DISCUSSION
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The cytogenetic effects of some steroidal hormones (sex hormone and corticosteroid) have been evaluated in vitro in the present study. The advantages and disadvantages of the test systems used, the parameters assayed and the effect of the steroids evaluated on these parameters are discussed below.

(A) General Considerations

All the results of mutagenicity (genotoxicity) evaluation of the steroids are eventually aimed at human safeguards. It is therefore ideal to evaluate them directly on humans who are exposed to these steroids or test them for genotoxicity on a system that has the same sensitivity of response as the human genome. The human peripheral blood lymphocyte culture system is a single system that provides us with a large number of dividing cells. These can be easily cultured to yield large number of mitotic figures from chromosome analysis. The lymphocytes also contain some metabolising enzyme systems that are able, to some extent, to activate certain promutagens into a mutagenically active state (Buckton and Evans 1982).

In vivo and in vitro evaluation:

It is ideal to study the effect of chemicals directly on persons exposed to these chemicals or drugs (Gebhart 1982, 1984). But ethical reasons preclude the experimental approach on evaluation of genotoxicity on humans. The in vivo study is
possible only in specific cases when individuals have been exposed to the agents either as a chemotherapeutic necessity (e.g. medicines) or occupational inevitability (e.g. exposure to styrene, aniline, polyvinyl chloride) or accidental reasons (e.g. exposure to radiation, atom bomb explosions etc.).

The genotoxicity evaluation among patients on therapy is difficult. Patients may be on multiple therapy and the effect observed may either be additive, synergistic or antagonistic to each other.

The in vivo cytogenetic evaluation also has its shortcomings. The cytogenetic abnormalities found in exposed persons represent the most drastic and obvious expression of genetic damage while the frequent and subtle genetic changes like mutations are not detected. Another limitation is that the peripheral lymphocytes which are cultured are not in the dividing cell cycle at the time the chemicals exert their influence in vivo. Drugs which can cause primary genetic lesions in the G_1 phase only can manifest their detrimental effects while agents that cause genetic damage in the S-phase may not exhibit cytogenetic damage in lymphocyte cultures of persons exposed to these agents (Gebhart 1982).

These shortcomings of the in vivo evaluations can be overcome by in vitro treatment of peripheral blood lymphocytes in cultures. The experimental approach can be used and the effect of drug/chemicals can be evaluated at a
range of concentrations (including sub-toxic and toxic level) on lymphocyte cultures of healthy individuals. This also permits the evaluation of effects of different time exposure to the drug and fate of lesion after consecutive cell division as well as the effect of drug during the $G_1$ as well as $S$-phase of the cell cycle. In vitro test, however, has its own shortcomings because is no metabolising system of the drugs and chemicals in it. But lymphocytes have been known to metabolise at least some compound, e.g. activation of polycyclic hydrocarbon benzo(a) pyrene (Whitlock et al. 1972, Okano et al. 1979). The presence of erythrocytes in cultures is also known to aid in metabolising compounds like aniline, HCl (Wilmer et al. 1984), sodium selenite (Ray and Altenberg 1978) and styrene and its analogues (Norppa et al. 1983; Norppa and Tursi 1984).

In the present study three sex steroid hormones, Estradiol 17-β, Estriol, Ethynylestradiol and three cortico-steroidal hormones (Hydrocortisone, Fludrocortisone, Dexamethaonse) were tested for their cytogenetic effects on human lymphocyte chromosomes in peripheral blood cultures in vitro. All the drugs were tested in the presence of metabolic activation (S9 mix).

Chromosome aberrations

Chromosome aberration assessment serves as a good indicator of the clastogenic effects of agents (Gebhart 1970, Buckton and Evans, 1982; Hsu 1982). The aberrations can be
classified into chromatid type aberrations which are caused in the latest $S_0$ or $G_2$ phase cell cycle and the chromosome type aberration which are caused by damage to the chromosome materials in the $G_1$ or early $S$-phase. The chromatid type aberrations include chromatid gaps, breaks and deletions. Chromosome type aberrations, isochromatid gap, and break out interchromosomal exchange resulting in ring chromosomes and acentric fragments, and interstitial deletions resulting in acentric fragments (Hsu 1982).

Inclusion of gaps in the quantitation of chromosome damage following exposure to drugs and chemicals is controversial. Many workers ignore gaps in their analysis (Bukton and Pike 1964, Goetz et al. 1975). However, others suggest that since an increase in the frequency of gaps is related to mutagen exposure, they should be included in the analysis (Gebhart 1977; Anderson and Richardson 1981, Hsu 1982, Brogger 1982).

The distinction between chromatid gap and break is very arbitrary. A gap cytogenetically is distinguished from a break depending on the length of the non-staining region in the chromosome. If the attenuated region is shorter than the width of the chromatid, it is classified as a gap and if longer than the width it is a break. But the problem in this classification is that chromosome at prometaphase are elongated with a small width of chromatid while at late metaphase they are condensed with larger width. This results in classification of more breaks in early metaphase and more
gaps in late metaphase.

Various studies have been done to look into the nature of gaps and breaks. A combination of studies with light microscopy, transmission electron microscopy and scanning electron microscopy of X-ray induced aberrations in _Vicia_ showed that gaps and breaks are different manifestations of the same event and that gaps may be completed breaks (Brecher 1977). Breaks, for example, are thought to be a complete loss of material at the non-staining region with displacement of the distal fragment, whereas gaps are thought to have a chromatin bridges. But electron microscopic studies (Brecher 1977) demonstrated that few breaks had chromatin material bridging the space while same gaps had no such chromatin bridges. Thus the distinction between the two is arbitrary at the light microscopy level and both are apparently different manifestations of the same phenomenon.

Comings (1974) offered a hypothesis regarding the nature of gap. According to him gaps and breaks represents the same basic lesions but differ in the time at which the lesions is induced. If a break in chromatin fibre occurs when the chromatin is fully extended (e.g. _G_1, _S_ and early _G_2 phase), subsequent condensation of chromosome results in a chromatid break. But if the chromosome is already partially condensed (e.g. _G_2 phase) and a break occurs, a few fibres still hold the chromosome together, resulting in a gappy appearance.
Silver staining technique of the chromatid core has shown that the chromatid core is not continuous in chromatid gaps (Satya Prakash et al. 1981). Some authors suggest that gaps are produced due to loss of despiralization of both DNA as well as chromosomal proteins (Stoain and Raicu 1973). An increase in constriction and gaps in human chromosomes following mercapto-ethanol exposure suggests that gaps may occur from damage to the protein moiety of the chromatid causing deficient folding of the chromatin fibre in metaphase chromatid (Brogger 1975).

However, under the influence of mutagen, the frequencies of both gaps and breaks increase. It has been suggested by many authors that gaps are indicative of toxic phenomena from genetic point of view and the aberrations could be useful sensitive indicators of chemically induced genetic damage (Anderson and Richardson 1981; Gibhart 1977, 1982, 1984; Goetz et al. 1975; Dipaolo and Popescu 1976; Hansteen et al. 1984; Hsu 1982). Schinzel and Schmid (1976) claim that gaps could be artefacts of staining and processing, and are most subjective parameters of analysis. But this claim does not appear to be correct because if they are really artefacts, a similar incidence would be expected in the control and treated groups.

Any damage altering the normal morphology of a chromosome (be it because of damage to DNA and chromatin or to the packing proteins of chromosomes) could interfere with
normal functioning of the chromosome causing genotoxicity. Therefore gaps were included in the present study.

Sister Chromatid Exchanges

Sister chromatid exchange (SCEs) represents the interchange of DNA strands between sister chromatid at apparently homologous loci. SCEs were first demonstrated by Taylor (1958) through autoradiographic technique using tritium labelled thymidine. The dilution of SCEs became simple and more precise with the incorporation of 5-Bromo-deoxyuridine (Brdu) into the DNA by culturing cells for two cell cycles to give one unifilary substituted and one bifilary substituted chromatid in a chromosome (Latt, 1973). Subsequent differential staining of these chromatids was obtained by fluorescent dyes (Latt 1981) or fluorescent plus giemsa stain (Perry and Wolf 1974).

The frequency of SCEs has been found to be greatly increased on exposure to mutagenic agents. Therefore, SCEs are considered to be sensitive indicators of DNA damage (Latt 1974; Perry and Evans, 1975; Carrano et al. 1978; Gibhart 1981, 1982, 1984).

A positive correlation between the frequencies of chromosome aberrations of SCEs has been found with most of the mutagenic chemicals (Latt 1974). But, some mutagenic agents cause an increase only in aberration frequency and not in SCEs, e.g. X-rays (Perry and Evans 1975), certain antitubercular drugs in combination in vivo (Madhuri et al. 1983)
and some antibiotics in vitro (Manjula et al. 1984). On the other hand there are other mutagenic chemicals that induce only SCEs but does not cause chromosome aberrations e.g. aniline (Abe and Sasaki 1977; Istudate and Odashima 1977). There is also a fourth category with a small number of mutagenic chemicals that neither cause chromosome aberrations nor induce SCEs e.g. sodium azide (Arenaz and Nilan, 1981).

Thus SCEs and chromosome aberrations are two different endpoints depicting cytogenetic damage due to mutagenic action. The mechanism of these end points is probably different. One system can not replace the other. Therefore both the endpoint should be scored to assess the genetic damage caused by drugs and chemicals.

**Cell Growth Kinetics**

Cell cycle kinetics analysis is based on the differential staining technique of sister chromatids. Scoring of preparation of cells at $M_1$, $M_2$ and $M_3$ (metaphase in 1st, 2nd and 3rd replication cycle) is a sensitive method for detecting delay or stimulation in human lymphocyte cultures (Tioe et al. 1976; Crossen and Morgan 1977; Morimoto and Wolff 1980).

The rate at which mitosis occurs is measured by the mitotic index. The index gives an idea about the stimulation of lymphocytes to divide and the mitotic activity in culture (Melarango and Smith 1984; Sinha et al. 1984). Any change in mitotic index following drug exposure compared to concurrent
control, provides information on mitostimulative or mitodepressive activity of the drug.

Treatment resulting in SCE formation have been found to induce cell cycle delay (Morimoto 1983; Craig-holmes and Show 1977). However, the relationship between the proliferation rate as measured by cell growth kinetics and the induction of SCEs is controversial. Santessen et al. (1979) found that SCE levels in B-lymphocytes (early proliferating cells) were significantly lower than those in T-lymphocytes from the same individual. Snope and Rary (1979) and Block et al. (1982) also found a lower SCE frequency in the more rapidly dividing cell populations. Lindblad and Lambert (1981) found that though the percentage of B and T lymphocytes did not correlate with SCE frequency, a significantly higher SCE rate was presented in slowly proliferating cultures. Becher et al. (1981) however found low SCE and longer cell cycle in leukemic cells while Heidemann et al. (1983) found increased SCE rate and shortened cell cycle time in trisomic cells of a Down syndrome mosaic. Many other workers however failed to find any relationship between SCE rate and cell cycle kinetics. For example, in second generation metaphases collected from different culture times (Beck and Obe 1979), Moritomo and Wolff (1980) and Morgan and Crossen (1981) found no significant differences in SCE frequency. Giulotto et al. (1980) also concluded that the incidence of SCE appeared to be independent of the proliferating properties of cultured lymphocytes, such as length of cell cycle, fast or delayed response to PHA and number of divisions performed in vitro.
Lamberti et al. (1983) set up repeated cultures in three tissue culture media and found a great variability in proliferation kinetics between tissue culture media and between donors. Speit et al. (1986) studied the SCEs frequency and proliferation of cultures in repeated human lymphocyte cultures. They also found no systematic relationship between the proliferation of culture and basal SCE values. Also, a great variation in the two parameters was observed in repeated cultures.

(B) Cytogenetic effects of steroidal hormones

I. Estrogens

Estrogens are mitotic poisons which at high concentrations, cause metaphase arrest, abnormal cell divisions and chromosomal aberrations (Wheeler et al. 1986).

The overall conclusions that emerge from the present results show that the use of estrogens (sex steroid hormones) are associated with a significant increase in the incidence of chromosome abnormalities per cell as well as with an increase in the number of abnormal cells when compared with normal controls. Of these sex steroids used for the present study, the estriol has the least effect on human lymphocytes in vitro. Even in the presence of metabolic activation, the drug did not induce any significant effect on the human lymphocyte. Estriol may be considered as weak estrogen. However, Estradiol 17-β and Ethynylestradiol are capable of inducing chromosomal abnormalities in human
lymphocytes and the effect is dose-dependent. The maximal effect was at 72 hrs of treatment, an observation in line with that for the majority of clastogens.

There are contradictory reports regarding the in vitro and in vivo mutagenic activity of sex steroids or oral contraceptives. Carr (1967) found that there is no evidence of an increase in congenital anomalies and multiple births in pregnancies which result after discontinuing oral contraceptives, although he found, in another investigation (Carr 1970), chromosome abnormalities in 6 out of 8 spontaneous abortions from women who had become pregnant after taking the steroids in the form of oral contraceptive. According to him a striking increase in triploidy took place which was considered highly significant statistically. Rohborn and Hansmann (1974) found a slight but not significant increase of non-disjunction due to 1 mg of norethisterone acetate immediately after treatment or after an interval without the drug application. Rice Wray et al. (1970) found no congenital defects and the studies of karyotypes and sex chromatin revealed no abnormalities in any of the children born from women who had taken the sex steroids before pregnancy. The same results were reported by Bishun and Mills (1971) and Van Leuven et al. (1974). Mills et al. (1975) found no significant difference in the frequencies of aberrations between the test and control mothers, although minor differences were found in the babies.
Of the various chromosomal aberrations induced by estradiol 17-β and ethynyle estradiol, chromatid and chromosome fragment and breaks were found more frequent. Similar observations were made in Anovlar treated plant chromosomes (Kabiraty and Mazrooei 1984). Hakeem and Amer (1965) found that the common effects of steroid hormone of chromosome were stickiness in diakinesis and metaphase, and bridges and sometimes lagging chromosomes in the anaphase. No material difference was observed in the percentage of abnormalities in diakinesis, metaphase I and metaphase II. Treatment with Conovid and Lyndiol affected significant low percentage of abnormalities. Anovlar caused chain of bivalents in addition to stickiness. This may be attributed to the sticking of the ends of the different bivalents with each other giving rise to such configurations.

Chromatid breaks were seen more frequently than chromosome breaks in the metaphase plates analysed. This indicates that the drug affects the DNA strand at its late S-phase or after the DNA has replicated or duplicated (Bird et al. 1982). The majority of the chemical agents induce measurable frequencies of aberrations only when the cell passes through on S-phase between treatment and observations. The peripheral lymphocyte is a G₀ cell, and so the first S-phase it will pass through is the in vitro one following mitogenic stimulation. It can be said that the frequency of chromatid type aberrations observed following exposure of G₀ will be related to the amount of DNA damage whose repair or
replication results in aberration formation, that remain at the time of replication (Preston 1986). This in turn will be influenced by several factors. It will, of course, be influenced by the amount of the specific DNA damage that results in aberration which will be dependent upon the agent and the exposure. It will be influenced by the amount of repair that can take place in G₀ and G₁ following stimulation with a mitogen. The amount of repair in G₀ will be dependent upon the rate of repair and the time between exposure and sampling - the longer the time, the more repair. The amount of repair in G₁ will likewise be dependent upon the rate of repair and the length of G₀ in vitro (Preston 1981). The present finding can be further corroborated by Goh in 1967, who reported an increase number of chromosome breaks and rearrangements in lymphocyte cultures of women taking oral contraceptives. This finding was confirmed by Badr et al. (1972), Littlefield et al. (1971) and McQuarrie et al. (1970), but was questioned by Shapiro et al. (1972). In in vitro studies Stenchever et al. (1969) and Timson (1969) failed to find any effect of these on human lymphocyte cultures. DeGutierrez and Lisker (1973) found that oral contraceptives do not seem to increase the frequency of chromosome rearrangements. Williams et al. (1968) stated that progesterone causes alterations in the meiotic chromosomes, which are chiefly sticky and show improper spreading with clumping of the chromosomes. Rao (1969) found that Estradiol 17-β induced reversible mitotic delay in Hela cells which
were localized at the beginning of mitosis. The duration of the mitotic lag was dose dependant. Only those cells that were in S or G₂ periods at the time of estradiol treatment suffered the delay but not those in the G₁ period. Shayma et al. (1991) studied on genotoxic effect on Anovlar, a combination drug containing the estrogen ethynyl estradiol and progestin norethisterone acetate. Statistically significant increase in chromosomal aberrations were observed in mouse bone marrow. But the drug did not induce a significant increase in number of micronuclei in bone marrow erythrocytes at any of the doses and time intervals studied.

The present data on the time response analysis revealed significant increase in chromosomal aberrations at 48 hrs of treatment and decreased later on which is supported by the study carried on by Shayma et al. (1991). The maximum frequency of chromosomal aberration were found at the 24 hrs. long treatment on mouse bone marrow while it decreased later on and gave no significant results. This suggests that the drug and/or its metabolites were active during this (48th hrs) period. The decline in the frequency of aberrations observed at the later time intervals may be due to some of or all of the following reasons (i) repairs of damaged genetic material, (ii) elimination of the drug and its metabolites; (iii) elimination of cells/chromosomes with damaged genetic material, (iv) or due to cell cycle delay and cell killing effect, (v) and inactivation of the drug or its metabolites.
Widmeyer and Shavar (1972) studied the chromosome complement of blastocysts recovered from rabbits injected with estradiol or progesterone during the time of ovum maturation or the first cleavage division. They found that the spindle fibre apparatus was disturbed due to the treatment. A lower mitotic index than normal was found in the whole mounts of these blastocysts.

On the other hand Gopalakrishna and Inamdar (1971) found that estrogen accelerates the mitotic activity immediately after administration of the first dose. Accelerated level however, never exceeds the normal level that is established in the target tissues in due course. The effect of estrogen on binuclearity in the cells of liver or mouse was also studied. The hormone affects the incidence of binucleates. The increase in the occurrence of binucleates could be due to mitotic effects exerted by the hormone.

The effect of estrogens (estriol, estradiol-17β and ethynyl estradiol) were studied in the presence of metabolic activation. Estriol was found to induce very low frequencies of chromosomal aberrations. This may be due to weak mutagenic potentiality of the drug even in the presence of $S_9$ mix (metabolic activation), as it did not produce significant abnormality. However, estradiol-17β and ethynyl estradiol induce chromosomal abnormality in the presence of $S_9$ mix. The time response analysis in the presence of $S_9$ revealed significant increase at 48 hrs of incubation and decreased later on. The study conducted by Banduhu and Obe (1985)
supports the present data. Estradiol binds to isolated DNA (Blackburn et al. 1974) and induces fragmentation in isolated DNA (Yamafuji et al. 1971). On the other hand, mutagenic studies, with and without metabolic activation, do not show positive results for steroid hormones (Lang and Redmann 1979; Drevon et al. 1981).

The present results show that the estradiol has the least effect among all the sex steroids for the study on the frequency of sister chromatid exchange (SCE). However this drug has affected the SCE frequency in the presence of liver enzyme ($S_g$ mix). The other sex steroids namely estradiol-17 $\beta$ and ethynyl estradiol do show marked increase in the incidence of SCE in peripheral human lymphocytes. These steroids also induced SCE in the presence of $S_g$ mix. The overall results on the induction of SCE by these steroids are in complete agreement with the study conducted by Prema and Murthy (1983) and Kochar (1988).

For a long period of time the scientific community has not been able to ensure the possible role of steroid hormones or hormonal contraceptive in causing chromosomal changes in mammalian cells. However, since the discovery of new high resolution techniques for detecting subtle changes in chromosomes, there have been a few studies to resolve this controversy. The study conducted by Prema and Murthy (1979) showed that women using estrogen-progestogen combination contraceptives showed significantly higher rates of mean SCE cultured peripheral blood lymphocytes compared to subjects
consuming no oral contraceptives or the pregnant women. Pregnant women were included in the study to assess the role, if any, of an altered normal hormonal profile *per se* on SCE. It is suggested that alteration in SCE in the cells of contraceptive - using women may be due to mutagenic activity of the estrogens or their metabolites. However, another group of workers, viz. Husum *et al.* (1982) found no evidence at all of the elevated SCE in women using a similar combination of contraceptives. The use of contraceptive had no influence on SCE in non-smoking or in cigarette-smoking women. This discrepancy could be due to slightly higher normal SCE rates in control women reported by them.

In contrast with these observations, women using progestrogen-containing contraceptives were found to have normal SCE rates. This may mean that the increased SCE rates observed in combination and contraceptive users could be due to potential mutagenic effect of estrogen.

The study performed by Kochar (1988) utilizing the SCE technique, does indicate that the steroid hormones are capable of producing elevated SCE in cultured Chinese Hamster Ovary (CHO) cells. This is in line with the present study that the estrogens namely estradiol, estrone, estriole and ethynyl estradiol are very effective in producing various types of chromosomal aberrations in SCEs in CHO cells. Also, a study conducted by Rao and Engleberg (1967) showed that similar estrogens affected a high degree of structural specificity in the induction of mitotic chromatid non -
disjunction that ultimately resulted in individual chromosomes in two daughter cells.

There exists a relationship between increased SCE rates and altered mutation frequency (Carrano et al. 1978), the long term persistance of increased SCEs in hormonal contraceptive users may have some ill effects on the health of these users. It may be important to find out whether the changes in SCEs associated with the use of contraceptive could be reversible. As per Prema and Murthy (1983) that the frequency of SCEs in 8 women during the use of combinational contraceptive showed significantly increased SCE rates and the SCE rate markedly decreased among those who discontinued the contraceptive. This may mean that cells with increased SCEs are being replaced with cells with normal SCE in these women. It can be suggested that sex steroid induced SCEs are a temporary phenomenon and there are no persistant mutations in contraceptive users.

The overall results of the present investigations on the cell growth kinetics, showed that the esteriol did not inhibit the in vitro proliferation of lymphocytes both with and without metabolic activation. However, both estradiol-17β and ethynyl estradiol strongly inhibited the proliferation of lymphocytes, these drugs affected the cell kinetics growth even in the absence of metabolic activation. Both these drugs are considered strong estrogens which have significant effect on the cell proliferation in lymphocytes and esteriol is considered a weak estrogen (Hill and Wolff 1983). Estradiol
may able to inhibit DNA and protein synthesis in mouse fibroblasts which may result in the inhibition of cell proliferation (Kuchler and Grauer 1962). The same study on mouse fibroblast indicated that estriol did not inhibit DNA and protein synthesis.

Proliferation of lymphocytes in culture is dependant on the time of phytohaemagglutinin (PHA) mediated transformation of small resting (GO) lymphocytes into blast cells and the length of the cell cycle after the transformation has taken place (Morimoto and Wolff 1980). It has also been observed that T-lymphocytes respond to PHA first (Greaves et al. 1974). As activated T cells are necessary to produce a later B-cell response, so it is possible that these estrogens slow down the T cell proliferation, thus B-cell transformation is delayed in treated cultures.

Inhibited cell growth after the estrogens is due to an extended G2 phase. The average length of time for G2 in activity dividing lymphocytes culture is 3.5 hr (Hill and Wolff 1983). Cells may become arrested in G2, because there is DNA damage or because a metabolic block is present. Such G2 blocks are well known after cells are X-rayed. When Chinese hamster ovary (CHO) cells are treated with nitrosourea compound, they also become blocked in G2 (Rao and Rao 1976). By using the premature chromosome condensation technique, they showed a linear relationship between the number of extensively damaged G2 chromosomes and the accumulation of G2 cells in treated cultures and suggested that chromosome
damage is a contributing factor to the $G_2$ arrest.

II Corticosteroids

The result of the corticosteroids namely hydrocortisone, dexamethasone and fludrocortisone on the frequency of the chromosomal aberrations showed that, hydrocortisone only affects the lymphocytes in vitro. Hydrocortisone also enhanced the frequency of chromosomal aberration in the presence of metabolic activation. The present study is in line with the study conducted by Bali et al. (1990). The other two corticosteroids did not prove to be genotoxic in the present study. The same was observed by Dhillon (1990) in the *Salmonella* test, that no evidence of any mutagenicity of these drugs either in the presence and absence of metabolic activation was seen. No significant increase was observed in the frequency of His$^+$ revertants/plate.

The genotoxic effect of the hydrocortisone on human chromosome is evident. The drugs caused the maximum number of aberrations at 25 mg/ml concentration. The higher dose of this drug (50 ug/ml) also exhibited clastogenic effect but with less magnitude. The reduction could be attributed to cell cycle and cell killing effect caused by hydrocortisone at the highest concentration. The continuous presence of the DNA synthesis inhibiting drugs in the medium may greatly reduce the mitotic index without increasing the frequency of chromosomal aberrations, as the severely damaged cells remain in interphase (Au and Hsu 1982). The same findings are
further corroborated by the reports of Navoma (1977) and Rubin (1982), indicating the suppression of DNA synthesis, prevention of protein binding of the drugs at its higher doses, reduction in mitotic cell population, and induction of genetic damage following hydrocortisone treatment in both animals and humans.

The effect of hydrocortisone on the appearance of aneuploid cells in bone marrow cells of mice has been observed by Longninova et al. (1970). The increased frequency of aneuploid cells with increasing concentration of the test compound has been observed. The phenomenon of aneuploidy gets increased at the expense of both hypoploid and hyperploid cells. Some disturbances of the hormonal equilibrium is associated with aneuploidy during early embryogenesis or gametogenesis. The deleterious effect of hydrocortisone on the frequency of chromosome aberration was also studied by Illinskikh (1979).

The frequency of sister chromatid exchange (SCE) also increased with higher dose, but with lesser magnitude at the highest concentration of hydrocortisone. The effect was same even in the presence of metabolic activation. The other two drugs (dexamethasone and fludrocortisone) did not enhance the SCE frequency, either with or without metabolic activation. SCE arise from DNA breaks and the reunion of broken exchanged fragments at almost homologous loci (Latt et al. 1981). The observed rise in the frequency of SCE could be attributed to the hydrocortisone's influence in increasing the number of
DNA lesions. The present study is in line with Loeb et al. (1973), Navoma (1977), and Illinskikh (1979) who observed DNA suppression, cell proliferation inhibition, and bone marrow deterioration effects of the hydrocortisone. The hydrocortisone found to be non-mutagenic both with and without S9 mix in the Ames/ *Salmonella* test system (Bali et al. 1990).

Among all the corticosteroids used for the present study, only the hydrocortisone could inhibit the proliferation of human lymphocytes. Thus it can be concluded that hydrocortisone is genotoxic and potent steroidal drug capable of directly attacking the genetic material.

Because of the apparent relationship between chromosome abnormalities, mutagenesis, carcinogenesis (Sandberg 1980), *in vivo* and *in vitro* screening for chromosome aberrations is an established tool in the evaluation of potential hazards to the genetic material due to environmental agent. However, the great limitations of this morphological approach is that only gross genetic damage is visible and negative findings do not exclude other types of genetic damage such as those resulting in point mutations.

The phenotypic expression of genetic damage either as visible chromosome aberrations or single point mutations, is dependent on the developmental time when the damage occurs, namely pre- or post natal life, and the type of cell, somatic or germinal, in which damage occurs (German, 1979). If such damage occurs in somatic cells of the developing embryo, it
can lead to malformations and abortion in extreme cases.

The theoretical possibility of genetic damage during post-natal life will apply directly to the health of the drug user. Any mutational effect on adult somatic cells could lead to cancer (Weiss, 1979).

One should, therefore, be mindful of the potential hazards of such drugs, especially if they are continuously used for such long periods.