories start testing these substances for mutagenicity it has become increasingly evident that very many of them are mutagens. Before we consider some specific examples, a rough classification of environmental mutagens might be made.

Naturally occuring mutagens

Physical Agents: Cosmic rays, heat, UVL, magnetic and electric activity etc.,

Chemical agents:

- Products *from higher plants* like the alkaloids (eg. colchicine) and the pyrrolizidines (eg. Heliotrine)
- *from ferns* (eg. cycasin and bracken toxin)
- *from fungi* (eg. afflatoxins from *Aspergillus* and patulins from *Aspergillus* and *Penicillium*).
- *Others* (eg. Nitrous acid and secondary amines as products of the nitrogen cycle)

Artificially synthesized Mutagens:

Physical Agents: X-rays, gamma-rays, neutrons, (from nuclear plants and bomb testing); heat (from thermal plants) UVL, etc.

Chemical Agents:

- Pesticides (like the chlorinated hydrocarbons, organophosphates and carbamates etc.)
- *Drugs* (like the cancer drugs, antibiotics and many others)
- *Food additives* (like the cyclamates, nitrites etc.)
- *Others* (like caffeine, nicotine, formalin, petroleum products etc.)

A naturally occuring substance is considered as an environmental mutagen if it becomes more widespread due to the activities of man than it would have been normally. Such mutagens are always present in the environment and perhaps have been present in the biosphere for millenia, but if with man's intervention the production of such substances increases and becomes more widespread then such increases and disseminations have to be viewed with concern, It is by man's actions alone that they can be reduced once more to the original levels. A good example of such a naturally occuring chemical is the potent mutagen, Beta-afflatoxin, which is a mycotoxin produced by the fungus *Aspergillus flavus* on ground nut, dessicated coconut and stored cereal. With the increases in the storage of such foodstuffs under hot, humid and ill ventilated conditions increases in the growth of fungi occur, and among them *A. flavus*. When these foodstuffs are distributed for consumption, or are used for extraction of oils for the food industry, the liver toxin and mutagen Beta-afflatoxin, is spread far and wide in the population.

Of the physical agents, radiations and isotopes increase in the atmosphere when nuclear devices are exploded. In the event of a thermo-nuclear war the radiation levels and the concentration of isotopes in the environment will increase to such an extent that its effects would be very long-lasting genetically. The third generation victims of the atom bombs at Hiroshima and Nagasaki do show the genetic effects of those terrible bombs, although the effects are supposed not to be so bad as expected. Apart from the actual increase in radiation by the explosion of nuclear devices, isotope production increases in thermonuclear power plants and some of these isotopes pose a serious disposal problem because of their long half-lives. Some isotopes of Ca and P if present in the environment can enter the metabolic processes of living organisms and produce damage. An artificial source of irradiations are X-ray machines which are used widely in hospitals for diagnostic work. If X-rays are used on the lower abdomens of young people their gonads will get radiation doses that damage the genetic material to produce mutations. Of course, doctors are aware of this danger and take the necessary precautions.

Apart from nuclear irradiations which pose the greatest mutagenic threat to mankind, synthetic chemicals are the most willingly and widely used substances in the whole world. Of these chemicals, some are by-products or waste-products which arise in the synthesis or the use of such chemicals. These are released into the environment and get churned in the biosphere due to physical factors like wind and rain or due to ecological factors such as carbon and nitrogen cycles or in food chains. For example the end-products of petrol combustion gets into the atmosphere, industrial pollutants are washed into rivers, lakes and seas etc. which get re-cycled by nature. Agro-chemicals are voluntarily used by the farmers and are involuntarily consumed by the population.

The chemicals we voluntarily swallow are the drugs and they are therefore the easiest to control by merely not using them. Not so with all the other chemicals, they are used by certain special categories of people, the industrialists the farmers and disease-preventing personnel. These chemicals which after they have been used at one place can be taken in by millions of unsuspecting people elsewhere.

Thalidomide was a drug used on pregnant mothers to relieve discomfort-but when their children were born they were hideous monsters with sort stumps in place of arms and legs. When it was discovered that it was the drug that had produced these teratologies (developmental abnormalities) it was withdrawn from circulation almost immediately. But it is not so with pesticides, for instance. In the

first place it is extremely difficult to link up a particular disease with a particular pesticide - and if such diseases arise many generations later as those caused by mutagens, they will never be linked to the mutagen. Even then some advanced countries which manufacture these pesticides have identified some to be dangerous and banned their use. But these same chemicals are yet being sold by some of those very countries to the less developed countries.

Pesticides, therefore, are a group of chemicals some of which have been shown to produce chromosome damage and which are used only by a very few persons but to which almost every single person is exposed. In Sri Lanka particularly, being an agricultural and malarious country, pesticides have been most extensively used and still continues to be used. It was because of its wide spread use that Mr. V. U. de S. Jayasuriya screened some of the more commonly used pesticides in Sri Lanka for possible mutagenic effects. He used 3 tests with *Drosophila melanogaster* and screened about fourteen pesticides. Although he was unable to show clearcut, definite increases in mutation frequencies with any of the pesticides he did show that many of them induced crossing-over in the male. This made him to conclude that most pesticides (he tested) did have an effect on the chromosomes, namely, the induction of single strand breaks in their DNA. The damage would be very slight, but could also be linked with the induction of cancers and, therefore, the use of pesticides have to be viewed with great caution.

Finally the next group of chemicals which are most throughly spread in human populations are the food additives. With the growth of the food industry - particularly the canning and pickling industry - all types of food preservatives are added to these products. These food additives range from simple inorganic chemicals like sodium nitrite to organic chemicals like butylated hydroxyanisole (BHA) and propyl gallate which are phenolic anti - oxidants and are "generally recognised as safe" (GRAS). Most of these substances, however, have proved to have synergistic action on other chemicals making them mutagenic.

CHAPTER VII

Tests for Mutagens

7.1 Introduction

Testing for mutagenicity of environmental chemicals is difficult as many of them may only be mildly mutagenic and determining statistical significance may involve large and laborious tests. In the last chapter it was mentioned that a study was made of the potential mutagenicity of pesticides commonly used in Sri Lanka and that no definite conclusions regarding mutagenicity could be drawn as the tests used did not show large increases in the mutation frequency after treatment with most of the pesticides. Two pesticides did show an increase, in fact, but it was so small that it was difficult to infer that an actual increase in mutation frequency had been induced by these two pesticides. The increases registered may have been due to experimental error. The numbers of Drosophila that were counted in these tests were too small to draw valid statistical conclusions. To increase the numbers counted would have been impossible as it would have made the tests very unwieldly and time consuming when screening a large number of chemicals. Of course, one of the tests did show chromosome breakage in that crossing over was induced in Drosophila males. But induction of crossing over does not prove that *mutations* are induced.

It is not possible to test chemicals for mutagenicity directly on humans for ethical reasons. Therefore, test organisms have always to be employed for such purposes. There are a whole range of such organisms from microbes to mammals. The results obtained from these test organisms have then to be extrapolated to man. This extrapolation is beset with many uncertainities. As was mentioned earlier, the increases of mutation frequencies by environmental mutagens may be so small that it would be difficult to draw definite conclusions regarding their mutagenicity to begin with. Extrapolating such doubtful conclusions to man makes it extremely difficult to conclude that such weak mutagens are actually mutagenic to man. This is because what is mutagenic to one organism may not necessarily be mutagenic to another. And what is mutagenic to a particular type of cell in one organism may not be mutagenic to another type of cell. Also what is mutagenic

to one sex may not be mutagenic to the other sex. We have seen such specificities operating with formaldehyde which is highly mutagenic only to the spermotcytic cells of Drosophila.

How are we then to extrapolate such results to man? This is indeed a very frustrating problem facing the scientists testing for weak mutagens. There seems to be no way out of the dilemma. Tests have to be made on test organisms and the results obtained from them have to be extrapolated to man. Therefore, the tests have to be repeated on the same organism to ascertain whether the results are consistent, tests have to be carried out on other organisms to confirm the results obtained from the first organism and finally some cytological tests on man himself have to be carried out on those persons who had been accidentally exposed to the chemical under test. Using this battery of tests will certainly reduce the uncertainities of extrapolation; but will not eliminate them altogether.

Before we look into the problems of extrapolation, however, let us first consider the tests themselves.

7.2 Mutagenicity Tests

The following is a very brief (and certainly incomplete) description of the mutagenicity tests that have been developed over the last few years and which are currently being used in laboratories all over the world. The reader is requested to consult books on such tests for details. (See Bibliography).

i) Microbial Systems

In these tests certain strains of bacteria are used to detect induced mutation frequencies after treatment with a suspected mutagen. Forward or back mutation frequencies may be employed. Detecting forward mutations are difficult. However, if strains susceptible to penicillin are used, then forward mutations to nutritional auxotrophs will prevent them from growing on a minimal medium containing penicillin in it. The prototrophs will start to grow on the minimal medium and will die because of the penicillin. The auxtrophs will not grow and will not die. These can then be isolated and their nutritional requirement determined. This test is, however, very laborious and elaborate.

It is much easier to detect reverse or back mutations. For this purpose, definite strains of bacteria with nutritional mutations on them are treated and plated on minimal media. Those bacteria which have

back mutated at those loci will grow and can be counted. In this way mutation frequencies can be determined. This is a quick, cheap and efficient method of detecting mutations.

The drawbacks of this method are of two types. One is a methodological drawback the other is with regard to extrapolating to man. The methodological drawback is that only reverse mutations can be detected and the more important gross mutational changes cannot be detected. Furthermore, mutations at a predetermined locus only will be detected and some chemicals may show allele specificity and may *not* show up as mutagenic, whereas in fact they may induce mutations at other loci. And, of course, reversions may occur either by correction of the mutation at the very site that produces the mutation or at some other locus, thereby suppressing the mutation, and to some extent, therefore, act at many points on the DNA and not only at a single point. The other drawback is that these test organisms are prokaryotes and it is difficult to imagine that the same metabolic processes that go on in the eukaryotic body is present in these lowly organisms as well. When both drawbacks are considered together, the extrapolation of results obtained from these tests to man becomes very dubious, if not entirely erroneous.

To get over the problem of the differences between prokaryotic cells and eukaryotic cells, the chemical under test can be pre-treated with liver extracts of mammals (like mice or rats). Then after this pre-treatment the chemical under test is used on the bacterial strains to detect mutagenicity. This is known as the *liver microsome assay* of Ames. Legator has suggested yet another method. He injects the bacterial strains into the abdominal cavity of mice which had been treated with the chemical under test. After a certain period of time the animals are sacrificed and their bacteria harvested and plated to detect mutations. This is called the *host-mediated assay*. Of course the strains of bacteria must be able to survive in the mice.

For all these assays, the bacterial strains have been produced by Bruce Ames of the Univerity of California, Berkeley. These strains of *Salmonella typhimurium* carry mutations in them at certain loci which can detect the exact nature of the reverse mutation, be it base-substitution or frame shift, and so on.

The liver microsome assay of Ames is, therefore, the most reliable and objective, the cheapest and quickest and, therefore, the most widely used method for detecting environmental mutagens. He has also shown very convincingly the relationship between mutagens and carcinogens because the test can also detect carcinogens with great accuracy.

Professor Auerbach of the Institute of Animal Genetics, Edinburgh, however, quite rightly maintains that the mutational process is a highly complicated one in that the changes in the DNA, which no doubt are fundamental and basic, are only just a few of the changes wrought by the mutational process. In the cell the membranes serve as sieves through which the mutagen must pass before reaching the DNA, and furthermore, there are the many metabolic processes with which the mutagen can interact. Even after 'hitting' the DNA the lesions produced can be repaired by a host of enzymes, the repair processes of which are dependent on many environmental factors like oxygen tension, heat etc.

So , in spite of the pre-treatment of the mutagen with the mammalian liver microsome fraction the conditions in an eukaryotic cell are not fully met to simulate even very roughly the actual conditions inside such cells. These tests can, therefore, be used only as a first approximation in a tiered series of tests.

In place of bacteria, eukaryotic lower organisms like *Saccharomyes cerevisiae* (yeast) or *Neurospora crass* (bread mould) can be used- the sophisticated strains as found with bacterial strains have not yet been obtained in these organisms and the scope of the tests are limited.

B. **Plant systems**

Maize (*Zea mays*) is the plant that has been most extensively used in mutation studies and can also be used therefore for testing environmental mutagens. Both genetic and cytological methods can be employed to detect chromosomal damage. Growing maize needs more space and time which makes these tests more expensive and time consuming. Soya bean (*Glyzine max*) onions (*Allium cepa*), *Tradescantia* are some of the other plants that are used in mutagen testing. Cytological tests are easily carried out with plant material. Chromosomal and chromatid aberrations can also be easily scored in plant material.

But the biggest objection to using plant systems for enviromental mutagenicity testing is their great difference from animal cells and hence from human cells. Extrapolating results obtained from plant systems to man is as uncertain, if not more uncertain, than doing so from microbial systems because of this difference.

C. **Insect Systems**

Insects are mid-way in size from microbes to man, their metabolic processes are somewhat similar, so that, their use in mutagenicity tests

are much more reliable than either plants or microbes. Being small organisms with comparatively short life cycles, tests with them can be carried out relatively quickly and fairly cheaply. Moreover, some of the insects, especially *Drosophila melanogaster* are the best studied organisms genetically where almost the whole of the mutation spectrum can be readily detected and identified. In Drosophila visible mutations can be detected by the specific-locus method (or the a *attached-X method*, or even the Muller-5 method). These are due to point or gene mutations. Chromosome aberrations can be detected by the *sex-linked recessive lethal test* (Muller-5) where two generations of crossings have to be carried out or by the *autosomal recessive lethal* test where three generations of breeding have to be made. The sex-linked lethal test is the most widely used as it is the easiest to carry out, is the most objective one, and takes only about one month to carry out. Of course this is about 10 times as long (and perhaps 10 times as expensive) to carry out as a microbial test. But reliability of the tests when extrapolating to man may be about 10 times more. A definite positive increase in the mutation frequency by a mutagen may more reliably indicate its mutagenic action in man than that obtained from a microbial test.

This test, therefore, forms the *second tier* in the mutagenicity testing protocol.

Auerbach is of opinion that if a weak mutagen repeatedly shows a *doubling* of the sex-linked lethal frequency with Drosophila then it is most certain that that chemical is a definite mutagen.

In addition to the sex-linked lethal test which mostly detects small deletions, Drosophila can be used to genetically detect non-disjunction, translocation, gene mutation, induced crossing-over in the male, and indeed the whole range of mutations. In addition inversions, deletions, and duplications can be easily detected cytologically in the giant polytene chromosomes of the salivary gland cells of the third instar larvae. Dominant lethals can be detected by counting the hatchability of eggs and the action of the various stages of gametogenesis can be easily carried out by means of a brood analysis.

Drosophila is, therefore, the most versatile test organism in use and if the doubling of the spontaneous mutation frequency is recognised as indicative of induced mutation, is the most reliable for extrapolating to man.

Apart from *Drosophila* which is a dipteran, *Habrobracon* and *Apis mellifia* (the bee) are hymenopterons while grasshoppers are orthopterans. They too can be used in mutagen testing. However, they are not as versatile as Drosophila.

(D) **Mammalian Systems**

Of the mammals, the mouse (*Mus*) is the most used test organism for mutagenicity testing. The dominant lethal test and the specific-locus test are the two tests widely carried out with mice. Mice are also used for cytological tests. Chemicals under test can be injected into them and their testes later studied cytologically for detecting chromosome aberrations. The erythrocytes in their bone marrows can also be used to look for chromosome defects (the nucleus is still present in these erythroblast cells and is lost only when they mature to become the red blood cells). Schmid of Switzerland has devised what is called the *micro-nucleus* test which detects chromosome aberrations (mostly fragmentation) by the presence of small nuclei in the erythroblast cells.

Using mice for tests is very expensive. But as they are mammals the problem of extrapolating results obtained from test organisms to man is greatly reduced. They are obviously the most reliable and form the *third tier* in the mutagenicity testing protocol.

Non-disjunction (particularly of the sex chromosomes) can readily be detected cytologically in mammalian cells by the presence of Bar bodies in their cells. These are relatively darkly staining (heteropycnotic) chromatin bodies of more than one X-chromosome. in cells of mammals stained with aceto-carmine or acetic-orcein. In females there is usually one such body but in individuals with XXX composition there will be two dark bodies. This is because according to Mary Lyon's hypothesis only one X-chromosome is active in a cell the other X 's becoming inactive or switched off. This inactivity makes them heteropycnotic and easily detectable.

These cytological methods can also be used directly on humans who are suspected of having been exposed to mutagenic chemicals. Sternal punctures can be made to extract the bone marrow from such subjects, although it is very painful, and their erythroblasts examined for chromosome aberration after culturing them *in vitro* with special chemically defined media. This method is a very useful way of monitoring chromosomal damage directly on humans in a population exposed to toxicogenetic chemicals. The abortion rates of a human population can also be used to monitor the ill effects of environmental chemicals and the cells of the foetal cadavers from abortions can be directly studied for chromosome defects.

(E) **Tissue-culture methods**

With the recent advances in tissue culturing it is now possible to maintain and grow cultures of mammalian (mouse, chinese hamster,

and even human) cells *in vitro*. The usual microbial techniques are used on them and like microbes they can be used directly for testing mutagenicity. At present only lethality studies and chromosomal assays are being carried out on them. But it is possible that very soon mutant stocks can be made from them just as with bacteria and they can be used directly for mutation tests. These tests, however, are difficult to carry out and are expensive. The question also arises as to whether cell clones grown in petri dishes have the same metabolic processes as in intact mammalian cells. These tissue culture cells are de-differentiated cells (like cancer cells) and are not the fully differentiated cells found in intact tissues in the living body.

The table on the next page gives a list of the tests mentioned above with their relative efficiencies in detecting various types of chromosomal and gene mutations.

7.3 **Relationship between mutations, cancers and teratologies**

There is a fairly well established relationship between mutagenesis, carcinogenesis and teratologies. Cancers are the wayward, uncontrolled proliferation of cells of a tissue. Chromosomes keep on repeatedly dividing which is followed by cell division. When chromosomes are destroyed by X-rays and cancer drugs (cytotoxic chemicals) the cells are unable to divide and cancerous cells are killed by the dominant lethal chromosome aberrations and the cancer is arrested. Such cancer arresting agents are called carcinostatic agents. By a great paradox of nature these same agents can also induce cancers. So carcinostatic agents can also be carcinogenic. The nature of this dual action is not fully understood. But the connection between carcinogenesis and mutagenesis is fairly well established. Similarly teratologies which are developmental abnormalities or disorders are caused by agents which are also carcinogenic or mutagenic. The epigenetic processes that go on in embryological stages is dependent on an ordered progression of gene action which can be upset by teratogens to produce abnormalities of development or teratologies. There are some similarities between mutagens, carcinogens and teratogens.

Cancers and teratologies are produced in the very generation exposed to agents which can induce them. However, mutagens produce their effects in later generations. In a way , the fact that sometimes the same agent induce all three types of cell defects may be the motive force which will galvanize the present generation to action to control

Table: **Types of genetic damage that can be detected by currently employed mutagen screening test organisms.**

(from Report of Committee 17 of the Council of the Environmental Mutagen Society, USA.)

Screening system		Type of damage						
		Chromosomal Aberration				Gene Mutation		Induced Recombination
System	Organism	Dominant lethal	Translocation	Deletion	Non-disjunction	Forward or Reverse	Specific locus	
Bacterial	*S. typhimurium*					+		
	E. coli					+		
Fungal	*N. crassa*			+	+	+	+	
	A. nidulans				+	+	+	+
	Saccharomyces	+			+	+	+	+
Plant	*Vicia faba*		+	+	+			
	Tradescantia		+	+	+	+		
Insect	*D. melanogaster*	+	+	+	+	+	+	+
	Habrobracon	+	+			+	+	+
	Bombyx mori (silk moth)	+				+	+	
Intact Mammal	*Mouse*	+	+	+	+		+	
	Rat	+	+	+	+			
	Man		+	+	+			
Tissue Culture	Chinese hamster		+	+	+	+		
	Mouse lymphoma		+	+	+	+		

the use of such agents. However, not all teratogens will be carcinogenic, nor will all carcinogens be teratogenic. But all mutagens can be carcinogenic and teratogenic.

Bruce Ames has shown a clear correlation between carcinogenesis and mutagenesis, in that definitely established carcinogens have all proven to be powerful mutagens in his bacterial test systems.

Certain recessive genes in homozygous condition may upset the delicate balance of the cell in undergoing cell division and may become cancerous. That certain chemicals in the cell, called chalones, are involved in the timing of cell division has been suggested. High concentrations of chalones are supposed to prevent cell division and when the cell increases in size their concentration drops so that at a particular low concentration the cell is triggered into cell division. Perhaps the production of the chalone is under genic control and recessive mutations may inhibit their production, thus depleting the cell of chalones. This will set off repeated cell divisions, thus producing cancers, If recessive genes are heterozygous for such mutations then they can become homozygous in some somatic cells due to somatic crossing over induced by carcinogenic agents and chalone free cancer cells may be produced. Of course, this is a purely conjectural picture of the origin of cancers. Cancers are produced by certain viruses and chromosome abnormalities as well. As no definite knowledge is yet available to explain the origin of cancers, I suppose it is not too unethical to conjucture.

7.4 Extrapolation of test results to man

The results of mutagenicity testing obtained from the test systems given in 7.2 have to be ultimately extrapolated to man in order to evalute the risks to him. Then only will the tests be meaningful. This extrapolation has been attempted in two ways with the defining of two basic units of conversion.

One such unit is based upon the spontaneous mutation frequency and a doubling of this spontaneous frequency is taken as indicative of the substance under test being mutagenic. The physiological concentration of a chemical which doubles the mutation frequency in a test organism for a particular period of time is called the rate doubling concentration (or human rate doubling concentration) expressed in mg/Kg/whole body weight when acting for the same length of time. We have already referred earlier on to this concept of a doubling of mutation frequency.

The second of the units is based upon radiation equivalents called rem-equivalent-chemical or REC which is in turn based upon the already available information concerning radiation induced mutagenesis whose standard of measurement is the radiation-equivalent-man (or REM). REC is the dose or the product of concentration and the time of exposure which produces an amount of genetic change equal to that produced by one REM of chronic irradiations.

Specialists are not yet fully decided as to which unit is more meaningful but Professor Auerbach, however, is strongly in favour of using the doubling dose concept. If a chemical were to induce a 100% increase (doubling) of the mutation frequency over the spontaneous level, especially in Drosophila then, according to her, that substance should be considered a mutagen. This conclusion cannot obviously be based on a single experiment even if it had been conducted on a large scale. Only if doubling of the spontaneous mutation frequency is obtained repeatedly is it safe to assume that a substance is truly mutagenic. Still, due to the lack of a clear dose-frequency relationship, due to the delayed production of mutations and due to specificity of action, chemicals which give negative results with one particular test for one particular test organism cannot be considered harmless. The case of formaldehyde should caution us with regard to such negative results.

Taking these facts into consideration it has been proposed that a 3-tiered testing protocol be adopted in mutagenicity testing.

Tier I - Preliminary screening of all chemicals using the Ames Test. If any chemical does not show mutagenicity in this test then it is classified as non-mutagenic and cleared for public use. The mammalian liver microsome pre-treatment of the chemical should be carried out.

Tier II - Those that prove to be mutagenic in the above tier should be then investigated further on an eukaryotic organism like *Drosophila,* Neuospora or Maize. If the substance proves to be mutagenic then extreme caution has to be exercised in its use.

Tier III - This is usually a mammalian test. If this test also proves positive then that substance is definitely mutagenic and legistative action should be taken to ban its manufacture and use.

For each tier, a doubling of the mutation frequency over the spontaneous level can be taken as indicating mutagenicity.

Even the above tiered series is not a perfect system. For one thing, to conduct all three tests routinely would be prohibitively expensive. For another, one may still, after having gone through the three tiers and got positive results be not certain whether that substance could be mutagenic in man. Research is being carried out to improve the methods

of testing. Until such time as a better method of tesing is obtained the tests already with us have to be used to test the thousands of new chemicals that are being synthesized every year all over the world.

CHAPTER VIII

Genetic Toxicology : Conclusions

8.1 Human Genetic diseases

Most mutations are deleterious and lead to disease and untimely death. An increase in the frequency of mutations in a population will increase the "genetic load" of that population. If back-mutations take place at a proportionate frequency then after an initial increase in the number of 'genetic deaths' their frequency will settle down to the former level. But most mutations are not gene mutations (which are the ones that revert) and are chromosomal aberrations like deletions, inversions, translocations and duplications (through rearrangements)' and aneuploidy (through non-disjunction). Once such aberrations occur they would lead to further aberrations. For instance, once aneuploidy arises through primary-non disjunction of chromosomes in the gonads of normal parents then in those aneuploid individuals secondary non-disjunction occurs producing more aneuploids in their progeny.

In human populations non-disjunction of the sex chromosomes produces individuals that are XO (Turner's syndrome) and XXY (Klinefelter's syndrome) both types of which individuals are sexually abnormal and are mentally retarded. Trisomy for the 21st autosome produces Mongol idiots (Down's syndrome). If the incidence of such abnormal humans were to increase in a population due to environmental mutagens the increase in human misery would be appreciable. The present incidence of such sex anomalies in Europe for men is 0.27 percent and for women 0.14 percent which as it is, is fairly high. Apart from these sex abnormalities which are due to increase or decrease of the sex chromosomes there is a whole series of genetic diseases present in human populations caused by gene mutations. They may be due to dominant mutations (Huntington's chorea, blindness, deafness, Achondroplasia, cleft lip or palate etc.) or recessive mutations (sickle cell anaemia, phenylketonuria, cystic fibrosis, adrenal hyperplasia etc) or sex-linked (muscle dystrophy, haemophilia, ichthyosis etc). These diseases are due to mutations which produce alterations in structural proteins or enzymes and cause metabolic disorders or diseases associated with the formation of organs. Apart from these disorders, of course,

there are the cancers and teratologies. All these diseases can increase in frequency in populations due to the action of environmental mutagens.

These diseases are incurable. Their action can be only suppressed or lessened by medical treatment. Those individuals that survive and reproduce after medical treatment spread their defective genes in the population and cause the increased incidence of these diseases in future generations. In order to get rid of such bad genes it is necessary to eliminate the individuals possessing them, which is to practice euthanasia and this is going against medical ethics. Even then, for recessive mutations elimination of individuals showing the disease will not work, for large numbers of the heterozygotes or 'carriers' harbour these defective genes and therefore such genes will persist in populations forever and also because recurrent mutations will add them to the population at each generation.

The only way, therefore, to reduce the human misery caused by genetic disease is to reduce the *chances* of more mutations arising in the population. This is possible only through the recognition and control of environmental mutagens for in this way we can keep the mutation frequency at least at the present level, New techniques in genetic engineering may revolutionize the curative approach to medical treatment of genetic disease by cutting away the defective genes and incorporating normal ones in their place. But such "operations" are not possible yet, and even if they can be carried out will be very, very expensive. Even if they become cheap, yet "prevention being better than cure" a concerted effort must be made to prevent the occurence of mutations which produce such defective genes. This can be done, as has now been oft repeated , only by controlling the use of environmental agents which are known to be mutagenic.

8.2 Situation in Sri Lanka

Very little research has been conducted in Sri Lanka on genetic diseases. Almost none have been conducted to ascertain the frequencies of such genetic diseases in the population. With regard to the testing of environmental mutagens in Sri Lanka, there is only one recorded survey that has been made. This was a survey for mutagenicity of the pesticides commonly used in Sri Lanka, and was carried out by Mr. V. U. de S. Jayasuriya at the then Vidyodaya Campus of the University of Sri Lanka between 1972 and 1976.

He made three tests each on thirteen pesticides using *Drosophila melanogaster* as the test organism and concluded that none of them

induced significant increases in their sex-linked recessive lethal mutations over the spontaneous level. Nor did they induce any visible mutations in the specific-locus test. But almost all of them (ten to be precise) did induce crossing-over in treated males. This would mean that almost all those pesticides that were tested (Endrin, Gammallin, Azodrin, Fenbar, Deenol, Nicotex, Antimucin, Gramaxone, stam and MCPA to use their trade names) did produce at least single-strand breaks in DNA. Furthermore, as they could induce crossing over in Drosophila males, this could mean that they perhaps could induce crossing over in the somatic cells of man as well. Somatic crossing over could produce cancers and that is a dangerous prospect.

The pesticides were administered to Drosophila larvae in their food which would assure that only a physiological dose would be given to the test organism. As most of the pesticides that were tested were insecticides, they were very toxic to the insect test organism that was used by Mr. Jayasuriya. Six insecticides proved too toxic to be used at all in the tests. But even these extremely toxic pesticides, as well as some of those which could be tested, produced developmental disorders at the very low physiological doses that were used. They can therefore be suspected of being teratogenic in action as well.

Pesticides are only one group of chemicals which can be classed as environmental pollutants as they are so extensively used in Sri Lanka both in agriculture and in public health programmes. Drugs and food additives and petroleum products are also very widely used. No tests have been carried out to find the mutagenic properties of these chemicals. In addition to these there are many chemicals like ayurvedic medicines (decoctions) and various waste products that are very widely dispersed in the environment.

Sri Lanka does not have the laboratory facilities to carry out the more sophisticated (but less expensive) microbial tests nor the more important (but very expensive) mammalian tests. The climate for research in Sri Lanka is not conducive to setting up such laboratories at the moment both for lack of trained scientists and for lack of finances.

Apart from the pesticides which are very extensively used in Sri Lanka as routine sprays and thus can enter our bodies there are a few instances where other mutagenic substances too may thus gain entry into us.

One such agent is Beta-Afflatoxin, the naturally occuring mycotoxin produced by the fungus *Aspergillus flavus*. Beta-afflatoxin can also produce liver damage. There are two possible ways in which this chemical can get into our bodies. One way is through paddy and

rice which is stored in huge ill ventilated stores where the ideal conditions for the growth of the fungus prevails. In this way Beta-afflatoxin can be spread in the population. The other route is via palm oil. Dessicated coconut has to be dried to a particular temperature before oil is extracted. But I gather that this does not always happen. Coconuts with a higher moisture content which can allow the growth of *A.flavus* are apparently being used to extract oil. Along with this oil is extracted Beta-afflatoxin which will again be widely distributed along with the contaminated coconut oil.

Another such proven mutagen that enters our bodies via our food is the yellow dye metanil which although it is not a food coloring agent is supposed to be used to colour 'pol sambol' in certain small hotels. Metanil is also recognised as a powerful carcinogen.

Due to lack of refrigeration facilities and scarcity of ice some fisherman are supposed to treat fish, which they want to store, with formalin. Formalin as we have discussed earlier on is a mutagen which is very specific in action.

Finally, almost all of us use drugs at least a few times in our lives. In Sri Lanka with the spread of western medical practices we are wont to use them rather indiscriminately (like pesticides on food crops) Aspirin has been shown by the Russians to be weakly mutagenic. X-rays are used very widely in hospitals. Many antibiotics are being used some of which exert their anti-bacterial (or bacteriocidal) action by poisoning the cellular machinery of bacterial cells. They are, therefore, cytotoxic and mutagenic. We, of course, do not know what is present in ayurvedic decoctions. Apart from the extracts of various medicinal herbs, it is known that various inorganic substances (even substances like mercury) also go into these decoctions. Even with regard to western medicines and drugs we obtain them cheap from certain countries where we cannot be altogether sure of their quality. These are possible ways by which mutagens can get into us with our medicines.

8.3 **Some proven environmental mutagens**

The above section dealt with some definite examples of mutagens that can be fairly widespread in Sri Lanka. There are a host of substances which have been shown to be positively mutagenic and whose use (and manufacture) have been banned in the advanced countries. We shall now look at some of them.

a) **Inorganic Compounds** - Arsenic, chromium, and nickel have been shown to be carcinogenic while arsenic has also been shown to be mutagenic.

b) **Asbestos** - Asbestos has been shown to be both mutagenic and carcinogenic. It is supposed that the mutagenic (carcinogenic) action is due to the size of its fibres (5-10 millimicrons in length and less than 0.5 millimicrons in diameter) for glass of the same size show similar mutagenic properties. It is possible, therefore, that these minute fibres physically pass into cells and get entangled with the chromosomes thus damaging them. In Sri Lanka we have a thriving coconut fibre (coir) industry. We do not know whether coconut fibres too can have such effects; perhaps they do.

c) **Polycyclic aromatic hydro-carbons (PAH)**

These are about the best known examples of environmental mutagens, (and carcinogens). Some particular PAH's like benzo (alpha) pyrene benzanthracene, dimethyl (alpha) anthracene, and dibenzo (dihydro) pyrene are the definite mutagens (or carcinogens) while others like benzo (e) pyrene, anthracene, phenanthrene are not mutagenic or carcinogenic.

The PAH substances are all pyrrolytic products of coal, wood, tar, asphalt, cigarettes, etc. and are present in the air as aerial pollutants. They can be found in the water and soil as well, being washed down by rain.

d) **N-nitroso compounds:** The N-nitrosoamines and the N-nitrosoamides which are produced from nitrites (added to food as preservatives) are the two most important groups that are both mutagenic and carcinogenic either (i) after activation by other chemicals or (ii) by themselves. Dimethylnitrosoamine and nitrosopyrrilidine are two products found in meat after nitrite treatment.

e) **Naturally occuring compounds:** The mycotoxins, especially afflotoxin from *Aspergillus flavus*, are the best known of the naturally occuring mutagens. Patulin and cyclochlorotin (from *Penicillium sp.*) and sterigmatocystin (from *Aspergillus sp.*) are other toxins from fungi. These have proved to be both mutagenic and carcinogenic in tests. These fungi are found in many stored food products but mainly in bean and corn.

Several plants produce pyrrolizidine alkaloids (eg. Heliotrine) that are carcinogenic and mutagenic. Safrole which is present in the oils of several spices like nutmeg, mace, ginger, cinnamon and black pepper (from 1-10% in the oil) produces tumours in rats and mice.

(f) **Vinyl chloride** which is the basic chemical substance used in many industries eg. the polyvinylchloride or PVC piping industry, has been shown to be mutagenic in the Ames microsomal assay test.

(g) **Pesticides:** Dichlorvos, Dimethoate, Bidrin, Dibromoethane, Folpet, Captan, and DDD (a metabolite of DDT) have been shown to be mutagenic in the same test.
(h) **Drugs:** The anit-Schistosomal drug hycanthone has been shown to be mutagenic. The anti-trypanosome drug Ethidium Bromide, LSD and even aspirin and alcohol are supposed to be mutagenic.
t(i) **Others:** A host of other substances found in the environment that are actively ingested by man like caffeine, benzene, toluene, ylenes, phenols, acrolein, chlorinated benzo-p-dioxins, coumarines, eic., have been suspected or shown to be mutagenic by themselves or after interacting with other chemicals in the environment.

8.4 Risk-Benefit Analysis

Once a chemical has been unambiguously identified as being mutagenic many other factors have to be considered before legislation can be enacted to ban its manufacture or use. The mutagenic risks of the chemical has to be weighed against its benefits. In the case of a pesticide, for instance, if its withdrawal means the spread of a killer disease like malaria and if no less harmful pesticide is available then it is unwise to ban the pesticide, Or, if the use of the pesticide on agricultural crops, especially cereals, is banned and yields drop due to pest infestations and people die of starvation, then obviously such a ban should not be imposed. *To evaluate the risk-benefits of a suspected mutagen specialists in various fields of study have to be consulted.*

8.5 Environmental Mutagen Societies (EMS)

Although Muller had been concerned with the problem of environmental mutagens (especially the irradiations from atom bomb tests) from the 1940s and Auerbach had been concerned with the addition of formaldehyde to pig-feed in the 1950s and both had the foresight to see all the implications of the presence of such mutagens in the environment with great clarity and vision, other geneticists - did not react to their call until much later. It was only in 1972 that the first EMS was formed in the U.S. Following this lead, other societies were soon formed in Europe (and Great Britain), Japan and India.

These societies hold regular meetings, symposia and seminars to discuss basic problems of mutagenicity testing as well as specific problems relating to their respective countries.

8.6. International Conferences

Members of the EM Societies and other individuals interested in this very important ecological aspect of genetics needed to exhange views and ideas among themselves and to co-ordinate their efforts on a global scale.

For this purpose the first International Conference on Environmental Mutagens was held at Asilomar in California in September 1973 where 372 delegates from all over the world congregated and discussed all problems associated with these genetically toxic agents.

The second International Conference was held four years later in July 1977 in Edinburgh, Scotland. More than double the number of delegates that came to the first conference attended this second one.*

8.6 Environmental Mutagen Information Centre (EMIC)

A centre for co-ordinating all the information relating to toxico-genetics has been set up in the USA at the following address:

The Environmental Mutagen Information Centre (EMIC)
Oak Ridge National Laboratory
Oak Ridge
Tennessee 37830
USA

The Director of this Centre is Dr. J.S. Wassom.

The main function of the Center is to collect all available literature on Mutagenesis and to produce annually a thoroughly cross-referenced bibliography of such a literature survey. All the information is stored in a special computer.

8.8 Concluding remarks

Mutagens, carcinogens and teratogens are found occuring naturally in the environment. They have existed on earth for a long time, too. But it is the recent activities of man that have increased their incidence in the biosphere. As this increase is due to the action of man, their control is also in his hands. Hence the active interest and research into genetic toxicology in recent times.

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* The Third International Cenference of Environmental Mutagens was held in 1981 in Tokyo.

Over 10 — 20,000 new chemicals are also being synthesized annually all over the world. Some of them come into general use each year if shown to have some useful property. These have, therefore, to be tested throughly before use. Over the last few centuries many such chemicals have been added to the environment due to their usefulness. Some of them are still being used. These have also to be tested rigorously, risk-benefits evaluated and necessary action taken to limit the use of those substances that are shown to be definitely mutagenic and carcinogenic.

Some substances may have to be banned. But even the worst substance may have some good use and hence should be manufactured and handled with great care like the radioisotopes, but those substances which are used by the public are the ones which have to be banned if proved to be mutagenic. Ways and means must be developed to reduce the spread of naturally occuring mutagens. As these substances are found mostly in our food due to improper storage conditions, all steps must be taken to improve storage facilities be it for paddy, rice, legumes or coconut.

To be able to do all this as stated earlier a well informed public is essential. Ultimately, therefore, it is to them that this booklet is really intended so that they may not be panic striken when the subject is discussed in the newspapers, but use this knowledge as far as possible to cope with the problem.

Further Reading

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