1. INTRODUCTION

Artificial production of mutation was started with late Prof. H.J. Muller (1927) who was pioneer to discover X-ray induced mutation in Drosophila. In the next year Stadler (1928) also reported the same thing in maize. Subsequently lot of works have been done on different aspects of the artificial production of mutation by ionizing radiations in several test organisms (See Sax, 1941; Hollaender, 1954; Russell, 1954; Lea, 1955; Bacq and Alexander, 1961; Evans, 1962; Sparrow, 1962; Manna and Mazumder, 1967; Mazumder and Manna, 1967; UNSCEAR, 1969; Savage, 1970; Bender et al., 1974; Searle, 1974). The effects of radiations on cells have drawn much attention of mankind because of their mutagenic and carcinogenic actions. Thus Muller (1927) opened a new approach which revealed many genetic facts. This area of research has gained special importance after the devastations caused by the explosion of atom bombs and for the gradual increase in the use of atomic energy. Consequently the hazards of radiation mutagenesis have been recognized and different countries have set up organisation like 'Atomic Energy Commission' or so to control the random use of radiations. In places where people are likely to be exposed to different radiation energies, the measure of safe-guarding them from their hazardous effects has been introduced. Public consciousness towards the deleterious effects of radiations has also been developed by now.
That chemicals can also induce gene mutation and chromosomal aberration has been shown just after the II World War by Oehlker (1943) and Auerbach and Robson (1944). This discovery of chemical mutagenesis extended the horizon of the field of artificial production of mutation. Subsequently a vast multitude of chemicals including several environmental agents have definitely been proved to be mutagenic in different test systems (See Kihlman, 1966; Vogel and Rohrborn, 1970; Fishbein et al., 1970; Hollaender, 1971). The effective compounds and combinations, the list of which now runs to several hundreds range from simple inorganic salt to highly complex organic compounds. The danger of exposing us to the chemical mutagens may be more than to the ionizing radiations since the sources are of varied nature. There is a growing concern over the possibility that future generations may suffer from genetic damage caused by mutation inducing chemical substances to which the population at large is deliberately or inadvertently exposed.

On the other hand, with the progress of civilization thousands of chemicals are now being produced and marketed at bewildering speed to combat with our needs. Widespread use of them as medicine, as drugs of habit, as pesticides, herbicides, fungicides, etc. in agriculture, as preservatives, as disinfectants and deodorants, as dye in textile and other industries, etc. causes a great concern. Over and above, there are other air and water pollutants like industrial wastes coming out in the stream and smokes coming out from automobiles and chimneys of the factories. Thus within the last 2-3 decades man has introduced
into his environment a variety of chemical substances and we are frequently exposed to those chemical and environmental agents. Actually we are now living in chemical age of ever increasing complexities.

No doubt the efficiencies of the compounds are tested thoroughly for the purpose for which they are meant before being marketed. However, their effects on the hereditary materials, the importance of which needs no emphasis, are hardly studied before being marketed. Unfortunately there is no organization parallel to 'Atomic Energy Commission' in any country to check the random use of drugs and other chemicals. The deleterious effects of chemicals and drugs have not drawn the attention of mankind to the same extent as that of the ionizing radiations. People are not also equally cautious about the indiscriminate use of the chemicals. This is perhaps due to our pre-occupation with the atomic age. Those new chemicals and drugs are produced and marketed at such a bewildering speed that it is not possible for the biologists and biochemists to keep pace with it unless a separate team is provided for testing their deleterious effects.

Thus chemical and environmental agents, like ionizing radiations, pose a great threat to human population. The increasing concern over genetic hazard from chemical and environmental agents leads to a challenging of more effort into mutagen testing. More recently attention has been focused on several drugs. During the early part of 1960s the thalidomide tragedy alarmed the general population as well as the medical community to the possible harmful potential of other chemical
agents. Concern was voiced in different quarters about the need to evaluate other drugs and environmental agents with regard to their possible antifertility, teratogenic, carcinogenic and/or mutagenic effects. In 1967 when Cohen et al. (1967a) and Irwin and Egozcue (1967) demonstrated lysergic acid diethylamide (LSD)—a widely used hallucinogen, induced chromosome damage in vitro and in vivo the phenomenon of chemical mutagenesis as it extended to the chromosome level was dramatically brought to the attention of an already alerted public.

By now a number of drugs including life-saving antibiotics and anticancerous drugs have been proved to be mutagenic. Almost all the anticancer drugs cause chromosome damage. Nevertheless since the majority of cancers are fatal, the risk of mutagenesis due to the use of these cytotoxic agents is justified. On the contrary, in case of non-fatal diseases like psychotropic disorders or so the justification of exposing a patient to drugs that are known to be mutagenic may be questioned, particularly if less mutagenic ones, as therapeutically effective are available. It is, therefore, highly important to study and evaluate those commonly used drugs for their clastogenic and mutagenic potentials. The present study deals with the evaluation of genotoxic and cytotoxic effects of some psychotropic and habit-forming drugs like diazepam, chlordiazepoxide and nitrazepam. Obviously, a review of works in this field would be useful to understand the present problem.

Before making a review of the results on the effects of drugs we would like to refer briefly to the living organisms—another known agent producing genetic damage. The living
organisms include viruses and bacteria (pending controversy with regard to viruses). It has been shown recently that besides physical and chemical agents, viruses and bacteria can also induce chromosome aberration and, therefore, possibly gene mutation. Hamper and Ellison (1961) for the first time recognised increased frequency of chromosome aberration in a heteroploid cell line of Chinese hamster treated with herpex simplex virus. This was followed by a number of works with different viruses in vivo and in vitro (See Aula, 1965; Nichols, 1966). The discovery of virus induced chromosome aberrations has drawn the attention of cancer cytologists to correlate the two theories on the etiology of cancer, viz. somatic mutation theory of Theodore Boveri and the viral theory of Peyton Rous (See Nichols, 1966). Manna and Bhowmick (1969) were pioneer to report similar types of effects in mice with bacteria. Since then Manna and his collaborators (Manna, 1971; 1973; Manna and Chakrabarti, 1970; Manna et al., 1973 Manna and Das, 1974; Manna and Gupta, 1977, 1978) have been studying different aspects of chromosome aberration induced by different bacteria as well as by their culture filtrate in mice. Therefore, the living organisms constitute the third agent – the biological agent.

Thus the mutagens can broadly be grouped under three heads:

1. Biological agents comprising viruses and bacteria.
2. Physical agents comprising radiations, temperature and pH.
3. Chemical agents of diverse nature.
1.1 Literature Review

So many workers have earlier reviewed extensively the effects of biological agents (Aula, 1965; Nichols, 1966; Manna, 1971, 1973), physical agents (Sax, 1941; Hollaender, 1954; Lea, 1955; Bacq and Alexander, 1961; Ray-Choudhury, 1961; Evans, 1962; Mazumder and Manna, 1967; Manna and Mazumder, 1967; Manna and Ray, 1964; Manna and Mukherjee, 1966; Sparrow, 1962; Manna, 1969; UNSCEAR, 1969; Savage, 1970; Russell, 1954; Sobels, 1963; Bender et al., 1974; Searle, 1974) and chemical agents (Sharma and Sharma, 1960; Kihlman, 1966; Auerbach, 1967; Vogel and Rohrborn, 1970; Fishbein et al., 1970; Hollaender, 1971, 1973, 1976, 1978; Leonard, 1972; Bishun et al., 1973; Palek, 1974; Matsuyama and Jarvik, 1975; Kato, 1977; Generoso et al., 1978; Sharma et al., 1978) on cells. So we refrain ourselves from making any review on them. Besides, it is beyond the scope of the present investigation. It would have been of considerable interest to review the works on drugs. However, the list of pharmaceutical compounds now in use is very extensive and their number runs to several hundred. Naturally it is difficult to make a comprehensive review on their effects at the cytological and genetical level. This aspect has however, been dealt with by several workers in connection with the effects of chemical and other environmental agents (Auerbach, 1950; Darlington, 1950; Biesele, 1958; Sharma and Sharma, 1960; Wilson, 1960; Rosen, 1964; Kihlman, 1961, 1966; Vogel and Rohrborn, 1970; Fishbein et al., 1970; Hollaender, 1971, 1973, 1976, 1978; Leonard, 1972; Bishun et al., 1973; Manna, 1969, 1971, 1973). Here we would like to restrict ourselves to make a review on the cytotoxic and
genotoxic effects of drugs and agents of abuse, which is directly related with our study.

The term 'drug' has been defined variously by individual scholars and organization. Traditionally it (drug) is defined as 'a medicine or a substance used in the making of medicine'. It is preferable to adopt the definition suggested by the World Health Organization that a drug is a 'substance that, when taken in the living organism may modify one or more of its functions'. This definition includes the dependence producing substances whose harmful consequences on individual and social life go beyond a socially or culturally acceptable level.

Among the drugs of common use the dependence producing drugs constitute the major bulk. They are commonly referred to as drugs of abuse and include hallucinogens, barbiturates, amphetamines, narcotics and opiates, tranquilizers, cannabis and its products, alcohol, stimulants, etc.

Drug abuse, a commonly used phrase now (many persons prefer to use the phrase as 'drug misuse'), is not a unique problem either to any particular country or to the present times. It has been a feature of all societies in the world since times immemorial. From time to time and from society to society the substances used for the purpose have varied. Initially, naturally occurring compounds served the purpose. But 20th century has witnessed a rapid growth of organic chemistry culminating in the introduction of many series of drugs with profound effects on behaviour and emotions. Thus to-day chemically synthesized agents have added greatly to the abundance and availability of
drugs. These substances provide a pathway of escape from the reality of life's stresses and strains. But important thing is - many of these drugs contribute much to the good medical care. In today's high pressure societies the number of youthful drug abusers is growing at an alarming rate in all the countries. Students, both male and female, of college, high school and junior high school levels are experimenting with a wide range of drugs and agents of abuse on a steady basis. Psychotropic drugs have arrived in the scene very recently. By gathering relevant literature it is hoped that an accurate information will be gained on the present knowledge, and the gaps in knowledge about the effects of these drugs on the hereditary material and the potential danger of present 'users' and to future generation.

By far the most intensively studied agents are the hallucinogens, in particular LSD (lysergic acid diethylamide), the most potent one. Initial report on LSD was made by Cohen et al. (1967a) who showed that LSD was capable of causing extensive chromosome damage when added in vitro and also in vivo. Since then variable claims have been made with regard to the chromosome breaking and mutagenic potential of LSD. It was reported to cause chromosome breakage in cultured human leukocytes (Cohen et al., 1967b; Jarvik et al., 1968; Corey et al., 1970), in leukocytes of LSD users (Cohen et al., 1967a, 1968; Egozcue et al., 1968; Nielsen et al., 1969; Gilmour et al., 1971) and in leukocytes of patients treated medically with pure LSD (Cohen et al., 1967b; Abbo et al., 1968; Tjio et al., 1969). However, it (LSD) is known to cross the placental barrier in mammal. Extensive
chromosomal damage was also noticed in the children whose mother used LSD during or before pregnancy (Cohen et al., 1968; Egozcue et al., 1968). In contrast, several negative reports were obtained in leukocytes of LSD users (Loughman et al., 1967; Sparkers et al., 1968; Bender and Sankar, 1968; Tjio et al., 1969), and in cultured human leukocytes (Sturelid and Kihlman, 1969; Genest, 1969). Its clastogenic effect was recorded in meiotic (Cohen and Mukherjee, 1968; Skakkeback et al., 1968; Skakkeback and Beatty, 1970) and bone marrow chromosomes (Cohen and Mukherjee, 1968) of mice. However, Egozcue and Irwin (1969) and Jagiello and Polani (1969) could not replicate these observations, both reporting negative results. None of the studies assessing clastogenicity of LSD in rats involving both meiotic (Goetz et al., 1972) and mitotic (Sato et al., 1971; Emerit et al., 1972) chromosomes, has yielded positive results. While Chinese hamster showed increased chromosomal aberration frequency (Moorthy and Mitra, 1979). Examination of leukocyte chromosomes of Macaca mulatta after in vivo and in vitro treatment showed significant increase in breaks; but meiotic chromosomes failed to exhibit significant increase (Egozcue and Irwin, 1969). In Drosophila after treating the males with LSD no increase in mutation and/or translocations (Grace et al., 1968) or lethality (Tobin and Tobin, 1969) was reported. Browning (1968), however, reported a marked induction of recessive lethality with high dosages of LSD in Drosophila. Similar positive (Singh et al., 1970; Kalia et al., 1970; Sadasivaiash et al., 1973) and negative (Sturelid and Kihlman, 1969; Riley and Neuroth, 1970) chromosome breaking effects
were reported in several different plant species exposed to LSD. Similarly literature provides conflicting results on the possible mutagenic potential of LSD. Although in Drosophila, Browning (1968), Vann (1969), Barnett and Munoz (1971) noted its mutagenic effect, Grace et al. (1968) and Sram (1971) were unable to detect it. Both positive (Vann et al., 1970) and negative (Zetterberg, 1969) results were obtained from microorganisms. Legator and Malling (1971) also failed to demonstrate its mutagenic effect in Salmonella with host-mediated assay as well as in vitro.

Clastogenicity, mutagenicity, teratogenicity, and oncogenicity of LSD have recently been reviewed very nicely by Matsuyama and Jarvik (1975) and Cohen and Shiloh (1978).

The use of marijuana (Cannabis) by college and high school students has increased tremendously in the last decade and it is spreading in other circles at an alarming rate. Several studies have indicated dependence, physical degradation and psychiatric disorders as consequence of long term cannabis abuse. Martin (1969) reported lack of effects of cannabis resin on chromosomes of rats when tested on leukocytes in culture, embryonic cells in tissue culture and cells cultured from rat embryos of mothers treated with a teratogenic dose. However, she found a decrease of mitotic activity with increasing concentrations. Gilmour et al. (1971) reported higher incidence of chromosomal aberration in the leukocytes of users, compared to non-users. Their observation was supported by Stenchever et al. (1974) from similar study. However, Rubin et al. (1973) and Dorrance et al. (1970) failed to obtain any difference in the incidence of chromosomal aberration of marijuana users and non-users. Leuchtenberger et al.
(1973) reported that lung explants exposed to marijuana smoke showed variability in DNA content and altered chromosome complements. But human chromosomes responded negatively when tetrahydrocannabinol, probably the active component of marijuana, was added to leukocyte culture (Neu et al., 1970; Stenchever and Allen, 1972). Geber and Schramm (1969) indicated an induction of congenital malformation in the fetal hamster and rabbit after high doses of marijuana treatment.

Caffeine occurs in coffee and tea and naturally a great number of people are exposed to it regularly. This alkaloid has some use in medical practice: for example, with antihistamine to combat motion sickness, with analgesics or ergot alkaloids for relief of tension and headache, as stimulant and as a coronary artery dilator. However, literature provides a large amount of conflicting evidences on its clastogenic and mutagenic effects (Tinson, 1977) and the contribution of Kihlman constitutes the major bulk (Kihlman, 1977). Caffeine induced chromosome damage was reported in plants (Kihlman and Levan, 1949; Yamamoto and Yamaguchi, 1969; Kihlman et al., 1971; Kihlman, 1977), in insects (Ostertag and Haake, 1966; Andrew, 1967; Clark and Clark, 1968) and in human cells in vivo and in vitro (Kihlman and Levan, 1949; Ostertag et al., 1965; Ostertag, 1966; Ostertag and Haake, 1966; Kuhlman and Ostertag, 1968; Lee Sung, 1971) and mice (Kuhlman et al., 1968). That caffeine is mutagenic was proved in E. coli (Demerec et al., 1948, 1951; Gezelius and Fries, 1952; Kubitscheck and Bendigkeit, 1961), fungus (Zetterberg, 1960) and phage (Kubitschek and Bendigkeit, 1964). However, Thyer et al. (1971) exposed HeLa cells to caffeine and found no significant increase
of chromosome aberrations. Meiotic chromosomes of laboratory
mammals did not also respond to caffeine (Adler and Rohrborn,
1969). Similarly negative results were obtained with micronucleus
test in mice (Schmid et al., 1971; Matter and Grauwiler, 1974;
Chaubey et al., 1976a), dominant lethal test in mice (Lyon et al.,
1962; Cattanach, 1962, 1964; Adler, 1969; Epstein et al., 1970a),
F₁ translocation test (Cattanach, 1962), specific locus mutation
test (Lyon et al., 1962) and host mediated assay using Salmonella
typhimurium (Gabridge and Legator, 1969). It failed to induce
change in sperm morphology also (Wyrobek and Bruce, 1975). Recently
caffeine was found to have no influence on the frequency of SCEs
induced by certain other mutagens in plants and animals (Kato, 1974;
Kihlman, 1975; Kihlman and Sturelid, 1978; Palitti and Becchetti,
1977; Basler et al., 1979).

Though alcohol is extensively used as a drink its
pharmaceutical utility cannot be ignored. Its possible mutagenicity
was doubted as early as 1930 by Bluhm (1930). However, its
chromosome breaking potentiality seemed to be published first by
Michaelis et al. (1959) in Vicia faba. Subsequently its chromosome
breaking capacity was reported in Vicia faba (Rieger et al., 1975),
in grasshopper (Manna and Mazumder, 1964; Mazumder and Das, 1969),
in human leukocytes in vitro (Bregman, 1971) and in vivo (Obe and
Herha, 1975; Torok, 1972; Obe et al., 1977, 1978). Lack of its
effect was not of course completely unknown (Obe et al., 1977).
Positive as well as negative reports are available so far ethanol
induced dominant lethality is concerned in rodents (Badr and Badr,
1975; Chauhan et al., 1976). No appreciable increase in the
frequency of micronuclei was obtained after alcohol treatment.
(Chaubey et al., 1977). Semezuk (1976, 1978) reported various types of sperm abnormalities in chronic alcoholics. Recently Ristow and Obe (1978) put forward the view that acetaldehyde, the first metabolite of ethanol, might be responsible for its mutagenicity.

A lot of works were done in an attempt to assess the active principle in cigarette smoke which is responsible for women giving birth to children of low birth-weight (Murphy and Mulcatty, 1971). Bishun and his co-workers (Bishun et al., 1972) studied the effects of nicotine in peripheral blood in vitro and in rats in vivo. The in vitro experiment showed that at high concentration the drug was toxic to blood cultures but failed to induce any chromosomal abnormalities. But with rats in vivo experiment it showed a high degree of chromosomal abnormalities. The active agent of cigarette smoke which may be mutagenic, has been postulated to be either cotinine or nicotine-1-oxide, metabolite of nicotine. Dominant lethal test with nicotine in mice gave also a negative result (Cattanach, 1964). Lambert et al. (1978) demonstrated increased frequency of sister chromatid exchanges among cigarette smokers; while Hollander et al. (1978) obtained negative response from similar studies.

The recent spurt in the use of narcotic analgesics for medical and non-medical purposes has involved a risk to man. The teratogenic (Iuliucci and Gautieri, 1971; Smith and Joffe, 1975) as well as mitodepressive (Kabarity et al., 1974) actions of morphine, the most widely used narcotic, are on record. It was shown to inhibit the growth of E.coli (Simon, 1964). Recently the clastogenic effect of both morphine and pethidine in the bone
marrow cells of mice have been reported from this lab from metaphase chromosome analysis (Swain et al., 1980) as well as micronucleus test (Das and Swain, unpublished). The chromosome breaking activity of morphine was recorded earlier also in plants (Oehlkers, 1952, 1956) and human addicts (Amarose and Schuster, 1971; Kushnick et al., 1972). But its effect in human leukocytes (Falek et al., 1972) and in Drosophila (Knaap and Kramers, 1976) proved negative. In view of the widespread use of narcotics Auerbach (1971) stressed on the need for further testing of their possible mutagenicity. An increased frequency of chromosomal aberrations was noticed in the leukocytes of heroin (a derivative of morphine) users (Gilmour et al., 1971) and in the leukocytes of infants exposed to heroin in utero (Abrams and Liao, 1974). However, Gendel (1974) failed to find an increased frequency of breaks in heroin addicted mothers as well as in their newborn babies. Similarly, both positive and negative results were obtained with methadone, a synthetic opiate-like drug. Falek et al. (1972) showed an increased incidence of chromosome aberrations in human \textit{in vivo}, but failed to do so \textit{in vitro}.

Teratogenic side effects in neonates of mothers being treated with phenytoin for a convulsive disorder were reported by Janz (1975) and Shapiro et al. (1976). Their use during pregnancy in human (Grosse et al., 1972) and mice (Roman and Caratzali, 1971) was associated with chromosome breaks in neonates as well as in mothers. However, phenytoin could not produce any chromosomal abnormality in rodents and human \textit{in vivo} as well as \textit{in vitro} (Munitz et al., 1969; Brogger, 1970; Stenchezver and Allen, 1973; Alving et al., 1976; Knuutila et al., 1977).
The tranquilizers are drugs which act primarily on the sub-cortical area of the brain. They are usually prescribed in the treatment of anxiety states, psychosis with agitation, hyperactivity, mild neurosis and other functional disturbances of the central nervous system. They are habit forming drugs and produce tolerance. They are now randomly abused and commonly called 'happiness pills'. Tranquilizers are at present the number one seller in the drug industry - they outsell even aspirin - about 30 billion meprobamate tablets alone are consumed in the U.S.A. each year (Green and Levy, 1976). Recent works on the cytotoxic and genotoxic effects of the tranquilizers give conflicting results. Talukder (1975) noted mitodepressive effects of phenothiazines but failed to obtain any chromosomal aberration in human leukocytes in culture. Significant increase in chromosomal aberrations was seen in the leukocytes of patients treated with chlorpromazine (CPZ), commercially known as Largactil (Cohen et al., 1967a; Nielsen et al., 1969; Jenkins, 1970; Gilmour et al., 1971; Donnini et al., 1976). In contrast, other in vivo studies gave negative results (Cohen et al., 1969, 1972). Non-human mammalian chromosomes were also found to be susceptible to CPZ (Siva Sankar and Geisler, 1971; Kelly-Garvert and Legator, 1973) as well as triflupromazine (Green et al., 1970) when they were added in vitro. In contrast, in vitro studies of Cohen et al. (1969), Nielsen et al. (1969), Kamada et al. (1971) failed to exhibit any chromosome breaking capacity of CPZ though that of Abdullah and Miller (1968) could do. Again CPZ (Epstein and Shafner, 1968) triflupromazine (Legator, 1971, 1972; Petersen and Legator, 1973), thioridazine (Sanjeeva Rao et al., 1973) have
been shown to cause dominant lethality in male mice. Sanjeeva Rao and his coworkers tested the mutagenicity of mellaril, thioridazine and eskazine in Drosophila and mice. The first two gave positive results (Prabhakar Rao and Sanjeeva Rao, 1976; Sanjeeva Rao et al., 1973) but the last one proved negative (Prabhakar Rao and Sanjeeva Rao, 1976). Murthy and Subramanyam (1976) showed also spermatocytic chromosome aberration in male mice treated with thioridazine hydrochloride.

Cohen et al. (1969) reported negative clastogenic effect of fluphenazine (permitil) and thioridazine (mellaril). However, these findings were based on 3 subjects in each case. Compazine and Stelazine were demonstrated to cause damage to barley (Polson and Adams, 1978) and human (Jenkins, 1970) Chromosomes. They also altered cell permeability and reduced cell division (Polson and Adams, 1978). Induction of teratogenicity by tranquilizers have been recorded in human (Mellin, 1964; Vince, 1969; Rumeau-Rouguette et al., 1977) and rodents (Padmañabhan and Singh, 1978). However, experimental works on tranquilizer induced teratogenicity have often shown contradictory results (Ordy et al., 1963; Brock and Kreibig, 1964; Kalter, 1971; Harvoth and Druga, 1975).

Reserpine, a major tranquilizer, produced chromosomal abnormalities in the bone marrow cells of mice (Subramanyam and Jameela, 1976). There are two reports on the clastogenic effects of perphenazine—a phenothiazine tranquilizer. Though Nielsen et al. (1969) found a significant increase of chromosome aberrations in the leukocytes of some patients using the drug Cohen et al. (1972) were unable to obtain it.
Jagiello et al. (1973) tested the effects of meprobamate, a commonly used minor tranquilizer, on the female meiotic chromosomes of mice. In vivo experiments gave negative results while a positive result was revealed when the drug was tested on the oocytes grown in vitro. In vitro treatment of leukocytes with various concentrations of meprobamate and for different durations of exposure also gave negative results (Kamada et al., 1971). While Manna and Das (1977) showed meprobamate associated chromosome aberrations in the bone marrow cells of mice.

Barbiturates are the main depressant drugs used in medical practice today to sedate or to calm patients. Some reports are already available on their clastogenic activity. They were shown to induce chromosomal aberrations in bone marrow cells of mice (Das, 1971, Manna and Das, 1973), in the cleaving cells of rabbit (Shaver, 1974) and in the meiotic cells of grasshoppers (Rajasekharasetty and Devi, 1966; Das and Mukherjee, 1977).

Chloral hydrate is another principal ingredient of sedative and hypnotic agents. Its toxicity (Goodman and Gilman, 1975) and mutagenicity (Barthelmess, 1956; Goldstein, 1960) were described. At high dose it was reported to increase significantly sperm shape abnormality in mice (Wyrobek and Bruce, 1978).

Medically urethan is employed as a mild hypnotic and sedative agent. It has been proved to produce a variety of chromosomal aberrations in the bone marrow cells of mice (Wakoning-Vaartaja, 1964), in Walker rat carcinoma (Boiland and Koller, 1954), in vitro cells of Chinese hamster and mice (Pogosyants et al., 1968) and in plants (Oehlker, 1953). Its mutation producing capacity
was proved in bacteria (Bryson, 1945; Demerec et al., 1951) and Drosophila (Vogt, 1948). But dominant lethal test failed to demonstrate any mutation in mice (Bateman, 1967; Epstein and Shafner, 1968). Wyrobek and Bruce (1975) could not also obtain any sperm abnormality in mice treated with urethan. However, its teratogenicity was recorded in a wide range of animals (Vesselinovitch et al., 1967; Nomura, 1974).

Bignami et al. (1974) tested the effects of a number of tranquilizers on the incidence of nondisjunction and crossing over in Aspergillus nidulans. Only chlorazepate was found to have significant effect, while oxazepam, temazepam, nitrazepam and dibenzepin were ineffective. Similarly chromosome analysis showed negative response for tranquilizers like medazepam, D-40 TA and MS-4101 in human and rodents (Schmid and Staiger, 1969; Staiger, 1970; Hitotsumachi and Kikuchi, 1974; Ishimura et al., 1975). Zolotareva et al. (1977) studied mutagenic effects of 14 psychotropic drugs: viz. tavor, ethosuximide, aminazine, fluphenazine, thioproperazine, reserpine, hexamidine, diazepam, oxazepam, thioaxine, dilantin, carbamazepine, benzonal and chlor Diazepoxide. Only the last three gave increased aberration frequency of chromosomes in cells of Allium fistulosum seeds. Matsubara et al. (1978) showed absence of mutagenic effect of clonazepam, a benzodiazepine tranquilizer, in bacterial tests in vivo and in vitro.

The works on diazepam, chlor Diazepoxide and nitrazepam with which the present investigation is concerned have been discussed in details in the respective drug chapters and these benzodiazepine tranquilizers also exhibit similar contradictory
reports (vide infra). From the foregoing paragraphs it is clear that the works on the cytotoxic and genotoxic effects of drugs and agents of abuse are very limited and fragmentary and that too highly contradictory.

Benzodiazepines are among the most commonly prescribed drugs in the world (Sellers, 1978). Approximately 10% Canadians, and 30% of hospitalised patients receive a prescription for a benzodiazepine each year. The prescription of benzodiazepines has been increasing steadily everywhere. Extent of use of psychotropic drugs including benzodiazepines in nine European countries has been reviewed nicely by Lader (1978). From a survey in south-east London it was reported (Lader, 1978) that about 1 in 3 females and 1 in 5 males had taken tranquilizer at some time. Recently extent of abuse of psychotropic drugs including tranquilizers in India has been discussed by the National Committee appointed by the Government of India (1977).

In view of the recent spurt in the use and/or abuse of the psychotropic drugs and contradictory reports on their potential mutagenicity we have reported here the cytotoxic and genotoxic effects of diazepam, chlordiazepoxide and nitrazepam in mice.

No doubt metaphase chromosome analysis is one of the most important procedure for testing potential mutagenicity of drugs or other compounds. But single test system is not sufficient for the evaluation of potential mutagenicity of a given agent. WHO (1971), Committee-17 (1975, appointed by the Council of Environmental Mutagen Society) also suggest to adopt different protocols for the purpose. Most of the earlier works on the three benzodiazepines we tested, however, were based on metaphase
chromosome analysis of somatic cells. The present investigation includes in addition to metaphase chromosome analysis of bone marrow cells, micronucleus test, metaphase chromosome analysis of germ cells and dominant lethal test. Besides, effects on the epididymal spermatozoal population and on sperm head morphology have also been encountered. Thus the evaluation will be based on the comparative studies of different test systems.

1.2 Selection of the Experimental Animal

For the testing of mutagenicity of such agents human subjects would have been ideal material. For human subjects cytogenetic studies involve the relatively simple procedure in which human lymphocyte or fibroblast cultures may be exposed to the given agent in vitro, or alternatively chromosomal damage resulting from the exposure of human subjects to the drug or agent can be observed periodically by culturing circulating lymphocytes or fibroblasts. The wider use of tissue culture material by the animal cytogeneticists can be justified because of the following advantages over the cells in vivo: (a) There is no need of killing the animal. (b) It is easy to treat the cells directly with the agent. (c) The treatment can be made in some synchronised condition of the cells.

In spite of the advantages mentioned above for the use of the in vitro cells in the study of the effects of certain substance on chromosomes there is one important draw-back too. It must be stressed that results derived from the in vitro studies, while important from the scoring point of view, do not necessarily reflect the action of the tested agent in vivo. In other words, the in vivo test provides the added advantage of taking metabolism
into account. There are certain agents which are harmless \textit{in vitro}, but harmful \textit{in vivo}. The best known example is cyclophosphamide. Probably here not the cyclophosphamide but its metabolites are mutagenic. Conversely, substances, effective on cells \textit{in vitro}, may not reach cells by normal channels in the whole organism.

The importance of \textit{in vivo} chromosome cytology to detect potential genetic damage is also documented by the Advisory Panel on Mutagenicity of Pesticides (1969), the Ad-hoc Committee of the Environmental Mutagen Society (1972), World Health Organization (1971), Committee-17(1975), Savage (1975), Hollaender (1971, 1973, 1976, 1978). But \textit{in vivo} cytogenetic study in human (i.e. study from the cultures of lymphocytes or fibroblasts of humans exposed to the agent) is not completely devoid of any limitation. It is practically impossible to obtain a person exposed to a particular drug or agent only. \textit{In vivo} cytogenetic studies from persons exposed to multiple drugs naturally create trouble in proper assessment of the mutagenicity of any particular drug. Therefore, it will not be unjustified to observe the effect on cells derived from experimental mammals which provide advantage of being more 'man like' and then extrapolate the results to man. Therefore, laboratory mammals constitute the best suited material for this sort of study and the present investigator has chosen mouse as experimental animal. The WHO report (1971) also recommends that all mutagenic tests of drugs should be done primarily on mammals.

There is sufficient reason to choose mouse as experimental animal. Though the mouse chromosomes in comparison to those of other laboratory mammals are less suitable for cytogenetic work,
most of our knowledge on the protocols adopted here has come from the experiments conducted on mice.

1.3 Methodologies Adopted

It will not be out of way if we now describe here the different methodologies adopted in the present investigation.

1.3.1 Cytogenetic assay of bone marrow cells (Metaphase chromosome analysis)

Since the chromosomes are the physical entity of the genetic system, it is naturally assumed that any agent which will affect the chromosomes, will also lead to 'heritable' genetic change. Hence, induction of detectable structural changes in chromosomes is a popular criterion for recognizing potential genetic hazards of a particular agent. Metaphase chromosome analysis is one of the oldest and most widely used method in this regard. With the recent advances in tissue culture techniques as well as in chromosome preparation such type of study is being carried out in most of the laboratories on different types of cells in culture exposed to a variety of external agents: physical, chemical and biological. Though cell lines of human origin like HeLa cells (Izutsu and Bieseke, 1966) or of other mammals like L cells of mice and chinese hamster cells (Hsu and Somers, 1961, 1962; Somers and Hsu, 1962; Hsu et al., 1964, 1977) are in use, short term cultures of leukocytes and fibroblasts (Cohen et al., 1967a,b; Staiger, 1969, 1970; Stenchever et al., 1970; Cohen and Hirschhorn, 1971; Talukder, 1975; Evans, 1976) are now commonly employed for the purpose.

Although such an approach by itself cannot provide unassailable proof of concurrent gametic involvement, it appears
likely that autosomal aberrations may indicate parallel gonosomal 
events of a similar nature. Bone marrow in mammalian system has 
been established as an ideal tissue to study the effect of a 
particular agent on somatic chromosomes \textit{in vivo}. Bone marrow cells 
are easy to handle and in the bone marrow a large number of 
dividing cells may be readily obtained. In the present 
investigation we have studied the effect of tranquillizers on the 
bone marrow chromosomes of mice.

1.3.2 Cytogenetic assay of bone marrow cells (Micronucleus test)

Lagging chromosome (s) or acentric fragment (s) is not 
capable of migrating to the poles of the spindle during anaphase. 
After telophase undamaged chromosomes and centric fragments that 
have migrated to the poles form daughter nuclei to be distributed 
in daughter cells. The lagging chromosome(s) and acentric 
fragment (s) may also be included in the main nuclei of the 
resultant daughter cells. But in most of the cases they form one 
or several secondary smaller nuclei. Owing to their smaller size, 
as compared to the principal nucleus, they are referred to as 
micronuclei.

Appearance of lagging chromatin material during cell 
division is caused due to either chromosomal breakage or 
disturbance in the functioning of the spindle apparatus. In the 
latter case micronuclei are of bigger size formed out of whole 
chromosome(s) loss. The micronucleus formed from acentric 
fragment resulted from chromosomal breakage is, as a rule, smaller 
in size. Under the influence of clastogens and spindle poisons 
micronuclei can thus be induced in different types of bone marrow 
cells viz: myeloblast, myelocytes, erythroblasts, etc (Boller and
The nature and occurrence of micronuclei have been known to cytologist since the beginning of the present century. Earlier these bodies were known as Howell-Jolly bodies in routine haematological practice. The occurrence of micronuclei in nucleated bone-marrow cells like myeloblasts, myelocytes, erythroblasts, etc. most often creates confusion as they cannot always be separable from the main nucleus and causes difficulty to score quantitatively. In the bone marrow of mammals the erythroblasts after the last mitosis expel the nucleus and thus anucleate erythrocytes (polychromatic) are formed. But for some unknown reason the micronuclei, if present, remain included in those cells, which can be easily recognized. These young polychromatic erythrocytes take about 24 h to be matured normocytes, after which they are released in the circulation. Since they are anucleate the micronucleus formed out of chromatin material appears prominently and it becomes easier to score without any confusion.

Though the occurrence of micronuclei in anucleate erythrocytes is known since long, its use as a protocol in the screening of potential mutagenicity of a given agent has greatly been emphasized only recently. Schroeder (1970), without performing any experiment, suggested scoring of nuclear abnormalities in the nucleated cells of bone marrow for the study of chemical mutagenesis in mammals. Schmid and his co-workers (Boller and Schmid, 1970; Matter and Schmid, 1971; Schmid et al., 1971; Ledebur and Schmid, 1973; Schmid, 1973; Maier and Schmid,
1975, 1976; Schmid, 1975, 1976) established the incidence of micronuclei as a test system after a series of experiments carried out with a large number of chemicals having diverse types of cytological effects during the period from 1970-1976 for the evaluation of potential mutagenicity of a chemical agent. Heddle (1973) who worked with radiation also proposed the micronucleus test as a simplified cytogenetic assay system. Since then lot of works have been done in this line (Matter and Grauwiler, 1974; Seiler, 1975; Richardson, 1974; Chaubey et al., 1976 a,b; Jenssen and Ramel, 1976; Friedman and Staub, 1977; Paik and Lee, 1977; Loan et al., 1977; Goodman et al., 1977; Hossack and Richardson, 1977; Fabry et al., 1978; Trzos et al., 1978; Wild, 1978) and the micronucleus test (MNT) has now been proved to be a simple, reliable and sensitive assay system for the analysis of cytogenetic effects in vivo in mammals. Recently Schmid (1976) has made a nice review on the simplicity and sensitivity of the MNT and there he also emphasized the importance of the test system in screening potential mutagenicity of different chemicals. Comparative studies of MNT and conventional cytogenetic methods (metaphase chromosome analysis) showed a good correlation between incidence of micronuclei and chromosomal aberrations obtained under identical conditions (Boller and Schmid, 1970; Muller et al., 1972; Heddle, 1973; Miller, 1973; Hess et al., 1973; Goetz et al., 1975).

Positive results were recorded even with very low doses of chemicals as well as irradiation (Ledebur and Schmid, 1973; Matter and Grauwiler, 1974; Jenssen and Ramel, 1976).

As an in vivo method for cytogenetic analysis of bone marrow cells, MNT is advantageous over the conventional metaphase
scoring. The following points can be said in support of the view:

1. MNT is less time consuming and naturally more number of cells can be scored within limited time, which is more relevant.

2. The spontaneous rate of incidence of micronuclei is very low and more or less constant.

3. Irrespective of karyotypic preference this test can be performed in any species (Matter and Schmid, 1971). Chromosome number of an organism has nothing to do with this test. On the other hand, in metaphase analysis a favourable karyotype is essential.

4. Cytological preparation of bone marrow cells for micronucleus test as well as scoring of the preparation are simpler than metaphase analysis. Again scoring does not require special training in cytogenetics.

5. It is suitable to detect whole chromosome loss due to abnormal functioning of spindle apparatus. Normochromatic forms are 24 hours older than the polychromatic ones; so agents inhibiting the normal mitotic process are capable of producing higher frequencies of normochromatic erythrocytes with micronuclei. Moreover, spindle poisons are capable of producing larger micronuclei which are formed out of whole chromosome(s) loss.

While in metaphase studies the aneuploidy is sometimes doubted as the result of technical shortcomings. Anaphase figures for the scoring of lagging chromosomes are not available due to colchicine treatment. Further, as the loss of chromatin leads to the dead end of the cell, these aneuploid cells hardly undergo second mitosis.

6. In metaphase scoring cytogeneticists often face difficulty to differentiate breaks from gap type aberrations. But in
7. Bone marrow proliferation and inhibition is quickly detected with MNT. In the later case reduction in number of polychromatic forms is an indicative of it. Any damage that causes inflow of peripheral blood into the lumen of bone marrow is clearly reflected in the observation for the presence of high number of normocytes and matured R.B.Cs.

Disadvantages: This test system is, however, not devoid of limitations which are as follows:

1. Agents producing point mutation are not expected to induce micronuclei except acridine and acridine like compound (Jenssen et al., 1974).

2. In MN analysis only the incidence of MN formation is taken under consideration irrespective of types or aberrations (eg. Chromosome and chromatid breaks, gaps, chromosomal re-arrangements, loss of whole chromosome, etc.).

3. Micronucleus in a cell may represent one chromosomal fragment or fusion product of more than one chromosomal fragments or chromosomes.

4. Conversion of polychromatic to normochromatic is a continuous process which takes about 24 h and there is no sharp demarcation. As such the cells closer to the boarder line need subjective decision of the observer whether to consider as poly- or normochromatic erythrocytes.

1.3.3 Cytogenetic assay of male germ cells

The testing of mutagens, as mentioned earlier, has mostly been performed on mitotic chromosomes. But there are other aspects which make it important to pay attention to meiosis in mammals.
Since the germ line cells are directly concerned with the heredity the aberrations induced in them may affect the progeny. Naturally such studies are of particular importance.

Differences between mitosis and meiosis indicate that the extrapolation from mitotic cells to germ cells is not always justified. The differential effects of radiation (Van Buul, 1973; Van Buul and Roos, 1977), progesterone (Williams et al., 1971; Klasterska et al., 1976), cyclamate (Rieger and Michaelis, 1967; Machemer and Lorke, 1975) etc. on mitotic and meiotic chromosomes can be cited as examples of differential sensitivity of these two cell types. Differential sensitivity of male and female meiotic chromosomes to mutagenic, teratogenic and carcinogenic agents is also evident (Klasterska et al., 1976; Wyrobek and Bruce, 1975).

However, study of mutagenesis on germinal chromosomes was long neglected for the want of suitable technique for their handling. Ingenious methods for the meiotic preparation described by Evans et al. (1964) and later by Meredith (1969) enable the present day cytogeneticists to study the male meiosis in laboratory mammals for induced mutagenesis. Investigations in females are somewhat more difficult to conduct.

1.3.4 Dominant lethal test

When a normal gamete is fertilized by a gamete having numerical or structural chromosomal aberration the resultant zygote may die at any stage of development. If such mutation is induced in the parental germ cells the frequency of zygotic death is expected to increase. Based on this principle dominant lethal (DL) test has been developed for the detection of mutagenicity of certain agent. So induction of structural and/or numerical changes of chromosomes in parental germ cells is considered to be the
genetic basis for dominant lethality (Bateman and Epstein, 1971). The DL test which is concerned with the incidence of zygotic lethality is an easy method to detect mutagenic effect of a given agent in parental germ line.

DL test is in practice since long in Drosophila system (Stromnaes, 1949) where the males are treated with the mutagen and at proper times they are mated with untreated virgin females. The frequencies of unhatched eggs arising from such matings over the control provide a measure of the mutation frequency. Since the middle of 1950s the DL test is being employed as one of the protocols for the detection of radiation induced chromosomal damage (Roy and Bose, 1953). Its application has subsequently been extended to chemical mutagenesis in mammals particularly in rats and mice (Bateman, 1958, 1960; Bateman and Epstein, 1971). During the last 10 years the DL test has extensively been employed to assess the mutagenicity of a wide variety of substances in mammals.

The standard procedure for DL test in mammals is to treat the adult male animals with acute or sub-acute doses of the agent to be tested. Each treated male is then allowed to mate with 2 or 3 untreated virgin females every week and this weekly mating of the same male with fresh females is continued for several weeks to encompass the entire spermatogenic cycle. Then the females are dissected in the late gestation period for the examination of the uterine content for dead and living implants and ovaries for the corpora lutea count. This protocol used in routine testing programme has been developed by Epstein and Shafner (1968), Epstein et al. (1970b), Epstein and Rohrborn (1971).
There are sufficient reasons to treat the males. These are as follows: (a) In females the treatment might disturb hormonal status and consequently ovulation, fertility and pregnancy. (b) The response in a treated female can be studied only in a single pregnancy. On the other hand, in case of treated males mutagenic response can be obtained from different females. (c) In the mature females all the germ cells remain in the oocyte stage, most of them being in resting, so called dictyate, stage. Naturally differential sensitivity is not expected. On the other hand, as the treated males are mated week by week with virgin untreated females, the chromosome breaking effect of a given agent can be encountered at all stages of spermatogenesis and it can be possible to conclude which stage is sensitive to the agent.

Treated females, instead of males, have however, been used in the DL test by Generoso and his coworkers (Generoso, 1969; Generoso and Russel, 1969; Generoso et al., 1971) and Rohrborn and Berrang (1967) and they have shown that the response in the female is just as important as the response in male. Generoso et al. (1971) have also found significant variation in the frequency of dominant lethality in different strains of mice as well as in different intervals between treatment time and mating. Recent comparative studies on dominant lethality with treated males and females also failed to exhibit differential results (Generoso et al., 1978).

Dominant lethality has two compartments: pre-implantation loss and post-implantation loss. When egg or blastocyst dies before implantation and it disappears without any trace nothing
is left in the uterus. This is what is known as pre-implantation loss. The only way to estimate the pre-implantation loss is to count the corpora lutea and subtract the number of total implants (dead + lining) from them. Unfortunately one major disadvantage of pre-implantation loss is that there is chance of errors in counting corpora lutea because they remain very close to each other and sometimes may also fuse (Epstein and Rohrborn, 1971; Epstein et al. 1972; Machemer and Lorke, 1973; Rytman, 1976). Besides, the pre-implantation loss of egg may be non-genetic in origin such as non-availability of the sperms for the fertilization due to the depletion of the spermatozoal population (Batsman and Epstein, 1971; Searle and Beechey, 1974).

The criteria for the post-implantation loss is the frequency of dead implants. It is simpler, easier and more rapid to get an account of post-implantation loss than its pre-implantation counterpart. It requires only examination of uterine contents for living and dead implants at the late stage of pregnancy. Early and late fetal deaths are also identifiable by distinguishing dead implants without and with placenta, where the former refers to early fetal death and the latter to late fetal death.

Soares (1972a,b) developed a new method where the females were dissected after litters had been weaned for the inspection of uterine scars, instead of dissecting females at pregnancy. The scar method is advantageous in the sense that the live offsprings can be saved. However, the main drawback associated with scar method is that it underestimates the number of dead embryos. Comparative studies of these two methods (classic and scar method) show that the classic method is more reliable
An excellent correlation between the dominant lethality and frequency of cells containing cytologically visible chromosomal abnormality has been found by Brewen et al. (1975) in the mouse ova fertilized with sperms from MMS treated males. Same type of correlation has been obtained when females were treated with MMS (Brewen and Payne, 1976). Similar observation has been made by Mätter and Jaeger (1975) with TEM. Their data clearly indicate that the incidence of pre- as well as post-implantation losses can be accounted for simple damage to the male chromosome complement. Not only the frequency of aberrant male chromosomes has correlation with the level of dominant lethality but the two parameters also have good correlation with the time interval between treatment and mating. This widely used DL test for the detection of mutagenicity is more reliable in mice compared to the Drosophila system, because in the former dead embryos (fertilized ova) are considered at an advanced stage of pregnancy thereby the scoring is more accurate.

The usefulness of this test system has been discussed in detail by several earlier workers (Ehling et al., 1968; Epstein et al., 1970b, 1972; Bateman and Epstein, 1971; Epstein and Rohrborn, 1971; Ehling et al., 1978). As DL test assesses chromosomal damage to the parental germ cells in vivo, which is more relevant, it is advantageous over the classical method of somatic metaphase chromosome analysis.

In spite of several advantages mentioned above, the sensitivity of DL test is sometimes doubted. The doubt relates to its interpretation and mutagenesis. It appears likely that
mutagens producing point mutation only, such as nitrous acid and purine analogues would go undetected in DL test (Auerbach, 1962; Ehling et al., 1968). Most of the mutagens of course produce both chromosome aberration and point mutation; and no agent has yet been recorded which breaks chromosome without producing point mutation. But it does not necessarily mean that DL mutations will always be accompanied by point mutation. Anyway, DL mutation which accounts for chromosome abnormality at the germinal level may be considered as evidence of general mutagenicity. It has also been recommended as one of the important currently available assay systems to detect the mutagenic effect of chemicals in mammals (WHO, 1971; Batsman and Epstein, 1971; Committee-17, 1975; Ehling et al., 1978).

1.3.5 Sperm count

One sperm is sufficient to fertilize an egg. However, successful fertilization of an egg requires a moderate size of the sperm population (Walton, 1927). It is of course not known at which level of the sperm concentration failure of egg fertilization starts to occur. In mammals the production of spermatozoa depends on gonadotrophic and steroid hormones. However, numerous physical and chemical agents are known to cause testicular atrophy and damage to the seminiferous tubules leading to total or partial arrest of spermatogenesis in mammals. With X-ray, an acute dose of 200 r or more has been shown to interrupt the fertility in mice (Schaefer, 1939, Bateman, 1958; Searle and Beechey, 1974). For some weeks after the treatment they remain fertile and then sterility starts and continues till the resumption of full fertility. With higher dose levels the sterile period lengthens at the expense of the initial fertile period.
It has been established that this sterility is resulted from spermatozoal depletion caused by the X-ray induced killing of spermatogenic cells (Searle and Beschey, 1974). Early spermatids are found to be most susceptible to X-ray (Cattanach, 1975).

Similarly various chemicals including certain metallic and rare earth salts have been reported to cause extensive damage of testis and arrest of spermatogenesis in several mammalian species (Sharma et al., 1973). Spermatogenic cell killing activity of triethylenemelamine (TEM) leading to sterility is well established (Cattanach, 1966). This sterility as judged by the sterile mating is due to its effect on the spermatogonial cells. Similarly spermatocytes and spermatogonia are sufficiently sensitive to mitomycin-C (MC) induced killing to give clearly recognisable sterile period with most doses (Ehling, 1971). Lead acetate, Yttrium nitrate, Lanthanum chloride* cause extensive damage and calcification of seminiferous tubules and arrest of spermatogenesis (Sharma et al., 1973). Mild to moderate damage is caused with formaldehyde (Sharma et al., 1973). Production of necrotic changes in the seminiferous tubules, epithelium as well as interstitial tissue leading to sterilization by cadmium salts has also been reported (Parizek, 1960; Kar and Das, 1962).

Thus the mode of action of the physical agents differs from that of chemical agents (Steinberger and Tjioe, 1969). Again different types of chemicals such as alkylating agents (Steinberger, 1962), amides (Steinberger and Sud, 1970), metal (Kar and Das, 1962; Chandra, 1971), cytotoxic agents (Nelson and Patanelli, 1965) act differently.
In contrast our knowledge on the effects of tranquilizers on spermatogenesis is very much limited (Burns, 1965; Hunter et al., 1967). Substances which reduce the fertilizing power of sperm or reduce the sperm count may not strictly cause genetic hazard. But in view of the widespread use, it is of great interest to study the effects of tranquilizers on the sperm production irrespective of the mode and site of action.

1.3.6 Sperm head abnormality

Male gametes generally have a species specific morphology. Different workers have shown in mice that the morphological characteristics of sperms are under genetic control (Illisson, 1969; Beatty, 1970, 1978; Wyrobek and Bruce, 1973; Krzanowska, 1976a,b). However, in normal healthy adult males even a fraction of sperm population is always with abnormal head morphology. Besides, different inbred strains of mice are known to differ widely in the proportion of their sperms with abnormal head structure (Beatty and Sharma, 1960; Krzanowska, 1972a, Bruce et al., 1974). Thus the incidence of these meiotic products with abnormal shape of their heads also seems to be genetically controlled. Several physical and chemical mutagens, teratogens and carcinogens have been reported to change the frequency of sperm abnormality of several strains of mice according to dose and time after exposure (Gillette, 1966; MacLeod, 1974; Bruce et al., 1974; Wyrobek and Bruce, 1975). Moreover, there is a remarkable similarity between the dose-effect curves for abnormal sperms and chromosomal abnormality after irradiation (Muller, 1954; Tate and Clelland, 1957; Wolff, 1961, 1963; Revell, 1963; Evans, 1963; Scott and Evans, 1967; Elkind and Whitmore, 1967; Report
of the UN Scientific Committee on the effect of Atomic Radiation, 1969). The foregoing facts strongly support the genetic control of normal development of spermatozoa.

Different workers have put forward different hypotheses to explain the genetic mechanism behind the production of abnormal sperms. Bruce et al. (1974) hypothesized the possible link between sperm abnormalities and their chromosome abnormalities. But Wyrobek et al., (1975) failed to obtain any correlation between the frequency of sperm abnormality and chromosomal aberration in mice homozygous and heterozygous for different Robertsonian and reciprocal translocations. They came to the conclusion that the induced sperm abnormalities were caused not for chromosomal abnormalities but due to gene mutation. Krzanowska (1974) claims that sperms with abnormal morphology are non-functional. If it is so, the possible mechanism of transmission of chromosomal imbalance, and also perhaps gene mutation, may well be the fertilization of an ovum with normally appearing sperm carrying abnormality. Krzanowska (1966, 1969, 1976a,b) from results of his studies on F$_2$ and the back crosses between two different strains of mice having differential frequencies of abnormal spermatozoa suggested that the head abnormality of sperms in mice is a polygenic character and it involved at least three genes one of them being located in Y-chromosome. Recently Godowicz (1977) from his autoradiographic studies concluded that in abnormal spermatozoa the biochemical composition of chromatin was also abnormal. So genetic explanations behind the production of abnormal sperms are not yet clearly known.
Sperm abnormalities, leaving aside the genetic background for their occurrence, may permit a simple quantitative assay for genetic damage in the male germ line. Over and above, out of all the germinal and embryonic cell types involved in mammalian reproduction sperms are by far the easiest to obtain. Head abnormalities of these germ cells can be scored easily and their proportion with and without abnormalities is reproducible in short time. With this background the measure of the proportion of sperms with abnormalities in their head morphology may well constitute a quick, simple and sensitive biological screen for drugs, chemicals and other agents having deleterious effects on the genetic material of the germ line cells in vivo.