Filariasis is a common tropical disease caused by the infection of filarial nematode, *Wuchereria bancrofti* or *Brugia malayi*. Infective larvae of *B. malayi* are transmitted to humans by the bite of certain *Mansonia* and *Anopheles* mosquitoes in southern India, Sri Lanka, southern China, and South-East Asian countries. *W. bancrofti* widely distributed in the tropics and subtropics, and in the Pacific islands is transmitted by *Culex* and *Aedes* mosquitoes. Adult worms mature in lymphatics and lymph nodes. Microfilariae (larvae of filarial worms) of *W. bancrofti* are found in the blood chiefly at night (nocturnal periodicity). *B. malayi* are usually nocturnally periodic but may be semi-periodic. While humans are the only vertebrate hosts for *W. bancrofti*, cats, monkeys and other animals may harbour *B. malayi*.

The early clinical manifestations of filariasis are inflammatory episodes of fever and lymphatic inflammation. The tissues of the affected part remain oedematous and soft initially; later with skin hypertrophy and sub-cutaneous connective tissue proliferation the part becomes hard and leads to the development of elephantiasis. The incubation period, i.e., from invasion of larvae to the development of clinical manifestation, is variable - 4 weeks to 8-16 months.

Filariasis in its various forms remains a public health problem of considerable magnitude in many tropical and sub-tropical countries affecting people living mainly in rural areas as well as in urban areas with poor sanitation. The disease is highly
prevalent in tropical and subtropical countries. It is estimated (WHO, 1934) that about 90 million people in the world suffer from this disease and more than 50% of the filaria patients live in three Asian countries: China, India and Indonesia; for India herself the figure turns to be 15 million.

Diethylcarbamazine (DEC) is the only drug of choice over past 33 years in the control and treatment of filariasis. DEC was earlier used as chloride but is now produced as dihydrogen citrate which contains only half of its weight as base. Centperazone, an analogue of DEC developed by the Central Drug Research Institute, Lucknow, India is now on its phase II trial in man.

We have studied the potential mutagenic effect of DEC.

5.1 Effect of Diethylcarbamazine:

5.1.1 Introduction

Diethylcarbamazine citrate is 1-diethylcarbamoyl-4-methylpiperazine dihydrogen citrate. It is a white crystalline odourless substance highly soluble in water, readily soluble in hot alcohol but sparingly soluble in cold alcohol. Its structural formula is as follows:

It is also used in tropical eosinophilia. It is marketed as Hetrazan by Lederly and as Banocide by Burroughs Wellcome. Its microfilarial action depends on the proper function of the humoral
and cellular immune mechanism of the host. The precise mechanism of action remains a subject of debate. The uniqueness of the drug is that it is highly microfilaricidal in vivo but has very little or no apparent action in vitro. It cannot also kill adult worms. The prescribed human therapeutic schedule (course) is 2 mg/kg thrice a day for a period of 12 days. As the drug is effective only on the microfilariae present in the circulation the blood of the patient needs to be checked for their presence at an interval of 3–4 weeks and the course of treatment should be repeated if any are still present. Cure may require such repeated treatment over a period of 1–2 years.

No DEC resistant case is reported. The side effects of the drug are headache, malaise, anorexia and weakness. Release of foreign protein by the death of the larvae may cause allergic reactions with fever, skin rashes, muscular pains and tender joints.

In India the total production of DEC for the year 1986–87 is 16.00 MT which can hardly meet the annual requirement that exceeds 40 MT (OPPI, 1987). From these figures one can easily have some idea about the widespread use of the drug.

Pharmacological aspects and metabolism of DEC have extensively been studied (Bangham, 1955; Chandrasekaran and Harinath, 1980). Unfortunately, so far as the author is aware, nothing is known about its genotoxicity. We do not know also if it has any carcinogenic or teratogenic effect. Courtney and Nachreiner (1976) studied the effect of DEC on sperm in male dogs and noted no statistically significant deterioration in quantity,
morphology, motility or viability of sperm even after six months of treatment with about twice the human therapeutic dose. Here we have evaluated the clastogenic potential of DEC by metaphase chromosome analysis and micronucleus test in bone marrow cells of mice.

5.1.2 Materials and Methods

Healthy young mice of either sex and of the age group 10-12 weeks constituted the experimental animals.

Base free sample of the drug (Batch No. 36099) was supplied by Burroughs Wellcome and Co. (India) Private Ltd. The drug was used as an aqueous solution.

The animals were treated with the drug once only via oral route – the human therapeutic mode of administration. The volume of the solution was always kept constant i.e., 0.25 ml. As the drug was fed as an aqueous solution untreated animals of the same age group were kept as controls. For evaluation of genotoxic potential of the drug the following two test systems only were followed.

I. Metaphase chromosome analysis in bone marrow cells.

II. Micronucleus test in bone marrow cells.

5.1.2.1 Metaphase chromosome analysis in bone marrow cells

The animals received a single oral dose of 40 mg/kg of the drug and were sacrificed at 8, 16, 24, 48 and 72 h post-treatment. For each sampling period 3-4 mice were employed and they received colchicine (4 mg/kg) 1½ h before being killed.
Details of processing of the bone marrow cells, preparation and staining of slides and scoring have been described in Materials and Methods (General) (vide 2.1).

5.1.2.2 Micronucleus test in bone marrow cells

A dose-response study was done for MT. Mice were fed with a single dose of 20, 40 or 80 mg/kg of the drug. They were sacrificed at 30 h post-treatment without colchicine pre-treatment. The details of the cytological procedure adopted have been described in Materials and Methods (General) (vide 2.2).

5.1.3 Results

5.1.3.1 Metaphase chromosome analysis in bone marrow cells

Qualitative - Most of the aberrations noted here were of chromatid type. The gaps and sub-chromatid type breaks were the most common type of aberrations. Next common in occurrence were the chromatid breaks. The site of break varied along the length of chromosomes and the broken acentric parts were variously placed (Figs. 18a-e). In a number of metaphases the broken acentric parts being resulted from terminal chromatid deletions were so small and so distantly placed that their origins could not be ascertained, and they were noted as fragments of untraceable origin (Figs. 18f,g). Iso-chromatid breaks were also noted (Fig. 18h) though rare. A paired minute fragment presumably arising from iso-chromatid or chromosome type break (NUP6 type) was recorded in a cell (Fig. 18i). In a metaphase plate a ring formed by two chromosomes involved in chromatid interchange was found to be
Explanation of Fig. 18

Photomicrographs of mouse bone marrow metaphase plates showing various types of structural aberrations induced by diethylcarbamazine.

a-c. Chromosomes showing chromatid breaks, fragments slightly displaced.

d, e. Chromosomes with chromatid breaks, the broken fragment displaced.

f. A metaphase plate showing fragment of untraceable origin.
Explanation of Fig. 18 (Contd.)

g. A metaphase plate showing fragment of untraceable origin.

h. A metaphase field showing an iso-chromatid break.

i. A metaphase plate with minute paired fragment.

j. A metaphase plate showing a ring formed by two chromosomes and an unpaired fragment lying apart.

k. A metaphase field with a centric ring.

l. A metaphase plate showing two chromosomes involved in chromatid interchange.
Fig. 18
accompanied with fragments lying a bit away from it (Fig. 18j). Ring chromosomes resulting from breakage and sister union (intra-arm intra-change) with and without accompanied fragment(s) were also noted in a few cases (Fig. 18k). Two chromosomes were found to be involved in a chromatid interchange (Fig. 18l). Cells with both break and gap type aberrations involving the same (Fig. 18a) or different chromosomes were also recorded. Incidence of aneuploidy was not at all appreciable.

Quantitative - Table 22 summarizes the data on chromosomal aberrations induced by DDC at different sampling periods following a single oral treatment (40 mg/kg). The frequency of breaks increased gradually with the lapse of time reaching the peak at 16 h post-treatment, then declined with further increase of time. Exactly the same picture was revealed for individual aberration types like chromatid breaks, fragments and gaps, as well as for total aberrations. Interestingly at 72h post-treatment the incidences of breaks as well as total aberrations came down to the control levels; at earlier sampling periods, i.e. at 8-48h post-treatment they remained at higher level. However, the increases were significant at 8, 16 and 24 h sampling periods for breaks, and at 8, 16, 24 and 48 h for total aberrations.

5.1.3.2 Micronucleus test in bone marrow cells

Qualitative - Occurrence of MN was more common in P. ecs than in N. ecs. No erythrocyte was found to contain more than one micronucleus. As usual the MN found in erythrocyte line or in nucleated cells varied in size and locations (Figs. 19a-k). Occurrence of mitotic figures in the smear was extremely rare.
Table 22. Frequency distribution of different types of structural aberrations of bone marrow chromosomes induced by a single treatment of diethylcarbamazine (40 mg/kg) in different sampling hours. Each of the iso-chromatid breaks, rings and exchanges was counted as two breaks.

<table>
<thead>
<tr>
<th>Sampling Set period (h)</th>
<th>Cells scored/br. animal</th>
<th>Chromat. Iso-chromat. Frag. Ring Exch. Total breaks</th>
<th>Gap + sub-chromat. br.</th>
<th>Total aberr. ± %SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>500/5</td>
<td>2</td>
<td>1</td>
<td>3*</td>
</tr>
<tr>
<td>8</td>
<td>T 400/4</td>
<td>5</td>
<td>4</td>
<td>12 + 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.75 ± 0.74b</td>
</tr>
<tr>
<td>16</td>
<td>T 400/4</td>
<td>11</td>
<td>1</td>
<td>17 + 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.75 ± 0.65a</td>
</tr>
<tr>
<td>24</td>
<td>T 400/4</td>
<td>8</td>
<td>2</td>
<td>12 + 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.50 ± 0.25a</td>
</tr>
<tr>
<td>48</td>
<td>T 400/4</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.50 ± 0.56</td>
</tr>
<tr>
<td>72</td>
<td>T 300/3</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.56 ± 0.27</td>
</tr>
</tbody>
</table>

$r = -0.433$

C = Control, T = Treated, * = absolute figure, ° = mean/100 cells ± S.E.
't' test: a = p < 0.001, b = p < 0.01, c = p < 0.05.
Explanation of Fig. 19

Cut-out photomicrographs of bone marrow smears displaying micromucleated erythrocytes and nucleated cell induced by diethylenearbamazine.

a, b. Monocytes with very small micronuclei.

c-j. Polychromatic erythrocytes, each with one micronucleus.

k. A nucleated cell with a small micronucleus.
Quantitative - Data on the incidences of HM and composition of bone marrow cells in mice treated with a single oral dose of DEC revealed some interesting results (Table 23). In the treated series for all the dose levels tested the Pecs, not the N.ecs, showed significantly higher frequencies of HM over the control. Frequencies of HM in N.ecs remained in the control range for all the doses. Naturally P. and N.ecs when considered together reflected the picture of P.ecs only. However, the frequencies of micronucleated P.ecs as well as P. + N. ecs for three doses remained very close to each other. The mitotic indices noted at different dose levels were very close to the control value indicating lack of inhibitory effect of the drug on the cell cycle. At no dose level the P/N ratio differed significantly from the control value.

5.1.4 Discussion

5.1.4.1 Metaphase chromosome analysis in bone marrow cells

Although DEC is now in use for the last 35 years or so no attempt had so far been made to evaluate its potential toxicity at the genetic level. Significantly higher incidences of chromosome aberrations in DEC treated mice compared to controls in the present study indicates positive clastogenic effect of the drug. The effects induced show a curve having the peak at 16 h post-treatment; individual aberration types as well as total 'breaks' and 'total aberrations' all exhibit this particular pattern. The value at 8 h was close to that of 16 h, thus it is clear that it causes an early effect. Such type of non-delayed effect is manifested by deoxyadenosine, diazepam and a number of other chemicals (see Kihlman,
Table 23. Results of the micronucleus test in bone marrow cells of mice obtained at 30 h following a single treatment of diethylcarbamazine with different doses. Values are mean per 100 cells ± S.E. Figures in parentheses are MN/cells scored.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>No. of P. ec. with animals</th>
<th>P. ec. with MN</th>
<th>P. + N. ec. with MN</th>
<th>% Nucleated cells with MN</th>
<th>P/N ratio</th>
<th>% of dividing cells (MN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 (Cont)</td>
<td>6</td>
<td>0.01 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.50 ± 0.06</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(10/6000)</td>
<td>(1/6000)</td>
<td>(11/12000)</td>
<td>(1/6000)</td>
<td></td>
<td>(63/12000)</td>
</tr>
<tr>
<td>20.0</td>
<td>4</td>
<td>0.05 ± 0.02</td>
<td>0.27 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.56 ± 0.05</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(20/4000)</td>
<td>(2/4000)</td>
<td>(22/8000)</td>
<td>(2/4000)</td>
<td></td>
<td>(42/5000)</td>
</tr>
<tr>
<td>40.0</td>
<td>4</td>
<td>0.05 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>1.06 ± 0.20</td>
<td>0.50 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>(13/4000)</td>
<td>(2/4000)</td>
<td>(15/8000)</td>
<td>(1/4000)</td>
<td></td>
<td>(41/8000)</td>
</tr>
<tr>
<td>80.0</td>
<td>4</td>
<td>0.12 ± 0.04</td>
<td>0.29 ± 0.03</td>
<td>0.01 ± 0.01</td>
<td>0.93 ± 0.02</td>
<td>0.49 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(13/4000)</td>
<td>(5/4000)</td>
<td>(23/8000)</td>
<td>(1/4000)</td>
<td></td>
<td>(39/8000)</td>
</tr>
</tbody>
</table>

*r = + 0.821

't' test: a = p < 0.001, b = p < 0.01.
1966; Das and Kar, 1977). It is not unreasonable to assume particularly in view of its lack of inhibitory effect on the cell cycle as revealed in the MTT that the drug affects late S and/or G2 phase chromosomes. Very high frequencies of gaps compared to the break type aberrations (Bender et al., 1974) at all the sampling periods corroborate the assumption.

5.1.4.2 Micronucleus test in bone marrow cells

That DEC is clastogenic is also evidenced from significantly high incidence of MN in polychromatic erythrocytes. Spindle poisons are also known to induce MN. But absence of spindle poisoning effect of DEC is documented by high incidence of MN in P. ecs and comparatively very low in N. ecs (almost in the control range). Besides, mitotic indices which remain almost unchanged in different post-treatment sampling hours, and non-availability of mitotic figures in appreciable frequency in the smear also document lack of spindle poisoning effect of the drug. Occurrence of tiny dot like MN in plenty also supports its clastogenic efficiency. P/N ratios show absence of any influence of the drug on the turn over of the bone marrow cells, and thereby indicate lack of mitodepressive effect. All these clearly reveal, on the one hand, clastogenic capacity of the drug, and, on the other hand, absence of spindle poisoning effect even with a single oral dose of 80 mg/kg; and, thus, support our findings based on the metaphase chromosome analysis.

All three doses tested increased the frequencies of micromucleated polychromatic erythrocytes and poly- + normochromat- ic erythrocytes significantly over their corresponding control
values. But the treated values fail to show any correlation with the doses \( r = +0.544 \) for P. ees, \( r = +0.821 \) for P + H. ees).

5.1.4.3 General

The factor(s) responsible for chromosome damage following DEC treatment is yet to be known. A look into the metabolism of the drug may help get the clue for it and explain our results. Metabolism and pharmacokinetics of DEC have extensively been studied in humans and experimental mammals like guineapigs, rabbits, monkeys, rats and sheep, though not in mice (see Bangham, 1955; Chandrasekaran and Harinath, 1960; Roy et al., 1982). Though in details the metabolism of DEC differs from species to species basically it is the same. It is rapidly absorbed through the gastro-intestinal tract, bio-distributed and almost completely eliminated from the body within 1-2 days. A number of metabolites have been identified in the urine samples of the individuals following DEC administration and the major metabolites are DEC-N-Oxide and ethlcarbamazine. A major portion of the drug is excreted out unchanged. The metabolism of DEC is slow in guineapigs and rabbits compared to humans. The percentage of excretion of DEC and its metabolites varies from species to species.

In human after an oral dose of 50 mg of DEC the peak blood plasma level reaches to 100 ng/ml within 1-2 h. Higher doses (800 mg) generally result in peak blood levels of 4-5 \( \mu g/ml \). It rapidly equilibrates in all tissues. In blood the half-life of DEC is 2-3 h and 60-80% of it is excreted unchanged.
within 48 h with urine, 10% appearing as DEC-N-oxide and 4-5% is eliminated with faeces (WHO, 1984).

In human and other mammals the amount of excretion of DEC and its metabolites decreases very fast with the lapse of time (Chandrasekaran and Harinath, 1980), and ultimately the drug is eliminated completely within a few days. The maximum clastogenic effect at early stage (16h), then gradual decrease and finally disappearance of the effect after 3 days as revealed from our metaphase chromosome analysis may be explained from the points of quick absorption, quick reaching of the peak plasma level and gradual and complete elimination of the drug. As DEC is metabolized quickly and a number of metabolites are formed in considerable amount it is not possible to ascertain if DEC or some metabolite(s) is responsible for its clastogenic capacity.

The treated values obtained in dose-response study in MNT did not virtually differ from each other. The reason remains unknown.