MATERIAL AND METHODS

Laboratory strain of white mice, Mus musculus, constituted the material for the present study. The bone marrow cells of the femur and humerus were taken out following the method described by Ford and Hamerton (1956). In each set of experiment generally four specimens were used unless otherwise mentioned in some specific cases. For cytological preparation each specimen was injected with 0.04% colchicine solution at the rate of 1 ml per 100 gm. of the body weight one hour before its bone marrow collection. Colchicine pretreatment was made both in control and experimental mice except in some cases which have been mentioned elsewhere. The bone marrow cells of each of the control and treated specimen were collected separately in warm (37°C) 1% sodium citrate solution by flushing the solution through the cavity of the bone from one cut end and collecting the same at the other cut end. The bone marrow cells carried along with the flush of the citrate solution were collected in a centrifuge tube. If needed a little amount of sodium citrate solution was further added to the bone marrow cell suspension. The material was flushed over and again by a pipette so as to detach the entangled cells and bring them into an uniformly suspended condition. After this the centrifuge tube containing

•X
the material was kept in an incubator at 37°C for about 15 minutes. The suspended material was then centrifuged for 5 minutes at a speed of about 500 rpm. The supernatant fluid was discarded. The tissue collected at the bottom of the centrifuge tube was fixed in acetic-alcohol mixture (1:3).

Two methods were employed for staining the fixed tissues:

(1) Air drying technique (Rothfels and Siminovitch, 1958) and (2) Smith's technique (Smith, 1943, with some modification (Manna and Talukdar, 1967).

1. Schedule for Air-Drying Technique:

Clean slides were stored in 50% alcohol chilled in the frozen chamber of a domestic refrigerator for the cytological preparation. The fixed bone marrow cells were resuspended in the fixative by means of gentle flushing. Then the chilled slide was taken out and the suspended cells in acetic alcohol mixture were dropped on the slide. Immediately the slide was passed over a flame so as to burn out the excess of alcohol and allow the material to spread as a thin film on the slide. The film was allowed further to dry in the air. It was then stained in Giemsa stain for about 45 minutes. The excess of stain was removed and the slide was rinsed.
in water. It was then allowed to dry in air for observation.

2. **Schedule for Smith's Technique**:

The cells fixed in acetic alcohol were centrifuged. The fixative was removed from the tissue settled at the bottom of the centrifuge tube. After this the tissue was mixed with a small drop of 45% acetic acid so as to make a thick emulsion like suspension. Small drop of the suspension was taken in the centre of a dry albuminized slide and the tissue was covered by a cover-glass. It was squashed between the slide and the cover-glass under the cover of a filter paper by gentle pressure of the thumb. The slide was then dried periodically by just passing it quickly over a small flame of a spirit lamp. After drying it was dipped into 50% alcohol for the detachment of the cover-glass. When the cover-glass was detached, the tissue was found adhered to the slide. The slide was processed through the down grades of alcohol to water. It was then ready for staining. Iron-alum haematoxylin and feulgen staining were made and after the staining the slide was processed through upgrades of alcohol for the permanent mounting in Canada balsam.
In the present study most of the preparations were made according to the air-drying technique. For some comparison occasionally Smith's technique was followed. The data were scored from each of the slides thus prepared of an individual. During observation whenever any type of aberration was encountered, it was drawn with the help of a camera lucida. Some photo micrographs were also taken to show the quality of the damages. In presenting the camera lucida drawings of the chromosome aberrations, the author has taken the liberty of drawing only the affected chromosomes without drawing the whole complement. This was followed for the sake of clarity in presentation and to minimise the time for drawing.

Since this is a sort of general account of the material and method used in the study, specific information about the treated series with regard to the concentration of the chemical used, hour of fixation, etc. have been indicated in connection with the particular chemical. The chemicals used and their groups are as follows:

<table>
<thead>
<tr>
<th>Chemical groups</th>
<th>Name of the chemical compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Antibiotics</td>
<td>a) Actinomycin-D</td>
</tr>
<tr>
<td></td>
<td>b) Na-Novobiocin</td>
</tr>
<tr>
<td></td>
<td>c) Dihydrostreptomycin</td>
</tr>
</tbody>
</table>
2) Phenols
   a) 2,4-Dinitrophenol
   b) p-Aminophenol
   c) Gallic acid
   d) Pyrogallol

3) Substituted amides.
   a) Hydroxylamine
   b) Phenylhydrazine
   c) Semicarbazide

4) Other chemical compounds.
   a) Caffeine
   b) Carbon tetrachloride
   c) Lithidium chloride.

For the radiation work mice were given a dose of 200r from an X-ray machine located at Barrackpore Jute Agricultural Research Institute. Grateful acknowledgement is made to the authorities for extending me the facilities of irradiation from time to time for the work.

CONTROL SERIES

In order to avoid repetition, the results of the control series have been presented in this separate section. They can be used as reference when necessary. As controls a number of mice were used. Befitting with the experimental conditions, different types of controls had been studied. Since in the experiments chemicals were dissolved in distilled water and for the chromosome preparation 0.14% colchicine solution was injected 1 hour before the collection of the bone marrow tissue. Good number of
metaphase complements in three types of the control were studied. They were (1) mice injected with specified amount of distilled water, (2) mice injected with specified amount of 0.04% colchicine and (3) mice injected with specified amount of distilled water followed by the injection of 0.04% colchicine solution 1 hour before a particular fixation time.

First type of Control:— In the distilled water injected control series 32 mice were used. They were injected with the same quantity of distilled water which was to be injected as solution of some of the chemicals in the experimental series. After the injection of distilled water the specimens were sacrificed at an interval of 4, 8, 24 and 48 hours. 500 metaphase complements were examined from the specimens fixed at each particular hour. In the total of 2000 metaphases besides some rarely occurring clumping and sticky effects, no other major types of chromosomal aberrations were encountered. Only one chromosome type and two chromatid type breaks were found in 2000 metaphases (0.15%). There were also four gap type aberrations (0.2%). Not a single instance of exchange type aberrations was found in this control series. The frequency of gaps and break type aberrations taken together was 0.35%. No palpable difference in the result was found in the materials fixed at different intervals. Thus, it may be said that the effects observed
in the experimental series (vide infra) were not
due to distilled water used as the solvent of chemicals.
Further, breaks were not induced in the bone marrow
chromosomes of mice for the injection of water as
reported in plant chromosomes (Sharma and Sharma, 1960).

Second type of Control :- It has become a regular
practice to apply low concentration of colchicine
solution, generally 0.04%, prior to the cytological
processing for the arrest of metaphase stage and better
disposition of chromosomes (Hsu and Pomerat, 1953).
Since it is known that the colchicine has some effects
on the dividing cells producing specially c-mitosis
and other types of anomalies (see Eigisti and Dustip,
1957; Biesebe, 1958; Manna and Parida, 1965a), it was
necessary to see if the colchicine pretreatment could
cause any chromosome aberration within the short period
of treatment. In order to verify this point, 10 mice
were injected with 0.04% colchicine solution at the
rate of 1 ml per 100 gm. of the body weight. The bone
marrow cells were collected and fixed one hour after
the treatment of colchicine solution for cytological
preparation. A study of 1000 metaphase plates revealed
that the pretreatment did not produce any break type of
aberration or stickiness of the chromosomes. The
morphology of the metaphase chromosome appeared to be
slightly shorter and the disposition of the complement was more clear than what was found in the distilled water injected control series. Rarely there was mild c-mitotic effect when the two chromatids at the centromeric region in some of the chromosomes were slightly separated. Very rarely one or two instances of polyploid cells were encountered. Variable condensation of the chromosomes was relatively common. Further, a comparison of the morphology of chromosomes of the normal and the colchicine treated mice indicated that the outlines of the former ones were less sharp in most cases. Since in the colchicine pretreated control series no true break type aberration was observed, the aberration types scored from the treated series and their quantitative data were not influenced for the colchicine pretreatment. The colchicine pretreatment just helped to have more metaphase complements with clear outline of the chromosomes.

Third type of Control:— Since in the experimental series after the treatment of the particular dose of the chemical, 0.04% colchicine solution was injected one hour before the fixation time of the bone marrow cells, in this type of control series 10 mice were injected first with the required amount of distilled water (equivalent dose as solvent of the chemical)
and then 0.04% colchicine solution was pushed one hour prior to the time of fixation. An examination of 1000 metaphase plates yielded 2 chromatid, 1 chromosome break and 5 gap type aberrations. Thus the frequency of break was 0.3% and all aberrations taken together was 0.8%.

Thus the three sets of controls in comparison to the experimental series (vide infra) yielded very negligible results. The data, however negligible, need to be subtracted from the experimental ones in order to have the correct assessment of the damages caused for the treatment of any particular chemical. Thus the control values have not been mentioned in the tables of the experimental series. There could be no doubt that the aberrations in the treated series (vide infra) were produced mainly due to the effects of the chemical and not due to water or the colchicine solution.

Besides scoring the data of chromosome aberration from the bone marrow cells of the control specimens, an analysis of the caryotype (Fig. 2) of some well placed metaphase complement (Fig. 1) of the bone marrow cell has been made according to the groupings adopted by Crippa (1964). Any how, the chromosomes are gradually seriated for which in good number of cases it appears rather confusing to recognize the group of a.
chromosome from a casual observation. Further, the groupings made by Crippa (1964) is an arbitrary one. Inspite of this difficulty an attempt has generally been made to find out the group of the broken chromosomes particularly in the treated series. This would enable us to know broadly if the breaks were non-random or not.

In order to determine the groups, chromosomes of several complements were measured and their relative percentage values were determined. Since this has already been done in our laboratory, the data obtained by Dr. R.K. Das have been quoted in the following table A. Anyhow first two pairs of autosomes could be recognised with less difficulty.

* **TABLE A**

The metaphase complement of 40 chromosomes of the female specimens grouped into five groups with their relative percentage length in micra.

<table>
<thead>
<tr>
<th>Group</th>
<th>Size</th>
<th>NO</th>
<th>Part of 2n Number</th>
<th>Relative % Length</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Long</td>
<td>2 pairs</td>
<td>1/10</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>Sub-long</td>
<td>4 pairs</td>
<td>1/5</td>
<td>21%</td>
<td>7 in male for one X</td>
</tr>
<tr>
<td>Group III</td>
<td>Medium</td>
<td>10 pairs</td>
<td>1/2</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>Sub-medi</td>
<td>3 pairs</td>
<td>3/20</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>Small</td>
<td>1 pair</td>
<td>1/20</td>
<td>4%</td>
<td>3 in male for the Y</td>
</tr>
</tbody>
</table>

* Quoted from Ph.D. Thesis of Dr. R.K. Das, Kalyani Univ. 1970.*
An attempt has also been made to make an idea if the breaks were localised in some particular region of the affected chromatid of the chromosomes. In order to verify this point, the length of the unaffected chromatid was considered into three equal parts like proximal, middle and distal and the region in which the break of the affected chromatid fell was noted. Thus the standard set for the comparison was determined from each of the affected chromosome. The method has been applied to the experimental series since the number of the chromatid breaks was negligible in the control series.

In order to minimise time and space, the author has taken the liberty of presenting the camera lucida drawings (x ca. 2100) of only the affected chromosomes. These diagrams have been presented along with the description of the effects caused by the chemical concerned.

A few photomicrographs have also been presented in the following (Plates 1-V, Pho. 1-30) to represent the quality of effects produced for the treatments of different chemicals and X-rays. Since the effects of different chemicals and X-rays were qualitatively not very different, too many photomicrographs have not been presented. Further, the effects were found to be more pronounced in case of 2,4-dinitrophenol, actinomycin D, X-rays etc. than most other chemicals.
Explanation of Plate

PLATE I

Photomicrographs of some metaphase chromosome aberrations in the bone marrow cells induced by chemicals. Aberrations have been indicated generally by arrows.

Pho. 1: An unstained gap in a chromatid of a group-1 chromosome and a ring-like chromosome formed due to the fusion of the ends of two chromatids. (Actinomycin D treatment and 24 hour fixation).

Pho. 2: A clear chromatid break with a small fragment lying apart in a group-3 chromosome. Another chromosome with a constriction in the terminal region of a chromatid. (Actinomycin D treatment and 8 hour fixation).

Pho. 3: A group-2 chromosome with a chromatid break in the proximal region and an exchange type aberration between chromatid of two non-homologous chromosomes. (Actinomycin D treatment and 8 hour fixation).

Pho. 4: A clear chromatid break in the mid-region of a group-2 chromosome with the fragment lying apart. (Novobiocin treatment and 24 hour fixation).

Pho. 5: A subchromatid break in a 'rabbit-ear' chromosome and a fragment of unknown origin nearabout a small new metacentric chromosome. Two other metacentric type chromosomes formed possibly due to centric association of two non-homologous chromosomes and a longer chromosome with twisted chromatids are also present. (Novobiocin treatment and 24 hour fixation).

Pho. 6: Chromosomes with uneven outlines. Ends of chromatids of some non-homologous chromosomes are attached due to stickiness. The attachment is also seen in the centromeric regions of non-homologous chromosomes giving rise to pseudo-metacentric and submetacentric chromosomes. A chromosome with a chromatid break is also present. (Actinomycin D treatment and 24 hour fixation).
Photomicrographs of some metaphase chromosome aberrations in bone marrow cells of mice induced by chemicals. Aberrations have been generally indicated by arrows.

Pho. 7: A chromatid break with the displaced fragment in a group-3 chromosome. (Dihydrostreptomycin treatment and 24 hour fixation).

Pho. 8: A chromatid break with the displaced fragment in a group-3 chromosome, a small fragment near a newly originated submetacentric chromosome and two other chromosomes with faint gaps. (2,4-dinitrophenol treatment and 24 hour fixation).

Pho. 9: A chromatid break with the widely displaced fragment in a group-2 chromosome and a translocation between two chromatids of the non-homologous chromosomes. (2,4-dinitrophenol treatment and 24 hour fixation).

Pho. 10: A chromatid break in the mid-region with the fragment lying apart in a group-1 chromosome, a 'rabbit-ear' chromosome with a subchromatid break in one of its stretched arms and a metacentric chromosome formed possibly due to centromeric association of two non-homologous chromosomes. (2,4-dinitrophenol treatment and 24 hour fixation).

Pho. 11: A chromatid break in the distal region with small fragment lying not very far and another chromosome with a constriction in the terminal region of a chromatid. (2,4-dinitrophenol treatment and 24 hour fixation).

Pho. 12: Metaphase chromosomes with eroded outlines and despiralised condition. (Pyrogallol treatment and 24 hour fixation).
Explanation of Plate

PLATE III

Photomicrographs of some metaphase chromosome aberrations in the bone marrow cells of mice induced by chemicals. Aberrations have generally been indicated by arrows.

Pho. 13 : A clear chromatid break in a group-1 chromosome with the fragment lying apart, a translocation-like configuration with a fragment and a group of sticky chromosomes. (P-aminophenol treatment and 24 hour fixation).

Pho. 14 : A fragment of the broken chromatid lying by its side and a ring-like chromosome formed due to the fusion of chromatid ends. (Pyrogallol treatment and 24 hour fixation)

Pho. 15 : A clear chromatid break in the middle of a group-1 chromosome. (Gallic acid treatment, and 24 hour fixation).

Pho. 16 : A chromatid break in the terminal region with the minute fragment displaced slightly. (Gallic acid treatment and 24 hour fixation).

Pho. 17 : A chromatid break with the fragment not much displaced and another minute fragment of unknown origin. (Hydroxylamine treatment and 24 hour fixation).

Pho. 18 : A chromatid break with the fragment lying not far in a group-2 chromosome. (Hydroxylamine treatment and 24 hour fixation).
Photomicrographs of some metaphase chromosome aberrations in the bone cells of mice induced by chemicals. Aberrations have generally been indicated by arrows.

Pho. 19: A chromatid break in the mid-region of a group-1 chromosome.
(Semicarbazide treatment and 24 hour fixation).

Pho. 20: A chromatid break in the distal region in a group-3 chromosome and two pseudo-metacentric type chromosomes formed due to centromeric attachments of two non-homologous chromosomes.
(Carbon tetrachloride treatment and 24 hour fixation).

Pho. 21: A chromatid break in the mid-region with the fragment lying apart in a group-2 chromosome and another chromatid break with the fragment not displaced in a group 3 chromosome.
(Caffeine treatment and 24 hour fixation).

Pho. 22: A clear chromatid break near the proximal region in a group-2 chromosomes and two other metacentric chromosomes formed possibly due to the association of centromeric and of two non-homologous chromosomes.
(Carbon tetrachloride treatment and 24 hour fixation).

Pho. 23: A metaphase complement with early separation of the chromatids in a number of chromosomes. Chromosomes are thin and shorter in appearance.
(Hydroxyamine treatment and 24 hour fixation).

Pho. 24: A metaphase complement with some chromosomes divided early at the centromeric region.
(Phenyl hydrazine treatment and 24 hour fixation).
Explanation of Plate

PLATE V

Photomicrographs of metaphase some chromosome aberrations in the bone marrow cells of mice induced by 2,4-dinitrophenol and X-rays. Aberrations have generally been indicated by arrows.

Pho. 25 : A translocation with three chromosomes involved. Some other chromosome struck together due to stickiness. (2,4-dinitrophenol treatment and 24 hour fixation).

Pho. 26 : A translocation involving three chromosomes, a ring chromosome and metacentric chromosome. (200r X-ray treatment and 4 hour fixation).

Pho. 27 : A translocation involving two chromosomes and a chromosome with isochromatid breaks. (200r X-ray treatment and 4 hour fixation).

Pho. 28 : A translocation showing a cross-shaped configuration which could be formed due illegitimate chromatid exchange and more of the same like that the chiasma in a meiotic bivalent. A second translocation involving the terminal ends of two chromatids, the other chromatids being free. (200r X-ray treatment and 4 hour fixation).

Pho. 29 : Metaphase with some translocations and sticky chromosomes. The stickiness also made some association of centromeric regions. (200r X-ray treatment and 24 hour fixation).

Pho. 30 : Polyploid cell with some chromosome aberrations in the form of isochromatid and subchromatid breaks and chromosome fusion. (200r X-ray treatment and 4 hour fixation)