5. EFFECTS OF NITRAZEPAM

5.1 Introduction

Nitrazepam (NZ), another benzodiazepine derivative, has recently been introduced in the clinical practice as a good hypnotic agent. Its trade name is mogadon. It is in wide use at present. Besides hypnotic action, it shares muscle relaxant and anticonvulsant properties of CDZ and diazepam. Its sedative effect has also been proved. Boethius and Westerholm (1976) made two surveys: One in 1968-69 and another in 1973, on the use of benzodiazepines in a town of Sweden. They reported 8 times or more increase in the use of NZ mostly at the expense of diazepam and combined products in 1973 as compared to 1968-69. This may give some idea on the recent spurt in the use of NZ. Similar reports on the extent of its use in other countries are not available. In several countries like U.S.A. (Breimer, 1976), Canada (Sellers, 1978) it was not marked at the time of report mentioned above. The present position is not known to the author, however.

It is claimed that mechanism of hypnotic action of NZ differs from that of other agents used to induce sleep (Goodman and Gilman, 1975). Recommended hypnotic dose of NZ for human is 10 mg. Higher dose produces more side-effects; 'hangover' is a common side-effect. The major clinical advantage of NZ is a greater safety and absence of serious drug interactions (Greenblatt and Shader, 1972).

Several recent reports suggest that NZ causes impairment of performance on the day following intake of therapeutic dose (Borland and Nicholson, 1975). In humans the average elimination
half-life of NZ has been demonstrated to be 25 h (Rieder, 1973). Sawada and Shinohara (1971) determined the urinary excretion of NZ after oral administration in man (10 mg) and rabbit (1,5,10 and 50 mg/kg). In man 14-23% of the amount administered was excreted while in rabbit 10-12%.

Although some amount of studies were carried out on the effects of diazepam and chlordiazepoxide on various cytological and genetical aspects it has been rather extremely limited for nitrazepam. Bignami et al. (1974) could not detect mutagenicity of NZ in Aspergillus nidulans as judged by the incidence of non-disjunction and crossing over. No chromosomal aberration was found in the bone marrow cells of mice treated with 200 mg/kg/day of NZ once or for five successive days (Hitotsumachi and Kikuchi, 1974). It is, therefore, a matter of interest to explore the cytotoxic and genotoxic effects of NZ in the somatic as well as germ line cells of laboratory mammals. We have studied the effects of NZ on the chromosomes of bone marrow and meiotic cells and on the sperms in mice the results of which are presented here. Due to paucity of time at the exposal of the author it was not possible to evaluate its effects with all the protocols used for other two drugs. Only five protocols (vide infra) were undertaken.

5.2 Material and Methods

For protocol nos. 1, 3, 4 and 5 same treated males provided the materials.

Altogether 16 males were treated with a single daily dose of 0.5 mg of nitrazepam for 15 consecutive days. After the last dose they were sacrificed at the end of 1, 3, 4, 6 and 8 weeks and...
different tissues were processed for different protocols given below. For the micronucleus test animals of either sex were treated separately, however. The same controls used for the earlier two drugs also served as controls here. Details are as follows:

5.2.1 **Cytogenetic assay of bone marrow cells (Metaphase chromosome analysis)**

Same as mentioned above and in Material and Methods (General) (vide 2.2.1). In addition, here single treatment series (set I and II) could not be done.

5.2.2 **Cytogenetic assay of bone marrow cells (Micronucleus test)**

Three dose levels (0.25, 0.50, and 1.00 mg) were tested for the micronucleus test and for each dose level 5 animals of both sexes were utilised. In each case drug was administered orally twice with an interval of 24 h and bone marrow was processed 6 h after the second treatment. Control data used for other two drugs mentioned earlier were also used here. Single treatment series (set II for 32 h) was not done here. Details of the control and processing were mentioned in Material and Methods (General) (vide 2.2.2).

5.2.3 **Cytogenetic assay of male germ cells**

Same as mentioned above and in Material and Methods (General) (vide 2.2.3).

5.2.4 **Sperm count**

Same as mentioned above and in Material and Methods (General) (vide 2.2.5).

5.2.5 **Sperm head abnormality**

Same as mentioned above and in Material and Methods (General) (vide 2.2.6).
5.3 Results

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

Qualitative: One aberration per affected cell was of common occurrence. However, in very rare cases two or three aberrated chromosomes in a cell were also recorded. Among the aberrations taken under 'breaks' chromatid type break was the predominant form. The broken piece of the chromatid was found to lie either near about its place of origin (Figs. 16a, c) or elsewhere in the field (Figs. 16b, g). In no cell a chromosome with more than one break was found. Two breaks involving two chromosomes were also altogether absent. In no field chromosome with unequal chromatids or iso-chromatid break was observed. However, there were some fragments of untraceable origin (Figs. 16e, f). In the treated series only one case of translocation was recorded.

Quantitative: The data on the nitrazepam induced structural aberrations of metaphase chromosomes at different weeks after 15 days repeated treatment are presented in Table 23. In comparison to other types of aberrations the incidence of gap type aberration was more. The minimum and maximum frequencies in the treated series were 1.00 and 5.66 respectively for total aberrations and 0.66 and 2.00 for 'breaks'. The frequency of the 'break' type aberrations was maximum at wk 1, but if the total aberrations were considered wk 8 data was the highest and this was influenced due to high frequency of gap type aberration in that week. For total aberration frequencies statistical analysis failed to indicate any significant difference between control and treated values in any week.
Explanation of Fig. 16

Photomicrographs of bone marrow metaphase plates, in part or full, showing some numerical or structural chromosomal aberrations induced by nitrazepam.

a. A chromatid break, fragment slightly displaced.

b. A chromatid break, fragment twisted.

c. Chromosome with a chromatid break.

d. Gap in a rabbit-ear chromosome.

e,f. Fragments of untraceable origin.

g. Chromatid break in a chromosome, fragment little displaced.

h,i. Intact metaphase plates with 39 chromosomes.

j. A metaphase with 41 chromosomes.
'breaks' the treated value of wk 1 only was significantly higher (p < 0.01) than its corresponding control value, but the increase was not so much striking.

Table 24 presents the data of numerical chromosomal abnormalities and centromeric separation of control and nitrazepam-treated mice at different weeks. The incidence of centromeric separation increased markedly over the corresponding controls during early weeks and the differences got reduced in later weeks. However, we have not done any statistical evaluation for this parameter; for that we do not like to comment anything on their significance.

Incidence of polyploidy in the treated series was comparable to that of control. So far aneuploidy was concerned we encountered cells with 38, 39 and 41 chromosomes (Figs. 16h-j). Their incidences were of the order 38 < 41 < 39 in the treated series and 42 = 41 < 38 < 39 in the control series. In the treated series none of the cells examined was found with 42 chromosomes. For convenience in statistical analysis all aneuploids were taken together and wks 3, 4 and 8 showed significantly higher (p < 0.05) incidences of aneuploidy after the treatment in comparison to their corresponding controls.

5.3.2 Cytogenetic assay of bone marrow cells (Micronucleus test)

Qualitative: After nitrazepam treatment no bone marrow cell was found to contain more than one micronucleus. In comparison to other two drugs mentioned earlier the size range of the micronuclei was very limited (Figs. 17 and 18). The shape was, however, as usual round. Here also for the nucleated cells only clear-cut cases were taken under consideration to avoid controversy over the
Explanation of Fig. 17

Cut-out photomicrographs of bone marrow smears of mice showing micronucleated erythrocytes induced by nitrazepam.

a-d. Polychromatic erythrocytes containing one micronucleus each. Note the size and location of micronuclei.

e-j. Normochromatic erythrocytes containing one micronucleus each. Note the size and location of micronuclei.

k,l. Polychromatic erythrocytes with one micronucleus each.
Explanation of Fig. 18

Cut-out photomicrographs of bone marrow smears of mice showing micronucleated cells induced by nitrazepam.

a-h. Nucleated bone marrow cells containing one micronucleus each.
nature of the micronuclei. Binucleate and trinucleate conditions, nuclear fragmentation, lagging chromosome(s) and unequal chromatin distribution were also associated with the nucleated cells, but with the highest dose level only. Another interesting point revealed at higher dose levels (0.5 and 1.0 mg) was the higher incidence, though not statistically verified, of metaphase figures in the smear and it appeared to be less in comparison to the other two benzodiazepines mentioned earlier.

Quantitative: The data on the occurrence of micronuclei in control and NZ treated (twice) mice are presented in Table 25. Except the normocyte line at 0.5 mg dose the incidence of MN at all the dose levels tested and in all the cell types remained at the control level. The frequency of micronucleated normocytes at 0.5 mg dose was marginally significant (p < 0.05) only. Similarly bone marrow depression as measured from the ratio of erythrocytes per 100 nucleated cells at the highest dose level was significantly higher, but marginally, than the control value. P/N ratios did not differ much from the control value.

5.3.3 Cytogenetic assay of male germ cells

Qualitative: Control and treated series did not differ much so far qualitative aspects of aberrations were concerned. Neither control nor treated series recorded any translocation. The breaks encountered were only of chromatid type (Fig.19d). No fragment was also recorded. Diakinesis and metaphase I plates containing 18, 19 and 21 bivalents were also noted in the treated series. The frequencies of cells with 19 or 21 bivalents were almost same. Among polyploid cells, tetraploids (Fig.19a,b) were of frequent occurrence. However, 6n and 8n cells were not complete.
Explanation of Fig. 19

Photomicrographs of diakinesis metaphase-I spermatocytes, in part or full, showing different types of chromosomal aberrations.

a, b. Tetraploid cells at diakinesis.

c. A diakinesis plate with early separation of X and Y, lying far away from each other.

d. Chromatid type break in a diakinesis bivalent.
Table 25. Results of micronucleus test after nitrazepam treatment at various dose levels. (Figures in parentheses are MN/Cells scored. For each dose level two treatments were given separated by 24 h).

<table>
<thead>
<tr>
<th>Dose of Animals (mg)</th>
<th>Nucleated Cells with MN Mean %±S.E.</th>
<th>P.e.c. Mean %±S.E. with MN</th>
<th>N.ec. Mean %±S.E. with MN</th>
<th>P. + N.ec. Mean %±S.E. with MN</th>
<th>P/N ratio Erythrocytes/100 nucleated cells Mean ± S.E.</th>
<th>P/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 5</td>
<td>0.290±0.025 (28/9700)</td>
<td>0.253±0.034 (25/9700)</td>
<td>0.272±0.019 (53/19400)</td>
<td>0.300±0.031 (30/10000)</td>
<td>12.394±2.357</td>
<td>0.936±0.055</td>
</tr>
<tr>
<td>0.50 5</td>
<td>0.370±0.057 (28/8200)</td>
<td>0.324±0.042 (26/8300)</td>
<td>0.346±0.043(54/16500)</td>
<td>0.230±0.041 (23/10000)</td>
<td>12.656±2.474</td>
<td>0.821±0.095</td>
</tr>
<tr>
<td>1.00 5</td>
<td>0.275±0.052 (23/7930)</td>
<td>0.239±0.080 (19/7625)</td>
<td>0.257±0.050 (42/15555)</td>
<td>0.221±0.014 (20/8800)</td>
<td>15.531±2.553</td>
<td>0.741±0.025</td>
</tr>
<tr>
<td>0.00 20 (Cont.)</td>
<td>0.297±0.029 (73/23850)</td>
<td>0.220±0.022 (55/23865)</td>
<td>0.262±0.018 (128/47715)</td>
<td>0.249±0.018 (65/25110)</td>
<td>11.383±0.989</td>
<td>0.968±0.099</td>
</tr>
</tbody>
</table>

'r' values =
\[ df = 1 \]

-0.33 -0.37 -0.34 -0.82 0.97 -0.96

't' test : c = p < 0.05
Early dissociation of autosomes as well as sex-chromosomes (Fig. 19b,c) was a common phenomenon. Among autosomes the smallest bivalent was involved in most of the cases. The dissociated X and Y chromosomes were variously placed in the field. In extreme cases as many as three bivalents were involved in the dissociation in a cell of the treated series.

Quantitative: The results of the cytogenetic analysis of spermatocytic chromosomes after repeated nitrazepam treatment are summarized in Table 26. In contrast to other two benzodiazepines mentioned earlier, here the frequencies of polyploidy in the treated series remained almost in the control range. Significant difference between the control and treated values was noted only in wk 1. The frequencies of aneuploidy increase due to treatment in almost all the test weeks, but wks 1, 6 and 8 only exhibited significant differences between control and treated values.

Regarding breaks, in fact, no marked difference was seen between control and treated values in any test week. However, wk 4 data showed significant difference and that significance was due to the zero value of its corresponding control, which was possibly for the low number of cells (155) scored in that particular week.

As regards univalent formation the data for autosomes as well as sex-chromosomes in all the test weeks except wk 8 for autosomes remained almost at the control level. Though the wk 8 data for autosomes was significantly higher than the corresponding control value the significance was marginal. However, the data for autosomes in the treated series showed an increasing trend with the increase of post-treatment period.
Table 26. Frequency distribution of different types of spermatocytic chromosome aberrations at different post-treatment weeks following 15 days repeated treatment of nitrazepam values are mean % ± S.E.

<table>
<thead>
<tr>
<th>Test wks.</th>
<th>Set Cells Scored/ Animals</th>
<th>Polyploid Cells</th>
<th>Aneuploid Cells</th>
<th>Breaks</th>
<th>Translocation</th>
<th>Early dissociation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treat. 200/3 9.52±1.13⁵⁺</td>
<td>6.00±0.71⁵⁺</td>
<td>0.49±0.40</td>
<td>-</td>
<td>4.97±1.03</td>
<td>6.00±0.71</td>
</tr>
<tr>
<td></td>
<td>Cont. 197/4 4.70±0.55</td>
<td>1.19±0.61</td>
<td>0.50±0.43</td>
<td>-</td>
<td>9.27±3.03</td>
<td>4.63±1.34</td>
</tr>
<tr>
<td>3</td>
<td>Treat. 200/3 8.93±1.24</td>
<td>3.03±0.75</td>
<td>0.47±0.38</td>
<td>-</td>
<td>9.96±0.94</td>
<td>7.50±1.45</td>
</tr>
<tr>
<td></td>
<td>Cont. 209/4 5.96±0.80</td>
<td>2.28±1.45</td>
<td>0.41±0.35</td>
<td>-</td>
<td>15.59±3.97</td>
<td>7.51±3.28</td>
</tr>
<tr>
<td>4</td>
<td>Treat. 200/3 9.92±1.25</td>
<td>4.50±0.73</td>
<td>0.98±0.40⁶⁻</td>
<td>-</td>
<td>10.51±0.75</td>
<td>9.08±1.99</td>
</tr>
<tr>
<td></td>
<td>Cont. 155/4 5.46±1.36</td>
<td>2.71±1.08</td>
<td>-</td>
<td>-</td>
<td>8.39±1.02</td>
<td>5.67±0.20</td>
</tr>
<tr>
<td>6</td>
<td>Treat. 200/3 9.00±0.49</td>
<td>9.50±0.82⁵⁺</td>
<td>1.00±0.49</td>
<td>-</td>
<td>14.00±0.70</td>
<td>11.00±1.11</td>
</tr>
<tr>
<td></td>
<td>Cont. 222/4 6.27±1.35</td>
<td>2.54±0.84</td>
<td>0.50±0.43</td>
<td>-</td>
<td>10.86±2.57</td>
<td>8.62±1.55</td>
</tr>
<tr>
<td>8</td>
<td>Treat. 200/4 8.00±0.70</td>
<td>11.50±2.68⁵⁺</td>
<td>1.00±0.49</td>
<td>-</td>
<td>18.50±1.97</td>
<td>12.00±1.22</td>
</tr>
<tr>
<td></td>
<td>Cont. 219/4 6.94±0.52</td>
<td>4.00±1.41</td>
<td>0.86±0.44</td>
<td>-</td>
<td>8.08±2.30</td>
<td>5.86±2.50</td>
</tr>
</tbody>
</table>

¹'t' test:  b = p < 0.01  ,  c = p < 0.05
5.3.4 Sperm count

Data on the sperm counts at five different post-treatment weeks are summarised in Table 27. At any particular week, the values for right and left epididymes were very close to each other. The lowest value in the treated series (71.21) was obtained at wk 1. With the lapse of time the values were found to increase gradually and the same picture was also revealed when values for right and left epididymes were considered separately. Upto wk 6 the values in the treated series were less in comparison to the corresponding control values, but the picture was just reverse at wk 8. Thus there was a general depletion in the sperm count upto wk 6 in the treated series. But the average value at wk 1 only was significantly less in comparison to the corresponding control value.

Table 27 Effect of nitrazepam on the epididymal sperm count in mice at different weeks after 15 days repeated treatment. Values are mean number of sperms (heads only) in one WBC chamber of haemocytometer. SE.3/4 animals were utilized in each week.

<table>
<thead>
<tr>
<th>Epididymes</th>
<th>Set</th>
<th>wk 1</th>
<th>wk 3</th>
<th>wk 4</th>
<th>wk 6</th>
<th>wk 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treat.</td>
<td>72.40</td>
<td>88.00</td>
<td>88.07</td>
<td>95.12</td>
<td>119.50</td>
</tr>
<tr>
<td>Right</td>
<td>Cont.</td>
<td>117.33</td>
<td>106.56</td>
<td>104.98</td>
<td>108.51</td>
<td>111.86</td>
</tr>
<tr>
<td></td>
<td>Treat.</td>
<td>70.02</td>
<td>88.22</td>
<td>90.15</td>
<td>92.05</td>
<td>110.17</td>
</tr>
<tr>
<td>Left</td>
<td>Cont.</td>
<td>109.61</td>
<td>109.74</td>
<td>99.85</td>
<td>108.21</td>
<td>103.60</td>
</tr>
<tr>
<td></td>
<td>Average of right and left</td>
<td>71.21</td>
<td>88.11</td>
<td>89.11</td>
<td>93.58</td>
<td>114.87</td>
</tr>
<tr>
<td></td>
<td>Treat.</td>
<td>±2.72</td>
<td>±6.12</td>
<td>±5.61</td>
<td>±8.04</td>
<td>±13.09</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>113.47</td>
<td>108.15</td>
<td>102.41</td>
<td>108.36</td>
<td>107.73</td>
</tr>
<tr>
<td></td>
<td>±10.41</td>
<td>±7.77</td>
<td>±9.27</td>
<td>±7.64</td>
<td>±8.22</td>
<td></td>
</tr>
</tbody>
</table>

't' test: c = p < 0.05
5.3.5 Sperm head abnormality

Qualitative: The abnormality in sperm heads in nitrazepam-treated series was of varied types. In addition to the common abnormal types like giant size, with flat base (Fig. 20a), amorphous, triangular, semilunar, (Fig. 20e, f), without hook, polyp form (Fig. 20b), with vacuole (Fig. 20c), bifurcated acrosomal end (Fig. 20d), etc. (recorded in control, diazepam and CDZ treated mice) some peculiar abnormal structures of sperm heads as depicted in Figs. 20g-1 were encountered in the NZ treated animals. Among the new abnormal types Figs. j-1 types were of frequent occurrence.

Quantitative: For statistical analysis the week-wise control data used for earlier two drugs were also used here. The percentage of abnormal sperm heads in different test weeks ranged from 7.83 to 10.87 in the treated series, while from 4.12 to 6.18 in control (Table 28). Interestingly, in every test week the value for treated animals was significantly higher than the corresponding control value.

Table 28 Effect of nitrazepam on the incidence of sperm head abnormality in mice at different weeks after 15 days repeated treatment. Values are mean % +S.E. 3/4 animals were employed in each week, treated and control and 400 sperm heads were examined from each animal.

<table>
<thead>
<tr>
<th>set</th>
<th>wk 1</th>
<th>wk 3</th>
<th>wk 4</th>
<th>wk 6</th>
<th>wk 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>7.83&lt;sup&gt;b&lt;/sup&gt; ±0.53</td>
<td>9.08&lt;sup&gt;a&lt;/sup&gt; ±0.29</td>
<td>9.00&lt;sup&gt;b&lt;/sup&gt; ±0.42</td>
<td>10.87&lt;sup&gt;a&lt;/sup&gt; ±0.85</td>
<td>10.12&lt;sup&gt;b&lt;/sup&gt; ±1.00</td>
</tr>
<tr>
<td>Control</td>
<td>4.62 ±0.36</td>
<td>5.18 ±0.35</td>
<td>4.81 ±0.51</td>
<td>4.12 ±0.59</td>
<td>6.18 ±0.32</td>
</tr>
</tbody>
</table>

*t' test: a = p < 0.001, b = p < 0.01
Explanation of Fig. 20.

Photomicrographs of normal and abnormal sperm heads in mice treated with nitrazepam. Arrows indicate abnormal sperm heads.

a. Sperm head with a flat base.

b. A polyp-type sperm head.

c. A sperm head with two vacuoles.

d. Sperm head with bifurcated acrosomal hook.

e, f. Semilunar type sperm heads.

g-l. Peculiar amorphous type abnormal sperm heads.
Fig. 20
5.4 Discussion

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

So far, metaphase chromosome analysis of bone marrow cells after repeated nitrazepam treatment is concerned, the data of all the test weeks except wk 1 show negative response. At wk 1 the treated value, though significantly higher than the corresponding control value, did not increase markedly over the pooled control mean value of all the test weeks. Thus the results of metaphase chromosome analysis of bone marrow cells after repeated treatment of NZ are at par with those of other two benzodiazepines tested. The similarity in the results of NZ and CDZ is more pronounced than that of diazepam and NZ. Results are in good agreement with those of Hitotsumachi and Kikuchi (1974) who also failed to obtain positive results in mice bone marrow cells after five days repeated treatment with 200 mg/kg/day. The dose used in the present study is equivalent to 20 mg/kg/day, which is 10 times less than that used by earlier workers. However, its clastogenic effect after single treatment has not been done in the present investigation due to want of time. So it is not known if single treatment can produce any effect on the bone marrow chromosomes like the other two benzodiazepines tested. Its pharmacokinetics and mechanism of action at the molecular level are still obscure.

Its polyploidising effect as revealed from metaphase chromosome analysis of bone marrow cells was also found to be nil. In this regard NZ also shows a close similarity with the other two benzodiazepines already tested. Slightly higher incidences
of aneuploidy in certain test weeks seem to be due to higher incidences of sub-diploid cells scored in those weeks, which may be attributed to the preparational shortcomings. The incidence of centromeric separation in the treated series does not reveal any marked difference from that of the control series.

5.4.2 Cytogenetic assay of bone marrow cells (Micronucleus test)

With all the 3 dose levels tested the incidences of MN in all the cell types were comparable to those obtained in the control animals, indicating lack of micronucleus producing capacity of the drug, at least with those doses. Absence of abnormal mitotic figures and presence of normal mitotic figures in the control range are indicative of the fact that NZ unlike the other two benzodiazepines has not any effect on the mitotic apparatus with even 1.0 mg dose. Similarly ratios of erythrocytes and nucleated cells as well as of poly- and normochromatic erythrocytes failed to demonstrate any appreciable difference between treated and control values, although one value, that of ratio of erythrocytes and nucleated cells for 1.0 mg dose, was only marginally significant over the control value. These data clearly demonstrate absence of any effect of the drug on the turnover of polychromatic erythrocytes in the mouse system at least with the doses tested.

This newly marketed benzodiazepine has not so far been tested for its mutagenicity except the work of Bignami et al. (1974) and Hitotsumachi and Kikuchi (1974). Its toxicologic action has not also been recorded. The highest dose (1.0 mg) used here was equivalent to 200 times the human hypnotic therapeutic dose. Our
findings on MNT support our results obtained from in vivo bone marrow and spermatocytic chromosome analyses in mice after repeated treatment. It is concluded that nitrazepam given maximally as an oral dose of 1.0 mg (equivalent to 200 times human therapeutic dose) twice with an interval of 24 h does not produce an increase in MN in the bone marrow cells of mice.

5.4.3 Cytogenetic assay of male germ cells

So far our knowledge goes the present study on the effects of NZ on spermatocytic chromosomes seems to be the first of its kind. Absence of breaks and translocations in all the test weeks indicate lack of its clastogenic effects at least with the dose tested. In contrast to the other two benzodiazepines tested, here the incidence of polyploidy remained almost in the control range, which was an indicative of the fact that it had least or no action on the spindle structure. Similarly no appreciable difference was obtained between the control and treated values when we considered univalent formation.

5.4.4 Sperm count

The sperm-count test reveals certain depletion in the epididymal spermatozoal population at least in wk 1. In other weeks, however, the data of the treated series did not differ strikingly from the control ones. Thus the results of sperm-count for diazepam and NZ show parallelism. The significant effect of nitrazepam on the sperm head morphology mentioned earlier may have some bearing with the lowering of sperm count at wk 1.

5.4.5 Sperm head abnormality

In contrast to the other tests of nitrazepam mentioned above analysis of sperm head morphology gives a completely different
picture. The incidence of abnormal sperm heads due to NZ treatment increased significantly at all the test weeks. Qualitatively also the abnormality varied in wider range in comparison to the other two benzodiazepines. As the peculiar forms depicted in the Figs. 20g-1 are found frequently in NZ treated mice and are absent in the control as well as diazepam and CDZ treated mice those forms may be assumed to be typical for NZ treatment. Negative response from the cytogenetic study and positive response from the analysis of sperm head morphology seem to be an indicative of the fact that chromosomal aberration has less or no role in the incidence of aberrated forms of sperm. Some genetic factor as it is thought by Wyrobek and Bruce (1978); Krzanowska (1969); Godowicz (1977); Hillman and Nadijcka (1978) may be responsible for the abnormal sperm heads. Similar type of negative response from cytogenetic study but positive response from sperm head analysis was also obtained earlier with chemicals like dichlorvos, EMS (Wyrobek and Bruce, 1975). However, its positive effect on sperm head needs further verification with different doses and in different species as well as with dominant lethal test.

5.4.6 General

Thus the results of bone marrow and spermatocytic chromosome analyses after repeated treatment and MNT reveal that nitrazepam is non-mutagenic. However, it should be taken with certain reservation. It needs further verification with other doses and other protocols like dominant lethal test, host-mediated assay and bone-marrow chromosome analysis after single treatment.