In the present investigation potential genotoxic and cytotoxic effects of two most widely used antileprotic drugs, Clofazimine (CLF) and Dapsone (DDS), and one antifilarial drug, Diethylcarbamazine (DEC) were evaluated by using different protocols in mice in vivo system.

Base free samples of the drugs procured directly from the respective manufacturers in the form of powder were used as test substances. Inbred Swiss albino mice aged 10-12 weeks constituted the experimental animals. As all these drugs are prescribed for oral consumption in human therapeutics they were always administered via oral route in this study. The drugs were either dissolved or taken in suspension in a suitable vehicle (alcohol, acacia or water) and administered by gastric intubation. Age and sex matched mice treated with corresponding vehicle, or untreated (where the solvent or vehicle is water) mice served the purpose of control (negative control).

The following assay systems were tested: (i) metaphase chromosome analysis in bone marrow cells, (ii) micronucleus test (MNT) in bone marrow cells, (iii) micronucleus test in regenerating hepatocytes, (iv) spermatocyte chromosome analysis, (v) sperm morphology assay, and (vi) sperm count assay. However, all the assays could not be tested for each of the drugs.

To standardize some of the test protocols and to fix up criteria for the analysis of various aberration types some positive control experiments were conducted with mitomycin C (MC) - a well
known mutagen-clastogen. It was not possible to run positive control side by side in each experiment. Dose-response analyses done following ip administration of a single dose (0.5, 1.0 or 2.0 mg/kg) of MC at 72 h post-administration for bone marrow metaphase chromosomes, spermatoocyte chromosomes and MN in regenerating hepatocytes exhibited distinct dose-related increases of the abnormalities in all the cases. The results were at par with those of earlier workers. The criteria adopted here to identify different aberration types were followed for test compounds also.

6.1 Effect of Antileprotic Drugs :

6.1.1 Effect of Clofazimine (CLF)

In an attempt to simulate human exposure repeated treatment schedule was followed for this drug. For bone marrow chromosome analysis mice were exposed to the drug either directly or through mother's milk. For the direct treatment series 2 sets of experiments were conducted with mice of both the sexes. In the first set (referred to here time-response analysis for convenience) mice were treated with a dose of 40 mg/kg/day for 1, 2 or 4 weeks and killed 24 h after the last dose. In the second set (dose-response analysis) the animals were treated with three different doses of the drug, viz. 4, 20 or 40 mg/kg/day for 1 week and killed 24 h after the last dose.

For the other mode of exposure the mothers were treated with a dose of 40 mg/kg/day for 4 weeks starting from the date of
delivery and the youngs which were allowed to take mother’s milk ad libitum were killed after 2 or 4 weeks of exposure.

The chromosome aberrations encountered for different modes of exposure, i.e. direct and through mother’s milk, did not differ qualitatively and were mainly of chromatid type. A few sub-chromatid type breaks were also noted. Despiralization of chromosomes was the general characteristic found in CLP treated individuals. In time-response analysis and for higher two doses in dose-response analysis ‘break’ type aberrations which included chromatid breaks, iso-chromatid breaks, fragments of untraceable origin, rings and exchanges increased significantly over the corresponding control values. The maximum breakage frequency (9.60%) which was more than 6 times the control value was obtained at week 1 with the highest dose (40 mg/kg/day) tested. The ‘total aberrations’ which included in addition to ‘break’ type aberrations, gaps and sub-chromatid breaks exhibited the same picture. The incidences of aberrations, ‘break’ types as well as ‘total aberrations’, showed highly significant positive correlations with the doses but not with the periods of treatment. The incidences of chromosome aberrations in youngs exposed to the drug through mother’s milk for 2 weeks increased significantly over the control value; but the values for 4 weeks of exposure remained in the control range.

For micronucleus test in bone marrow cells a dose-response study was performed. Adult animals of both the sexes were fed with a dose of 4, 20 or 40 mg/kg/day of the drug for 1 week and their bone marrow cells were processed 24 h after the last treatment for MIT. The analysis of MN in bone marrow cells revealed
increased incidences of micronucleated poly- and normo-chromatic erythrocytes, taken separately or together, over the respective control values. The increases were significant with two higher doses. A decline in P/N ratio was marked. The mitotic indices calculated from the smear decreased greatly in the treated series and the decreases were significant compared to controls at higher two dose levels; further, the decreases showed a strong negative correlation with the doses.

For micronucleus analysis in hepatocytes a batch of mice received a daily dose of 40 mg/kg for one week. On the 5th day of the treatment period the animals were hepatectomized and after 72 h of hepatectomy, i.e. on the 8th day of treatment (24 h after the last treatment) their regenerating liver cells were processed for MNT following a simple method developed by us. Only the hepatocytes, both uni- and bi-nucleate, were examined for the presence of MN. The incidences of uninucleate and uni- plus bi-nucleate cells with MN increased significantly over the controls. The frequencies of cells with other type of nuclear anomalies remained within the control range.

A total of 12 adult males were treated with a dose of 40 mg/kg/day of the drug for different periods, viz. 1, 2 and 4 weeks and their testes, epididymes and vasa deferentia were processed for spermatocyte chromosome analysis, sperm count and sperm morphology assays respectively. Meiotic chromosome alterations noted in diakinesis-metaphase I stages were scored under three heads: numerical changes, structural changes and univalent formation (behavioural anomaly). In no case occurrence of aneuploidy and polyploidy was remarkable. Structural aberrations which
included chromosome and chromatid breaks and fragments increased significantly in all the test weeks. The highest frequency (5.2%) which was about 10 times the control value (0.5%) was noted at week 1. Occurrence of multivalents or exchanges was totally absent in treated as well as control series. Though control individuals contained a few chromatid breaks only, chromosome and chromatid type breaks and fragments were available in the treated series.

CLF enhanced the incidence of univalent formation involving autosomes as well as sex chromosomes. The increases were statistically significant for sex chromosomes and for sex chromosome plus autosomal bivalents.

Vas deferential sperm exhibited numerous types of abnormalities in their head morphology and qualitatively they were categorized under some arbitrary 'types'. The 'types' like spherical, twin and granular were encountered only in the CLF treated series. The frequency of occurrence of mis-shapen sperm (taking all the 'types' together) increased in all the test weeks in the treated series. However, the increase was significant only at week 1.

Haemocytometric count of epididymal sperm did not reveal any remarkable effect of CLF on sperm production though a tendency of suppression was noted in all the test weeks.

Thus, bone marrow chromosome analysis, spermatocyte chromosome analysis and micronucleus tests in bone marrow cells and regenerating hepatocytes clearly demonstrate clastogenic capacity of CLF following chronic treatment. The clastogenic efficiency is dose-related, but seems to have no relation with
the period of treatment. Its adverse effect, though mild, on
the induction of mis-shapen sperm is also documented. Our finding
which turns out to be the first report on the genotoxic effect
of CLF in eukaryotic system contradicts the earlier reports based
on bacterial system. Cases have been discussed where such type
of contradictions are known. Discussions have also been made
on the probable role of the drug itself (not of its metabolites)
and on its antimitabolic effect for causing chromosome aberrations.

6.1.2 Effect of Dapsone (DDS)

As the drug is recommended for long term use in human
therapeutics here also we used repeated treatment schedule for
evaluation of its potential genotoxicity. A batch of adult male
mice were fed with the aqueous suspension of the drug at the
rate of 40 mg/kg/day for 2, 4 or 8 weeks. This regimen was
referred to as time-response study. Similarly a dose-response
study was conducted with three different doses of the drug,
viz. 20, 40 and 80 mg/kg/day fed to adult male mice for 4 weeks.
In both time-response and dose-response studies the animals
were killed 24 h after the last treatment, and from each animal
bone marrow cells, testes, vasa deferentia and epididymes were
collected and processed for bone marrow chromosome analysis,
spermatocyte chromosome analysis, sperm morphology test and sperm
count assay respectively. Thus, for 4 different assays mentioned
above both time-response and dose-response analyses were done,
and materials were provided by the same animals. For micronucleus
test (MNT) in bone marrow cells a dose-response study was done
and mice of both the sexes were fed with a dose of 20, 40 or 80 mg/kg/day for 2 weeks. The animals were killed 24 h after the last treatment. For the entire DDS series untreated age matched mice served as controls.

Qualitatively the chromosome aberrations noted in bone marrow cells in time-response and dose-response studies did not differ from those of CLF treated series. The frequencies of break type aberrations increased significantly at all the dose levels and for all the treatment periods; while total aberrations showed significant elevations at two higher dose levels and for two longer periods of treatment. Individual aberration types also showed higher frequencies than the corresponding control values in all cases.

The KMT also revealed clastogenic effects of DDS as evidenced by significantly high incidences of MN in poly- and normochromatic erythrocytes, taken separately or together. However, doses had no influence on the incidences of MN. P/N ratios seemed to be little influenced by DDS. Mitotic indices showed a little decline, but it was not so striking.

Though the structural changes of meiotic chromosomes which included chromatid and chromosome breaks and fragments were more common in treated individuals of both time-response and dose-response studies their incidences were significantly higher only at the highest dose level (80 mg/kg) and for the longest period of treatment (8 week). The occurrence of aneuploidy was not at all remarkable. Similarly the incidence of univalent formation was found to be significantly high only for 8 week treatment schedule and that too marginally.
Like CLF, DDS also induced various 'types' of abnormalities in the shape of sperm head but failed to show any significant rise in the incidence of mis-shapen sperm over the control values either in time-response or in dose-response analysis.

DDS was also found to have no influence on sperm production as documented from epididymal sperm count.

The results obtained from bone marrow metaphase chromosome analysis, FNT in bone marrow cells and spermatocyte chromosome analysis reveal a positive, though mild, clastogenic effect of DDS with the doses tested and periods of treatment chosen. But sperm morphology assay and sperm count assay demonstrate lack of its effect on quality and quantity of sperm. Our findings are in good agreement with those of earlier workers who noted clastogenic effect of DDS in human cells in vivo as well as in vitro, but do not corroborate the findings based on bacterial assay system. The reason for differential responses of two systems - chromosome analysis in eukaryotes and bacterial assay system, remains unknown. Discussion has been made with reference to such differential responses noted for other chemicals. As the metabolism of the drug is not clearly understood it is not possible to explain if the drug itself or its metabolite is responsible for its genotoxic effect.

6.1.3 Relative Effectiveness of Clofazimine and Dapsone

In the subsequent section data of CLF treated series were compared with those of DDS treated series just to have an
Idea on the relative effectiveness in producing genotoxic effects. Data obtained following common treatment schedules only were considered. Bone marrow chromosome analysis following identical treatment schedules (40 mg/kg/day for 2 and 4 weeks) revealed higher incidences of breaks as well as total aberrations in CLF treated series than in DDS treated individuals. Identical picture was noted when spermatocyte chromosomes were analyzed. Comparative analyses of the data following identical treatment schedules on mg/kg basis show higher clastogenic efficiency of CLF than DDS in mouse in vivo system.

6.1.4 Effect of Clofazimine plus Dapsone

As recommendation of the triple drug regimen which includes both CLF and DDS is the current practice in the treatment of leprosy an attempt was made to analyze genotoxic potential of these two drugs when given simultaneously. Only bone marrow chromosome analysis was done for that.

Mice of both the sexes were treated via oral route with a dose of 40 mg/kg/day of CLF as well as with a dose of 40 mg/kg/day of DDS for 4 weeks. The animals were killed 24 h after the final treatment and bone marrow cells were processed for the protocol. Values from age matched untreated controls from DDS series (as DDS was used as an aqueous suspension) and from age matched 0.2% alcohol treated controls from CLF series (as CLF was used as 0.23 alcoholic solution) were pooled, and when this pooled control value was compared with the data of combined treatment significant increases in both break type aberrations
and in total aberrations were noted in the treated series. A comparison of the data of combined treatment with those of separate treatments and with the expected values (i.e. additive values for separate treatments for CLF and DDS), revealed an antagonistic effect of these two drugs when given simultaneously in the production of breaks as well as total aberrations. In view of genotoxic effects of these two drugs and involvement of risk to human combined treatment of CLF and DDS seems to score better (i.e. less harmful) over monotherapy.

6.2 Effect of Diethylcarbamazine (DEC) :

Potential genotoxic effect of the antifilarial drug, DEC, was evaluated by two assay systems: metaphase chromosome analysis and MNT in bone marrow cells. For metaphase chromosome analysis mice of either sex were treated orally with a single dose of 40 mg/kg of DEC and cytological preparations of their bone marrow cells were made at 8, 16, 24, 48 and 72 h post-treatment. For the MNT a dose-response study was done and mice of both sexes were fed with a single dose of 20, 40 or 80 mg/kg of the drug and the bone marrow cells were processed at 30 h post-treatment. For both the tests controls were run with untreated age and sex matched mice.

Metaphase chromosome analysis revealed the peak breakage frequency at 16 h post-treatment, then it declined gradually with the lapse of time and ultimately reached the control level at 72 h post-treatment. Exactly the same picture was obtained for individual aberration types as well as for total aberrations.
Significantly higher incidences of breaks compared to controls were noted at 8, 16 and 24 h.

The incidence of MN was significantly high in *P.* ecs for all the dose levels, but not in *N.* ecs. The *P.* + *N.* ecs line reflected the picture of *P.* ecs. The incidences were not dose related. For any dose level neither the mitotic index value nor the P/N ratio virtually differed from the control value.

Significantly high incidences of breaks in bone marrow metaphases and of MN in *P.* ecs (but not in *N.* ecs) clearly indicate clastogenic efficiency of DEC. That it has no spindle poisoning effect nor cell cycle inhibitory effect is evidenced from MNT and mitotic index analysis. This seems to be the first report on possible mutagenic effect of DEC. It is not clear if the drug itself or some of its metabolite(s) is the causative factor. Coming down of the breakage frequency at control level 72 h after treatment is supposed to be due to complete elimination of the drug and its metabolites from the body.