EFFECT OF SOME CHEMICALS AND X-RAYS ON THE CHROMOSOMES OF BONE MARROW CELLS OF MICE, MUS MUSCULUS

By

Ardhendu Bikas Mitra, M.Sc.*
C.S.I.R. Fellow
Department of Zoology
Faculty of Science
University of Kalyani
Kalyani
West Bengal
India

INTRODUCTION

The mechanisms of hereditary changes are better understood by their study from artificial means than natural ones. Studies of artificial production of chromosomal aberrations and gene mutations are directly connected with various fundamental and applied problems in biology. Mutation is an event which occurs within

* Present Address : Research Officer, Department of Pathology, S.N. Medical College Agra, U.P. (India).
the cell. It brings changes in the somatic as well as in the germinal cells. It has been encountered in all organisms, from virus to man. It forms the basic source of all heritable and few somatic variations. In spite of all these facts a precise definition of mutation is wanting because it embraces various types of manifestations. The mutation may produce on the one hand lethal effect having various degrees of penetrance and expressivity and on the other, visible effects of various categories. Cytogeneticists, in some broad sense, group mutations in the following two main categories: (1) "Point mutation" or change in the molecular composition of the genetic material undetectable by ordinary cytological means but revealed by the phenotypic changes of the organism and (2) "Chromosomal mutation" or change in the chromosome which is generally detectable under microscope. In the diploid organisms the latter ones are manifested in the form of (a) changes in whole set of chromosomes e.g. haploids and polyploids; (b) changes in whole chromosomes as nullisomics, monosomics and polysomics; (c) changes in relations of parts as inversions and translocations and (d) changes in amounts of portions of chromosomes as duplications and deficiencies. Any of these changes may be termed as mutation because in the phenotypic expression
no sharp distinction between the point mutation and chromosomal mutation can be made. When the chromosomal changes are very minute and reach beyond the resolving power of light microscope a distinction between point mutation and minute deletion is not possible. This fact has led some early workers to cast doubt on the reality of the occurrence of point mutation. According to Stadler (1932) point mutation is nothing but the structural changes of very minute nature and the difference between gene mutation and chromosomal mutation is not always very sharp. Goldschmidt (1951) was also in favour of considering all mutations as due to structural rearrangements of chromosomes. However, Dobzhansky (1947) and other workers believe that many mutations are caused by the changes within the gene itself and the heritable materials possess an eternally mutable chemical composition. In the present time biochemical aspects of mutations are known better than the chromosomal abnormalities. But both of them have some common features too. According to Freese (1963) point mutations result from the changes in DNA component of the gene. These changes occur in the number and nature of the base composition. The changes in the base composition possibly occur in two ways - transition and transversion. In the transition, a purine is replaced by another purine or a pyrimidine is replaced
by another pyrimidine and in transversion a purine is replaced by a pyrimidine and *vice versa*. Thus in the point mutation, since change occurs in the base pair, no visible change would be expressed in the chromosome structure. In the case of chromosomal mutation, alteration in the chromosomal structure of very minute to large detectable breaks may be caused due to the involvement of the DNA strands or/and some constituent parts of the chromosomes. The chemical architecture of chromosome is more complex than that of the genes. Thus it is very likely that point mutation and chromosomal mutations may be basically different as well as similar. Structurally and functionally they are not easily demarcable. Anyhow, in the present study we are concerned with the detectable changes in the chromosomes produced by various chemicals and x-rays.

The study of the artificial production of mutation or chromosome aberration will have special significance because it would reveal similar phenomenon occurring spontaneously in Nature. Before we pass on to review some literatures regarding the production of chromosomal aberration or mutation by artificial means, we would better examine briefly the phenomenon of spontaneous mutation and chromosome aberration. It is well known that all the Mendelian factors are nothing but the mutated
genes. The frequency of various types of spontaneous mutations has been studied in various organisms amongst which Drosophila can be cited as the classical example. However, we are restricting our review more on the spontaneous chromosomal aberrations because they are related to the present study. These have been found to occur both in plants and animals. Various types of manifestations of them can be grouped into two major categories - (1) physiological effect like stickiness, pycnosis, despiralization, etc. and (2) structural alteration and variation in number of chromosomes. Stickiness is quite common in many materials and they are produced under various physiological conditions of the materials. In coleopteran insects it is a matter of common experience that the spermatocyte chromosomes of old adults are sticky in comparison to those of newly hatched or young adults. Physiological effects of different categories are very commonly met within cancer cells. It is a matter of common experience that on some occasion the stickiness of the chromosomes can be seen in the cytological preparation of some of the individuals of a species.

Vast literatures have accumulated with regard to the spontaneous aberrations of chromosomes. In the following
we will be citing some of the well-known examples. It is not the intention of the present author to make a comprehensive review of the spontaneous chromosomal aberrations since it will be beyond the scope of the present study. In plants several examples of polyploid species have been discovered. Darlington (1937) considers that about half of the species of angiosperms owes their origin due to polyploidy as evidenced from the chromosome number. There can be no doubt about the great role played by polyploidy in the evolution of caryotypes in different plant species but it is less so with the species of animals (Muller, 1925; White, 1954). Since animals generally reproduce by sexual means, the occurrence of polyploidy may cause some hindrance in the process of gametogenesis. The number of well accepted polyploid species in animals is very limited (see White, 1954). They are more common in the groups of animals where parthenogenesis or hermaphroditism is present. White (1954) has objected to the claim on the occurrence of polyploidy in some animal species made by some workers because these were not based on sound reasonings. The phenomenon of polysomy and its cytogenetical implication has been well documented in Datura by Blakeslee and his co-workers (Blakeslee, 1922, 1928; Blakeslee and Belling, 1924). Amongst animals polysomy is more frequent than polyploidy.
The cytogenetical studies have been fruitfully made with respect to the IV chromosome (Bridges, 1921) and the X chromosome in *Drosophila* (Bridges, 1914) and in recent years with regard to various congenital diseases in man (see Ashley-Montagu, 1961). Hundreds of chromosomal anomalies in relation to human diseases are being reported every year after the improvement and introduction of various techniques for better handling the chromosomes. However, the variation in chromosome number due to change in whole set or the whole chromosome has, most likely, not played a very significant role in the evolution of caryo-types in animals like plants (Manna, 1969). Probably inter- and intrachromosomal structural changes have played the most significant role. Such changes are often expected to lead to manifold types of sterility and inviability. In spite of these handicaps, there are abundant evidences that some of them manage to establish in Nature. They form the basic source in the evolution of caryo-types in various species. The types of structural rearrangement and their consequences have been excellently reviewed by several workers (Darlington, 1937; Dobzhansky, 1951; White, 1954; Swanson, 1957; Manna, 1969). The spontaneous structural rearrangements leading to the evolution of the caryo-type in a species in a natural population is established through chromosomal polymorphism. However
in comparison to animals, because of the various types of propagative methods occur in plants, structural heterozygotes are commonly met with in plant species. Spontaneous chromosomal aberrations have been recorded from lower to higher groups of plants by various workers (McClintock, 1939, 1941, 1942a, b, 1943, 1944; Darlington and Upcott, 1941; Olenov, 1949; D'Amato, 1948). Nichols (1941) observed the spontaneous breakages of chromosomes in Allium cepa. Spontaneous aberrations have also been studied in Nothoscordum (D'Amato, 1948) Trillium (Sparrow and Sparrow, 1950), Bromus (Walters, 1950, 1951) Antirrhinum and Vicia (Kihlman and Levan, 1951; Sharma and Bhattacharya, 1956a); Hyacinthus (LaCour, 1953), Scilla (Rees, 1952), etc.

Lewis and John (1966) have studied the meiotic consequences of spontaneous breakages. Translocation type of structural rearrangements have been evidenced in different groups of animals. Some workers like Robertson (1916) Matthey (1931, 1933), Smith (1950), White (1954) etc. have shown that translocation in the form of whole arm transfer has been the main source of changing the chromosome form from rods to V. In such cases two acrocentric chromosomes gave rise to a metacentric one and a supernumerary chromosome originated as a bye product. The latter in course of time was lost. This type of change has been
recorded in different species of grasshoppers, gryllids, beetles, lizards, mammals etc. Manna (1967) has estimated the number of species possessing metacentric chromosomes due to the whole arm transfer in grasshoppers. The change in caryotypes due to "Robertsonian principle" (Robertson, 1916) in the form of \( A + A = M \) (whole arm transfer) has been claimed in different species of urodale amphibians by White (1961) and in lizards by Matthey (1931, 1933, 1939, 1945). The data of latter group have also been extended by Asana and Mohabale (1941), Makino and Momma (1949) and others. Large number of evidences have also been obtained in mammals (see Matthey, 1949, 1962, 1963, 1964, 1965, 1966, 1967, 1968a,b,; Manna, 1969; Hsu and Benirschke, 1967, 1968). Similar phenomenon has also been recorded in other groups which has been reviewed by White (1954). This has been the main way in the evolution of caryotypes in Gryllidae (Ohmachi, 1935). In Coleoptera, evidences of fusion mechanism have been obtained in two very well known genera *Pissodes* (Manna and Smith, 1959) and *Chilocorus* (Smith, 1956, 1957, 1958). The evolution of caryotypes due to fusion has been evidenced in large number of species of *Drosophila* (Patterson and Stone, 1952). Origin of metacentric type due to fusion has also been observed in different plants (see Darlington, 1937). Besides the phenomenon of whole arm transfer, translocation of
other types has been evidenced in different groups. However, the examples of heterozygotes for reciprocal translocation among animals are rather limited. In *Drosophila* the frequency of translocation heterozygotes has been found to be much less than the inversion heterozygotes (see Dobzhansky, 1951; White, 1954; Manna, 1969). Translocation heterozygotes have been obtained in natural populations of *Drosophila ananassae* (Dobzhansky and Dreyfus, 1943; Ray Chaudhuri and Jha, 1966), in various species of scorpion (Piza, 1939, 1947, 1948, 1949, 1950), grasshopper (Carothers, 1913, 1931; Helwig, 1942; White, 1949, 1951, 1956) gryllid (Manna and Bhattacharjee, 1962, 1964) and so on. Translocation heterozygotes has also been evidenced in human beings (Ashley-Montagu, 1961). In plants translocation heterozygotes are quite common in different species of the genera *Tradescantia* (Belling and Blakeslee, 1924), *Vicia*, *Allium*, *Oenothera* etc. (see Darlington, 1937).

The occurrence of inversion heterozygotes has been found to be very common in the genus *Drosophila* and on this basis some phylogenetic relationship has been established in the different populations of *D. pseudobscura* and *D. persimilis* by Dobzhansky (1951). Inversion heterozygotes have also been observed in some species of
The structural heterozygosities due to deletion and duplication have also been studied cytogenetically in Drosophila (see White, 1954). Similar phenomenon also exist in other species of animals but in those cases cytogenetical correlations have not been so clearly demonstrated as in Drosophila. Spontaneous chromosome aberrations have been recorded in good number of tissue culture strains (Manna 1961, 1962; Harris, 1964).

Most of the examples referred to above represent the permanent changes in the chromosome structure which could manage to establish in natural populations. Spontaneous large number of aberrations like simple chromatid breaks, bridges and gaps are also produced. As a matter of fact only a very small fraction of the total amount of aberrations could be traced because most of them are lost during cell division. They may also undergo restitution. On the whole the frequency of spontaneous chromosomal aberration is extremely low. Therefore, for obvious reason their study in the artificial condition would enable us to have more insight into the problem.

**Induced Chromosome Aberrations:**

At present different agents which are known to
cause chromosomal aberrations and mutations can be grouped into three categories. They are (1) living agents - viruses and bacteria, (2) physical agents and (3) chemical agents. Since our study is connected with the effects of some chemical mutagens and X-rays, a very brief survey on the actions of different mutagens has been made in the following.

**Virus and Bacteria induced chromosomal aberrations**:

It has been discovered in recent years that the pathogens like virus and bacteria can induce chromosomal aberrations. So far as the present author is aware chromosome breakages due to virus infection was reported for the first time by Hampar and Ellison (1961). Since then several cytologists have been working on the virus-induced chromosomal aberrations. Mazzone and Yerganian (1963) have shown the chromosomal damages in the cells of Chinese hamster which were infected with **Herpes simplex** virus. Fjelde and Holtermann (1962) have studied the effect of measles virus on the chromosomes of HEP-2 culture cells. Nichols (1963) has studied the effect of SR strain of Rouse sarcoma virus in producing chromosomal breakages in diploid embryonic cells. Similarly, Stich et al (1964a, b) working with adenovirus, Boue et al (1964) with rubella virus, Saksela et al (1965) with Sendai virus obtained chromosomal aberrations of various nature. Works of

The mechanism of virus induced chromosomal aberration has not been so elaborately studied as compared to the radiation or chemical induced one. This is due to the fact that this field of study has a recent origin. In spite of that, this field is receiving due attention of many cytologists. Some probable modes of action by which viruses may produce effect on the chromosome have been suggested by different workers. There may be a direct "attack" of viral agent on chromosomes or it may combine with components of chromosomes. On the other hand, it may interfere in the DNA synthesis either by inhibiting cellular enzymes or by competing with the nucleic acid building blocks. It may also produce its effect mediated through the cytoplasmic organelle - lysosome. Their effect on lysosome in turn will release DNase which may act upon the chromosome structure causing aberrations.
In order to determine the mechanism or pathogenesis of virus induced chromosomal aberrations various methods have been employed. Some are based on comparative data. For example like viruses there are chemicals which inhibit the DNA synthesis and cause similar chromosomal aberrations. Preliminary studies on radioactive viruses have also been made. Whether the mechanisms of actions between the virus induced chromosome breaks and those associated with the inhibition of DNA synthesis are the same or not are not known but some similarities between the two systems are quite apparent. These include (1) similarities in the morphology of the chromosomal aberrations, (2) effect as mitotic inhibition (Kihlman et al 1963; Nichols, 1964; Nichols and Heneen and Peluse, 1965), (3) the ability of both to produce chromosomal aberrations shortly after the exposure (Kihlman et al 1963; Nichols and Heneen 1965), (4) the localization of breaking point (Nichols, Levan, Aula and Norrby 1964) Nichols, Heneen and Peluse (1965), (5) the long term effects and virus transformation (Nichols and Heneen, 1965) and (6) the synergism between RSV-SR and CTP (Nichols, Heneen and Peluse (1965). But the similarities are by no means indisputable in all cases. It is assumed that in a virus infected cell there are two potential
mutagenic events. The first is the addition of the virus genome to the infected cells, and the second is the alteration of the genome of the cell itself. On the other hand, according to Temin (1964) the conversion of a benign cell to a malignant one by the virus occurs in three steps - (1) formation of new genes, (2) activation of new genes and (3) the expression of new genes. The chromosomal damage induced by virus can be inhibited by the introduction of excess amount of normal deoxyribosides or deoxyribonucleotides (Nichols, 1963).

The different mechanisms discussed above are only a few of the several potential mechanisms capable of inducing chromosome breaks. It has been demonstrated in bacterial systems that infections of some bacteriophage are capable of producing an increase of DNase (Pardee and Williams, 1953). This possibility has been followed in mammalian cell systems by Allison and Paton (1965) to explain the chromosome breaks with the activation of lysosomal enzymes. Any mechanism alone or in combination with other may produce the observed chromosomal effects. Before we pass on to the effects of physical and chemical mutagens, a brief survey on the effect of another biomutagen, the bacteria, on chromosomes will not be very much unwarranted in our present study.
Although some advancement in the field of virus induced chromosomal aberrations has already been made, the field of bacteria induced chromosomal aberrations remains almost unexplored. Truly speaking it has just started. As far as the present author is aware Kaplan (1948) was the pioneer in starting this field of study. Later on Montezuma-de Carvalho (1955) reported the radiomimetic effects of a number of bacteria and their products in the root tips chromosomes of *Vicia faba*. Unfortunately no progress was made in this new field.

Further, until recently the effects of bacteria on animal chromosomes were unknown. It has been reported by Manna and his collaborators from our laboratory that bacteria like viruses can induce chromosomal aberrations in mammals (Manna and Bhowmick, 1969; Manna and Chakrabarti, 1970a,b; Chakrabarti and Manna, 1969, 1971; Manna, Ghosh and Mishra, unpublished). Since the discovery, they have been studying various aspects of bacteria induced mammalian bone marrow chromosome aberrations. They have been able to show that the pathogenic bacterium, *Staphylococcus aureus*, when injected into the body of the mice, could induce various types of chromosomal aberrations in the bone marrow cells. The aberrations have been found in the form of C-mitosis, erosion or condensation of chromosome, chromatid break, translocation, etc. Further, it has also been shown that
the bacteria free culture filtrate when injected into the body of the mice similar types of effects were produced. On the other hand, isolated bacteria mixed with distilled water, if injected into the body of the mice produced similar chromosomal aberrations. The injection of inactivated bacteria also produced chromosomal aberrations as those of other series but the effect was mild. The bacteria induced chromosomal aberrations have been observed as early as 5 minutes after the injection and they continued for several days. It has not been possible for the authors to make any definite discussion as to the cause of aberrations produced by the bacteria in culture medium, bacteria free culture filtrate isolated bacterial cells mixed in water, and by inactivated bacteria since the work is at present in the explorative stage. Anyhow, they are searching for the causative factor. Further comment is, therefore, not desirable. Only it is pointed out that there might have some relation of this study with the chemically induced chromosome aberrations because we will find in the following pages that a good deal of similarity exists between the aberration types produced by the two groups of mutagenic agents.

**Physical agents:**

The study on the effects of ionizing radiations on cells dates as far back as the beginning of this century.
(Bergonie and Tribondeau, 1904, 1906; Perthes, 1904). Its real implication could be realised only after the pioneering work of Muller (1927) and Stadler (1928). Since then a vast amount of literatures have been accumulated on chromosome aberrations induced by various types of ionizing radiations like X-ray, $\beta$-ray, $\gamma$-ray etc. Because of the vastness of the data it is not possible to make a precise review within the present limited scope and space. Moreover, excellent reviews in this field have been published by various workers from time to time (Carlson, 1941; Sax, 1941; Hollaender, 1954; Bacq and Allexander, 1955, 1961; Kihlman, 1961; Ray Chaudhuri, 1961a, b; Evans, 1962; Wolff, 1957, 1960; Wolff and Luippold, 1955, 1956a, b; Sparrow, 1962; Bender, 1964; Manna, 1969 etc). Studies on various aspects of ionizing radiations on animal cells have frequently been carried out on Drosophila, grasshoppers and mammalian materials. Some are directed to the verification of the target hypothesis, some with the mechanism of induction of breakages and rearrangements, some with the applied aspects like therapy, crop improvement, etc. The effects of X-ray on chromosomes can broadly be classified into two categories; they are (1) the primary or physiological effect during which the chromosomes are generally pycnotic and sticky and (2) the secondary effect when really the damages done to the chromosome could be
studied (Alberti and Politzer, 1923, 1924). The effect of radiation has been studied on the somatic as well as the germinal cells. The importance of these studies is fundamentally different. The study of the effect on the somatic chromosome will be more related to the therapeutic use, the somatic mutation, radiosensitivity, etc. while the same on the germinal chromosomes will primarily be related to the hereditary changes.

The effect of X-rays on mammalian cells have been studied from various standpoints. The problem of radiosensitivity has been studied in animals belonging to different groups using various indices like lethality test, somatic mutation rate, growth inhibitory effect, aberrations of chromosomes, stages of division, etc. The employment of cellular constituents such as nucleus, chromosomes etc. in testing the radiosensitivity is all the more important to us for their hereditary implications. Some materials used in the study are protozoa (Halberstaedler and Back, 1942), Drosophila (Pontecorvo, 1942; Sonnenblick, 1940) grasshopper (Manna and Mazumder, 1968) amphibia (Piatt, 1962; Bardeen, 1911) chick (Lasnitzski, 1943a,b) various mammals (Spalding and Brooks, 1962 and vide infra) etc. More of the mammalian examples will be referred to hereunder. The effect has been studied on mice embryo by a number of workers (Furth and Furth, 1936; Quastler, 1945).
Russel and Russel, 1950; Abrams, 1951). The dividing cells of testes of rodents exhibit differential radiosensitivity (Snell, 1935, 1941; Henson, 1942). Effect on oogenesis has also been studied (Beaumont, 1962). Differential radiosensitivity depending on the age factor in mammals including man has been studied by several workers (Wilson and Karr, 1950; Lindop and Rotbliat, 1962; Shapiro et al, 1963). At the chromosomal level this aspect has been studied in different types of cancer because it is intimately connected with its radio-therapy. It has been shown that the chromosomes of Walker-256 are more sensitive to X-rays and neutrons than those of lymphosarcoma of rats (Marshak and Bradley, 1945). Not only the chromosomes of cancer tissue but also of the normal one have been employed in the study of radiosensitivity. The chromosomes of different normal tissues in culture condition have been found to be differentially radiosensitive. The responses between the epitheloid and fibroblast cells of Chinese hamster, man and monkey to X-rays have been found to be different (Bender, 1960). The differential radiosensitivity has also been studied in the spermatogenetic stages of various rodents (Snell, 1941, Hertwig, 1941; Henson, 1942). Besides the above studies, the effect of whole body radiation on mice and man has been studied by some workers (Conkite and Bracher, 1952; Wahrman and Robinson, 1963). The present author, in order to make a comparison of the nature of
breaks caused by ionizing radiation and chemical mutagens as well as for the study of some other problems, irradiated mice with X-rays. The effect of X-rays on the chromosomes of bone marrow cells of laboratory mice has been studied both qualitatively and quantitatively (Chapter I). Further the effect in conjunction with some chemical has also been studied (Chapter II).

Chemical Agents:

The study on effect of chemical mutagens is equally important, if not more than that of ionizing radiations because of their wider uses in practical life. The knowledge can be utilised in (a) chemotherapy, (b) radioprotection (c) improvement of agriculture and live stocks and so on in the practical side and on the other, for understanding some fundamental problems relating to (a) chemistry of chromosomes, (b) nature of gene (c) mechanism of chromosome breakages (d) relationship between physical and chemical mutagens, etc. It is generally observed that the chemicals which possess chromosome breaking activities have also the ability to induce gene mutation. Further, since there is the general agreement between the types of breaks produced by X-rays and some chemicals, the latter ones are called radiomimetic chemicals. The possibility of artificial production of
mutations by the treatment of chemicals has been extensively explored by numerous workers (Oehlkers, 1943; Auerbach and Robson, 1947; Levan and Tjio, 1948a,b; Levan, 1951; Darlington, 1953; D'Amato and Avanzini, 1954; Koller, 1954; Eigsti and Dustin, 1957; Biesele, 1958; Sharma and Sharma 1960; Wilson, 1960; Rosen, 1957, 1964; Rieger, 1965; Schoneich, 1965; Kihlman, 1961, 1966; Auerbach, 1967; Manna, 1969) after the discovery of mutagenic action of radiation. In general, the basic idea is that the mutagenic agent whether chemical or physical produces active radicals which in turn act on the genetic material. It is generally accepted that organic peroxide is responsible for the mutagenic action of ionizing radiation but for chemical mutagens other mechanisms may also operate. Anyhow, regarding mutagenic action of chemical the positive evidence was obtained by earlier workers. They found that the application of nitrous acid caused mutation in *Aspergillus*. This finding like some other reports, escaped attention of many workers. Later Oehlkers (1943) made an extensive study on the radiomimetic action of urethane on plant chromosomes. Since then radiomimetic effects of various chemicals have been tested on plant and animal chromosomes. The mutagenic action of chemicals on animal material was clearly demonstrated at first in *Drosophila* by Auerbach and Robson (1947). They established that the
treatment of nitrogen mustard was as effective as the ionizing radiations in the artificial production of mutations in *Drosophila*. Hundreds of chemical compounds are at present known which show radiomimetic activity. These radiomimetic chemicals are of diverse nature. Their range extends from simple inorganic salts to the highly complex organic compounds (see Sharma and Sharma, 1960). These chemicals have been tested on different experimental materials.

It seems that in the study of chemicals mutagenesis the plant materials have been employed more than the animal materials. This may be due to the fact that the plant materials are rather easy to be employed for experimentation. Root tips, pollen mother cells, leaf buds, etc. have generally been used because they serve as the ready source of dividing cells. Root tips serve as the best source of obtaining mitotic chromosomes while the pollen mother cells for meiotic chromosomes. Plants most commonly used are (1) *Vicia faba* employed by Ford (1949) Darlington and McLeish (1951); Tjio (1951); Read and Kihlman (1956); Rieger and Michaelis (1960, 1962, 1963, 1964); Kihlman (1960, 1963a,b, 1964); Natarajan and Upadhya (1964); Herich (1965) and others and (2) *Allium* sp. employed by Levan (1938, 1940); Nybom and Knutsson (1947); Levan and Loffy (1949); Novick and Sparrow (1949); Nygren (1949).
D'Amato (1950); Kihlman (1950); Wilson (1950); Levan and Tjio (1951); Smith and Lotfy (1954); Sharma and Mukherjee (1955) and others. Besides them, other plants used are Zea mays (Ericksson and Rosen, 1949); Pisum sp. (Rosen, 1957, 1964, Nethery and Wilson, 1966), Hordeum vulgare (Ramana and Natarajan, 1966), microspores of Tradescantia (Steffensen, 1953, 1955); Nakahara and Komoto, 1957) and so on. Since the beginning of the study of chemical mutagenesis regular reports appeared on plant materials (see Oehlkers, 1943; Ambrose and Gopal Ayengar, 1953; Darlington and LaCour, 1938; Darlington and McLeish, 1951; Darlington, 1950; Levan, 1938, 1940, 1951; Levan and Tjio, 1948a, b, 1951; Kihlman, 1949, 1950, 1952a, b, 1955a, b, 1956, 1957, 1961, 1962, 1966; Loveless, 1953; Sharma and Sharma, 1960; Wilson, 1960; Wilson and Morrison, 1958; Rosen, 1957, 1964; Yakar, 1952) than on animals (see Biesel, 1958) which left a wrong impression that plant chromosomes are more vulnerable than animal chromosomes to the effects of chemicals (Ray Chaudhuri, 1961a). In recent years, the effects of a large number of chemicals on the meiotic and mitotic chromosomes of grasshoppers and mammals have been studied in our laboratory (see Manna, 1969). The response of plant and animal chromosomes to chemical mutagens may differ. It has been seen in some cases that chemicals which were active in producing chromosome breakage
in mammalian cells, were ineffective in plant cells and vice versa. Some chemicals like diaminopurine, purine riboside, BUdR, cytosine arabinoside, hydroxylamine etc. are effective in producing chromosome breakages in tissue culture cells of mammals (Bieselev et al 1952, 1955; Hsu and Somers, 1961, 1962; Somers and Hsu, 1962; Kihlman et al 1963) but they have been found to be ineffective in the similar sense in plant cells (Rieger and Michaelis, 1962; Kihlman, 1962, 1966). Again maleic hydrazide and 8-ethoxycaffeine produce chromosome breakage in plant cells but they have no marked effect on mammalian cells (see Kihlman, 1966). The differential response to a particular chemical has also been found in the different species of grasshoppers (see Manna, 1969) or in plants (see Kihlman, 1966). Ethyl alcohol has been found to cause chromosome aberration in Phloeoba antennata but it failed to cause similar effect in the X chromosome of Oxya velox (Manna and Mazumder, 1964).

As mentioned before amongst animals Drosophila have extensively been used for experimentation. The effects of mutagens have been tested on the different stages in the life cycle of Drosophila beginning from the egg to adult (Glass, 1956). Several workers have used a wide variety of chemicals administering them with the food or by injection into the larva or the adult to study
their effects as mutagen (Alderson, 1964; Auerbach, 1947, 1950, 1953, 1967; Pelecanos, 1965; Rohrborn, 1959; Beermann, 1965; Clark, 1960; Fedoroff and Milkman, 1965; Jenkins and Simsons, 1968; Khan, 1968; Mazar, 1968; Sram and Ondrej, 1968 etc.).

The mitotic and meiotic chromosomes of grasshoppers have been used to test the effect of various chemicals by several workers because cytologically they are considered to be very ideal material (Yosida, 1950; Gaulden and Carlson, 1951; Sarkar, 1957, 1958; Manna and Mazumder, 1964; Manna and Parida, 1965a,b, 1967, 1968 and unpublished; Manna and Lahiri, 1966). Mammalian materials have also been extensively used by a large number of workers to test the effects of various chemicals (see Biesele, 1958; Rosin, 1951; Kihlman, 1966; Manna, 1969). In these materials chemically induced chromosome aberrations have been studied both in in vivo and in in vitro condition. In the earlier days studies were mainly carried out on tissue culture cells (see Biesele, 1958). Two types of cell lines were used. They are of (1) normal and (2) cancer tissue. Sometime past we have been using the chromosomes of bone marrow cells in the study of the effects of various chemical mutagens (see Manna, 1969). It must be kept in mind that cells in culture do not necessarily respond in the same way as cells in in vivo condition. This may be due
to the different environmental conditions prevailing in the two situations. It has been found by many workers that the chromosomes in culture condition underwent changes even when they were not treated with any chemical. The best example is the L-strain and its derivatives. Now this long-established cell line neither have the normal diploid number of chromosomes nor the original caryotype (Manna, 1962). Various other cell lines have also been found to deviate from their normal diploid chromosome constitution. In Jensen sarcoma and its cell line derivatives very high frequency of translocations and chromosome breaks have been reported (Hsu and Manna, 1959; Manna, 1961). Thus in one sense the effect of the chemicals on the chromosomes in *in vivo* condition would furnish a more reliable data than those obtained from *in vitro* condition. As mentioned before chromosome aberrations induced by chemicals have been extensively studied on tissue culture cells (see Biesele, 1958) and *vide infra*. Kihlman, Nichols and Levan (1963) have studied the effects of cytosine arabinoside and deoxyadenosine on the cultures of peripheral white blood cells. Normal and cancer tissues from different parts of various animals mostly of mammals, have been employed to study the effectiveness of the chemicals on the chromosomes (Biesele et al 1952, 1955; Makino et al, 1953 1955, 1963; Biesele, 1958, 1962; Tanaka et al, 1955;
Arrighi and Hsu, 1965; Shojt and Kimura, 1964; Banerjee and Ray, 1967; Das and Manna, 1968, 1969, 1970; Mitra and Manna, 1968, 1969; Manna, 1969). With the progress of leukocyte culture technique human chromosome aberration induced by chemicals has been studied in recent years by many workers from different parts of the world (Cohen et al, 1963; Palmer and Funderbruck, 1964; Stich and Yohn, 1964b).

If a generalization is desired of the different cytological effects induced by the treatment of chemical mutagens, they can be classified in different ways. According to some authors they can be divided into three categories viz. (1) cytotoxic effect - involving mainly the spindle apparatus, (2) nucleotoxic effect - involving mainly the chromosomes and (3) the combined effect - involving both the spindle apparatus and the chromosomes. Other workers have tried to group the chemicals according to their chemical properties. On the whole the classifications put forward by different workers as to the effect of different chemicals on cells differ considerably because the approaches were different. Moreover the properties of the chemicals and the experimental materials were different. Kihlman (1966) has discussed the actions of chemicals on the dividing cells from various aspects. He has treated the problems of mitotic inhibition and the production of chromosomal aberrations in Part II of his book.
The process of mitotic inhibition has been considered in terms of (i) cells prevented from entering mitosis and (ii) inhibition acted upon some stages of division when the cell has already entered into mitosis. Further, he (Kihlman, 1966) has divided the chromosome breaking chemicals most of which were used in his own experiments, into five groups viz. (a) DNA precursors and related compounds, (b) antibiotics, (c) alkylating agents, (d) ribose compounds and (e) miscellaneous chemicals. However, cytologically this classification can not be fully appreciated because the chemicals belonging to the same group may not produce similar cytological effect. Thymidine analogues - BUdR, IUdR, FUdR etc. have been found to have different cytological effect in different materials, e.g. BUdR induces characteristic type of aberration in animal cells (Hsu and Somers, 1961, 1962; Manna and Basrada, 1968) but it is ineffective on the chromosomes of the root tip cells of V. faba (Kihlman, 1966). Some other cytologists have also tried to classify the chemical mutagens on the basis of the cytological effects produced irrespective of their diverse nature. But this too was found to be inadequate. Biese (1958) has made a review on the classifications adopted by different workers as given in the following : Bauch (1947) divided the mitotic poisons
into three groups like (1) spindle poison, (2) cell
division poison and (3) chromosome poison, while Ahlstrom
(1951) classified the effects into four categories like
(1) spindle poison, (2) interphase poison (3) caryoplastic
poison and (4) other poisons of ill-defined nature. These
two classifications have very little difference except
the grouping as "other poisons of ill-defined nature"
proposed by Ahlstrom (1951). Levan (1951) attempted
to classify the effects of chemicals in a different way.
This was based on the actions of chemicals on the chromosome.
They were (1) lethal and toxic reactions like nuclear
fragmentation, pycnosis, stickiness, etc. which are partly
irreversible types, (2) reversible physiological reaction
like metaphase arrest and (3) mutagenic reaction including
structural changes of chromosomes. Hughes (1952)
has tried to classify the effects of mitotic poisons
according to their action on the stage of division which
can broadly be said as inhibitory effects and aberration
effects. He has classified as (1) prevention of cell
division, (2) inhibition of division at prophase,
(3) inhibition of spindle formation at metaphase,
(4) breakages of chromosomes at different stages, and
(5) inhibition of cytokinesis. Dustin (1951) has grouped
the chemicals for their common effects into three categories
viz. (1) spindle or metaphase poisons involving extra
Ludford (1953) has tried to classify the cytological effects of the mitotic poisons as (1) metaphasic arrest due to the inhibitory action on the formation of the spindle, (2) prolongation of metaphase, (3) irregular separation of chromosome due to stickiness or some other reasons and (4) true chromosome breaks. The classification presented by D'Amato (1954) has some points common to the previous classifications. He considered the mitotic poisons mainly as inhibitors of (1) preprophase, (2) spindle apparatus and (3) cytokinesis. This classification does not include the effects specifically on the chromosome rather it narrates the events as a whole. Lettre (1954) has tried to restrict the classification without making it very broad. He objected to the classification of some of the previous workers because they included some chemicals which would interfere the synthetic period of the DNA in the interphase nucleus. He has taken into account those mitotic poisons which would act upon the dividing cells and not an interphase. He broadly grouped the effects into two categories (1) reactions with chromosomes and (2) reactions with the acromatic spindle apparatus. The approach made by Koller (1954) was more or less on the same line of Lettre.
He distinguished the mitotic poisons as agents which would effect the cells in division from those which would act upon the cells in interphase condition. However, such a distinction in all practical purposes is difficult to maintain because there are chemicals which show mixed types of effects. Due to this fact many authors have not maintained this sort of distinction in their classification. Koller himself was aware of this difficulty and therefore, for practical purposes he has classified the effects into two very generalized forms like (1) nucleotoxic effects and (2) cytotoxic effects. Biesele (1958) in his book has treated the mitotic poisons as (1) poisons of prophase and earlier stages, (2) poisons of the chromosomes and (3) poisons of metaphase and later stages. Wilson (1965) has classified the antimitotic agents as (1) spindle poison (2) prophase poison (3) mitotic inhibitor and (4) chromosome breaking agents. In other words he did not maintain the separate groupings as "mitotic poisons" and "radiomimetic poisons" as defined by Dustin (1947). In an earlier review on the drug effect at the cytological level he (Wilson, 1960) classified them into two groups viz. (1) those which do not penetrate the cell but are presumed to exert their effects by modification of the cell surface and (2) those which penetrate either actively or passively and disrupt or modify metabolic actions usually through some type of interference in enzymatic activity. In
principle the effects are seen just like chemical mutagen. Fragmentation of chromosomes and antimitotic activity are produced by them. Sharma and Sharma (1960) in their review on the chemically induced chromosome aberrations made different approach in grouping the mutagens. They are grouped as special drugs, antibiotics, plant products, plant hormones, vitamins, growth regulators, organic compounds belonging to mustard groups, phenols and heterocyclic bases, inorganic salts and so on. Grouping of mitotic poisons on the basis of chemical properties has been attempted by Rosen (1957, 1964).

It would appear from the above paragraphs that the classification of the chemical mutagens or mitotic poisons or radiomimetic chemicals advocated by different workers have in each case some justifications as well as some pit-falls. The pit-falls are of course not with the classification but the overlapping effects produced by the chemicals. Further, the chemicals have more than one property and the author made a choice of them. Thus the classification based either on cytological effects or their chemical nature would very likely be partly subjudice. In spite of this limitation a classification is helpful, at least, to know the preliminary nature of the chemical. The effects of the chemical compounds as reviewed by Biesele (1958); Sharma and Sharma (1960); Wilson (1960, 1965);
Khlman (1966) and others on the dividing cell can broadly be generalized as:

1) Inhibitory effect on cells preventing them to enter division.
2) Acceleratory effect on cells enhancing the activity of cell division.
3) Prophase poison affecting the formation of the spindle apparatus.
4) Metaphase poison or spindle poison distorting or disorganizing the metaphase or anaphase spindle apparatus.
5) Nucleotoxic effect (i) on the general morphology of the chromosome in the form of stickiness, general disorganization of the matrix despiralization, etc. and (ii) true breaks of simple nature or exchange type ones in the chromosomes.
6) Failure of cytokinesis and restitution at the different stages of division.

The thesis has been presented in three chapters. In the following chapter (Chapter I), the qualitative and quantitative effects of 13 chemicals and X-rays on the chromosomes of the dividing bone marrow cells of mice have been presented.
The chemicals used in the present study are:

1. Actinomycin-D
2. Na-Novobiocin
3. Dihydrostreptomycin
4. 2,4-Dinitrophenol
5. P-aminophenol
6. Pyrogallol
7. Gallic acid
8. Hydroxylamine
9. Phenylhydrazine
10. Semicarbazide
11. Caffeine
12. Carbon tetrachloride
13. Lithium chloride.

In chapter II of the thesis the result on the effect of sodium azide and adenosine triphosphate on the chromosomal aberrations of bone marrow cells of mice induced by X-rays and actinomycin-D has been presented.

The chapter III contains the result of the effect of pH in relation to the action of hydroxylamine, phenylhydrazine and semicarbazide producing chromosome aberrations in bone marrow cells of mice.

There are some justifications in selecting the chemicals for the present study. The knowledge on their
mutagenic action has been reviewed briefly while presenting data of each chemical. Chemicals used in the present study have not been tested before as to their effects on the chromosomes of bone marrow cells of mice. This information will be helpful in correlating the results with other published data obtained in other types of material. Further, some of the chemicals in the present study are used as drugs. Their mutagenic action on bone marrow chromosomes may serve as a warning against their indiscriminate use. Further, the knowledge on the effect of phenols will have indirect bearing on the cancer problem. Over and above the implication of the present study in the basic problem is none the less important.