CHAPTER I

THE QUALITATIVE AND QUANTITATIVE ASPECTS OF CHEMICALLY AND THE X-RAY INDUCED BONE MARROW CHROMOSOME ABERRATIONS IN MICE
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A. ANTIBIOTICS

1.

Actinomycin-D (AMD)

It needs no mentioning that the discovery and the use of antibiotics in the field of chemotherapy has been a great achievement of this century. Since actinomycin has been very extensively used in various types of studies beside our present investigation, a general knowledge about the antibiotic will be of some interest to us.

The nature, formation and activities of actinomycin, reviewed by different workers, are contained in the book entitled "Actinomycin" edited by S.A.Waksman (1968). The editor has presented a historical account on the success of isolation of actinomycin.

Actinomycin is brightly coloured (crystals are red and the solution is yellow-organs in colour) peptide containing antibiotic. It was discovered by Waksman and Woodruff (1940a). It was the first antibiotic to be isolated in crystalline form from a culture of actinomycetes. The antagonising properties of actinomycetes on other microorganisms were, however, reported much earlier by Casperini (1890). Since its discovery it has
been tested on various mammalian tumors including man. Tumors which have been found to be sensitive to actinomycetin-D are 'Wilms' tumor, rhabdomyos sarcoma, Earling's sarcoma, choriocarcinoma of testes and uterus, lymphosarcoma and bronchiogenic carcinoma. The Council of Drug of the American Medical Association recognised actinomycin-D (Dautinomycin) as an anti-neoplastic agent in the report of the 25th April, 1966 (see Waksman, 1968). Recently it has also found a place as adjunct suppressor of the immune response to kidney homograft in man. Its inhibitory effects upon 11 gram-positive and 10 gram-negative bacteria are known (Waksman and Woodruff, 1940b). The substance designated as actinomycin A is primarily active against gram-positive bacteria including tubercular one. Johnson, Todd and Vining (1952) named actinomycin-D but the biological properties of A and D are identical. Brockmann and Grubhofer (1949) isolated actinomycin C from a culture of Streptomyces chrysomalles. The report that actinomycin possess anticancer property led Waksman and his group (Manaker et al 1955) to produce actinomycin D from a culture of S. parvulus. The molecular formula of actinomycin-D is C_{60}H_{70}O_{15}N_{13}H_{2}O. This antibiotic is very much toxic to experimental animals and reduces the weight of the spleen.

Besides chemotherapeutic use, actinomycin-D has been employed in the study of numerous important
have studied its effect on development and Perez-Davila and Baker (1967) on early imaginal disks of eye in *Drosophila*. Thus we find from examples cited above that actinomycin is very important biologically.

Kawamata (1964) has suggested the mechanism of action of actinomycin D and it has recently been described in detail by Reich, Cerami and Ward (1967). This antibiotic has been used as a very important biochemical tool.

The biological activity of actinomycin presents elements of unusual selectivity and specificity as given in the following:

(i) It supresses the growth of DNA viruses, but permits normal or even supernormal growth of most RNA viruses. (ii) In intact cells as well as with purified enzymes, actinomycin inhibits selectively the DNA-directed synthesis of RNA, but not of DNA although the templet remains the same for both the synthesis. Thus actinomycin D has the ability to supress RNA and protein synthesis but it would not interfere with DNA synthesis. (iii) Actinomycin forms complexes with DNA, but not with RNA (Kawamata and Imanishi, 1960). This fact suggests that the biological basis of actinomycin action is probably unitary, and very likely it depends on the formation of complexes with DNA. (iv) The complex formation between actinomycin and DNA shows an absolute specific requirement.
the mechanisms of the DNA function. It will help in the formation of concrete idea related with the problem of the control of gene action.

Actinomycin-D has been used in the study of the genetic code because of its specificity of action referred to above. The current idea on the role of nucleic acid in protein synthesis prevails that the genetic information contained in chromosomal DNA is transferred to the site of protein synthesis by the specific messenger-RNA. This m-RNA is synthesized on a DNA template for which it will have a base composition complementary to that of the template. Inhibition of RNA synthesis would lead to block the protein synthesis because m-RNA decays rapidly. Since actinomycin-D is specific inhibitor of DNA dependent RNA synthesis, its application will cause inhibition of DNA-primed RNA synthesis in a specific way by combining with DNA template. Reich and Goldberg (1964) have made a review on the biochemical activities of actinomycin in relation to nucleic acid function. The blocking of the genetic information by actinomycin-D will prevent the transport of gene product from nucleus to cytoplasm. Because of this, actinomycin has been applied in the analysis of gene action and the role of RNA metabolism in various materials.
The actinomycin-D has been shown to bind to DNA (Kirk, 1960; Rauen et al 1960) provided the DNA contains guanine (Goldberg et al 1962). Actinomycin interacts in solution with deoxyguanosine and guanosine to a lesser extent with adenosine and not at all with other nucleosides (Kersten, 1961). A model for the structure of the complex has been presented in which the chromatophore of actinomycin-D is hydrogen bonded to the amino group of guanine and to the ring oxygen of deoxyribose and the cyclic peptides of actinomycin-D lie along with the narrow groove of the DNA helix.

The pharmacology of actinomycin in mammals has been studied by Philips et al (1960). Its presence in the serum within three minutes after the intravenous injection has been proved beyond doubt. Actinomycin is excreted out in unchanged condition chiefly with the bile and to some extent urine. It appears that liver can accumulate it.

Relatively few studies have been carried out on the effect of actinomycin-D at the chromosomal level. But the importance of such studies is obvious because the DNA is the most important constituent of the chromosome. A number of physical changes are associated with the binding of actinomycin to DNA. For example the viscosity of DNA solution is increased for its presence. Isolated lumpbrush chromosomes lose their
flexibilities and become rod like with their characteristic loops shrunked when they are exposed to the antibiotic (Izawa et al, 1963). The typical banding pattern of the giant salivary gland chromosomes of dipteran larvae disappears in the presence of actinomycin (Sirilin et al, 1963). It also produces contraction of chromatin. Deitch and Godman (1967) studied the cytology of culture cells which survived the treatment of actinomycin-D. Bal and Gross (1963) studied its action on mitosis. Whilson and Podilla (1964) studied its effects on stomatogenesis and cell division in temperature synchronised condition. Monesi and Veronesi (1955) reported the chromosome breakages produced by actinomycin M. Arrighi and Hsu (1965) observed the effect of actinomycin-D on the metaphase chromosomes of the chinese hamster in cultured cells. Recently Manna and Bhunya (see Manna, 1969) have studied the effect of this antibiotic on the meiotic chromosomes of grasshoppers. So far as known the effect of actinomycin D has not been tested on in vivo mammalian chromosomes. The present study was undertaken in order to gather more data on the effect of this chemical on mammalian chromosomes.

The inhibitory action on cell division is suspected to be initiated only when a higher concentration is used and that acts on DNA polymerase. However, the inhibitory action could be assumed as due to the suppressed RNA and protein
synthesis than due to DNA synthesis. Sirlin, Tandler and Jacob (1963) reported that the treatment of actinomycin D completely blocked the nucleolar RNA synthesis but it acted partially upon the chromosomal RNA synthesis.

**EXPERIMENTAL PROCEDURE**

Two doses, 25 µg and 125 µg of actinomycin D, dissolved in distilled water were intraperitoneally injected to individuals. Each mouse was of 6-8 months old and about 23 gm. in weight. The group of mice which were individually, treated with 25 µg of AMD, thrived well for longer period but specimens of the other set which were treated with 125 µg. of AMD could not survive beyond 16 hour after the injection. Bone marrow cells of specimens treated with 25 µg of AMD were fixed at 4, 8, 24 and 48 hour while those of 125 µg series only at 4 and 8 hour after the injection. The cytological preparations were made according to the schedules described before. Control specimens were also studied, the date of which have been presented earlier.
Fig. 3a Graphical representation of the dividing cells in the bone marrow fixed at different intervals of the control and 25 μg actinomycin-D treated mice. Dotted line (c) represents control and solid line (T) represents the treated series.
RESULTS

The effect of actinomycin D studied on the mitotic index and on the structure of the chromosomes of the bone marrow cells are being presented in the following:

A) Mitotic Index:

The frequency of dividing cells at the intervals of 4, 8, 24 and 48 hour in the bone marrow tissue in the control specimens was 2.75%, 2.60%, 2.90% and 2.70% respectively. At each of the fixation time, the number of the dividing cells was determined from 2000 counts made from random areas. The number of dividing cells were 55 in 4 hour, 52 in 8 hour, 58 in 24 hour and 54 in 48 hour.

The mitotic index was also determined in the bone marrow tissue of the mice injected with 25 µg of AMD and fixed at 4, 8, 24 and 48 hour. In the determination of the frequency of dividing cells, as done in the control series, different areas in several slides were taken randomly and the number of dividing cells encountered in a total 2000 in each fixation time was recorded. In the treated series the frequencies were 1.4% (20 in 2000) at 4 hour, 1.5% (30 in 2000) at 8 hour, 1.0% (20 in 2000) at 24 hour and 2.6% (52 in 2000) at 48 hour. As the data of the control and
experimental series are plotted graphically (Fig. 3A), it would appear in the treated series (T) that the frequency of dividing cells was dropping down up to 24 hour after the treatment. When compared with the control data (C), it would appear that even at 4 hour there was 1.35% drop. The value was reduced at 8 hour because the difference was 1.1%. But this difference was negligible. We can neglect the slight rise at 8 hour as due to sampling error. In the treated series at 24 hour the frequency dropped down to 1% as compared to a rise (2.90%) in the control series leaving a difference of 1.90% drop with the control series. However, at 48 hour in the treated series the frequency of dividing cell was found to be 2.6% which was very close to the frequency of the control series (2.70%). Since the tissues were not fixed at regular intervals, we can not specifically say how long the effect continued to be present and what was the time of the maximum inhibitory effect. Anyhow, from the present data one can suggest that effect was very quick since it was palpable at 4 hour after the treatment. The effect reached the maximum at 24 hour. After this time the effect must have gradually been removed and at the 48 hour the frequency almost reached to the normalcy. The inhibitory effect of AMD, therefore, lasts for a shorter duration.

B) Effects on Chromosomes

Qualitatively the effects of two doses of AMD,
EXPLANATION OF FIGURE
(3 A - L)

(3 A - L)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by 25 ug and 125 ug actinomycin D. Only Affected Chromosomes Drawn).

(A) A chromatid break near the proximal region in a chromosome of group I.

(B) A chromatid break near about the mid-region of a chromosome of group I.

(C) Chromatid break in the proximal part of a chromosome of group II.

(D) A subchromatid break in group III chromosome.

(E) A chromatid break in a group II chromosome. The fragment lying apart in a rotated position.

(F) A chromatid break in group III chromosome. The fragment lying in a slightly displaced position.

(G) A chromatid break in a group II chromosome.

(H) A chromatid break in a group II chromosome with the fragment lying apart.

(I) A fragmented chromatid of unknown origin.

(J) Two group II chromosomes in a plate, each with a broken chromatid.

(K) Ends of two chromatids of two chromosomes attached.

(L) A translocation in the terminal ends in two chromatids of two chromosomes.
e.g. 25 \( \mu \text{g} \) and 125 \( \mu \text{g} \) on the chromosomes of bone marrow cells fixed at different intervals have been found to be not markedly different. Thus the author has not attempted to present the qualitative effects of two doses separately. The effects on the chromosomes, for the sake of simplification, could be generalized into two forms viz. (1) on the general morphology and (2) on the structural integrity. The former ones may be called as physiological effect and the latter ones as true breaks.

The general effect was found in the form of wooly or despiralized appearance of the chromosomes (Plate I, Pho. 6). The general effects were not encountered in all the metaphase complements. The treatment of AMD did not produce too much clumping or stickiness in the chromosomes. It was interesting to note that the breaks were mainly chromatid-type (Fig. 3A-G) and not a single clear instance of chromosome type break was encountered in a total of 1000 metaphases examined for the aberration study (Table 1). Rarely one or two ring-like chromosomes were found but they could have been produced due to fusion of terminal ends of the chromatids (Plate I, Pho. 1). Exchange type aberration was also very rare (Plate I, Pho. 3).

The chromatid type breaks (Fig. 3A-C, E-J) were most prevalent but in one or two instances the entire chromatid was not broken. In such a case half of it was broken and the
broken area was in a little stretched condition. It has been designated as the subchromatid break (Fig. 3D). The disposition of the fragment in case of the chromatid break was of variable nature. More often the broken part of the chromatid showing the clear indication of breaking point remained in its normal position (Fig. 3A-C). Such a type of disposition sometimes led to a confusing situation in considering it a break or gap (Fig. 3B). The broken part of the chromatid in good number of cases was also found to be lying in the completely displaced condition (Plate 1, Pho. 2) but its origin could, however, still be followed because it was not lying too far (Fig. 3F-H). The fragmented part was also found to be lying in twisted (Fig. 3E), bend (3G), or in some other condition (Fig. 3, I). In some cases when the small fragment was lying in a completely isolated position, the chromosome of its origin could rarely be traced (Fig. 3, I). Not more than one break in a chromatid of the chromosome of any group was encountered. Generally only one break was found in a metaphase plate. Deviation from this was extremely rare. In an analysis of 45 metaphase complements with chromatid breaks, two instances with two breaks in each plate (Fig. 3, J), and one instance with four breaks were found. However, in this type of analysis only the simple breaks were considered and the translocation types were excluded. In some instances exchange type aberrations in the form of
translocation between chromatids of non-homologous chromosomes were encountered (Fig. 3L). However, sometimes the author was left to some confusing situation because in some instances it could very well be explained as due to the fusion or close association of the two ends of two chromatids of two non-homologous chromosomes (Fig. 3K). In such case one could not decide with conviction if this was the result of the association of two ends or they are held together due to translocation at the terminal end. The possibility of simple terminal association could not be ruled out and a critical observation was made for a decision. Besides a few controversial situations there were definite cases of the translocation between the chromatids of two non-homologous chromosomes. Apart from the true break type aberrations and translocations, some constrictions and gap-like structures were found. They have not been taken into account in the present study. Only the unambiguous cases of breaks have been accounted for. If all types of aberrations were taken into account, the frequency would be much higher than what has been presented in the table (Table 1).

It appeared from the analysis of the chromatid type breaks that the region of their occurrence was not very randomly distributed. The distribution of the breaking points was more frequent at the middle to the distal half of the affected chromosomes. In order to have a more precise
idea of region-wise distribution of the breaking point, the length of the affected chromosome was artificially demarcated into three regions viz. proximal (near the centromere, Fig.3A,C) middle (Fig. 3B,E,G,H) and distal (free end side; Fig.3F) and the number of breaking points were adjudged in these three parts. This type of analysis would reveal the region which was more susceptible to break if the effect of AMD was non-random in nature. In an analysis of 99 chromatid breaks obtained in the present study, 50 were in the distal part, 40 at the middle and 9 at the proximal part of the affected chromosomes. On the other hand, due to the difficulties of correct identification of the group of a particular affected chromosomes it was not possible to say definitely whether the chromosomes belonging to some particular group or groups were broken more often than others. Inspite of these difficulties some approach was made. It has been found that the breaks were more common in the two larger group of chromosomes (groups I and II) than the rest. The first two groups form about one-fourth of the diploid number of chromosomes (excluding the X) according to the groupings shown already. Thus the author finds that the chromatid breaks were non-random in distribution with regard to the place of origin and the chromosome concerned.

Quantitative study - In order to have an idea of the amount of damages caused by the treatment of two doses of AMD, the
frequency of the break type aberration was only taken into consideration (Table 1). In each set of data the number of chromatid breaks in 200 metaphases was scored. Very rarely more than one break in a metaphase plate was present and the frequency would hardly exceed 2%.

**Table 1**

Frequency distribution of Chromatid Type Breaks at Different Intervals in the Metaphase Chromosomes of Bone Marrow Cells at Different Intervals in Mice Treated With Two Doses of Actinomycin D.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Fixation Hour</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 hour</td>
<td>8 hour</td>
</tr>
<tr>
<td>25 µg</td>
<td>16/200</td>
<td>17/200</td>
</tr>
<tr>
<td>Percentage</td>
<td>8.0</td>
<td>8.5</td>
</tr>
<tr>
<td>125 µg</td>
<td>22/200</td>
<td>23/200</td>
</tr>
<tr>
<td>Percentage</td>
<td>11.0</td>
<td>11.5</td>
</tr>
</tbody>
</table>

An analysis of the data (Table 1) would indicate that the frequency did not vary much in the tissues fixed at different hours after the administration of a particular dose of AMD. In 25 µg it was 8% at 4 hour, 8.5% at 8 hour and 10.5% at 24 hour. If the total number of chromatid breaks of the different fixation time of 25 µg dose was taken into consideration, it became 9% (54 breaks in 600 cells). Thus
the deviation on either side of the average in different hour of fixation was not very significant. It can, therefore, be suggested that the effect did not vary significantly with the lapse of time say upto 24 hour. The frequency of chromatid breaks with the dose of 125 µg AMD at 4 and 8 hour also did not differ to any great extent. It was 11.0% at 4 hour and 11.5% at 8 hour. In this series no material at 24 hour could be fixed because the specimens did not survive upto that time. A comparison of the aberration data of the two doses at any particular fixation time would reveal that the frequency was higher in 125 µg than that of 25 µg dose. However, the increase was not proportional because the difference in the two doses was 5 times while that of the aberration frequency was not even 1.5 times. It seems very likely that the effect of AMD is dependent on some threshold concentration. Since in the present study no data of the tissue fixed at 48 hour were scored, we could not say how long the effect of AMD in producing the aberration persisted.

COMMENTS

The present study on the effect of two doses of actinomycin D in the bone marrow chromosomes of mice was directed mainly in the following four lines (1) the effect on dividing cells, (2) the physiological effect on chromosome;
(3) the chromosome breaking effect, and (4) the non-random
distribution of breaks.

It has been reviewed from the data that due to the
treatment of 25 μg of AMD the frequency of the dividing
cells was reduced upto 24 hour after the treatment beyond
which the inhibitory effect was disappearing. At 48 hour
the frequency was almost equal to the control data. Before
the question of decrease in the frequency of dividing cells
for the treatment of AMD is taken up, some other factor in
the present study, if involved in any way, needs to be
considered. In the control and in the treated series
colchicine solution was injected into the specimens for the
better cytological preparation. Therefore, the action of
colchicine needs be considered before we can think that the
effect was entirely due to the treatment of AMD. In all
cases, colchicine solution was injected one hour prior to
the fixation of the tissues. The colchicine is known to be
a mitotic arrestor because of its effect on the mitotic
spindle (Eigsti and Dustin, 1957). It has also been shown
that there is some inhibitory action of colchicine on cell
division which is dependent on the state of the DNA in the
interphase nucleus (see Gelfant, 1963). The colchicine is
claimed to have inhibiting action if the DNA is in the
presynthetic or G1 phase. But it has no action on the
synthetic (S-phase) or post synthetic phase of DNA (G2 phase) for which the cell will proceed division up to metaphase. The action of colchicine will again be seen on the spindle apparatus to produce C-mitotic effect. These facts may partially stand in our way to consider that the inhibitory effect was not solely due to the treatment of AMD. However, we may rule out such a possibility on some ground. Firstly we have compared our data with the control one and in both the cases colchicine pretreatment was done one hour before the fixation. Therefore, the effect due to the colchicine, if any, is expected to be the same in both cases. Secondly, in comparison to the control series, the treated series, up to 24 hour after treatment, showed a drop in the mitotic frequency, which indicates that a number of cells fail to enter mitosis. This cannot be explained as due to the effect of colchicine 1 hour before the fixation. If this is true, we should not expect the regaining of the normal frequency at 48 hour with the same type of colchicine treatment. Thus the retardation in the frequency was due to the treatment of AMD. In what way it caused the retardation is a matter of speculation. Since it is known that AMD inhibits DNA dependent RNA synthesis at concentrations which have no effect on DNA polymerase (see Reich, 1964), it would have effect on protein synthesis. At higher dose of AMD the DNA polymerase may be affected. With these facts in view, the inhibition
of cell division very likely is due to the suppressed RNA and protein synthesis rather than the inhibition of the DNA synthesis. Elliott (1963) also considered that the cell division is inhibited by concentration below the dose required for the inhibition of DNA synthesis. The chemicals which have an affect on chromosome, are likely to act on some of its constituent parts, DNA, RNA, proteins and other binding substances (Manna, 1969). It may have either multiple effects or specific effect on some particular constituents as the effect of BUdR on A-T pairs suggested by Somers and Hsu (1962). The action may take place directly or indirectly on the constituents. Since AMD has been shown to be a strong inhibitor of DNA dependent RNA synthesis (Perry, 1963; Reich, 1964) and it is also hydrogen bonded to guanine of DNA molecule (Reich, 1964), its action would very likely affect the chromosome reproduction. Certain messenger-RNAs which are normally required to initiate the cells to undergo mitosis, may be blocked by the action of AMD. Arrighi and Hsu (1965) planned their study on this basis. It was found in the present data that the inhibitory effect of AMD was on the waning after 24 hour which would indicate that the damage was not of a permanent nature and the action did not last too long. Arrighi and Hsu (1965) studied the inhibitory
action of AMD on the tissue culture cells of Chinese hamster and they followed the mitotic frequency in the cells treated with a dose of 5 μg/ml AMD upto 240 minutes. In this set they did not use colchicine in the cytological preparation. In another set of experiment they used 10 μg/ml AMD and studied the mitotic frequency upto 250 minutes using colchicine in the preparation. In latter case, like the previous one, the inhibitory effect was obtained but in the colchicine treated cell the frequency was higher than the other. The result obtained by them, if correlated with our findings, the frequency which we obtained might be little higher. However, the effect would not be of the same magnitude as obtained by Arrighi and Hsu (1965) because they tried with tissue culture cells and colchimid was used along with AMD. Further, the effect of AMD on the mitotic index was studied by them only upto four hours which would not reveal how long this effect could continue as has been shown in the present data. Therefore, the present data indicate that AMD has inhibitory effect on the cell division and this effect could last for a limited period. The effect is reduced with the lapse of time.

The second type of effect produced by the treatment of AMD on the chromosomes of bone marrow cells was of physiological nature. It was in the form of erosion or woolly appearance and despiralization of the chromosome. However, the effect was not so drastic as observed by
Arrighi and Hsu (1965) on the chromosomes of tissue culture cells of Chinese hamster. The difference in the degree of the effect could mainly be accounted for the difference in the materials used. In tissue culture since the cells were exposed more directly to AMD than those of bone marrow cells, the effect was severe in previous case. It was to enter the bone marrow cells through metabolic pathway. Because of the indirect mode of penetration the strength and the action could have been modified to some extent. Whatever be the difference in degree, there was no disagreement as to the overall effect. The effect of actinomycin D studied on the spermatocyte chromosomes of grasshopper (Manna and Bhunya, unpublished) was more severe than what has been found on the chromosomes of bone marrow cells under the present study. There was tremendous sticky and clumping effect in the grasshopper chromosomes. Most likely the different degrees of physiological effects were produced due to the interference of AMD in the protein synthesis. The physical effect might lead sometimes to produce true breaks (Biesele, 1958) if the effect was severe. Besides some physiological effect, the treatment of AMD also produce the chromatid type breaks and very few exchanged type aberrations. Let us examine some of the chemical properties of AMD. There is no disagreement as pointed out before that the treatment of AMD causes selective inhibition of DNA dependent RNA synthesis and it is
bonded to guanine of DNA molecule, these properties are more concerned with the gene action. Since the break type aberrations have been obtained by the application of AMD, our present problem is, therefore, related with the chemistry of the chromosome. As mentioned before chemically the chromosome is made up of constant amount of DNA, histone, variable amount of non-histone protein and a small amount of RNA and metal ion. Broadly it consists of nucleoprotein blocks bounded by metal ions. But controversies exist as to their molecular arrangements, number of strands, back bone, etc. (see Srivastava, 1968; Manna, 1969). The break type aberration has been caused due to the application of various types of mutagens in the above mentioned components besides other things (see Biesele, 1958; Sharma and Sharma, 1960; Kihlman, 1966; Manna, 1969). The cellular metabolic disturbances may lead to chromosome break and many chemicals act in that way. Usually DNA synthesis of the cell takes place during S phase of the interphase nucleus (Howard and Pelc, 1953). Generally the lower concentration of AMD has no effect on DNA but at higher concentration it may affect DNA polymerase activity. What should be the optimum concentration in living form has not been determined. In the present study we have used two concentrations, one was very high, 5 times higher than the other. Both of them produced mainly the chromatid type breaks. On the other hand, the effect of AMD has been compared with that of the ionizing
radiation by Kihlman (1966). In that case the cytological
effect will depend not only on its effect on DNA but also on
DNA associated components. The DNA-protein complexes if
altered, we will not only observe some physiological effect
but also some after effects as breaks and exchange type
aberrations. Kihlman (1966) considers that the physical
properties of DNA are changed by actinomycin D because it
inhibits separation of the strands of DNA like ionizing
radiation. According to him due to this alteration of the
state and properties of chromosomes aberrations are produced.
Therefore, the chromosome aberration produced by AMD may be
explained on the physio-chemical basis rather than only on
chemical basis. Kihlman (1966) has not totally excluded
the possibility of chemical basis. How, it really affects
the cellular metabolism leading to the production of
chromosome breaks is not fully understood. Kihlman (1966;
page 179) remarks "It may be concluded that although it seems
likely that the ability of ethoxycaffeine, streptonigrin and
actinomycin D to cause chromosome aberration at least partly
is the result of the ability to combine with DNA and/or to
alter its physical properties, very little is known about
the mechanism by which the aberrations are produced". In
the present study the breaks were mainly the chromatid type
which would definitely indicate that the chemical acted
after the duplication of chromosomes. If the breaks were
due to its effect on DNA, AMD must have affected at the late S phase or G₂ phase of DNA synthesis (Evans and Scott, 1964). It is, however, not very clear why only one chromatid of a chromosome but not both the chromatids were affected. Even the chromatids could have been affected in different sites but not in the form of chromosome type breaks. Arrighi and Hsu (1965) observed that the effect was conditioned by the replication time of the chromosomes, since the Y chromosome in their material which has a different condensation cycle was less affected. It has been assumed that AMD competed for binding sites with histones. The explanation is not sufficient to cover our present data. We have no other plausible explanation.

It has been revealed in the present study that the breaks were non-randomly distributed with respect to the region as well as the size of the chromosome. The chromosomes of the larger groups (Groups I and II) were more susceptible to breaks. Further, the breaks were located mostly in the middle and in the distal region of the chromosomes. Thus the proximal region was most resistant to the action of the chemical. Kihlman (1966) pointed out that breaks induced by chemical agents were mostly non-random in distribution. Extreme localization of breaks has been observed in the root tip chromosomes of *V. faba* for the treatment of EOC, AdR and MH (see Kihlman, 1966; page 148) and in region 7 (near the
centromere) of the chromosome number 1 of Chinese hamster cells by BUdR (Hsu and Somers, 1961), near the chromosome ends of human leucocyte culture (Kihlman, Nichols and Levan, 1963) produced by AdR, CA, etc. These localization of breaks have been explained due to the specific absorption of the chemicals to the regions concerned. In the present study such specific localization could not be suggested because of some inherent difficulties lying with the material. It was also not possible to assign the exact point of breaks. Since middle and distal regions have been found to break often with chemicals of non-related nature (Manna and Das, unpublished and vide infra), it is suspected that there could be some weaker regions in the chromosome which are liable to break more easily by odd type of chemicals. The occurrence of predominantly chromatid type breaks, however, leaves again to some confusing state. If it was the question of weaker regions, why the breaks were confined to one of the chromatids of the affected chromosomes? We have not yet found out the answer but the fact was there.
The antibiotic, Novobiocin was isolated independently in three pharmaceutical research laboratories of different companies and was named differently (see Goldberg, 1959) as Cathomycin (Merck), Streptonovicin (Up John) and Cardelmycin (Pfizer). It is available in the form of white monosodium, disodium, monocalcium and dicalcium salts. Monosodium salt of Novobiocin used in the present investigation was donated by Merck, Sharp and Dohme Research Laboratories, U.S.A. to Prof. G.K. Manna. The chemical formula of novobiocin as given by Goldberg (1959) is $C_{31}H_{36}N_{20}O_{11}$. It is the first clinically employed antibiotic which has been found to inhibit nucleic acid synthesis and is active mainly against gram-positive bacteria. The action of this antibiotic has been tested on various bacteria. Brock and Brock (1959) studied the effect of NB on the cell permeability of *E. coli*. Wishnow et al (1965) observed its biochemical effect on *Staphylococcus aureus*. Smith and Davis (1965a) reported the inhibitory effect of this antibiotic on the nucleic acid synthesis in *E. coli*. The other aspects of the action of NB on bacteria have been studied by other workers (Morris and Russel, 1968; Smith and Davis, 1965a). Although various
types of action of NB have been studied on bacteria but its effect on the chromosomes practically remained unexplored. Recently Manna and Bhunya (unpublished) along with other antibiotics have been studying the effect of novobiocin on the spermatocyte chromosomes of grasshopper and some of their preliminary observation has already been communicated (Bhunya and Manna, 1969). Since the effect of this chemical on the mitotic chromosomes of mammals has not been taken up by others, the present study was undertaken.

**EXPERIMENTAL PROCEDURE**

2.25 mg of novobiocin mixed with distilled water was intra-peritoneally injected into each specimen. The experimental mice were sacrificed at 4, 8, 12, 24 and 48 hour after treatment. Cytological preparation of their bone marrow cells fixed at 4, 24, 48 hour were made for aberration study according to the methods described earlier while tissues fixed in all the five intervals were examined for the determination of mitotic index.

**RESULTS**

A) **Effect on the Mitotic Index**:

Novobiocin has been found to have inhibitory effect on the cell division, but it was less pronounced.

The value were only 10.0% in the novobiocin and 1.70% in the AMD series. Further, the AMD treatment had relatively quick inhibitory as well as recovery action than that of novobiocin. The rise in the frequency of dividing cells at 4 hour after the treatment of novobiocin appeared to be very interesting.
Fig. 4 - Graphical representation of the frequency of the dividing cells in the bone marrow tissue fixed at different intervals in the control and 1.25 mg. Novobiocin treated mice. Dotted line (C) represents the control and the solid line (T) represents the treated series.
B) Effect on Chromosome

Qualitative - Novobiocin has been found to have practically no effect on the general morphology of the chromosome. It manifested generally in the form of eroded outline, stickiness and despiralization. The antibiotic, however, produced a number of chromatid type breaks (Figs. 5A-C,E-H; Plate 1, Pho. 4) and a few other type of aberrations (Fig. 5H; Plate 1, Pho. 5). A chromosome having more than one break in one of its chromatid has not been observed in 32 aberrations obtained from 750 metaphase complements (Table 2). The breaks were not very restricted to any particular region but some preferential occurrence was found. In the artificial regional demarcation of the chromosome as proximal, middle and distal, the survey of 32 chromatid breaks revealed that 14 were in the distal (Fig. 5C), 14 in the middle (Fig. 5E-H) and 4 in the proximal region (Fig. 5A,B). Further, due to the treatment of antibiotic, 60% of the chromatid breaks were in the longer groups of the chromosomes. The chromosomes of the longer groups (Groups I and II) from \( \frac{3}{4} \) of the diploid number and 36% of relative percentage length. Therefore, they had more breaks than the others. Amongst the longer groups, breaks were mostly at the middle and in the distal region of the chromatid of the chromosome concerned. The frequency of breaks between the middle and distal region
in the longer groups of chromosomes was almost equal. Since the distinction between the chromosomes of the longer groups could not be made, it was not possible to say definitely if some particular chromosome was liable to break more often than others. Roughly it appears that the longest pair had the highest frequency of breaks. Amongst the smaller groups, the 'rabbit-ear' chromosomes sometimes serve as the marker chromosome. Out of 32 chromatid breaks analysed, one was in this type of chromosome (Fig. 5H). Sometimes confusion arose about some of the chromatid breaks because the fragment was lying almost in its original position and unstained region was very narrow (Fig. 5F). Such confusing cases were not accounted in the quantitative data. In most cases there could be some doubt about the reality of the breaks in the chromatid because the broken fragment was lying further apart (Figs. 5A, B) or in a displaced position (Fig. 5C, G). However, on the whole the effect of novobiocin on the somatic chromosomes was found to be less than that of actinomycin-D. Further, the treatment of novobiocin did not produce more than one break in a metaphase complement. At least such type of incidence must be very rare because not a single instance was observed in an examination of 750 metaphases (Table 2). Besides the chromatid breaks, no other true break-type aberrations was observed. Not a single instance of exchange type aberration was encountered in the present study.
Quantitative - The quantitative study of the chromatid breaks inflicted due to the injection of novobiocin at different intervals of time (Table 2) revealed that there was an optimum hour of the effect after injection. At 4 hour and 48 hour the frequencies were lower and more or less close to one another (2.4% and 2.8% respectively), while it was highest at 24 hour, the value being 7.5%. It might be revealed, on the other hand, that inhibitory effect had been shown to be the maximum at the same 24 hour. Thus it seems very likely that the chromosome breaking effect and the inhibitory effect were not related and they were independent of one another. The frequency of aberrations if taken together, irrespective of their time of fixation, it would be 4.3%. Since the frequency of chromatid breaks in the control series referred to before was very negligible, the value obtained in the present study was due to the treatment of novobiocin.

Table 2

Frequency Distribution of Chromatid Breaks in the Bone Marrow Cells fixed at 4, 24 and 48 Hour after the Injection of 1.25 mg of novobiocin.

<table>
<thead>
<tr>
<th>Time of Fixation</th>
<th>No. of Metaphases Examined</th>
<th>No. of Chromatid Breaks</th>
<th>Percentage of Chromatid Breaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hour</td>
<td>250</td>
<td>6</td>
<td>2.4%</td>
</tr>
<tr>
<td>24 hour</td>
<td>250</td>
<td>19</td>
<td>7.5%</td>
</tr>
<tr>
<td>48 hour</td>
<td>250</td>
<td>7</td>
<td>2.8%</td>
</tr>
</tbody>
</table>
EXPLANATION OF FIGURE
(5 A - H)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by 1.25 mg of Novobiocin. Only Affected Chromosomes Drawn).

(A) A chromatid break nearabout the proximal region of a group I chromosome.
(B) A chromatid break in the same region as in A. The fragment is more displaced.
(C) A chromatid break in the distal region of a group III chromosome. The fragment lying in displaced condition.
(D) A terminal constriction or gap in a chromatid of group III chromosome.
(E) A chromatid break in the mid-region of group II chromosome. The fragment is lying apart.
(F) A chromatid break nearabout the mid-region. The fragment is not at all displaced.
(G) A chromatid break in a group III chromosome.
(H) A chromatid break in a rabbit-ear chromosome.
It has been mentioned before that some investigations on the mechanism of the action of novobiocin at the biological and biochemical level have been carried out on bacterial cells but only one on the chromosomes. The knowledge about the mechanism of action derived from bacteria is also far from complete. Sometimes contradictory reports appeared (see Brock, 1956; Morris and Russell, 1968; Smith and Davis, 1966). Anyhow, Morris and Russell (1968) have recorded several types of effect of novobiocin on E. coli and S. aureus. Some of their observations could be applied in our attempt to explain the probable mechanism of the inhibitory action on cell division and chromosome aberrations obtained in the present study. They are (a) the inhibitory action on the cell-wall formation, protein and nucleic acid synthesis; (b) the enhancement of cell permeability; (c) the induction of intracellular deficiency of Mg$^{++}$ ions; etc. Let us take up first the inhibitory effect of novobiocin on cell division. Smith and Davis (1965a) studied the action of novobiocin on the inhibition of cell division in certain strains of E. coli. The earliest effect produced by the treatment of novobiocin was the inhibition of DNA synthesis. There was also some amount of inhibition of RNA synthesis. Subsequently the drug also impaired the protein synthesis and the cell
wall formation. It was found further that all the nucleotide triphosphates were accumulated with the inhibition of the nucleic acid synthesis.

Smith and Davis (1965a) showed that the treatment of novobiocin inhibited the DNA synthesis by inhibiting the enzyme template system responsible for DNA polymerization, but with the inhibition of DNA synthesis, the synthesis of four deoxynucleoside triphosphates were not inhibited and the template DNA was not degraded. Again as novobiocin does not inhibit the synthesis of any of the four ribonucleoside triphosphates, it affects the RNA synthesis by inhibiting the RNA polymerase - DNA template complex. Brock (1962) found that Mg++ overcame the inhibitory effect of novobiocin against E. coli. and not with S. aureus. It was suggested by Smith and Davis (1965b) that novobiocin has two negative charges. It binds magnesium and inhibits certain magnesium-dependent enzyme systems. Magnesium deprivation of ML-35 cells leads eventually to impair membrane integrity and RNA degradation. Brock (1956) proposed that the effects of novobiocin are the consequences of binding intra-cellular magnesium. Furthermore, the effect of novobiocin on DNA polymerization in vitro is dependent on the magnesium concentration. However, Steffensen (1953) was able to induce chromosome breakage at meiosis in Tradescantia by the use of magnesium deficient medium.
As it is not known that novobiocin does not inhibit the synthesis of nucleotide triphosphates or degrades DNA or affects the energy metabolism immediately, it would very likely inhibit the nucleic acid synthesis by its direct action on the templet of polymerase complex. Further, as it is known from the bacterial material that novobiocin damages primarily the growing cell membrane, it will lead to affect the cellular metabolites. Subsequently some other effects like an increased intracellular uridylylate pool and inhibition of RNA synthesis, cell division and DNA synthesis would also be seen. The inhibitory effect of NB produced in the synthesis of both DNA and RNA has been studied with C$^{14}$ thymine and C$^{14}$ uracil incorporation method while that of protein synthesis by C$^{14}$ leucin incorporation. The protein synthesis inhibited by NB was mediated through the action on RNA synthesis. The above findings on the action of NB on nucleic acid and protein synthesis on bacterial cells cannot be cited fully in explaining the inhibitory action of NB on cell division of bone marrow cells in mice. In the present study no doubt the application of NB caused some inhibition of cell division but it was not an immediate effect as found with the treatment of AMD. Thus some time must have been taken by the drug to enter inside the interphase nucleus. It did not have
immediate entry. The effect was noticeable, in fact, at about 12 hour after the administration. The inhibitory effect was very likely produced due to its effect on DNA and protein synthesis.

The treatment of novobiocin has revealed, besides the inhibitory effect on cell division, the chromosome breaking activity in the bone marrow cells of mice under the present study. The inhibitory action has been explained as due to its action primarily on DNA synthesis. In this respect the chemical perhaps acted upon the $S$ phase of DNA in the interphase nucleus for which newer cells could not enter division at the normal rate, till the action of the chemical was over.

The inhibitory action on DNA synthesis has also affected the protein synthesis. However, it seems that the chromosome breaking activity of NB was although dependent on DNA synthesis but did not have much effect on the protein frame work of chromosome. The basis of this supposition was that the morphology of the chromosome was not affected by the treatment of NB. Thus the breaks produced by the chemical could be interpreted for its action on DNA of the nucleoprotein blocks or on metal ions. As mentioned before it has affinity for the magnesium ion. It has been claimed that NB inhibited
certain magnesium dependent enzyme in DNA polymerization. This might be the cause of the chromosome break. It is further supported from the fact that when Mg\textsuperscript{++} are supplemented along with NB the frequency of chromosome breaks was found to be reduced in grasshopper (Manna and Bhunya, unpublished). The role of metal ion binding the nucleoprotein blocks of the chromosome structure has been discussed by various authors (Steffensen, 1961; Kihlman, 1966). Some metal chelators have also been found to break the integrity of the chromosomes.

In the present study the breaks were of chromatid type and they were present in the tissues fixed at various intervals. The presence of only chromatid type breaks would certainly lead to consider that they were produced after the duplication of the chromosomes, presumably at the G\textsubscript{2} phase. The effect of the antibiotics with regard to its chromosome breaking activity was non-delayed type according to the criterian proposed by Kihlman (1966), because, the breaks were found at 4 hour after the treatment. However, the frequency reached the maximum at 2\textsuperscript{4} hour. The chemical had effect on the cell which were in dividing condition on the one hand and the inhibitory effect on the cells which have not entered the division on the other. The treatment of NB on the spermatocyte chromosomes of grasshopper has shown some
interesting results (Bhunya and Manna, 1969; Manna and Bhunya, unpublished) which might be compared with our data. Of the two species of grasshoppers employed in *Phloeoba antennata* the antibiotic produced localised form of breaks in the X chromosome and in an autosomal bivalent. Eleven out of 14 breaks in the X chromosome were very near to the centromere while in *Spathosternum prasiniferum* no such localization was found. This has been explained by the authors as due to the differential chemosensitivity of the species concerned. Differential response to some chemical mutagen by different organisms is not very uncommon (see Kihlman, 1966). If we compare our data with those of the spermatocyte chromosome several differences could be pointed out. Both chromosome and chromatid type breaks were found in grasshopper but in mice it was only the chromatid type break. Thus the effect of NB can take place any time of the S phase of DNA synthesis in the interphase nucleus instead of what has been produced for the chromosome of mice. Localization of breaks was found in the X chromosome which was heterochromatic but such thing was not obvious in the bone marrow cells. But in the present study the breaks were non-randomly distributed in the middle and distal regions of the longer chromosomes. They might have been produced due to the presence of some weaker regions in the chromosomes rather due to some specific chemical reaction in the regions concerned.
3.

Dihydrostreptomycin (DHSM)

Chemically streptomycin is an optically active, triacidic base having the empirical formula $\text{C}_{21}\text{H}_{39}\text{N}_{7}\text{O}_{12}$. A variety of organic and inorganic salts of the antibiotics have been prepared. Streptomycin is composed of three moieties - streptidine, streptoes and N-methyl-L-glucose amines joined by glycosidic bonds. Reduction of aldehyde to an alcoholic group gives dihydrostreptomycin. It is produced by a species of microorganism named *Streptomyces humidus*. DHSM posses a biological activity comparable to streptomycin. Unlike streptomycin it is relatively stable to alkali (see Goldberg, 1959).

Mutagenic action of streptomycin in $T_2$ bacterio-phage was shown by some workers. Sager and Trubo (1962) observed it as a mutagen for non-chromosomal gene of *Chlamydomonas*. The effects of streptomycin on plant chromosomes have been reported by various workers (Wilson and Bowen, 1951; Monesi and Veronesi, 1955; Craveri and Veronesi, 1956). Recently the effect of DHSM on germinal chromosomes of grasshoppers have been studied in this laboratory (Manna, 1969; Parida and Manna, unpublished).
EXPERIMENTAL PROCEDURE

DHSM prepared by Glaxo Laboratories (India) Pvt. Ltd., was obtained from the market. Three concentrations 1%, 3% and 5% aqueous solutions, were prepared for the injection. Each of the concentrations at the dose of 0.25 ml was injected in a specimen. The specimens injected with 5% solutions died within few hours for which no cytological studies could be made for the use of this concentration. 0.25 ml of 1% solution contained 2.5 mg and that of 3% solution 7.5 mg. of DHSM. The experimental mice were sacrificed at 4, 12, 24 and 48 hour after the treatment. The aberration frequency was studied from the materials fixed at 24 hour while the inhibitory effect was studied at all intervals.

RESULTS

A) Effect on the Mitotic Index

The effect of DHSM on the dividing cells has been found to be more or less similar to that of NB. The data of the control series were taken from the previous one. They were 2.75% at 4 hour, 2.80% at 12 hour, 2.90% at 24 hour and 2.70% at 48 hour. In the treated series mitotic index in each case was determined from 2000 cells of the bone marrow fixed at 4, 12, 24 and 48 hour. At 4 hour after the treatment the frequency was
Fig. 6 - Graphical representation of the frequency of dividing cell in the bone marrow of control and 1% dihydrostreptomycin treated mice fixed at different intervals. Dotted line (C) represents control and the solid line (T) represents the treated series.
present case. Of the three antibiotics, the inhibitory action was most pronounced in AMD.

B) Effect on Chromosome:

Qualitative study: For the lack of time and also due to good amount of physiological effects in the early period, the effect of dihydrostreptomycin on the chromosomes of bone marrow cells, at present, has only been studied from the materials fixed at 24 hour after the treatment. The effect of DHSM on chromosomes could be classified into two categories but the differences were not at all rigid. There were the effect on the morphology and the break type aberrations.

In comparison to the effect on the general morphology of the chromosomes produced by the two antibiotics described before specially that of AMD, it was more pronounced in the present study. The chromosomes developed a good amount of stickiness for which they were clumped together. At 24 hour in 200 cells of 2.5 mg DHSM treated individual, 11 cells were with stickly effect (5.5%) and with 7.5 mg it was in 19 cells out of 200 cells examined (9.5%). The sticky effect if considered to be produced due to physiological action, its presence at 24 hour after the treatment of DHSM was little unusual. However, we have not put special attention to this type of physiological effect.
In the metaphase plates which did not have sticky effect, the break type aberrations could be studied better. Like two other antibiotics studied previously, DHSM caused mainly the chromatid-type breaks (Fig. 7A-H; Plate II, Pho. 7). Sometimes a confusing situation was noticed to distinguish an aberration as a break or gap when the broken fragment was lying in its original position and the unstained gap was very narrow (Fig. 7H). In the scoring of quantitative data, such confusing cases were not taken into consideration. As in novobiocin, not more than one break-type aberration in a metaphase plate was observed. Since this chemical produced some amount of stickiness, sometimes due to this effect the ends of two different chromosomes were found to be attached with one another giving an impression of a translocation. On careful observation we could not be convinced to consider them as true translocation, they were not included in the data. Not more than one chromatid break in a chromosome was observed for the treatment of this antibiotic. The chromatid breaks were found to be present at various regions of the chromosomes—proximal (Fig. 7C); middle (Fig. 7A,E,F) and distal (Fig. 7D,G). In an analysis of 27 chromatid breaks, 13 were found to be present in the distal, 10 in the middle and 2 in the proximal region of the affected chromosomes. The
breaks were not present in the chromosomes belonging to some particular groups. Out of these 27 breaks, 14 were present in the longer chromosomes (groups I and II) and none in group V. Further, no rabbit-ear chromosome was found to have any chromatid break. Out of the 14 chromatid breaks in the groups I and II, 1 was in the proximal, 4 in the middle and 9 in the distal region. It was however, not possible to determine further, if one and the same chromosome belonging to a group was affected more than others. Anyhow, the breaks were non-randomly distributed and they were located more in the distal region than in the middle or proximal region. In this respect there was not much difference in the result obtained for the treatment of AMD and novobiocin.

Quantitative study: A quantitative study on the frequency of chromatid breaks in 200 metaphase plates from each of the concentrations (Table-3) would reveal that there was very little increase in the frequency for the administration of the higher dose of DHSM.

**TABLE - 3**

Frequency of the chromatid aberrations induced by DHSM at 24 hour after the treatment.

<table>
<thead>
<tr>
<th>Quantity injected</th>
<th>No. of metaphase cells</th>
<th>Chromatid breaks</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mgs</td>
<td>200</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>7.5 mgs</td>
<td>200</td>
<td>15</td>
<td>7.5</td>
</tr>
</tbody>
</table>
At 2.5 mgs it was 6% while at 7.5 mgs it was only 7.5%. No dose-frequency relationship was therefore, observed for the treatment of AMD.

COMMENTS

The streptomycin is generally regarded to be relatively impermeable to mammalian cells. But Richardson and Halt (1962) has shown that streptomycin can penetrate into the mammalian cells as evidenced by its ability to inhibit the multiplication of bacteria growing within them. Newton (1965) in his review on antibiotic actions has grouped different antibiotics with regard to their pronounced effect on microbial organisms. He has placed penicillin, cycloserine and griseofulvin as antibiotics affecting cell-wall synthesis; cyclic peptide antibiotics and polyenes as antibiotics affecting cell membrane permeability; actinomycin A, gramicidin and valinomycin acting as uncoupling agent and inhibitors of electron transport; tetracyclines, streptomycin etc. acting as chelator and inhibitor of metallo-protein synthesis; azaserine, DON, cordicepin, hadacidin and psicofuranine as inhibitors of purine and purine-nucleotide synthesis; mytomycins, profiromycins, phleomycin, edeine as inhibitors of DNA synthesis; actinomycins and daunomycin as antibiotic
affecting transcription of genetic information; puromycin, chloramphenicol, the streptomycin group, actidione, tetracyclines etc. as antibiotics which inhibit protein synthesis. It has been found that the type of action designated above can deviate but this classification is quite helpful to know the primary effect. In the above review Newton (1965) has put the primary action of streptomycin as inhibitor of protein synthesis. Davis and Feingold (1962) have cited that streptomycin can precipitate nucleic acids in vitro. It has also been found to cause damages on cell membrane, inhibit protein synthesis and break down RNA. Controversies exist as to which one could be the primary effect. It seems very likely that the effects on respiration, oxidative phosphorylation and breakdown of RNA are secondary to the inhibition of protein synthesis and alteration of cell permeability behaviour. Again how the action on the inhibition of protein synthesis and alteration of cell permeability related, is a matter of controversy but it seems that two processes are inter-dependent. Further, it is suggested that the association of streptomycin with ribosome may interfere with the normal movement of mRNA over the ribosome surface or may link with the mRNA to the ribosome in an abnormal manner (see Newton, 1965). Kruger (1966) demonstrated that $H^3$ DHSM at a concentration
of μg/ml was rapidly taken up by the intact splenic cells and bound to the 40S ribosomal subunits. He suggested that DHSM and presumably streptomycin bind the splenic ribosomes. The mechanisms of action of streptomycin referred to above did not throw much light to interpret the result of our present study. The mechanism of actions of three antibiotics azaserin, mitomycin C and streptonigrin isolated from *Streptomyses* on the chromosomes have been discussed by Kihlman (1966). He stated that azaserin as an inhibitor of purine biosynthesis causes disturbances of amino acid biosynthesis. It may act as an alkylating agent which may be responsible for the induction of chromosome aberration. The effect is dependent on oxygen and oxidative phosphorylation. The chromosome breaking activity of mitomycin C may be due to its action on the inhibition of DNA synthesis, degradation of DNA or alkylation while streptonigrin also inhibits DNA synthesis and degrades DNA. Different types of antibiotics have been isolated from *Streptomyses* and they do not possess the similar property. Thus it is not possible to explain the chromosomal aberration in this way.

At the present moment it is not possible to suggest a definite mechanism of chromosome aberration produced by DHSM. It may be suggested that DHSM enter
the cell and act upon the chromosome structure indirectly via ribosome, as ribosome is involved in protein synthesis. As nucleo-protein blocks form the essential constituents of the chromosome, the action of DHSM may ultimately be reflected on it in causing the aberration of chromosome. It is very likely that the chromosome aberration is resulted due to the indirect action of DHSM.

The present study on the chromosome has shown three types of effect produced by the treatment of DHSM. It showed inhibitory effect on cell division. Although the inhibitory effect produced by other antibiotics has been claimed by some workers, but none claimed similar action of DHSM. From the present result it can be suspected that DHSM has effect on the nucleo-protein blocks of the chromosome structure. The effect is perhaps dependent on the state of interphase nucleus. If it reaches the nucleus prior to DNA synthesis (G₁ phase), it will probably act upon it to prevent the DNA synthesis. This would ultimately lead to affect the frequency of mitotic index. Besides the inhibitory effect, the antibiotic also produced only chromatid breaks at 24 hour. This would indicate that the antibiotic may break the chromosomes after its replication. Similar dual effects, inhibition of cell division and the chromosome aberrations have also been found with the treatment of AMD and novobiocin.
All of them could be due to some similar mode of indirect action. Anyhow, that the post synthetic period of DNA could be affected by DEHM is proved from the fact that the aberrations were only chromatid type. A third type of effect observed in the present study was stickiness in the chromosomes. It is assumed that the sticky effect is generally produced due to the depolymerization of the nucleo-proteins of the chromosome. It is considered as a physiological effect. The stickiness has been found to be produced by certain other agents. Darlington (1942) views that the stickiness is produced due to the break down of chromosomal nucleic acid to a depolymerised and fluid condition. The temporary liquification of chromatin in the production of stickiness has also been suggested by Selman (1952). However, Himes (1950) shares a different view. Anderson et al (1960) suggests that stickiness is caused by loose strands of DNA which are cast off from the main bundle of DNA. Stickiness can be produced due to the action on chromosomal proteins. Biesele (1958) considered that sometimes stickiness causes the chromosome to break at different points.

Streptomycin induced chromosome aberrations have been reported in root tips of Tradescantia. The chromatid breaks produced by this chemical is most probably due
to its accumulation at some specific region of chromosome. This assumption has been made from the fact that the breaks were localized at some particular regions of the affected chromosome. In the present study the breaks were also non-randomly distributed. But they could be explained on the same ground of the specific absorption effect of the chemical in the region concerned because no such relationship could be established. The non-random occurrence of breaks in the present material have been suspected as due to the presence of some weaker regions in the chromosomes.

The effect of streptomycin on the spermatocyte chromosomes of grasshoppers has been studied by Manna and his collaborators (see Manna, 1969). The effect of 5 mg of DHSM was found in the form of stickiness of chromosomes in all the dividing stages and gaps and breaks in the diplotene bivalents to anaphase I. Breaks were both chromosome and chromatid type. There it was found that out of 53.8% cells with some kind of aberrations 42.2% were due to stickiness. Thus, we find that responses of the chromosome of the bone marrow cells under the present study and those of the meiotic cells of grasshopper qualitatively and quantitatively differ.
Metaphase Chromosome Aberration in Bone Marrow Cells of Mice Induced by 2.5 mg and 7.5 mg of dihydrostreptomycin. Only Affected chromosomes Drawn.

(A) One chromatid of a group II chromosome broken in the middle with the non-displaced fragment.
(B) Distal chromatid break in a group III chromosome.
(C) A proximal chromatid break in a group I chromosome.
(D) A distal chromatid break in a group II chromosome with the non-displaced fragment.
(E) A distal chromatid break in a group III chromosome with fragment lying widely apart.
(F) A chromatid break in the middle of a group II chromosome with the fragment lying in a displaced condition.
(G) A distal chromatid break in a group I chromosome.
(H) A chromatid break nearabout the middle of a group III chromosome.
GENERAL COMMENTS ON THE ACTION OF ANTIBIOTICS

The mutagenic action of different antibiotics on chromosomes of animals is very important for obvious reasons. However, so far the effects of very few antibiotics on chromosomes have studied. The classification of antibiotic made by Newton (1965) on the basis of their primary action would not fully satisfy the effects obtained with regard to the chromosomes. However, we have, like most other authors tried to explain the mechanism on the same basis as experimented on bacteria or other microorganisms. The classification made by Newton is quite useful to cytologists because it will help at the initial stage to advocate some explanation on the mechanisms of chromosome aberrations induced by antibiotics. In this respect the groupings adopted by Goldberg (1959) with regard to their various uses are less attractive. Wilson (1960) made an attempt to group the antibiotics very broadly on the basis of their mechanisms of action as (1) those which do not penetrate the cell but exert their influences by changing the environment of the cells and (2) those which enter the cell and disrupt or modify the metabolic action through some kind of interference of enzymatic activity. Anyhow, all the antibiotics under present study belong to the second category. Biesele (1958) grouped the antibiotics into three types on the basis
of their cytological effect (i) prophase poison-
terramycin, actinomycin etc. (ii) chromosome poison-
streptomycin and actinomycin etc. and (iii) and spindle
poisons- penicillin. None of the classifications can
be considered too rigidly because the same antibiotic
may possess various properties and can produce varieties
of actions. In the present study actinomycin D,
novobiocin and dihydrostreptomytin produced similar nature
of effects on the dividing cells and on the chromosomes.
But Newton (1965) according to the primary action has put
actinomycin D as an antibiotic inhibiting DNA dependent
RNA synthesis while streptomycin into the group of
antibiotics which inhibit protein synthesis. When more
data on the antibiotic induced chromosome aberrations will
be available, we will be in a better position to judge the
mechanism of chromosomal aberrations in a definite manner
than what has so far been attempted by different workers
(see Kihlman, 1966) and the present author.

In our laboratory Manna in collaboration with
Bhunya (unpublished) studied the effects of oleomomycin,
neomycin, novobiocin, actinomycin D and griseofulvin and
in collaboration with Parida (unpublished) studied the
effects of chloramphenicol, aureomycin, terramycin,
streptomycin and penicillin on the spermatocyte chromosomes
of grasshopper. They have studied the effects both
qualitatively and quantitatively. Three of these antibiotics actinomycin D, novobiocin and dihydrostreptomycin have been treated by the present author on the chromosomes of bone marrow cells of mice. The results in two different kinds of animals were not the same. In the grasshoppers all these antibiotics produced chromosome and chromatid type breaks and a good deal of stickiness, besides some other type of aberrations while in the mammalian cells they produced only the chromatid type of breaks. Thus much of the actions of the antibiotics seem to depend on the physiological condition of the experimental animals. The problem of chemical mutagenesis is difficult to solve because in the living state many factors are interlinked. Anyhow, inspite of the diversities in the effects, the three antibiotics under present investigation did produce some common types of effect on chromosomes and division frequency. Since they produced effect of similar nature it may broadly be considered that their effect was mediated indirectly in the same way possibly through the cellular metabolism.

The problem of inhibition of cell division can be tackled in different parts and the part with which the present study is concerned is the prevention of the cell from entering mitosis. It is generally assumed that the agents which prevent cells from entering division will
also inhibit division of cell, nucleus and chromosomes. In the present study these problems can not be treated as separate entities because the antibiotics also produced chromatid breaks. Thus it seems that they had two different modes of action. One might be independent of the other, namely the inhibitory action was perhaps related to the G, or S phase of DNA synthesis in the interphase while the chromosome breaking activity was related to the G₂ stage. This type of action has so far been emphasised by the present author. But alternatively it might be assumed that the antibiotics acted as what may be termed as 'prophase poisons' by D'Amato (1952). In this case all the actions were supposed to have taken place at G₂ phase. In such case the antibiotic concerned inhibited generally the cell to enter mitosis and some of them which followed the course had only chromatid type breaks. All the antibiotics under present investigation were indirectly connected with the DNA metabolism. Kihlman (1966) has cited the effect of azaserine as an inhibitor of biosynthetic reaction for cell division. Similar data on the antibiotic under present study are lacking but some of the probable mechanisms have been presented before.

The three antibiotics produced almost invariably the chromatid type breaks and they were non-randomly distributed. Kihlman (1966) remarked that the breaks
induced by chemical mutagens are generally non-randomly
distributed. He also cited some specific cases of
localized breaks induced by maleic hydrazide, BUdR
hydroxylamine, ethoxycaffeine etc. from his own as well
from other data. It has already been pointed out that
no such explanation could be advocated in support of the
occurrence of non-random breaks by the antibiotics on the
bone marrow chromosomes of mice. The present author
considers that there may be some weaker regions in the
chromosomes because these regions are found to break
by odd types of agents. Manna (1969) also made such a
suspicion in explaining the localised breaks found in the
grasshopper inflicted due to the treatment of X-rays and
some chemical mutagens. However, this is just a surmise.
It needs to be verified with critical evaluation.
B. PHENOLS

4.

2,4- Dinitrophenol (DNP)

This is yellowish crystal with very little solubility in water (0.6 gm/100 ml water at 25°C). It is acidic due to the electron attracting substituents like NO₂.

The effects of DNP have been studied on the mitotic cell division (Krahl, 1950; Nethery and Wilson, 1966) and also on the mitotic chromosomes in plants (Kihlman, 1966). The effect has been tested on the cleavage division of the Echinoderm eggs. The chromosome breaking activity has been studied specially on the root tip cell of Vicia faba. Manna and Parada (unpublished) have studied the effect of DNP on the spermatocyte chromosomes of grasshoppers. This chemical has been employed in the cytological investigation for various reasons (see Kihlman, 1966). Inhibition of P³² release from human red cells is effected by DNP (Omachi, Scott and Glader, 1968).

EXPERIMENTAL PROCEDURE

In the present study different doses 0.25 ml, 0.50 ml and 1 ml of the saturated solution of DNP were
Fig. 8 - Graphical representation of the frequency of dividing cells in the bone marrow of the control and 0.50 ml DNP injected mice fixed at 4, 24 and 48 hour. Dotted line (C) as control and solid line (T) as treated series.
(Fig. A-M), few translocations (Figs 9N, P), ring chromosome (Fig. 90), etc. The physiological effects in the form of stickiness, despiralization, etc. were not very commonly observed. Anyhow, the effects of DNP appeared to be more severe than those of antibiotics described earlier. The breaks were invariably in one of the two chromatids of a chromosome except on a very rare occasion both the chromatids were found to be broken but they were not exactly in the identical position (Fig. 9K). Unlike the cases of antibiotics where not more than one break in a chromatid was found, in the present study chromatid with more than one break was quite common. In an analysis of 60 metaphase complements having chromatid type breaks, 9 were found to have more than one chromosome with chromatid type breaks. Further, an analysis was made to locate the region of break since the chromatids with single break were found to occur at different regions. For this kind of analysis an examination of 50 chromatids with single break were taken randomly in which 23 were found at the distal part, 23 in the middle and 4 at the proximal region. Another type of analysis was also made. In this case the breaking points of the affected chromosomes were considered by dividing the length of chromosomes into 4 parts. In such cases they would be found to occur in maximum quantity in the third quarter beginning from the centromeric end.
If the distribution of breaks in different groups of the chromosomes is considered, out of these 50 breaks 26 were found in the larger groups. Thus there was some specificity of action in the longer group than the remaining ones. Further, these 26 breaking points were located near about the mid-region of the chromatid of the larger groups. Thus the above analysis would reveal some obvious facts. They are: (1) breaks were non-random, (2) chromosomes of groups I and II were most susceptible, (3) middle and the distal regions were almost equally vulnerable to break. However, since there were more than one break in a chromatid, those cases could not be included to determine the regions of the breaks in them.

Unlike the effects of the antibiotics reported earlier, the action of 2,4-dinitrophenol produced in a number of metaphase plates more than one broken chromosome (Fig. E-H,J,L,N-P; Plate II, Pho. 8,10). Over and above in a good number of cases a single chromatid was broken at more than one point (Fig. 9F, G, M,N). The maximum number of breaks found in a chromatid was 3 (Fig. 9F) but the frequency of chromatid with two breaks was much higher than that with three. It is very interesting to note that whenever more than one break was observed in a chromosome, they were almost invariably in the same chromatid. It was rather extremely rare to have their distribution in the
two chromatids (Fig. 9K). The broken fragment of the chromatid was most often seen to be situated near about its place of origin (Fig. 9, A,B,H) but in some instances they were seen to be lying in a completely detached condition from the parent body (Fig. 9D, J; Plate II, Pho. 9,10,11). Sometimes even the fragment was untraceable and the broken chromosome had two very unequal chromatids (Fig. 9E).

Besides the simple break type aberrations, in one or two percent cases translocation-type configurations were observed (Fig. 9N, P; Plate II, Pho. 9; Plate V, Pho. 25). In such cases the ends of the two chromatids of two non-homologous chromosomes were held together while the others were remaining free. Very likely they were produced by the fusion of the broken distal ends of the two chromosomes involved. The fragment produced by the translocation could not be demarcated from the normal chromosomes of smaller size. Sometimes this type of translocation seemed to have been formed due the fusion of terminal ends of two chromosomes when no fragment was found. Sometimes the ends were also seen to be terminally associated. The latter cases were not considered as translocations. After considering all the possibilities, we were convinced that there were some instances of real
translocation between two chromosomes and they were not just terminal association of two ends of two chromosomes. Besides the translocation type of aberration, the treatment of DNP also produced some ring chromosome (Fig. 9,0), the frequency of which was very low.

Quantitative :– Differences in doses did not show any demarcable qualitative changes but the quantitative study revealed some differences (Table 4).

<table>
<thead>
<tr>
<th>Dose in ml.</th>
<th>No. of Metaphases.</th>
<th>No. of Metaphases with Aberration.</th>
<th>No. of Chromatid break.</th>
<th>% of Aberration.</th>
<th>In affected cell br./chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>290</td>
<td>20</td>
<td>20</td>
<td>6.9</td>
<td>0.025</td>
</tr>
<tr>
<td>0.50</td>
<td>230</td>
<td>34</td>
<td>45</td>
<td>14.5</td>
<td>0.033</td>
</tr>
<tr>
<td>1.0</td>
<td>250</td>
<td>42</td>
<td>57</td>
<td>17.2</td>
<td>0.034</td>
</tr>
</tbody>
</table>

An analysis of the data (Table 4) would reveal that the frequency of metaphase chromosome aberration increased with the use of higher doses of the solution but the increase was not proportional. From the use of 3 doses we have not been able to find out the maximum.
limit of increase because the frequencies were on the increase with the higher doses e.g. 6.9% at 0.25, 14.5% at 0.50 and 17.2% at 1 ml of DNP solution. With the use of 0.50 ml of the chemical the frequency was about two times more than that of 0.25 ml. Thus the increase was not proportional with the dose as it was very disproportionate with regard to 0.50 ml and 1.0 ml doses. The increase was 2.7% with the use of 1.0 ml solution in comparison to 0.50 ml data. In this respect the increase for the injection of 0.50 ml was 7.6% from the value of 0.25 ml injection. This would clearly indicate that there was no linear relationship between the frequency of metaphases chromosome aberrations and the doses. However, one may think that the application of higher doses produced more breaks in the affected cells. In this case the disproportionate increase in the frequency of abnormal cells at different doses might be expected. For this reason the frequency of break per chromosome at different doses was calculated. It was found to be 0.025 in 0.25 ml, 0.033 in 0.50 ml and 0.034 in 1.0 ml dose. This type of analysis also indicated the same conclusion that the increase was disproportionate to the doses. The increase of breaks was quite appreciable at the dose of 0.50 ml in comparison to 0.25 data while it was negligible between 0.50 and 1.0 ml.
EXPLANATION OF FIGURE

( 9 A - P )

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Saturated Solution of 2,4-Dinitrophenol. Only affected chromosomes Drawn).

(A) A chromatid break in the proximal region of group I chromosome.
(B) A chromatid break in the proximal region of group III chromosome.
(C) A chromatid with a break and a gap in a group II chromosome.
(D) A chromatid break near about the proximal region of a group III chromosome with the fragment lying quite apart.
(E) Two broken chromosomes, each with a chromatid break and the fragment in one not traceable present in the same metaphase complement.
(F) A metaphase with 3 broken chromosomes. Each of them with one of the two chromatids broken. One chromosome with 1, the second with 3 and the third with 2 chromatid breaks.
(G) A metaphase with two broken chromosomes, one with 1 and the second with 2 breaks in one of the chromatids.
(H) A metaphase with 2 broken chromosomes, each with one chromatid break near about the middle region.
(I) A group I chromosome with 2 breaks in one chromatid.
(J) A group II chromosome with a chromatid break at the middle and a group III chromosome with a distal chromatid break.
(K) A group I chromosome with the 2 chromatids broken in non-identical regions, one in the middle and the other at the distal region.
(L) A metaphase with 2 broken chromosomes each with a single chromatid break.
(M) A group II chromosome with 2 breaks in a chromatid.
(N) A metaphase with a translocation and a chromosome with a chromatid broken at 2 places.
(O) A metaphase with 2 ring chromosomes.
(P) A metaphase with a translocation and a chromosome with a chromatid break.
COMMENTS

It is more or less an accepted fact that DNA synthesis and oxidative phosphorylation are required for cell division. Inhibition of any one or both the processes would be reflected in the inhibition of mitosis. Thus the strong DNA synthesis inhibiting chemicals like FUdR, AdR, cytosine arabinoside and aminopterin are potent mitotic inhibitors. On the other hand the respirators like CO, cyanide, and uncoupling agent DNP also inhibit mitosis.

The present author has studied broadly the effect of DNP on mitotic index because the materials were fixed at 4, 24, and 48 hour after the treatment. As the time at disposal of the author was limited a detail study was not possible. Moreover, from the experience gathered with the effects of the antibiotics the above three fixation times were selected to get an over-all idea. The result clearly indicated the inhibitory action of DNP on cell division in bone marrow as compared to the untreated series. In the present study the inhibitory action was found already in the material fixed at 4 hour after the treatment and reached the maximum at 24 hour. The frequency came back to alcy at 48 hour (Fig. 8), Since the data were obtained from three points no other critical consideration could be made. It has been mentioned before that the effect of DNP on the mitotic index was more or less the same when compared with the effect
of AMD. As to the mechanism it has also been said that AMD primarily inhibits DNA dependent RNA synthesis which is connected with the genetic information transfer. Other actions have also been discussed. Thus the mode of inhibitory action was, very likely, not the direct one. It has been argued previously that the inhibitory effect produced by the antibiotics was mostly due to their suppressing action on RNA and protein synthesis which was indirectly connected with DNA metabolism. The inhibitory action shown by the injection of DNP, on the other hand, needs to be considered from a different standpoint. This chemical has specifically an indirect action on DNA synthesis because energy requirement is very important during DNA synthesis in the interphase nucleus. The action of DNP is mainly related with the cellular metabolism affecting the cellular structure. It has been mentioned in the beginning that oxidative phosphorylation and DNA synthesis are important factors for cell division and, therefore, inhibition or impairment of them would naturally affect mitosis. Since DNA synthesis inhibiting chemicals and other chemicals which disturb the oxidative phosphorylation or cellular respiration are known to act as mitotic inhibitors, it may logically be argued that DNA synthesis is dependent on energy liberating processes. In sea urchin eggs, the rate of mitosis has been found to be closely related to the level of ATP maintained by oxidative phosphorylation. The process of mitosis no doubt requires energy. Thus, agents which are supposed to inhibit glycolysis, respiration and oxidative phosphorylation would also
inhibit mitosis. The inhibitory effect obtained by the use of DNP in the present study was produced by its action on the respiration coupled phosphorylation. It would have indirect action on DNA synthesis. Kihlman (1966) listed a number of phenols like 4,6-nitro-2 phenol; 2,4-6triiodophenol; 4,6-dinitro-0-cresol; 2,4-dinitro-napthol and DNP which are highly effective in blocking oxidative phosphorylation inhibiting the cleavage division of sea urchin egg. The decrease in the inhibitory action after 48 hour of the treatment, observed in the present study, would indicate that with the dilution of chemical agent in course of time, the impairment of oxidative phosphorylation was removed gradually for which normal frequency of division was regained at 48 hour. Shacter (1955) reported that the increasing concentration of DNP progressively inhibited the respiration in Ehrlich tumor cells. He was able to show that the mitotic activity was independently related to the oxygen consumption by the cell as well as the availability of high energy phosphates. DNP is known to be an uncoupling agent. It inhibits oxidative phosphorylation. It is known that two oxidative enzyme systems are considered to provide energy for cell division. They were cytochrome oxidase system and the oxidative phosphorylation. According to Krahl (1950) both the systems could be affected by the treatment of DNP. The effects of DNP on cells have been found to be associated mainly with cellular metabolism including division, cytosis, etc. The respiration coupled phosphorylation is important for the cell. By this process the cell can generate high energy phosphate bond.
DNP because of its chemical nature would likely to affect this normal physiological process which is manifested in the form of inhibitory action of cell division. The action of DNP on cell has been found to be pH dependent. The effect is stronger at lower pH than that of higher pH. It has been shown by Stenlid (1949) that in the wheat roots the absorption rate of DNP was faster at pH 4.5 than at pH 7.0. That DNP disturbs the oxidative phosphorylation has been proved from the following fact. The use of this chemical decreases the ATP level like other chemical, CO₂, azide (Epel, 1963). Epel (1963) has also shown that the rate of mitosis was related to the resultant ATP level. It has been reported that DNP inhibits the formation of energy rich phosphate bond and it prevents the accumulation of polysaccharides and RNA in nucleus. From the above discussion it is clear that the inhibitory action produced by the application of DNP is primarily due to its effect on respiration coupled phosphorylation and this process in turn is associated with the DNA synthesis.

In the present study the second type of effect for the treatment of DNP in bone marrow cells of mice was the break type aberration. The aberrations in most cases were chromatid type breaks and very few cases of translocation. The infrequent occurrence of the translocation could be explained as due to the (a) rarity of breaks in different chromosomes in a cell, (b) broken ends when more than one might not be close enough, (c) the time of occurrence might not be synchronous, etc. These factors are also applicable as explanation for the absence of
translocation in connection with all other chemicals under the present study.

The application of DNP producing good number of breaks in more than one place in the same chromatid of the chromosome would rather draw some special attention. In order to have the chromatid type break it was obvious that the chemical very likely acted after the reproduction of the chromosomes, otherwise we would expect to have some chromosome type breaks. Even then it would not solve the question as to the absence of some isochromatid breaks. Along with this the fact that more breaks were produced in certain particular regions of the chromosomes as well as in some particular groups of chromosomes would need to be answered. As far as we are aware, no specific action of DNP with any component part of DNA has been established which could be considered in the same way as it was done with the case of localized breaks observed in the chromosomes of Chinese hamster for the treatment of BUdR (Hsu and Somers, 1961; Somers and Hsu, 1962). On the other hand, it seems very likely that the chromosomal aberrations produced by DNP was due to its antimetabolite action. The chromosome breaking activity of some respiratory inhibitors is to some extent known. This would mean either the involvement of oxidative phosphorylation or the chemical in question underwent oxidative degradation to become active in causing the aberration. Therefore, the breaks obtained by the application DNP were due to its action as antimetabolite on the
structure of the chromosome after its reproduction. The action is no doubt an indirect one but how it could be so much localized as observed in the present study. This form of action would not explain the somewhat region-wise localized form of breaks in the chromosome. In such case we may suggest that this was due to the presence of some weaker places in the chromosomes which are likely to be affected more easily with the disturbance caused by various chemical agents. With this supposition, it would be expected to have atleast some isochromatid breaks because weaker spots in the identical region of the unaffected chromatid should also be present. Even some breaks in the non-identical positions of the two chromatids of the affected chromosomes are also expected. Except in one instance it was not found. We have really no plausible explanation for the specific type of action in only one of the two chromatids of the affected chromosome. It could be due to some chance factor which we have not carefully analysed at present.

Although the mechanism by which DNP could induce chromosomal aberration has not been specifically dealt with by other workers but this chemical has largely been used as a chemical influencing the production of chromosomal aberration (see Kihlman, 1966) or inhibiting cell division at various stages (Nethery, and Wilson, 1966). Since the action of DNP is associated with oxidative phosphorylation it has been used in conjunction with other chemicals to study the effect of oxygen
in the production of chromosome aberration. The treatment of DNP has been found not to influence the frequency of chromosomal aberration when tested with 1-Methyl-3-nitro-1-nitrosoguanidine and N-Methyl phenyl-nitrosoamine but a different kind of effect was found with FUdR, AdR (see Kihlman, 1966). The treatment of DNP in irradiated tissue has been found to reduce the frequency of chromosomal aberration (Wolff and Luippold, 1955). Thus results of the additive action of DNP with some chemicals or physical agents would strengthen the view that the chromosome breaking effects observed in the present material was due to its indirect action as an antimetabolite in the cell. Manna and Parida (unpublished) studied the effect of DNP on the spermatocyte chromosomes of grasshoppers. The dose used by them was rather high (5 mg/individual) for which there was too much sticky effect and the chromosome structure sometimes degraded to appear just as some pycnotic blocks without regular outline. This prevented the above authors to determine accurately the damages in the individual chromosome. Insipite of the extreme clumping and sticky effect some instances of chromatid and chromosome breaks were observed by them. However, because of the excessive physiological effects found in the grasshopper testis our present data could not be compared with those of the spermatocyte chromosome. On the other hand the effect of DNP on the bone marrow chromosome was found to be more than that of the antibiotics used in the present study. But a real comparison is not possible.
since the doses were not the same. If only the qualitative expression were compared, some very striking differences would be found among some common type of aberration. In both the types of chemicals the aberrations were chromatid type but all the antibiotics produced not more than one break in a chromatid while DNP produced breaks in more than one place in the chromatids. This would certainly indicate that DNP was more active than antibiotics in the production of chromosome aberration. However, the occurrence of multiple breaks in the same chromatid would further support this view.
5.
P-Aminophenol

The chemical is a crystalline solid dark brown in colour. It has a low solubility in water. It is a weak acid possibly because of the amino group - the electron releasing substituent. Not much work on the effect of p-aminophenol on chromosome has been carried out so far. Levan and Tjio (1948a,b) were pioneer to report the chromosome breaking activity of p-aminophenol along with the number of phenols and other related compounds. Besides the report of Levan and Tjio (1948a,b), no other work on its effect either in plant or in animal chromosome has been carried out. In this respect the present study may be taken as a first attempt to study its effects on animal chromosome.

**EXPERIMENTAL PROCEDURE**

0.25 ml and 0.50 ml. doses of freshly prepared 0.1M and 0.5 ml. of 0.02M aqueous solution of p-aminophenol were injected separately into the mice each weighing about 23 gm. The specimens were sacrificed at 24 hour after the treatment for fixing the bone marrow cells.
RESULTS

Effect on Chromosomes

Qualitative - The two concentrations did not produce any difference in qualitative effect. There was not much physiological effect in the chromosomes except some stickiness. The effects produced by the treatment of the chemical was the chromatid type aberrations (Fig. 10A-H; Plate III, Pho-13). Besides the chromatid type breaks some instances of gaps and constrictions were encountered. In an analysis of 64 metaphase complements with chromatid breaks only one plate contained two breaks (Fig. 10F) and two plates contained three breaks (Fig. 10H) in different chromosomes while the remaining ones had only one break in each complement (Fig. 10A-E). In general not more than one break in a chromatid of affected chromosome was observed. In the present analysis out of 64 metaphase complements only in one instance one chromatid of a chromosome had two breaks (Fig. 10G). The positions of the breaking point were found to be variable and they were non-randomly distributed as found in the previous cases. In order to have an idea how breaking points were distributed, an analysis was made. It was found that in 64 single breaks 29 were at the distal region (Fig. 10C,H), 27 at the middle (Fig. 10A,D) and 8 were in the proximal region (Fig. 10B). Thus the breaks were more frequent from the middle to the terminal
Further, analysis was made to find out if the breaks were frequent in some particular group of chromosomes. In this case 17 were found in the larger group. This fact indicated that the larger chromosomes were more susceptible to the effect of the chemical. The breaking parts of the chromatids in good many cases were lying in its normal position (Fig. 10A, C, F). There were also instances when they were driven further apart from the parent body and were situated in various places (Fig. 10B, D, E, H). Very rarely some exchange type aberrations were encountered (Plate III, Pho. 13). Since the author was not very much convinced about its reality, it has not been accounted.

Quantitative - An analysis of the data of chromatid aberrations for the use of two concentrations (Table 5) would reveal that

<table>
<thead>
<tr>
<th>Strength</th>
<th>Quantity of sol. injected per individual</th>
<th>No. of metaphases</th>
<th>No. of Chromat. break</th>
<th>Percentage of chromatid break</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01M</td>
<td>0.25 ml</td>
<td>200</td>
<td>7</td>
<td>3.50</td>
</tr>
<tr>
<td>0.02M</td>
<td>0.50 ml</td>
<td>450</td>
<td>31</td>
<td>7.11</td>
</tr>
<tr>
<td>0.02M</td>
<td>0.50 ml</td>
<td>320</td>
<td>28</td>
<td>8.75</td>
</tr>
</tbody>
</table>

the increase in the frequency in the higher concentration was
very negligible. However, injection of 0.25 ml and 0.5 ml. of 0.01M P-aminophenol solution produced different frequency of breaks. It was 3.50% in the former and 7.11% in the latter. The injection of 0.5 ml. of 0.02M solution produced slightly higher frequency of breaks and it was 8.75%. But it was not at all proportional in comparison to 0.01M solution.

**COMMENTS**

According to Levan and Tjio (1948a,b) the chromosome aberrations produced by p-aminophenol in the plant material was due to the oxidizable nature of the compound. So far as we are aware, none of the phenols has been claimed to have direct action on DNA of the chromosome. It is not possible to comment definitely whether the breaks were induced due to the indirect action on DNA or on nucleoprotein of the chromosome. Since the action of the chemical was an indirect one, the effects were produced due to its action on nucleoprotein blocks of the chromosome. Since phenol has got an oxidative action, the chromosome breaking effect can be suspected as due to the formation of peroxide. DNP induced breaks could also be interpreted in the same way. It is also known that hydrogen peroxide can produce cellular lesion which may be manifested as a sort of radiomimetic action (Lea, 1962; Dustin and Gompel, 1949). Since the phenols and its related compounds are readily oxidizable, the chromosome breaking activity could more readily be seen after the treatment. Unfortunately the effect of the chemical in the early hour of fixation has not been studied at present.
The result obtained here if compared with that of DNP, some similarities could be drawn. These were in the nature of the breaks, non-randomness in their distribution, etc. Both the chemicals almost always produced chromatid type aberration. However, in DNP treated series the effect was more pronounced with different varieties while in p-aminophenol it was stereo-type. There was mainly the single chromatid type break in a chromosome of the whole complement. However, no difference was found with regard to the nature of the non-random distribution of the breaks. Thus the explanations given for the DNP treatment about the occurrence of (1) chromatid type breaks (2) non-random distribution of breaks could also be applicable for the present case. The occurrence of only chromatid type breaks would suggest that the chemical was effective after the reduplication of the chromosomes. The non-random distribution would suggest that the middle and the distal regions of the chromatids of chromosomes, specially of groups I and II, possess some weaker spots which are more fragile than other places. The localization was not due to any specific chemical reaction in the region concerned since these regions were found to break more frequently with the treatment of antibiotics and DNP.
FIG. 10

EXPLANATION OF FIGURE
(10 A - H)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by p-aminophenol solution. Only affected chromosomes Drawn).

(A) A chromatid break in the middle of a group III chromosome.
(B) A chromatid break with the fragment displaced in a group III chromosome.
(C) A chromatid break at the distal region of a group II chromosome.
(D) A chromatid break at the middle with the fragment highly displaced in a group I chromosome.
(E) A chromatid break at the distal region with the fragment highly displaced in a group III chromosome.
(F) A metaphase with 2 chromatid breaks in two chromosomes
(G) A chromatid broken at 2 places of a group III chromosome.
(H) A metaphase plate with 3 chromatid breaks in 3 regions of 3 chromosomes.
6.

Pyrogallol

It is colourless solid soluble in water, ethenol and ether. The aqueous solution of pyrogallol oxidises very rapidly on exposure to air. Pyrogallol is oxidised to a complex mixture containing among other things—carbon monoxide, carbon dioxide, acetic acid, oxalic acid, etc.

Some works on the effects of pyrogallol have been carried out by various workers on the root tip chromosomes of Allium and Vicia (Levan and Tjio, 1948a,b; Sharma and Sharma, 1960; Therman-Soumalainen, 1949). No study has been reported as to the effect of this chemical on animal chromosomes. Besides the present study, the author also observed chromosome aberration produced by this chemical in the spermatocyte cells of grasshopper; (Mitra, unpublished).

EXPERIMENTAL PROCEDURE

0.25 ml of freshly prepared 0.01, 0.02 and 0.03 M aqueous solution of pyrogallol were injected into different mice and the specimens were sacrificed at 24 hour after the treatment for the study of their effects on the bone marrow chromosome.
RESULTS

Effect on the Chromosome

Qualitative - The effect of this chemical has been found to be more varied in nature than that of the other phenolic derivative studied. This chemical produced a good deal of sticky effect along with despiralised and eroded outlines of chromosomes (Plate II, Pho. 12). There were also break type aberrations. The sticky effect was not present in every dividing cell. In the affected ones it generally prevented to study the chromosome morphology in greater detail. In many cases it led to the clumpir of chromosomes. Among the affected metaphase complements the aberration in the individual chromosome structure could be followed in few cases. As regards true break type aberration, one of the chromatids of a chromosome was mostly broken (Fig. 11A-G). Besides the chromatid breaks, extra fragment, clumped chromosomes and other type of aberrations like translocation and ring chromosome were rarely found. The exchange type aberrations were less convincing because of the general sticky effect (Plate III, Pho. 14).

In an analysis of 17 chromatid breaks, 11 were in the terminal region (Fig. 11A, B), 4 in the middle (Fig. 11 D, F, H) and 2 in the proximal region (Fig. 11C, G). Quite often along with break type aberration in some chromosome, clumping effects in other chromosomes were observed. Metaphase complements containing chromosome with chromatid breaks and several extra fragments were also observed (Fig. 11H). Metaphase complement with more than one chromatid break was also found (Fig. 11G) but the
frequency was negligible. The frequency of breaks seemed to be not very high in the longer groups of chromosomes. On the whole the effect of the chemical could not be systematically studied because of the marked sticky and clumping effects on the chromosomes.

Quantitative - The frequency of chromosome aberrations obtained for the use of different concentrations (Table 6) had some limitations. The data represent only a part of the affected cells. Only the metaphase complements containing the chromatid aberrations without other effect have been analysed. Thus the frequency data obtained here would represent only part of the total effect.

<table>
<thead>
<tr>
<th>Strength</th>
<th>Dose in ml</th>
<th>No. of metaphases</th>
<th>No. of Chromat. breaks</th>
<th>Percentage of Chromat. breaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01M</td>
<td>0.25</td>
<td>150</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.02M</td>
<td>0.25</td>
<td>150</td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td>0.03M</td>
<td>0.25</td>
<td>150</td>
<td>10</td>
<td>6.6</td>
</tr>
</tbody>
</table>

An analysis of 150 metaphase plates from each concentration reveals that 4% metaphases were with sticky chromosomes for the use of 0.01M, 6.7% for 0.02M and 9.3% for 0.03M solution.
No true chromatid break was obtained in 150 metaphase plates in individuals treated with 0.01M solution. The stickiness, however prevented the correct determination but the frequency would be very low. The application of 0.02M solution induced 4.7% chromatid breaks. Some other complements also contained them but the correct determination was not possible because of the stickiness. The frequency was found to be still higher when 0.03M solution was used. At this concentration the frequency was 6.6%. The present data, would, therefore, indicate that very low concentration like 0.01M of the chemical was unable to induce breaks to a great extent but it caused good amount of physiological effects.

**COMMENTS**

In the present study two kinds of effects have been observed for the treatment of pyrogallol solution of different strength. They were stickiness and clumping of the chromosomes and chromatid type breaks. The stickiness observed in the present study was not of the same magnitude as observed by Sharma and Bhattacharya (1956b) in the plant material. In order to explain the sticky effect produced by the treatment of pyrogallol solution on plant chromosomes Sharma and Bhattacharya (1956b) opined that it was due to the depolymerization of the nucleoprotein of the chromosome structure. The present author
also supports their view. The difference in the degree of the effects was, however, due to the difference in the materials as well as the concentrations used by the different workers. The action of the pyrogallol solution would be more direct in the plant material since the roots were immersed into the solution. On the other hand, in the present study the chemical must have reached the cells through the metabolic pathways and did not have a direct access to come in contact with the bone marrow cells. This difference would naturally bring some difference in the concentrations of the chemical in reaching the cells.

Pyrogallol is readily oxidizable and this property has been assumed to have played a role in inducing the chromosome breakages. This chemical is indirectly connected with the oxidation-reduction process of the cell. Levan and Tjio (1948a,b) have suggested that the oxidation effect played the major role due to addition of oxygen or removal of hydrogen from the cell for the treatment of pyrogallol. The X-ray induced chromosome aberrations are also considered to be produced due to the oxidation-reduction process (Lea, 1962). The oxidative products of pyrogallol includes CO, CO₂, acetic acid, oxalic acid, etc. with other things. Of these CO and CO₂ are known to have inhibitory effect on cell respiration which would affect the cellular metabolism. On the other hand, acetic acid has also been shown to produce chromosome aberration in the spermatocyte cells of grasshoppers by altering the ionic activity of the cells.
(Manna and Mukherjee, 1966). Whatever way the author treats the action of pyrogallol, he finds that it is indirectly causing the metabolic disorder in the cell which very likely reflected in the production of chromosomal aberration. It has not been shown that this chemical can directly affect some chromosomal constituent like DNA. Levan and Tjio (1948a,b) were first to report the chromosomal breaking activity of pyrogallol in the root tip cells of Allium cepa. They obtained chromosome fragmentation by the use of this chemical. They considered that the breaks were due to the formation of pseudochiasmata. In the present study this type of mechanism could not be ascertained but the possibilities can not be denied. Therman-Soumalainen (1949) reported that the breaks in the chromosomes in certain plants were found more in the region of the secondary constriction with the treatment of pyrogalol. However, this has been contradicted by Tjio (1951) because he failed to observe such localized breaks in the chromosomes of V. faba. The chromosome complement of mice contains some rabbit-ear chromosomes which possess clear constricted regions. In our present study not a single break was found to have been produced in this particular region in 450 metaphases examined. Therefore, the present result is also not in agreement with the claim made by Therman-Soumalainen (1949) that breaks are localized in the constricted region. On the other hand it is also not fully agreeable with that of Tjio (1951). Although no very specific breakage points could be obtained in the present study but they were located more commonly in the middle and in the
distal part of the affected chromosomes. It was not possible to say definitely if the breaks were related with the intercalary heterochromatic part of the chromosomes. However, no heteropycnotic behaviour was observed in these regions in normal individuals. Since the above mentioned regions also broke frequently with non-related chemicals, it has been assumed that these were perhaps the weak points in the chromosomes. Further, as found with other phenols under present study the breaks were of chromatid type. This would naturally lead to conclude that they were produced after the duplication of the chromosomes. It has been pointed out before that the stickiness was produced due to the action of the chemical on the nucleoprotein of the chromosome.

It may be assumed that the severe effect in some particular regions might have led to the chromatid breaks. Biese(1958) suspects most of the aberrations are resulted from the stickiness of the chromosomes. It was, however, not clear why only one chromatid should be affected and only one break should be present in a chromosome. Thus the explanation forwarded by Biese (1958) would have limited applicability.
A chromatid break in the terminal region with the fragment lying quite apart in a group II chromosome.

A chromatid break in the terminal region with the fragment not much displaced in a group II chromosome.

A chromatid break in the proximal region in a group III chromosome.

A chromatid break in the proximal region of a group II chromosome with the fragment lying quite apart.

A chromatid break in the middle of group IV chromosome with the fragment lying quite apart.

A metaphase with 2 chromosomes each with a chromatid break.

A metaphase with several fragments unknown origin and 2 chromosomes, each with a chromatid break.

EXPLANATION OF FIGURE

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Pyrogallol. Only Affected chromosomes Drawn).

(A) A chromatid break in the terminal region with the fragment lying quite apart in a group III chromosome.

(B) A chromatid break in the terminal region with the fragment not much displaced in a group II chromosome.

(C) A chromatid break in the proximal region in a group III chromosome.

(D) A chromatid break in the middle of group IV chromosome with the fragment lying quite apart.

(E) A chromatid break in the proximal region of a group II chromosome with the fragment lying quite apart.

(F) A chromatid break in the middle of group III chromosome with the fragment lying apart.

(G) A metaphase with 2 chromosomes each with a chromatid break.

(H) A metaphase with several fragments unknown origin and 2 chromosomes, each with a chromatid break.
Gallic acid (3:4:5 trihydroxybenzoic acid)

It is white powder-like in nature. It occurs in free state in tea and in other plants. It is known to be a powerful reducing agent and readily soluble in water. Cytological studies on the effect of gallic acid on chromosomes are rather limited (Sharma and De, 1954). So far it has been used in plant chromosomes. Along with the study of the present author, Manna in collaboration with another worker have studied (Manna and Bhunya, unpublished) the effect of this chemical on the spermato-cyte chromosomes of grasshopper. The present study, therefore, would add to our knowledge the effect of this chemical on the somatic chromosomes of mice.

**EXPERIMENTAL PROCEDURE**

0.25 ml of 0.02M, 0.03M, 0.04M and 0.05M solution of gallic acid was injected into different individuals and the treated mice were sacrificed at 24 hour after the treatment for the cytological preparations.

**RESULTS**

**Effect on the Chromosomes**

**Qualitative** - The effects of different molar solutions of
gallic acid could be put into two categories, viz., general effect and the effect on the individual chromosome. In some cases both of them were found to be present in the same cell. The former type of effect was generally the stickiness and the later ones were the break type aberrations. However, the stickiness produced by the treatment of the chemical was not so pronounced as that of pyrogallol. The stickiness sometimes led to form groups of clumped chromosomes. We have not scored the quantitative data for this type of effect. Just like all other chemicals referred to above aberrations were mainly of chromatid type breaks (Fig. 12A-P, Plate III, Pho. 15, 16). Some instances of subchromatid type breaks were observed but not a single chromosome type break was encountered in 2000 metaphase plates examined from the specimens of the treated series. Rarely some translocation type of configurations were also observed. Most of them were not very convincing for which they were excluded from the present record. The frequency of metaphase complements containing one chromatid break in each plate (Fig. 12A-F) was higher than others. An analysis of breaks from this point of view reveals that out of 191 metaphases containing chromatid breaks, 6 contained 3 breaks (Fig. 12M, N) 16 had 2 breaks (Fig. 12I-L) while the rest contained only one chromatid break. The frequency of chromosomes having more than one break in one of the chromatids was extremely rare and in an examination of 140 chromosomes with clear chromatid breaks only one had one
from the materials fixed after the treatment of each molar solution and the number of metaphase carrying chromosomal abnormalities like breaks and stickiness has been recorded. Besides this type of scoring, the number of chromatid breaks produced for the use of each type of molar solution was also recorded. The difference between the number of chromatid break and the abnormal metaphases would indicate the number of metaphase which contain only sticky chromosomes.

**Table - 7**

Frequency of the Chromosomal Aberration induced by Gallic Acid at 24 Hour After the Treatment of 500 metaphases were studied in each case.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No. of Meta. with aberration</th>
<th>Percentage of Meta. with aberr.</th>
<th>No. of Chromat. break</th>
<th>Percentage of Chromat. break</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02M</td>
<td>32</td>
<td>6.4</td>
<td>22</td>
<td>4.5</td>
</tr>
<tr>
<td>0.03M</td>
<td>85</td>
<td>17.0</td>
<td>78</td>
<td>15.0</td>
</tr>
<tr>
<td>0.04M</td>
<td>44</td>
<td>8.8</td>
<td>26</td>
<td>5.2</td>
</tr>
<tr>
<td>0.05M</td>
<td>30</td>
<td>6.0</td>
<td>14</td>
<td>2.8</td>
</tr>
</tbody>
</table>

If the frequency of metaphase abnormalities (Table 7) is considered, it was maximum (17%) at 0.03M while at 0.02M it was 6.4%, at 0.04M 8.8% and at 0.05M 6%. Thus no relationship could be established between the doses and the frequency of metaphases with chromosomal abnormalities. Similar result was obtained when the chromatid breakage data were considered.
It was maximum (15\%) at 0.03M while at 0.02M, 0.04M and 0.05M the frequencies were 4.5\%, 5.2\%, and 2.8\% respectively (Table 7). It was rather very interesting to note that 0.03M was proved to be the optimum condition.

COMMENTS

Sharma and De (195*) obtained chromosome fragmentation and stickiness with the application of gallic acid on onion root tip cells. Manna and Bhunya (unpublished) also observed severe type of stickiness and clumping effect besides breaks, gaps and chromosome bridges in the spermatocyte chromosomes of gasshoppers treated with the gallic acid solution. In other words, the effect of gallic acid on plant and animal chromosomes was basically the same. It had affected the general morphology as well as the structural integrity of the chromosomes. In the present observation the stickiness was also observed but to a lesser degree. The sticky effect produced by the chemical could be interpreted in the same manner as was previously done in connection with the effect of pyrogallol. They are produced due to the depolymerization of nucleoproteins of the chromosome. As regards the mechanism of breaks induced by gallic acid it can also be interpreted in the same line as was done in connection with the action of pyrogallol but in a different way. In both the cases the oxidation-reduction system has played a part in bringing the chromosomal aberration. However, pyrogallol is
an oxidizing agent while gallic acid is a powerful reducing agent. In case of pyrogallol it has been proposed that organic peroxide might be formed because of its oxidizing nature which could have been the source for induction of chromosome aberration. On the other hand, since gallic acid is a reducing agent it might have disturbed the oxidation process by removing the oxygen from the cell. This would naturally cause disturbance in the oxidative metabolism. It has been found that the effect of a number of chemical mutagens in the production of chromosomal breakages was oxygen dependent (Kihlman, 1961, 1966). Chemicals which inhibit oxidation process are also found to be generally mutagenic (Kihlman, 1961) in action. Since the gallic acid would affect on the oxygen supply, it can also inflict chromosomal aberration. If it is assumed that gallic acid acts on the respiratory enzyme because of its reducing action, it would ultimately affect the metabolism. Thus the effect produced by this chemical could be generalized as due to its antimetabolitic action.

In the present study, the gradual decrease in the frequency of chromatid breaks with the use of 0.04M and 0.05M solution in comparison to 0.03M solution appeared to be very peculiar. If the chemical has the optimum effect at 0.03M, it is expected that on reaching the maximum limit of the effect by the particular concentration, the frequency should not drop so drastically with the use of still stronger dose. It should have at least maintained the optimum frequency. The X-ray effect on
the lethal mutation rate to establish the dosage frequency relationship sometimes depicts similar result of a decline in the linearity curve. In such a case after reaching the maximum limit (optimum), the curve showed a decline (Lea, 1962). A similar tendency was shown by the present data. In order to explain our data it may be assumed that the concentration beyond 0.03M produced greater amount of damages which brought lethality to the cells. Most of them, therefore, could not enter into division while the less affected cells had no such bar. This might have led to the present anomalous result. Unfortunately, we have no data in support of the above explanation. We have not estimated the frequency of degenerated cells due to the treatment of different molar solutions of gallic acid. Unless we can present proof that higher concentrations bring more cell lethality, our speculation is to be taken with reservation. Although there are no specific data in this line with regard to gallic acid but there are other reports on the action of phenols which will support our proposition made above. Levan and Tjio (1948a,b) tested 40 phenols and related compounds on onion root tips. It has been shown by them that some of these compounds kill the cell at higher concentration but at lower concentrations the chromosome aberration of milder nature could be followed. The intermediate concentrations produced C-mitosis. In the light of the above data our assumption would get enough support.

The types of chromosomal aberrations produced by the treatment of different molar solutions of gallic acid were
basically the same as found for the treatment of different phenols and even the antibiotics under the present study. Breaks were predominantly chromatid type. In majority of the cases it was 1, sometimes 2, rarely 3 in the same chromatid of the affected chromosomes. Chromatid having more than one break was found to be produced by gallic acid and DNP while such types were not found for the treatment of \( p \)-aminophenol and pyrogallol. Therefore in the present series DNP and the gallic acid may be considered as powerful mutagens. Pyrogallol and gallic acid produced fragmentation of similar nature. Like all other chemicals referre to above, gallic acid did not produce a single chromosome or isochromatid type of break. However, very rarely two chromatids of a chromosome were broken in the non-identical regions and such a type was obtained not only for the treatment of the gallic acid but also with DNP. The occurrence of only chromatid type breaks could be explained in the same way as was done before, viz. the effect of the chemical was produced after the reduplication of interphase chromosome.

As found with the treatment of all other chemicals, the breaks were non-randomly distributed. The middle and distal regions were more susceptible. Further, chromosomes of group I were also more susceptible to the action of the chemical. Since similar results have been obtained with all the phenols and antibiotics under this study, the present data would also support our general contention that the chromosome possesses some weaker regions which are more fragile than the rest. Breaks caused in these regions appear in the localized manner.
EXPLANATION OF FIGURE

(12 A - P)

(Metaphase Chromosomes in Bone Marrow Cells in Mice Induced by Gallic acid Solutions. Only Affected chromosomes Drawn).

(A) A chromatid break in the distal region of a group I chromosome.

(B) A chromatid break in the middle of a group III chromosome.

(C) A chromatid break in the distal region of group II chromosome.

(D) A chromatid break in the middle with the fragment lying apart in a group III chromosome.

(E) A chromatid break with the fragment attached by a thin thread like connections or it could be a subchromatid break.

(F) A chromatid break in a group II chromosome with its highly displaced fragment.

(G) A chromatid break with its highly displaced fragment and the 2 other fragments of unknown origin.

(H) A double break in the chromatid of group I chromosome. Both the fragments are connected by a thin connection.

(I) Two chromosomes of a metaphase plate, each with a chromatid break.

(J) Two chromosomes of a metaphase plate, each with a chromatid break, the fragment is not displaced.

(K) Two chromosomes of a metaphase plate each with a chromatid break, one fragment is lying widely apart.

(L) A group I chromosome with a chromatid break and an extra fragment.

(M) 3 chromosomes of group I, II and III in a metaphase plate, each with a chromatid break.

(N) 3 chromosomes of groups I, II and IV in a metaphase plate each with a chromatid break.

(O) A group I chromosome with two chromatids broken in the non-identical position.

(P) A group I chromosome with 2 chromatids broken in the non-identical position.
GENERAL COMMENTS ON PHENOL-INDUCED CHROMOSOME BREAKAGE

The effect of phenols and their related compounds could be put under three categories with regard to their action on cell division and on chromosomes as done by Biesele (1958). They are: (1) poisoning effect on prophase and earlier stages because they primarily act as inhibitors of metabolic process required for the onset of cell division; (2) poisoning of the chromosomes which leads to form the stickiness and breaks and (3) the poisoning of metaphase and later stages which are caused due to their inhibiting or destroying action on the spindle structure. Among four phenols used in the present study, the inhibitory action on cell division has been studied only with regard to 2,4-dinitrophenol. In the life cycle of the cell the interphase condition is taken generally as a preparatory stage for further cell division. At this time synthesis of materials must take place to make the cell ready for entering into the divisional process. In this type of cell of animal and plant, the period of interphase has been divided into three phases with regard to the DNA synthesis. They are $G_1$ or presynthetic period extending between telophase and the beginning of $S$ phase, $S$ phase or period of DNA synthesis and $G_2$ or post synthetic period extending from the end of $S$ phase and the beginning of the prophase. The time taken by the three stages was studied by Howard and Pelc (1953) with $^{32}P$ levelling. It has also been determined in
different materials by other workers as well (Dewey and Humphrey, 1962; Hsu, Dewey and Humphrey, 1962; Wimber and Quastler, 1963; Evans and Scott, 1964). Since all cells in interphase condition may not enter division, Lajtha (1963) has suggested such a true resting stage as $G_0$ in contrast to the stages, $G_1$, $S$ and $G_2$ of interphase nucleus which will undergo division. In order to have inhibitory action of phenols and their related compounds they may act on any stage of interphase which will prevent the cell to enter mitosis. The action may also sustain injury which will be manifested when the cell will enter division at a later period. The phenols and their derivatives have been found to affect the cellular synthesis in different ways. In other words they have not been found to have a direct action on the component part of the chromosome. The effect is produced in an indirect way, because they are mostly affecting the metabolic pathways of the cell which in turn are related with the cell division. Chromosomal histones undergo synthesis along with DNA. Alpert (1955) found the amount of histone doubled before cell division simultaneously with the DNA. Other materials which need to be synthesized or mobilized in the preparation for the cell division are lipid, lipo-protein, etc. The synthesis of all the chromosome constituents are no doubt essential for cell division, but this does not mean that the cell will at once enter division. It is suspected that there is some trigger mechanism which has not been clearly identified. The reason of such a suspicion is that the synthesis of all essential constituents does not lead to the
cell division (Mazia, 1956; 1961; 1963; Mosses and Taylor, 1955). Thus the inhibitory action of different chemicals may operate on any one of the factors to upset the dividing activity of the cells. The factors are inhibition of the synthesis of essential constituents on the one hand and prevention of the trigger action on the other. 2,4-dinitrophenol has been found to inhibit the cell division. It was due to its action on oxidative phosphorylation upsetting the energy supply. Different phenols have been found to act in different ways but they ultimately lead to the inhibition of cell division. Similarly various antibiotics under present study having different modes of action on cell were found to produce inhibitory action on cell division.

The phenolic derivatives in the present study have in all cases produced chromosome aberrations. Therefore, following the classification of Biese (1958) their action may be termed as the poisoning of chromosomes. The effects were found to be stickiness and breaks of variable degrees produced by the treatment of different chemicals. The effect on the chromosomes of the dividing cells indicates that the chemical concerned did not produce inhibition of cell division in all the cells. Quite a good number of them escaped from their complete influence. Thus some of them had the chromosome aberrations. In these cells either the chemicals affected the synthesis of DNA and nucleoprotein, or they acted upon the chromosome after the synthetic phase was over when the cell was at the G_2 stage. In order to understand the chromosome
aberrations we need a good idea about the structure of the chromosome. We know that the chemical constituents of the chromosome consists of DNA, RNA, histones, more complex protein and metalions. Amongst them nucleic acids and proteins are considered to be the principal constituents. But how the chemical constituents are arranged is still an enigmatic problem (Taylor, 1962; 1963; De, 1964; Srivastava, 1968; Manna, 1969). Chromosome aberrations have been produced not only due to the effect of the chemical on nucleic acid but also on other constituents (see Manna, 1969). Stickiness is a kind of effect which could be produced by the physical and chemical agents. It has been considered as the primary effect or physiological effect (Carlson and Harrington, 1955). Darlington (1942) considered that the stickiness is resulted due to the break down of the chromosomal nucleic acid to a depolymerised and fluid condition, while others (Beadle, 1937; Ostergren, 1948; Kaufman and Das, 1955), forwarded different reasons for stickiness. Anyhow, stickiness could be produced mostly due to the denaturation of nucleoprotein. Chemicals affecting nucleic acid may ultimately lead to the denaturation of nucleoprotein. This will be revealed in the form of the sticky effect in the chromosome. Various phenols have been suggested to be protein denaturing agents for which they produce the sticky effect (Levan and Tjio, 1948a,b). Kopac(1947) suggested that the stickiness may be resulted due to denaturation of the protein moiety of the chromosomes. Even excess of histones might produce stickiness by cross linking
DNA strands of neighbouring chromosome (Anderson, Fisher and Bond, 1960). Thus the stickiness is a kind of effect which can be produced by the action of chemicals on nucleic acids, proteins, nucleoproteins, etc. directly or indirectly by affecting their metabolism. The phenols and their derivatives: produce their effect mostly in an indirect manner by hampering the metabolic pathway.

The probable mechanism of action of chemicals under present study have already been mentioned. Krahl (1950) considered that the enzymatic oxidative phosphorylation is affected by a series of substituted phenols. Ambrose and Gopal-Ayenger (1953) showed that the polytene chromosome is composed of nucleoprotein micels which cohere longitudinally by H-bond. Phenols may attack their H-bonds and may cause longitudinal swelling of the chromosomes. They also showed that the chromosome breaking effect by radiomimetic agents was attributable to the interference in the synthesis. According to the thermodynamic principle compounds with a thermodynamic activity greater than 0.05 are considered by convention to act by physical means while compounds with thermodynamic activity less than 0.05 would probably act chemically. However, Gavaudan, Dode¹ and Poussel (1944, 1945) applied the concept to the radiomimetic chemicals and showed that phenols in general fall in the latter order since their thermodynamic activity was lower than 0.05. Levan and Tjio (1948a,b) studied many phenols which fell into this group. In the
present study also similar results have been obtained. Nethery and Wilson (1966) have shown that more electron introducing substances have higher activities when incorporated into phenolic compounds. On the other hand, the electron releasing substances by displacing the electrons into the ring and and increasing the electron density at the available carbon centres, activate them for electronphilic attack. Blackman et al (1955) proposed that the rings inactivated by electron attracting substituents to attack by positive ions are still free to bind two negative cellular sites. Further, the phenols activated by electron releasing substituents to electronphilic attack may be more susceptible to hydrolysis, metabolic degradation or detoxification. In the present study the action of 2,4-dinitrophenol may be interpreted as the electron attracting substituted phenols while that of p-aminopheno as the electron releasing substituted phenols, according to the proposition made by Nethery and Wilson (1966). It was also found in the present study that the effect of 2,4-dinitrophenol was more than that of p-aminophenol, and that was in agreement with the suggestions made above. In the present study the effect of phenolic derivatives has been found to be relatively less than what has been obtained in the plant materials by other workers. Of course, a very definite conclusion cannot be drawn because the studies were independently done and there were differences in the experimental set up. The greater action in plant material stated by the present author was considered from the general impression and not on real comparative
data. The reason for greater action could be that in case of plant material the chemical would reach the cell more directly since the absorption was done by root tip cells and these cells were studied for the effect. In animal tissue even when the testing was made on tissue culture cells, the effect was found to be more than in vivo condition. In the present study the injected chemical most likely have reached the bone marrow tissue through circulation. So by the time the chemical reached the tissue, the strength might have been reduced.

In the present study it has been found that all the phenolic derivatives produced breaks which were of chromatid type. The occurrence of chromatid type breaks would invariably lead to the consideration that the breaks took place after the duplication of chromosomes but it would not explain how this can take place. Various explanations have been given by different workers as to the production of chromatid type aberration. Biesele (1958) considered that the poisoning effect on the condensation of the chromosomes would lead to the chromatid type aberration. Levan and Tjio (1948 a,b) working on the action of various phenolic compounds on chromosome breaks came to the conclusion that the breaks were due to the formation of pseudochiasmata as a result of irregular and delayed division of certain segments of chromosomes. The occurrence of stickiness and pseudochiasma due to irregular reduplication would cause hindrance to the normal duplication.
of the adjoining segment. The tension thus created may break one of the chromatids at those loci. In the present study the explanation put forward by Levan and Tjio (1948 a,b) could not be fully substantiated. In our materials breaks were found even when there was no pseudochiasma-like structure. It has been assumed that the breaks were produced not due to the mechanical tension of above nature but due to some other reason. The indirect action of the chemicals on the nucleoprotein parts of the chromosomes induced the chromatid breaks. The possible mechanism has, however, been discussed before in connection with the different chemicals. Besides the pseudochiasma hypothesis, it has been generalized by some workers that the chromatid breaks might have been resulted due to the severe action of stickiness (see Biesele, 1958). But in the present study we have obtained chromatid breaks when there was practically no sticky effect produced by the chemicals. On the other hand, it can be assumed that the breaks were produced due to their indirect action on chemical bonds of the chromosome which inflicted the breaks. Anyhow, it seems very likely that the stickiness, formation of pseudochiasma and breaking of the chemical bond due to chemical action all may contribute to the production of the chromatid break. They are all interlinked but in some material one process may predominate over the other.

Together with the chromatid type breaks their localized appearance need be considered. Due to the application of the chemicals the breaks under present study were, to some extent, localized between the middle and the terminal parts of the
affected chromatid. They were also non-randomly distributed in the chromosomes belonging to different groups. Levan and Tjio (1948 a,b) have also indicated that treatment of phenols produced some locus specific breaks. We donot know really what could be the reason for such types of break. It has been suggested that some of the chemicals producing the breaks for their effects on some enzymatic system. If such enzymatic activity is localized more in the affected region, it may lead to localized break but we have no specific data in this line. This matter may be investigated in future. We may attempt to explain it in a different way. Therman-Suomalainen (1949) obtained more breaks in the secondary constriction region in her material. But in the present material atleast no such cytologically demarcable regions could be found in the normal individuals except in the 'rabbit-ear' chromosomes. In the experimental result practically no break was found in the 'rabbit-ear' chromosomes. Therefore, the explanation given by Therman-Suomalainen (1949) is not applicable to the present case. The breaks concentrated more in the distal half than in the proximal half in the affected chromosomes may be considered as a general feature because this type of effect was found not only with the treatment of phenols but also with various other types of inducing agents under present study. Localized breaks have been obtained by various chemicals like BUdR, MH, EOC, hydroxylamine, etc. due to their some specific action (see Kihlman, 1966). The explanation given by different authors on the mechanism of the induction of
localized breaks is very attractive but needs further generalisation. Why the same chemical fails to show similar action when applied to other materials? It is yet to see whether some mechanical factor of something else is operating in these cases. In the present study the action of the chemicals was found more pronounced towards the distal half in affected chromatids. As mentioned before, it could be related with some enzymatic action. According to this view the affected regions in general would have more enzymatic action than any other part. If the suspicion could be materialised, it would also be helpful for giving an explanation of localised breaks in the present material. But there is not the least evidence in support of this view. The non-random distribution of breaks can not be explained with this hypothesis. Rather the present author is more inclined to some mechanical reason. Since the same group of chromosomes and the same regions were often broken by non-related chemicals, it has been assumed that the chromosomes in question may have some weaker regions which are easily broken.

In the present data all chemicals produced mostly the simple chromatid type break and multiple configuration was absent or the frequency was very negligible. The absence of the second category of aberrations was not an unexpected one. In order to have an effective translocation, various conditions must be satisfied which have been mentioned before. Even in Drosophila the frequency of spontaneous translocation has been found to be very low in comparison to that of inverson
(see White, 1954; Dobzhansky, 1951; Manna, 1969). For an effective translocation two potential breaks must take place at the same time, at the same place according to 'breakage - first' hypothesis given by Sax (1941). In case of X-ray induced chromosome aberration, the frequency of translocation was found to be lower than that of non-exchange type break. The reasons for low frequency of translocation have been put forward by Manna and Mazumder (1967).

The genuinity of the chromatid breaks obtained by the application of phenolic chemicals and other chemicals in the present study may be questioned because in most cases the broken fragments were lying close to their normal position. In such cases they may be treated as the unstained gaps than true breaks. In making the record of chromatid breaks, the doubtful cases were rejected. If the gap-type aberrations were included, the effect would have been more than what has been recorded. Anyhow, we have not put undue stress on the quantitative data because some limiting factor could not be fully avoided. But this would not mean much difference. It can definitely be said that most of them cannot be explained without considering them as true breaks. Further, it was not a fact that all the broken fragments were lying in undisplaced condition. The fragments were also lying widely apart. Furthermore, no such aberration was present in the control series.

In the present study metaphase plates with a particular chromosome having more than one break were relatively rare but
not totally absent. They were found when the higher concentrations were used or were related to the chemical used. This fact would suggest that most of the chemicals used were not very effective mutagens in causing aberrations. If the breaks were dependent on some mechanical factor, the presence of one may influence the production of the other in different places in the same chromatid. Non-random distribution of inversions in different chromosome as well as in different species has also been documented in *Drosophila* and some such explanation has been given in that connection (see White, 1954).

The aberrations produced in the chromosomes of mice by phenols, antibiotics or other chemicals under the present study were overwhelmingly chromatid type. The cause of such type of aberration has been discussed before in a scattered manner and from different standpoints. Therefore, a general discussion on the hypothesis in their production has been attempted here and it would not be repeated in details in explaining mechanism in connection with each of the chemical. There are two main hypothesis, one put forward by Sax (1941) and the other by Revell (1953; 1955) which has been advanced by Evans (1962) to account for the 'primary event' produced by the chromosome breaking agents. The hypotheses are basically opposite to one another. The 'breakage first' hypothesis was originally proposed by Sax (1941) and it was successively accepted by many workers. According to this hypothesis it is considered that due to the effect of the chromosome breaking agent, as the primary event, a chromatid
or a chromosome breaks in its continuous structure during the interphase nucleus. The nature of breaks either chromatid or chromosome type depends on the state of chromosome at which the effect takes place. If it is before duplication, it will be chromosome type and if after duplication it will be chromatid type. It has been assumed that, as a rule, the ends at the breakage point rejoin to restore the original configuration which has been termed as restitution. However, the end may remain open or illegitimately rejoin with other broken ends depending on time and space. The illegitimate joining may result in various types of configurations including exchange type ones. It is generally assumed that the fate of the broken ends is determined within short time. White (1954) has reviewed elaborately the mechanism of structural changes on the basis of the 'breakage first hypothesis'. However, in recent years evidences have been presented that the broken end may remain open for a considerable period. The 'exchange hypothesis' proposed by Revell (1955; 1959) and supported by Evans (1962) is gaining ground as a counter part of the 'breakage first' hypothesis. According to the 'exchange hypothesis', the primary event is some kind of 'lesion' on the chromosome and not the 'break' as proposed by Sax. This lesion has a shorter durability and it will decay with time. But within the specified period it may revert to another state incapable of taking part in exchange formation or to normal. But if the two primary events are close enough in time and space, they may succeed by another stage designated by Revell as 'exchange initiation stage'.
This exchange initiation is stable and of considerable duration. However, no genetic change takes place at this stage but in subsequent stage it is transformed into a real chromatid exchange. Thus, at the initial stage the hypothesis of Sax is diagonally opposite to Revell. According to Revell's hypothesis, all types of chromatid aberrations arise as a result of exchange process and the isochromatid break is resulted from one of the four ways of complete chromatid intra-exchanges. On the other hand, the chromatid break is the incomplete intra-exchange. Further, according to this hypothesis the true chromatid break is a rare type of aberration and may be occurring twice the number of isochromatid breaks. Of the two hypotheses, Kihlman (1966) is little inclined towards the exchange hypothesis because of its special features. He finds that during the last few years good amount of evidences have been presented by different authors in support of this hypothesis from qualitative as well as quantitative standpoint. This hypothesis helps to explain how unrelated chemicals are able to initiate an exchange process. He further adds that the chromosome breaking agents create a situation in the mitotic cell like that of pachytene stage of meiosis. However, there is some difference. Since the induced exchanges are not under cellular control, the exchange usually occurs between heterologous chromosomes and in the asymmetrical way. The similarity between the meiotic crossing over and the chemically induced exchanges has also been pointed out earlier by Marquardt (1950), Ostergren and Wakonig (1954), and Manna (1961).
The types of aberration obtained in the bone marrow chromosomes for the treatment of various chemicals under present study can be explained by both the hypotheses but any one will not fulfil all the points. Since some of the chemicals are unreactive to initiate a break in the complex structure of the chromosome, the exchange hypothesis would appear to be more attractive. These chemicals after the production of some kind of lesion in the chromosome might have succeeded by 'exchange initiation stage'. As a result of this, different types of chromatid aberration were observed. Inspite of this some of the events could not be explained properly with the hypothesis. In the present study no isochromatid break was encountered which according to Revell should have been half the number of chromatid breaks. The number of chromatid break was considerable but not a single isochromatid break was found. Further, on this basis it is rather difficult to explain the localised breaks. The 'breakage first hypothesis' on the other hand would pose no difficulty in explaining all the aberration types but the chromosome breaking agents used in the present study would hardly be able to break the chromosomes directly. On the other hand by the exchange hypothesis it will not be so easy to explain the origin of subchromatid break but it is less so with breakage first hypothesis. Thus we find that none of the hypotheses can fully explain all aspects of aberrations induced by various chemicals in the bone marrow chromosomes of mice. Further, much of the merit in the acceptibility of any hypothesis rests on the detection of the primary events are not very different in both the hypotheses.
C. SUBSTITUTED AMIDES

8.

Hydroxylamine (HA)

The chemical is a crystalline solid easily soluble in water. The molecular formula is $\text{NH}_2\text{OH}$.

Freese and his collaborators (Freese et al., 1961a,b; Freese and Stracks, 1962) have shown that hydroxylamine (HA) is a mutagen with higher degree of specificity of action in $T_4$ phage. The action of HA on $T_4$ phage, at low salt concentration, is lethal while at higher salt concentration the mutagenic effect becomes more striking. They have further shown that the time factor has some role with respect to the appearance of different mutant forms. The genetic damage produced by HA is thought to be due to its initial action on cytosine. This is supported from the biochemical study. The direct action of HA on free DNA bases has shown that it has a preferential action on pyrimidine cytosine. HA has some reaction with hydroxy-cytosine. It has very little or no reaction with 5-methyl-cytosine. Further, it has no reaction with bases, thymine or adenine. Treatment of thymus DNA with HA leads to a loss of cytosine but not the thymine. The chromosome breaking effect of HA has been studied by some workers. Somers and Hsu (1962) has shown the chromosome breakage due to the treatment of HA in Chinese hamster while Borenfreund, Krim and Bendich (1964) have reported the effect of HA derivatives on mammalian chromosomes. Cohn (1964) has shown that HA can also break the plant chromosomes.
EXPERIMENTAL PROCEDURE

Different concentrations like $10^{-3}$M, $10^{-2}$ M and $10^{-1}$M of HA solution were used. 0.25 ml of each solution was injected intraperitoneally into the mouse weighing 23 gm. The specimens were sacrificed at 24, 48 and 72 hour after the injection for obtaining the tissue for the cytological preparation.

RESULTS

Effects on Chromosomes:

Qualitative: Qualitatively the different concentrations of HA produced two kinds of effects e.g. 1) on the general morphology of the chromosome and 2) the chromatid aberrations. The effect on the general morphology of chromosomes manifested no pronounced sticky effects but on the outlines of the chromosomes in many cases which was found to be uneven. Sometime the chromosomes appeared to be relatively long. On the whole the general type of effect seemed to be associated with the despiralization of the chromosomes.

The aberrations induced by the treatment of HA as in most other chemicals described earlier were mainly chromatid type breaks (Fig. 13 A-H; Plate III, Pho. 17, 18) and a very few translocations (Fig. 13 H). The simple aberrations could primarily be demarcated into two categories viz., 1) across the centromeric region or (2) some other region of the chromatid. There was some doubt about the occurrence of the first category.
of aberrations. In such cases a good number of chromosomes in the affected metaphase were found to have the two chromatids separated as found with C-mitotic effect (Plate IV, Pho. 23). There was no clear indication that the separation was due to breakage in the centromere. It could have been due to the precocious separation of the centromere. The number of affected chromosomes was variable from cell to cell but in no case, as found in C-mitosis, the two chromatids of every chromosome were separated. Thus it was rather uncertain whether the separation of the chromatids of large number of chromosomes was due to the breaks induced in the centromere by HA or due to the asynchronous separation of chromatids of the metaphase chromosomes initiated by the chemical. Since similar phenomenon has not been found so widely in the materials treated with other chemicals in present study we with some hesitation, have considered that the separation was due to the break of dissociation in the centromeric region. Further, it is to be mentioned in this connection that in the control specimen no such effect was present.

There was no doubt about the occurrence of the chromatid aberration in regions other than the centromere. We can safely consider most of the chromatid aberrations as true breaks produced by HA. In an analysis of 41 chromatid breaks, 30 were present in the middle (Fig. 13 A, D, F, G), 7 in the distal (Fig. 13 B, C) and 4 in the proximal region (Fig. 13 E). Amongst 41 breaks in the chromatids 15 were in the longer groups of
chromosomes. The distribution of these 15 chromatid breaks in the longer chromosomes was found to be 11 in the middle, 2 in the proximal and 2 in the distal region of the affected chromatids. Further, in the present study in only 2 instances a chromatid was broken in two places (Fig. 13 E, H), while the remaining ones had one break in each of the affected chromatids. Among 41 metaphase complements containing chromatid breaks 2 plates had two breaks, one in each chromosome (Fig. 13G) and one plate had 2 breaks and a translocation (Fig. 13 H). The occurrence of such a plate was extremely rare. Very few instances of translocation were encountered for the treatment of HA. In some cases it was hard to distinguish between a true translocation and a terminal association of two chromatids when it was located in the terminal region. In such cases the broken fragment could not be traced. In the present study no instance of both the chromatids of a chromosome was found to be broken either in the identical position (isochromatid break) or in the non-identical position. As found with the treatment of other chemical, the fragment of the chromatid originated due to the break mostly close to the affected region (Fig. 13A, B, C, G) but it was also found to lie elsewhere (Fig. 13 D, F).

Quantitative: The frequency of chromatid aberration produced by different concentrations of the chemical in the bone marrow cells fixed at different hour has been scored (Table 8).

An analysis of the frequencies of chromatid breaks and centromeric dissociations at different concentrations and
in the tissues fixed at 24, 48 and 72 hour indicates that in general both the types were in higher frequencies with the use of higher concentrations of the chemical (Table 8). The frequency of chromatid aberrations at the non-centromeric regions at 24 hour was 0.026 (4/150) in $10^{-3}$M; 0.040 in $10^{-2}$M and 0.054 in $10^{-1}$M solution. Similarly the frequencies of

**TABLE - 8**

Frequency of the Chromatid Aberration and Centromeric Dissociation induced by HA. The Number of Metaphases Affected with Centromeric Dissociation and Chromatid Breaks in 150 Metaphases Examined in Tissues at Different Intervals.

<table>
<thead>
<tr>
<th>M. Sol.</th>
<th>Dose in ml</th>
<th>24 Hr.</th>
<th>48 Hr.</th>
<th>72 Hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Chromo Abr</td>
<td>No. of Chromo Abr</td>
<td>No. of Chromo Abr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centr</td>
<td>Non-Centr</td>
<td>Metaphage</td>
<td>Centr</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>0.25</td>
<td>150</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>0.25</td>
<td>150</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>0.25</td>
<td>150</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

centromeric dissociation per cell at 24 hour were none at $10^{-3}$M, 0.033 at $10^{-2}$M and 0.060 at $10^{-1}$M. Thus there was no proportionality in the occurrence of centromeric dissociation per cell. In the tissue fixed at 48 hour after the treatment, the frequencies showed an increase with the higher dose. They were 0.014, 0.046 and 0.113 for non-centromeric and 0.020, 0.067 and 0.173 for centromeric region. The same tendency was found in the tissue fixed at 72 hour although no aberration
was found with the concentration of $10^{-3}$M solution. In other two concentrations the frequencies were 0.006 and 0.020 for non-centromeric and 0.014 and 0.046 for centromeric region. If the frequencies of chromatid breaks in the non-centromeric regions at different hour of fixation are considered at $10^{-3}$M, it was more at 24 hour than that of 48 hour while there was none at 72 hour. However, the results obtained for the frequency of centromeric dissociation with the above concentration were erratic because no effect was found at 24 and 72 hour but at 48 hour the frequency was 0.020. With the use of $10^{-2}$M solution the frequencies of non-centromeric chromatid aberrations were not at all different at 24 and 48 hour (0.040/cell) but it was quite low at 72 hour (0.006). On the other hand, the frequencies of centromeric dissociation were found to be 0.033 at 24, 0.067 at 48 and 0.014 at 72 hour. In this case the frequency was highest at 48 hour. At $10^{-1}$M solution the frequency of chromatid aberration in the non-centromeric region was highest at 48 hour (0.112) while at 24 hour and at 72 hour it was considerably low (0.54 and 0.020 respectively). A similar situation was found when the frequencies of centromeric dissociation was considered. It was highest at 48 hour. Thus in general, there was a trend in the increase of the frequency at 48 hour irrespective of the concentration used. The effect was quite reduced or absent at 72 hour. However, no definite proportionality was maintained in the frequencies with regard to concentration or the fixation hour whether the centromeric
dissociation or chromatid aberrations in the non-centromeric region was considered.

**COMMENTS**

The effect of HA on the chromosomes of tissue culture cells of Chinese hamster was studied by Somers and Hsu (1962). They found multifarious types of aberration which were very interesting for various reasons. The aberration types they observed were constriction, despiralizations of chromosomes as the general effect and chromatid and chromosome type breaks and interchromosomal exchanges. The most interesting observation they recorded was the high incidence of aberration at the centromeric region than in any other parts of the chromosome. Borenfreund, Krim and Bendich (1964) also obtained the chromatid type aberration in the tissue culture cells of Chinese hamster and embryonic cells of mice not only with the treatment of HA but also with its derivatives and hyponitrites. The effect of $10^{-3}$ M HA on the chromosomes of root tips cells of *V. faba* and *A. cepa* was in the form of a scattering of chromosome, exchange type aberrations, etc. We have used the same dose on the bone marrow cells but the effect was not severe as recorded in plant material. If the chromosome aberrations produced by different concentrations of HA of the present study are considered, it will be found that the higher concentration did not produce higher degree of damages like multiple breaks in a chromatids or more of the exchange type aberrations. The frequency of higher aberrations at higher concentrations was due to the
effect on the morphology of chromosome. Further, it has been recorded in plant materials (Cohn, 1964) that the maximum effect was found in one to two days which was also found in our materials and it continued even up to 72 hours. Further, the effect of HA in the present study has been found to be less severe than what has obtained in tissue culture cells of Chinese hamster or mouse embryo. In the present material chromosome or isochromatid breaks were completely absent and the frequency of exchange type aberrations was negligible. The reason for the reduced effect obtained in the present study could be explained as due to different mode of application. In tissue culture cells or in onion root tip cells the chemical could come in contact with the cells more directly than in the present method of treatment. The effect could get diluted by the time it has reached the tissue through different pathways. However, a true comparison is not possible because sometimes the doses were different and the standard for comparison was not the same. Somers and Hsu (1962) found that HA acted more specifically in the centromeric region. In the present study we could also support their observation to some extent. It has been mentioned that due to the treatment of HA, a high frequency of chromatid separation at the metaphase stage was noticed. It has also been pointed out before we could not substantiate definitely whether it was due to break in the centromeric region or only premature centromeric separation. Anyhow, whatever be the origin, the separation of chromatids must have been due to some action on the centromeric region.
by HA as seen in Chinese hamster chromosomes. Besides this, in our study we also found clear evidence of the chromatid breaks localized more in the middle region and lesser in the proximal and distal region, as found with the treatment of other chemicals. The occurrence of somewhat localised breaks would again lead to suspect some weaker regions in the chromosomes which has already been advocated. It is suggested that HA induced aberrations are produced due to its primary reaction with cytosine moiety of DNA. Further, Somers and Hsu (1962) recorded that the localized breaks produced by HA in the chromosomes of Chinese hamster were in the centromeric region but they were not in the same region which was sensitive to BUDR. As reported by the same authors (Hsu and Somers, 1961) they were near the telomeres and the region 7 of chromosome no. 1 of Chinese hamster. In order to explain BUDR induced localized breaks in the chromosomes of Chinese hamster, it has been suggested that since BUDR is a thymidine analogue, it was incorporated into DNA by replacing thymidine. The greater amount of its incorporation in the specific region would indicate the repeated occurrence of A-T pairs (Hsu, 1963) than in other regions. The breaks inflicted in region 7 behaved in a telomere-like way because they were quite stable and rarely joined with other broken ends. The stability of the broken ends in region 7 as well as that of normal chromosome ends has been argued by Hsu (1963) was a consequence of their A-T content. On the other hand, the localized breaks in the centromeric regions in the chromosomes of Chinese hamster
have been explained by Somers and Hsu (1962) as due to the high content of C-G pairs because HA reacts with cytosine and altered mainly the cytosine moieties of DNA. These attractive hypotheses can be questioned on various ground. In the present study the localized breaks were in the middle part of the chromatids and the effect on the centromeric region was the case of early separation. Kihlman, Nichols and Levan (1963) have observed the cytosine arabinoside induced breaks in the chromosomes of human leukocytes which were localized at the ends but it did not affect the centromeric region. Since the chemical is known to cause deficiencies in cytosine deoxyribonucleotides, it would very likely affect the regions of chromosomes which are rich in C-G pairs. Thus according to the two sets of arguments the centromeric regions of chinese hamster chromosomes (Somers and Hsu, 1962) and ends of human chromosomes are rich in C-G pairs (Kihlman et al 1963). Hsu (1963) has also claimed richness of A-T pairs in the telomeric regions in Chinese hamster chromosomes. Either we are to consider that the same structure might differ in base composition in different species or else the localized breaks were due to some other unknown factors which have not been solved as yet. In our material we have suspected some weaker region. The interpretation given by Borenfreund et al (1964) suggested that the chromosomal aberration induced by HA was due to main chain scission of DNA rather than due to reaction with cytosine in DNA. In other words it acts on linkers of nucleoprotein blocks of chromosomes.
A chromatid break in the middle of a group I chromosome.

A chromatid break at the distal region of a group III chromosome.

A chromatid break at the distal region of a group II chromosome.

A chromatid break at the middle of a group IV chromosome with the displaced fragment.

2 breaks in the same chromatid of a group I chromosome.

A chromatid break in a group III chromosome with the displaced fragment.

A metaphase plate with 2 chromatid breaks, one in each chromosome.

A metaphase with a translocation and 2 chromosomes with chromatid breaks. One of them had two breaks in the same chromatid.
Phenyl hydrazine (PH)

Phenyl hydrazine is a white powder with the chemical formula \( \text{C}_6\text{H}_5\text{NH NH}_3\text{Cl} \). The aqueous solution changes its colour when exposed to air. Thus freshly prepared solution was used always for the experiment.

So far as we are aware, the chromosome breaking activity of PH was not tested before. However, the mutagenic action of hydrazine has been reported by some workers (Brown et al, 1966). It has been shown that hydrazine reacts on uracil of nucleic acids. It has been pointed out before that HA reacts on cytosine moiety of DNA. Thus hydroxylamine and hydrazine react with certain pyrimidine bases but they have different chemical specificity. It is stated that hydrazine breaks the ring of uracil and cytosine giving rise to pyrozolone and 3-amino-pyrozole respectively. Anhydrous hydrazine when treated on RNA produced 'riboapyrimidinic acid' free of pyrimidines and on DNA produces the corresponding apyrimidinic acid. It is also said that the action of hydrazine is pH dependent. Thus we have some knowledge on the biochemical action of hydrazine on nucleic acid, the basic constituents of chromosomes, but its action in living state has not been tested. Moreover, the biochemical action of PH on nucleic acid is not known. The present study would reveal that PH has the capacity to induce the chromosome aberration in the bone marrow cells of mice.
EXPERIMENTAL PROCEDURE

0.25 ml of freshly prepared 0.01 aqueous solution was injected into each experimental mice. The specimens were fixed at 24 and 48 hours after the treatment. In another set 0.1M solution was injected but the individual could not survive. Thus no study was possible from this group.

RESULTS

Effects on Chromosomes:

Qualitative: The treatment of 0.01M solution of PH produced some physiological effect as well as aberrations in the chromosomes. As a result of physiological effect the chromosomes were found to be in slightly despiralized condition or with the eroded outline. The chemical produced mainly chromatid type of aberrations (Fig. 14A-H) and some centromeric dissociation. Like hydroxylamine this chemical also acted upon the centromeric region of the metaphase chromosomes which caused the two chromatids to dissociate earlier (Plate IV, Pho. 24). The degree of effect was variable. Sometimes all the chromatids were separated as found in case of C-mitotic metaphase while at others only a few of them. We have not designated them as centromeric breaks because so many breaks at a time in the centromeric region seemed to be highly improbable. The treatment of PH produced besides centromeric dissociation some chromatid aberrations but the effect was less severe. Out of 400 metaphases only 10 chromosomes had chromatid aberrations. Out
of these 10, 5 were in the middle (Fig. 14 A, C, D), 3 in the distal (Fig. 14 F, G) and 2 in the proximal part (Fig. 14 B) of the affected chromatids. Not more than one chromatid in a metaphase complement was seen to be broken. As found with other chemicals, the fragments were either situated near to their places of origin (Fig. 14 A-C) or lying quite apart (Fig. 14 D, E, F). Sometimes a thread-like connection was seen between the fragment and the chromatid concerned (Fig. 14 H). Since the data were very inadequate, no attempt was made to indicate whether the aberrations were common to some particular group of chromosomes. Out of these 10 breaks, 3 were in the longer chromosomes. No chromosome type or exchange type aberrations were observed for the treatment of PH.

**Quantitative**: Analysis of the data would reveal that the frequency of the chromosome aberration was very low. It was reduced further with the extension of the fixation hour because the chromatid aberration was 3% at 24 hour and 2% at 48 hour. Similar reduction in the frequency of dissociation of chromatids was also observed. It was 4.5% at 24 hour and 2.5% at 48 hour. Therefore, the effect of PH was waning out gradually with the lapse of time after the treatment.
TABLE -9

Frequency of Chromatid Aberrations induced by 0.01M Solution of PH on the Bone Marrow Cells. In each Experiments 200 Cells were Examined.

<table>
<thead>
<tr>
<th>Dose in ml.</th>
<th>FIXATION HOUR</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hour</td>
<td>48 hour</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% of Chroma-</td>
<td>% of Centro</td>
<td>% of Chroma-</td>
</tr>
<tr>
<td></td>
<td>tid break</td>
<td>dissoci</td>
<td>tid break</td>
</tr>
<tr>
<td>0.25</td>
<td>3</td>
<td>4.5</td>
<td>2</td>
</tr>
</tbody>
</table>

COMMENTS

The chemical has been found to have some chromosome breaking activity but the frequency was very low in comparison to that of some chemicals described earlier. The mechanism of breakage cannot be properly suggested because the biochemical action of this chemical on nuclear component has not been studied. It has been mentioned before that hydrazine has been found to act on the pyrimidine bases of the nucleic acid, it may be suspected that this chemical also acted in that way on the chromosomes. However, hydroxylamine is claimed to have specific action on cytosine while hydrazine acts more on the uracil. The chromosome breaking effect of PH, if considered to be the same as that of hydrazine, it will not be able to break the DNA strands of chromosomes directly. It should act indirectly by impairing RNA synthesis. In case of HA induced chromatid breaks Borenfreund et al (1964) have suggested that they were
EXPLANATION OF FIGURE

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Phenyl Hydrazine. Only Affected chromosomes Drawn).

(A) A chromatid break in the mid-region of a group I chromosome.

(B) A chromatid break in the proximal region of group I chromosome.

(C) A chromatid break in the mid-region of a group IV chromosome.

(D) A chromatid break in the mid-region of a group I chromosome with the well displaced fragment.

(E) A chromatid break in a group III chromosome with the well displaced fragment.

(F) A chromatid break in a group II chromosome with the displaced fragment.

(G) A chromatid break in the proximal region of a group III chromosome.

(H) A chromatid break with the fragment attached by thin thread like connection in a group IV chromosome.
Semicarbazide (SC)

The action of semicarbazide (SC) on chromosome is practically unknown. Rieger and Michaelies (1962) made a review of chemical mutagens having actions on the chromosomes of *V. faba*. It has been reported there that the chemical has no action in the production of chromosome aberration in *V. faba*. Besides this negative result, the present author is not aware of any other work carried out with this chemical on other materials. Thus the present study will be of much interest because positive result has been found on bone marrow chromosome

**EXPERIMENTAL PROCEDURE**

0.25 ml. of 0.1M aqueous solution of semicarbazide was injected into each experimental animal and they were sacrificed at 24 and 48 hour for the study of effect in the bone marrow chromosomes.

**RESULTS**

*Effect on Chromosomes* :

Qualitative : The action of SC on the chromosomes of bone marrow cells was found to be even milder than PH. It produced very rarely some erosion effect on the metaphase chromosomes. This chemical did not produce centromeric dissociation leading to the early separation of chromatids as found with the
treatment of HA and PH. Besides some rare instances of general effect, some chromatid aberrations were seen (Fig. 15; Plate IV, Pho. 19). The number was very low and the nature was simple. Out of 400 metaphases, only 13 chromosomes (3.25\%) were found to have chromatid breaks. In case of PH treatment the frequency was 2.5\% (10 out of 400). Usually one break in an affected cell was present. Very rarely an exception to this was found. In the present data 13 chromatid breaks were found in 11 affected metaphases, i.e. 2 plates were with 2 breaks each and the rest with one. The chromatid aberrations were found to be occurring mainly in the mid region (Fig. 15A-D, G and a few in the distal part of the chromatid (Fig. 15 E, F, H). The longer chromosome seemed to have relatively more breaks than others. However, because of the limited data, a critical analysis has not been attempted. The fragments were lying sometimes close to their place of origin (Fig. 15 D-F) but often they were situated in various displaced conditions (Fig. 15 A, B, C, H). No exchange type aberration or chromosome break was encountered in the present study.

Quantitative: An analysis of the data (Table 10) would show that at 24 hour the frequency of chromatid breaks was only 4\% whereas it was 2.5\% at 48 hour. The effect naturally decreased with the extension of the fixation hour.
TABLE - 10

Frequency of Chromatid Aberrations Induced in the Bone Marrow Cells of Mice Treated with Semicarbazide Solution and Fixed at 24 and 48 Hours. In Each Experiments 200 Cells were Examined.

<table>
<thead>
<tr>
<th>Dose in ml</th>
<th>24 hour</th>
<th>48 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**HOURS OF FIXATION**

- % of Chroma-tid break
- % of Centro-dissociation

**COMMENTS**

It is very difficult to suggest any specific mechanism of action by which this chemical could induce chromatid aberrations. From the cytological point of view it is proved beyond doubt that the treatment of this chemical induced chromatid breaks comparable to those of other chemicals under present study. In case of the other two substituted amides described before it has been considered that the breaks might have been produced due to biochemical reaction on the pyrimidine bases. In those cases it was suspected that they induced aberrations by their action on linkers of DNA. Being a chemical of the same group we donot know if it was also reactive in the same way for inducing the aberration. Rieger and Michaelis (1962) obtained negative result for the use of this chemical. It was not very unusual because it has already been reported that a particular mutagen may fail to induce similar effects.
A chromatid break in the mid-region of a group II chromosome with the fragment lying widely apart.

A chromatid break in the mid-region of a group II chromosome, with the displaced fragment.

A chromatid break nearabout the middle of a group III chromosome.

A chromatid break in the mid-region of a group II chromosome.

A chromatid break in the terminal region of a group II chromosome with the fragment lying displaced.

EXPLANATION OF FIGURE
(15 A - H)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Semicarbazide. Only Affected chromosomes Drawn).

(A) A chromatid break in the mid-region of a group II chromosome with the fragment lying widely apart.

(B) A chromatid break in the mid-region of a group III chromosome with the displaced fragment.

(C) A chromatid break nearabout the middle of a group III chromosome.

(D) A chromatid break in the mid-region of a group II chromosome.

(E) A chromatid break in the distal region of a group I chromosome.

(F) A chromatid break in the mid-region of a group IV chromosome.

(G) A chromatid break in the mid-region of a group I chromosome.

(H) A chromatid break in the terminal region of a group II chromosome with the fragment lying displaced.
D. MISCELLANEOUS COMPOUNDS

11. Caffeine

This chemical is an important purine compound and highly soluble in water. The effect of caffeine have been studied mainly on the root tip cells and animal tissue culture cells by various workers. The inhibitory action of caffeine on cell division has been found in different materials. On the other hand exposure of tissue culture cells to caffeine supressed mitosis to a greater extent in Sarcoma-180 cells than mouse embryonic skin cells. This indicates the differentially inhibitory effect of caffeine in different tissues. A partial reversal of the inhibitory action of caffeine on the conidial growth of Opisthoma multiannulatum with the addition of adenine has been found (see Bieseke, 1958). Kidder and Dewey (1949) reported the inhibitory effect of substituted purines on the growth of Tetrahymena geleii. The inhibitory action of caffeine could partly be reversed by guanine or other purine. However, some authors found that tryptophan, alanine, procain could also modify the effect of caffeine as are antagonistic to it. It has been inferred from this finding that caffeine does not necessarily produce inhibitory action by competing with purine specific enzyme.

The chromosome breaking activity of caffeine was discovered by Kihlman and Levan (1949) after testing the
chemical on the root tip cells of onion. Kihlman (1949) also extended his study on the effects of xanthin and other related purine on the root tip chromosomes in plants. According to him the purines which were found to produce effects on chromosomes could be put under two groups. A member of the first group 8-ethoxy-caffeine has been found to be very active mutagen. Members of this group in general are more lipid soluble and can penetrate the lipoidal layer of the interphase cell membrane. The members of the other group like caffeine, 1,3,7,9-tetramethylylaric acid etc. are more water soluble. They depend for their action on mitotic periods because they can act upon the chromosomes when they are unguarded by cellular membrane (Kihlman, 1961). The suggestion made by Kihlman that caffeine can break the chromosome only during mitosis was put forward earlier by Östergren and Wakonig (1954). The frequency of chromosome breakage produced by caffeine has been found to be modified by the treatment of nucleoside. It has been reported that the frequency of chromosome breakages in the root tips of *Vicia faba* induced by caffeine could be reduced in the presence of guanosine (see Biesele, 1958). However, it has not been confirmed by other workers. Kihlman (1956) was unable to prevent 8-Ethoxy caffeine induced chromosome breakages in *Vicia faba* with the addition of guanosine but he succeeded the same with the use of equimolar adenosine. This has been explained by Kihlman (1966) that the blocking effect of adenosine or caffeine on ethoxycaffeine
might result from the formation of molecular complexes. Caffeine was found to be more effective antagonist.

Besides the chromosome breaking effect, the action of caffeine has been tested on the spindle structure. Nakahara (1952) found that caffeine has no action on the elongation of spindle in spermatocytic cells of grasshopper but it could prevent the early anaphase movement of chromosomes. However, the inhibition of anaphasic movement by caffeine did not prevent cleavage furrow. It was said to be controlled by the mitochondrial structure (Nakahara, 1952). Other workers reported that caffeine arrested mitosis in metaphase and there was also failure in cytokinesis. Gosselin (1940) reported that pea and oat root tips when treated with 0.1% caffeine for 1 to 6 days, some inhibition of cell wall formation, binucleate cell and giant spindle were produced. Mangenot and Carpentier (1944) studied the effect of caffeine on the root tip of onion and wheat and reported that it did not produce C-mitosis. However, some chromatin bridges between the daughter nuclei were formed. Kihlman and Levan (1949) found with this chemical that its treatment supresses the cell wall formation and the stronger concentration even showed the tendency of the induction of C-mitosis.

Caffeine has not only been tested for their action on chromosome or on cell division but it has also been applied to the study of the gene mutation. Webb and Kubitschek (1963) have shown that caffeine can increase the mutation frequency.
in *E. coli*. This property was suspected to be connected with its ability to decrease the temperature for denaturation of DNA.

Although the chromosome breaking activity of caffeine has been tested in some plant materials, so far as the present author is aware similar studies on animal chromosomes are still lacking. The present work would partially fill up the lacuna.

**EXPERIMENTAL PROCEDURE**

0.25 ml. of 0.1% and 1% aqueous solution of caffeine was injected separately into the each of the experimental mice. The specimens were sacrificed at 4, and 24 hour after the treatment.

**RESULTS**

**Effect on Chromosomes:**

**Qualitative:** The treatment of caffeine practically did not induce any physiological effect on chromosomes. It mainly produced chromatid aberrations of simple nature (Fig. 16A-H, Plate IV, Pho. 21). An analysis of 30 chromatid aberrations in different chromosomes revealed 17 in the middle (Fig. 16C, D,E,F,G,H,), 12 in the distal (Fig. 16A,B) and 1 in the proximal region of the affected chromatids. The frequency of breaks in the proximal region of the chromatid was significantly low. Further, all the affected metaphases did not contain more than 1 chromatid break in a complement. The broken part of the chromatid was found to be situated mostly nearabout
its original position (Fig. 16 A,B,D,E), but in some cases it was lying further apart (Fig. 16C, F,G). A critical examination further revealed that out of these 30 breaks obtained from the examination of 800 cells, 8 were in the longer chromosomes. In these long chromosomes 6 were located in the mid-region of the affected chromatid. Thus the longer chromosomes were affected relatively more and their mid-region was most susceptible to break than any other region. Besides the chromatid breaks, not a single instance of chromosome or exchange type aberration was observed.

Quantitative: The frequency of chromatid breaks in the material fixed at two different hours with the use of different concentrations has been studied (Table 11).

**TABLE - 11.**

<table>
<thead>
<tr>
<th>Solution</th>
<th>4 hour fixation</th>
<th>24 hour fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cells</td>
<td>No. and % of Chromatid breaks</td>
</tr>
<tr>
<td>0.1%</td>
<td>200</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>1.0%</td>
<td>200</td>
<td>7 (3.5%)</td>
</tr>
</tbody>
</table>

An analysis of the data (Table 11) would show that the increase in the frequency of chromatid breaks at 4 hour for the use of 0.1% to 1.0% concentrations was very negligible. It was also the same at 48 hour. The ten times difference in the higher concentration yielded only an increase of 0.5%.
aberrations at 4 hour and 1.5% at 24 hour. The frequency of
breaks at two different times of fixation for a particular
concentration has also been found to differ slightly. In
0.1% solution the increase was 0.5% from 4 to 24 hour and
in 1.0% solution it was 1.5%. On the whole higher concentration
with late fixation yielded highest frequency of breaks (5%).
Since the data was too meagre, we refrained from making further
analysis.

**COMMENTS**

In the present study the effects of caffeine has
been tested for its chromosome breaking properties. It was
found to be rather milder in comparison to most other chemicals
studied before by the present author. The aberrations were
only of chromatid type. In the report on the chromosome
breaking activity of caffeine Kihlman and Levan (1949) did not
mention whether the breaks in their materials were localized
or not. In the present study even with the meagre data it
was rather evident that the longer chromosomes were more
susceptible to break due to the action of caffeine. The breaks
were also located mostly in the mid region of the chromatids
concerned. It was really not known what caused to induce this
specific type of effect. Since only chromatid type breaks
have been observed, it could be said that the caffeine acted
during the condensation process of the chromosomes after
the onset of division or atleast after the reduplication of
chromosome. The localized breaks observed in the present study
could be explained in one way as due to the specific action of caffeine on purine bases specially on adenine. In the affected regions A-T pairs could have been present repeatedly and the occurrence of localised breaks could be suspected in the same way as the BUdR induced localized breaks explained by Somers and Hsu (1962). However, such an explanation may not be valid when we consider similar breaks produced by other chemicals in the present series. The localized breaks seem to have been produced due to some mechanical reason rather than some localized biochemical action. It was thought so because these regions were also affected by other odd type of chemicals and X-rays (vide infra). However, it remains still paradoxical why the two chromatids in the identical region were not affected?

Kihlman (1966) has discussed the chemical, physiological and biochemical properties of some chromosome breaking agents which are likely to be responsible for inducing the cytological effect. According to him some chemicals have the properties of (i) inhibiting DNA synthesis and DNA precursors (ii) some degrade and cause denaturation of DNA, (iii) some remove DNA bound metal and (iv) some produce abnormal DNA alkylation and/or incorporation of abnormal precursor. The action of caffeine can be included into the second category. The mechanisms by which caffeine could induce aberration have been discussed by Biesele (1958), and Kihlman (1966). It has been revealed before that caffeine was very likely active in the late interphase, after the duplication of chromosomes. Thus
the chemical was not active during the synthesis of DNA. However, it has some affinity for DNA. Thus if the breaks were due to the involvement of DNA, it must be considered in a different way. This might bring an alteration in the state and the properties of the nucleoprotein block of the chromosome. In support it may be cited that some workers have found that caffeine combines with DNA and alter the physical properties of the latter (Ts' o et al., 1962; Ts' o and Lu, 1964). Caffeine is known to be an efficient solubilizing agent (Weil-Meitherber, 1946; Booth and Boyland, 1953) and that would enable it to form molecular complexes with DNA. It is probably caused by their electron donor properties (Pullman and Pullman, 1958). Kihlman (1952a,b) has made a correlation between the chromosome breaking activity and the solubilizing power of methylated oxypurines.

It has been suggested above that caffeine can denature DNA, and in the present study it appeared that it must has taken place after the chromosome reproduction. However, in the introductory part it has been pointed out that the effect of caffeine can be seen in three different ways - as the inhibitor of cell division, as a poison of the chromosome causing breaks and as a poison for metaphase and other stages. The effect of this chemical at different conditions of the cell must, therefore, be dependent on certain other factors too. It has also been shown that the effect could be modified by the treatment of guanosine (see Kihlman, 1966). Thus it will be fair to consider that caffeine can act on chromosome structure in different ways and different mechanisms may operate according to the physiologic condition of the cell.
EXPLANATION OF FIGURE
(16 A - H)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Caffeine. Only Affected chromosomes Drawn).

(A) A subterminal chromatid break in a group III chromosome.
(B) A chromatid break in the mid-region of a group III chromosome.
(C) A chromatid break in the mid-region of a group III chromosome with the fragment lying apart.
(D) A chromatid break in the mid-region of a group IV chromosome.
(E) A chromatid break in the mid-region of a group III chromosome.
(F) A chromatid break in a group III chromosome with the fragment lying apart.
(G) A chromatid break in the middle of a group I chromosome with the fragment lying apart.
(H)* A chromatid break in the middle of a group II chromosome.
12.

Carbon tetrachloride

So far as the author is aware the chromosome breaking activity of this chemical has not been tested on any cytological material. This chemical is known to be a lipid solvent and, therefore, it was tested to find out its effect on chromosomes.

EXPERIMENTAL PROCEDURE

0.25 ml of concentrated carbon tetrachloride (E. Merk) was injected into each mouse. The specimens could not survive long after the injection. Bone marrow cells were, therefore, fixed only at four hour after the treatment.

RESULTS

Effect on Chromosomes

Two kinds of effect have been observed for the use this chemical. The first one was the erosion effect for which the outline of the chromosome was uneven, and the second one was the chromatid aberrations (Fig. 17 A-H; Plate IV, Pho.20,22). The second type of effect was of simple nature. In an examination of 200 metaphases 9 were found to have chromatid aberration, one in each plate. Most of the breaks were found near about the mid
region (Figs. 17 A, B, E, F, H) and the rest in the distal region (Fig. 17 C, D, G). The fragmented part of the chromatid of the affected chromosomes was mostly lying not far off from its place of origin (Figs. 17 A-D, F, H). Sometimes it was also found to be situated elsewhere (Figs. 17 E, G). The frequency of chromatid aberration determined from the present study was 4.5% which was even higher than those of semicarbazide on caffeine. Rarely some chromosome other than acrocentric type was found but doubt existed about their genuinity (Plate IV, Pho. 20, 22).

**COMMENTS**

There could be no doubt that the carbon tetrachloride induced chromatid aberration was of similar nature as produced by other chemicals under the present study. Further as found with the treatment of other chemicals, the breaks were also somewhat localized in the central and the distal part of the affected chromatids. The longer chromosome seemed to be more affected as found in other cases. Lastly only one of the two chromatids was affected. Thus qualitatively the chromatid aberrations produced by this were in no way different from those of other chemicals. Therefore, no special comments on them is desirable.

The mechanism by which carbon tetrachloride could induce chromatid breaks remained fully unexplored because this chemical was not employed in such type of study by previous workers.
Even biochemical action of this chemical on isolated nuclear constituents has not been tested. Anyhow, chemically its action on lipid is known. Apparently it has not much significance because the lipid is present in the nuclear membrane but traces of lipid are also claimed to be present in the chromosome structure. Its role and importance in the structure of chromosome is totally unexplored. In this situation let us try to explain the probable mechanism of action on the basis of its effect on cellular membrane. The cellular envelope can be dissolved or destroyed by this chemical but how it could affect the structure of the chromosome is not known. But the fact remained that the structure was affected. Therefore, it possibly has acted in some indirect way. The cytoplasmic DNase and RNase are found to be located in the lysosome. Their unnatural release may be caused by the action of carbon tetrachloride on lysosomal membrane. On the other hand it has been shown by MacGregor and Callan (1962) and Gall (1963) that DNase can break the chromosome. These authors have shown that isolated lampbrush chromosomes are fragmented by DNase. DNase has also been shown to be released from microsomes and sRNA as a result of mitomycin C (Kersten, 1962; Kersten et al, 1964). Kihlman (1966, page 185) stated that "It would not be unreasonable to assume that the release of large amount of DNase in the cell at a moment when the chromosomal DNA is unprotected by other substances (the S period ?) would result in chromosomal aberrations". Allison and Paton (1965) also shared the same view regarding the DNase hypothesis.
According to them the production of chromosome aberration in human diploid cells by visible light in the presence of various photosensitizing agents was caused primarily due to their photosensitizing effect on lysosomes. They concluded that damage to lysosome means the release of DNase. It would lead to structural alteration in chromosome by breaking the DNA chain which is the backbone of uncoiled interphase chromatid. They went on further to suggest that some other chemicals such as alkylating agents, maleic hydrazide, etc. produce the chromosomal aberration in the same way, i.e. they release DNase from lysosomes. However, Kihlman (1966) objected to such type of generalization. Effects of odd types of chemicals could be transmitted by various mechanisms, some of which have already been discussed. Anyhow, in the absence of any other evidence the mechanism of chromatid aberrations produced by carbon tetrachloride could possibly be mediated through the release of DNase and it was less so due to its action on chromosomal lipid structure. However, it must be admitted that due attention was not paid to the second mode of action referred to above. The knowledge on the role of lipid in the chromosome structure is practically nil. Thus the possibility of the chromosome aberration induced by the lipid solvent carbon tetrachloride due to its direct action on lipoidal constituent of chromosome structure, however impractical it sounds, cannot be totally rejected. It would be taken as a possible suggestion for future verification.
(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by Carbontetrachloride. Only Affected chromosomes Drawn).

(A) A chromatid break in the mid-region of a group III chromosome.
(B) A chromatid break in the mid-region of a group I chromosome.
(C) A subterminal chromatid break in the distal region of a group I chromosome.
(D) A chromatid break in the subterminal region of a group III chromosome.
(E) A chromatid break in the middle with the fragment lying apart.
(F) A chromatid break nearabout the middle part of a group I chromosome.
(G) A subterminal chromatid break with the fragment lying quite apart.
(H) A chromatid break in a group III chromosome.
Lithium Chloride

Deufel (1951) obtained the chromosome aberration in root tip cells of Vicia when he treated them with this chemical. Lithium chloride is a weak metallic salt. This nature of action may be supposed to cause their chromosome breakages or exchanges. The chromosome breaking activity of salts of heavy metals e.g. tellurium, cadmium, copper, etc. and weakly acting group like zinc, lithium, etc. and the inactive group like sodium, potassium etc. have been found by various authors (Deufel, 1951; Michaelis and Räeger, 1959; 1963; Glass, 1955, 1956; Kihlman, 1950, 1961; Manna and Parida, 1965b; Manna, 1969). Since the effect of lithium chloride has been studied on plant chromosome, the present study was undertaken with a view to finding out if it could also produce similar effect on mammalian chromosomes. Two concentrations like \(10^{-3}\)M and \(5\times10^{-1}\)M solution were used for the experiments. 0.25 ml of each solution was injected into the experimental mouse. However, in an examination of 400 mitotic metaphase plates, no chromosomal aberration was observed. The concentration used in the present study was much lower than the concentration used by Deufel (1951) which induced chromosome aberration in Vicia faba. We tried higher concentration but specimens could not tolerate that dose when the injection of 1.0M solution was administered the animal died almost immediately after the injection. The negative result obtained in the present study does not fully substantiate that the chemical has no
effect in the production of chromosomal aberration in bone marrow cells. The lack of tolerance for the high dose prevented our present study. This needs to be extended for some confirmatory result.
E. EFFECTS OF X-RAYS ON THE CHROMOSOMES OF BONE MARROW CELLS OF MICE

With a view to comparing the nature of chromosome aberrations produced by 12 of the 13 different chemical mutagens referred to earlier in the bone marrow cells of mice with that of ionizing radiations, a set of mice was irradiated with X-rays. The result obtained after the use of whole body irradiation with a dose of 200r have been presented in this part. There was also some other reasons for the present study which will be revealed in the following chapter of the thesis (Chapter II).

A comparative study of chromosome aberrations and the mitotic activity by physical and chemical agents has been made by some workers (Conklin and Upton, 1963; Kihlman, 1966; Russell and Michelini, 1951). The effects of ionizing radiation on chromosomes of plants and animals have been studied by a large number of workers. They have been ably reviewed by different authors from time to time (Sax, 1941; Sparrow et al., 1950; Sparrow, 1951, 1962; Hollaender, 1954, 1960; Bacq and Alexander, 1961; Evans and Sparrow, 1961; Ray-Chaudhuri, 1961a,b; Evans, 1962; Lea, 1962; Borstel and Amand, 1963; Russels and Sylors, 1963; Manna and Mazumder, 1967; Mazumder and Manna, 1967; Manna, 1969). The mammalian cells were treated with ionizing radiations for various types of studies. In this respect rats and mice have generally been used as experimental animals. The problem of
radiosensitivity has been studied on various mammalian tissues. The differential radiosensitivity of the spermatogenic cells were studied by Snell (1935, 1941); Hertwig, (1938, 1941); Henson (1942); Monesi (1962); etc. in the monkey, Maccaca mulata (Bakulina and Orlova, 1963) certain types of spermatogonia was more radiosensitive than others. Beaumont (1962) studied the stage sensitivity of oogonia and oocyte cells in the ovary of rat. Radiosensitivity with regard to the volume of chromosome has been worked out in tumor by Marshak (1937, 1939).

The qualitative and the quantitative studies on the chromosome aberrations in bone marrow cells induced by whole body irradiation have not been extensively done. Sharman (1959) has studied the bone marrow chromosome aberrations in marsupials due to whole body irradiation. The X-ray induced chromosome aberration has however, been studied more widely in different mammalian tissue culture cells (Bender and Gorch, 1961; Bender, 1964; Gorch and Bender, 1963) inducing human leucocyte culture (Bell and Baker, 1962; Ohnuki, Awa and Bomerat, 1961). The chromosome damages caused due to therapeutic roentgen treatment have also been studied by some workers (Lindergren and Norryd, 1963).

**EXPERIMENTAL PROCEDURE**

Mice of the age of three to four months weighing about 23 gms. were subjected to X-rays of a dose of 200r. The required dose was administered in 7 seconds and the specimens
were sacrificed at 4 and 24 hour after the irradiation for the preparation of bone marrow chromosomes. The radiation dose was obtained from a 100 KV, 11.2 mA X-ray machine with an output of 1667r per minute.

RESULTS

Effect on Chromosomes

Control Series - Non-irradiated mice injected with 0.04% colchicine solution one hour before the scheduled time of fixation served as the controls. The colchicine solution was injected both in control as well as in treated specimens for better cytological preparations. In the control specimens an examination 1000 metaphase plates revealed only 2 break type aberrations. This negligible frequency (0.2%) is to be deducted from the value obtained in the irradiated series to obtain the net value.

Irradiated Series - As expected the X-ray treatment produced some physiological effect on the chromosomes which appeared mostly in the form of stickiness and despiralization in the material fixed at 4 hour. However, the primary physiological effect was absent at 24 hour. In the present study we have concentrated more on the break type aberrations. The break type aberrations have been classified into three categories e.g. chromatid breaks, chromosome breaks
(Plate V, Pho. 30) and exchange type aberrations (Plate V, Pho. 26, 27, 28, 29). In the present study 47 out of 250 metaphase plates (18.8\%) were found to contain break type aberrations. Most of the aberrations were of chromatid type (Fig. 18A-I). Out of 47 metaphases 37 had only one chromatid aberration in each of them (Fig. 18 A-E), 4 had 2 (Fig. 18F-I). Metaphase plates with more than one type of aberrations was rare (Fig. 18 L). Besides the chromatid breaks, very rarely a translocation (Fig. 18) or a ring chromosome was observed. In 47 metaphases with aberrations not a single instance of chromosome or isochromatid breaks or two chromatids broken in non-identical regions of a chromosome or a chromatid of a chromosome broken at more than one place was encountered. The breaks in the chromatids were localized more in some particular regions. When they were grouped into three regions, it was found that out 43 simple chromatid breaks in different chromosomes 22 were in the middle (Fig. 18B,G), 14 in the distal (Fig. 18A,F) and 7 in the proximal region (Fig. 18C,D). Further, out of these 43 chromatid breaks 10 were located in the longer chromosomes and they were broken in 5 cases at the middle, 3 cases at the distal and 2 cases at the proximal region of the chromatid. In the present study out of 47 cells with aberrations only in four cells had more than one type of aberrations (Fig. 18 K,L). Some affected metaphases contained an extra fragment along with a translocation (Fig. 18K,L or a chromatid break (Fig. 18 I) or with both (Fig. 18 L). As found with the chemical treated series, the fragment of the
chromatid either remained almost to its place of origin (Fig. 18 C,F,G,H) or somewhere else (Fig. 18 A,B,D,E,H,I). Sometimes the fragments was so much displaced that its place of origin could not be traced.

Table - 12
Frequency Distribution of Chromosome Aberration in Bone Marrow Cells of Mice Treated With 200r of X-rays and Fixed at 4 and 24 Hour.

<table>
<thead>
<tr>
<th>Hour of Fix.</th>
<th>No. of Meta.</th>
<th>Number of Affected Chromosomes with Type of Aberr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single Chromatid break</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>24</td>
<td>150</td>
<td>10</td>
</tr>
</tbody>
</table>

An examination of the data (Table 12) would reveal that at 4 hour out of 100 metaphases examined, 24 cells contained one chromatid break in each plate, and 4 plates contained 2 chromatid breaks, one in each chromosome of each plate. There were six cells with translocation and 5 cells with 6 extra fragments. If the translocations were considered as two breaks and the simple chromatid break or the extra fragment as one break, the total number of breaks at this hour would be 50.
The frequency of chromosome aberrations produced by the same dose and examined at 24 hour has been found to be greatly reduced. It was only 16 effective breaking points out of 150 cells examined. Thus a good deal of restitution must have taken place with the lapse of time.

**COMMENTS**

As indicated before that it was not the intention of the author to study the mechanism of ionizing radiation in the production of chromosome aberrations in bone marrow cells. It was planned just to make a comparison of the aberrations produced in bone marrow chromosomes by chemical and physical agents. It would reveal from the present study that there was a good deal of similarity between the types of breaks produced by X-ray and the chemical mutagens. However, the frequency was much higher in the irradiated series than that of the chemically treated series. This was quite natural because the energy behind the X-ray photons was much higher than that of the chemical reaction. As a result of indirect action of X-rays in tissues, hydrogen peroxide is ultimately produced which is considered to play an important role for the induction of mutation and aberration (see Lea, 1962). The chemically active radicals produced by X-rays have much higher energy than the reactive groups of the chemical mutagens. Moreover, the amount of damages would also vary in chemical mutagens because they might act
differently. These have already been discussed in the previous part. Anyhow, we are not in a sound position to compare the frequency of chemically induced breaks and the breaks produced by X-ray since the equivalence of doses of the two kinds of mutagens could not be determined. But we would surely make a precise comparison of the qualitative effects produced for the treatment of X-rays and chemical mutagens.

Kihlman (1966) has made some comparative study of the X-ray and chemically induced chromosome aberrations. He pointed out some striking differences. At first he compared the effects of alkylating agents with X-rays. He found that the (i) alkylating agents had only the delayed type of effect whereas X-ray induced breaks were non-delayed in occurrence, (ii) The delayed effect of alkylating agents was manifested mainly in the form of chromatid aberrations while X-radiation induced subchromatid exchanges in prophase, chromatid aberrations in the middle and late and chromosome aberrations in early interphase, (iii) Chemical induced aberrations might be localized but it was not the case with X-rays, (iv) Chemically induced breaks were non-randomly distributed between nuclei but it was less so with X-rays, (v) Effects of alkylating agents were oxygen independent but X-ray effects were oxygen dependent. However, Kihlman (1966) also pointed out that chromosome breaking chemicals represented by alkylating agents when compared with X-rays cannot be taken as typical because EOC induced breaks were more like X-rays as it produced (a) non-delayed type of effect, (b) induced sub-chromat
exchange in prophase, chromatid aberration in the middle and late and chromosome aberration in early interphase. Moreover the effect was oxygen dependent. The only difference found was the non-randomness in the distribution of breaks within and between chromosomes.

It has been suggested by Kihlman (1966) that X-ray induced breaks in chromosomes are produced due to the actions on proteins. The chemical mutagens, on the other hand, mostly act on the DNA strands of the structure of chromosomes. This suggestion is debatable. The present data have indicated several modes of actions. If the quality of breaks induced by X-rays and chemical mutagens at different fixation hour is compared, it will be found that the types of break remained more or less the same. The difference in the types of aberrations produced by different chemicals under the present study was also negligible. It was quite surprising that in both types of studies with the chemical and physical agent the chromatid type breaks were most common. The exchange type aberrations were relatively rare in X-ray series and it was practically absent in most cases of the chemical series. Isochromatid and chromosome breaks were practically absent in both the series. The absence of chromosome type breaks could be explained in an unusual way. We might assume that the cells at G1 stage of DNA synthesis in the interphase nucleus affected by the chemicals and the X-rays of the present study did not enter mitosis at all. This effect prevailed for a long time.
The recovery could not take place within the time interval used for the fixation of the tissue. In support of the above assumption it may be cited that all the antibiotics and DNP under present study showed an inhibitory action on cell division. The inhibitory action of X-ray has also been claimed but it has not been widely accepted. However, we do not think that the complete absence of chromosome type breaks could entirely be due to the complete inhibitory effect on those cells in which the reduplication of the chromosome did not take place. On the other hand all the chemicals which were tested for inhibitory action, recovery was observed after 24 hour. The absence of chromosome break in X-ray series was all the more peculiar. Of course in general the frequency of chromatid breaks might be higher, but the complete absence of chromosome type breaks have not been encountered in any other material. In a study of the X-ray induced chromosome breaks in grasshoppers (Manna and Mazumder, 1962, 1967; Mazumder and Manna, 1967) both chromosome and chromatid type breaks were observed. Amongst them the frequency of chromatid type breaks was much higher. Thus we cannot really explain what caused almost the complete disappearance of chromosome type breaks in our present material. If the frequency happened to be very low, they might not have been encountered because the data were not sufficient. Anyhow, there could be little doubt about the major role played by the inhibitory action for the absence of chromosome type breaks in the present study.
The present materials when treated with different chemicals and X-rays, mainly a single chromatid break per affected metaphase plate was very common. In the irradiated tissue of course there were some cells each with more than one breaks. The same was true for phenols used in the present study but the frequency was lower than that of X-rays. Thus on the whole in both cases, a similarity was observed.

The X-ray data were also in agreement with that of the chemical treated series because the frequency of aberration dropped down with the enhancement of the fixation time after the treatment. This could be explained either in terms of restitution of broken ends (Sax, 1941) or the exchange initiation stage did not lead to break the chromosome (Revell, 1955). It could also be thought that many damaged cells did not survive to complete the division.

A comparison of the exchange type aberration produced by the different chemicals and by the X-ray would indicate that those produced by X-rays were more convincing and varied types than other. However, it was beyond doubt that some true exchange type aberrations were also produced by some of the chemicals which were of similar quality as those produced by X-rays. Since the frequency of multiple breaks in a cell was negligible, the exchange type aberration was either absent or the number was insignificant. Besides this the question of time and space would also arise in the origin of this type of aberration. Thus the difference in the data obtained for the treatment of physical and chemical agents were not at all unexpected.
It was very interesting to note that the regions of breaks in the chromatids were mainly localized in the middle or distal regions for the use of chemical and X-rays. In both types of agents it was further found that the longer chromosomes were more susceptible to break than others. Of course, we have not been able to locate any specific spot because of some of the inherent difficulties existing in the chromosomes of mice used for the present study. A gross examination has, however, revealed that both chemical and physical agents produced chromosome aberration mostly in the mid-region or in the distal segment. Breaks were more prevalent from mid-region to the half of the distal region than in the other sites. Centromeric region and its vicinity was found to be more stable to the action of X-rays or chemical mutagens because the frequency of breaks in this region was relatively very low. It was not clear why the breaking points were non-randomly distributed in both the types of treatments. It is logical to consider that since the breaks produced by X-rays and chemical mutagens were due to different mechanisms, the regions of breaking point would naturally differ unless some common factor has a greater influence. The non-randomness in the distribution of breaks induced by various chemicals could, in a way, be thought as due to the non-random distribution of the chemical constituents in the chromosomes, with which they selectively reacted. Kihlman (1966) has discussed some reasons for the non-randomness in the breaks induced by some chemicals. A few of them have been referred to earlier. However, a similar explanation could not be suggested
for the non-random distribution of breaks in our material because chemicals of unrelated groups induced similar kind of non-random breaks. We have tried to explain it on the basis of some physical phenomenon. In the present study we have assumed that some weaker spots in the chromosomes of mice might be present. They would naturally be more susceptible to break than other places. Whenever, any disturbance was caused due to the treatment of physical or chemical agent, these weaker regions would be first to break. In making this generalised statement, the author is quite aware of its pitfalls. Therefore, critical study is needed to arrive at a conclusion. However, the present data have given sufficient indication to pursue this line of approach. Some sort of localized breaks in the X chromosome of grasshopper induced by the treatment of some chemicals and X-rays was also reported by Manna (1969) and Manna and Mazumder (1962, 1967). The presence of weaker regions in the chromosomes is not very unexpected but their demonstration in normal individual would be a very difficult task.

It has been pointed out before that some particular group of chromosomes was more susceptible of break for the treatment of X-rays and chemical mutagens. It was mainly the group I according to the classification of caryotype made by Crippa (1964). Since the groupings are not very well defined, in the present some less ambiguous groups or marker chromosomes were taken into consideration. The first four long chromosomes of group I and the "rabbit-ear" chromosomes belonging to
shown by Manna and Mazumder (1968). But in the present study since we have seen the response of X-rays and odd types of chemical mutagens was the same, we are in favour of explaining the fact in terms of weaker spots in the chromosomes which has been discussed earlier.
EXPLANATION OF FIGURE
(18 A - L)

(Metaphase Chromosome Aberrations in Bone Marrow Cells of Mice Induced by 200r X-rays. Only Affected chromosomes Drawn).

(A) A subterminal chromatid break with the fragment lying apart.
(B) A chromatid break in the middle of a group II chromosome with the displaced fragment.
(C) A chromatid break in the proximal region of a group I chromosome.
(D) A chromatid break with the highly displaced fragment.
(E) A chromatid break in the middle of a group II chromosome.
(F) A chromatid break in a group II chromosome.
(G) A plate with two chromatid breaks in two chromosomes.
(H) A plate with two chromatid breaks in two chromosomes of which one fragment is lying apart.
(I) A plate with two chromatid break both the fragments lying apart.
(J) A metacentric chromosome due to translocation.
(K) A translocation and a fragment.
(L) A translocation, a chromatid break and a fragment of unknown origin.
SUMMARY

1. Controls

As controls of different treatments the chromosomes of three sets of untreated mice as given in the following were examined, (1) Specimens were injected with an amount of distilled water which was used as solvent of the chemical in the treated series, (2) specimens were injected with 0.04% colchicine solution at the rate of 1 ml/100 gms. body weight one hour prior to the fixation of the tissue and (3) the specimens were injected with the known quantity of distilled water and then one hour before fixation they were again injected with 0.04% colchicine solution. The bone marrow cells of the control specimens were fixed at 4, 8, 24 and 48 hour and studied with regard to (i) the mitotic index and (ii) chromosome aberrations. Mitotic index in 2000 bone marrow cells of 4 control series 2, fixed at 4, 8, 24 and 48 hour was determined. They were 2.75%, 2.60%, 2.90%, and 2.70% respectively. Further chromosome aberrations in 2000 metaphases from control series 3, of which 500 were taken from each fixation hour were determined. In 2000 metaphases 1 chromosome break, 2 chromatid breaks and 4 gap type aberrations were found. The control data have been presented in a separate section to avoid repetition.

2. Actinomycin D (AMD)

Mice were injected individually with 25 µg of AMD. They were sacrificed at 4, 8, 24 and 48 hour after the treatment.
for taking out the bone marrow cells for cytological preparation. Another set of mice were also individually injected with 125 μg AMD. They were fixed at 4 and 8 hour because they did not survive beyond 8 hour.

The mitotic index of the treated specimens was determined at 4, 8, 24 and 48 hour from 2000 cells in each fixation time. In AMD treated series inhibitory effect was quite appreciable at 4 hour (1.4%) and it reached the minimum at 24 hour (1.0%). It recovered to normalcy at 48 hour (2.6%).

The AMD induced breaks were mainly chromatid type. Some amount of despiralization effect was also found. No chromosome type break was encountered and the frequency of exchange type aberration was negligible. An analysis of the distribution of chromatid breaks in different regions of the affected chromatids has been made. The middle and the distal regions were found to be most vulnerable because out of 99 chromatid breaks 35 were in the former and 55 in the latter region. The frequencies of chromatid breaks at different fixation hour as well as in different concentrations was determined. The increase of the aberration frequency at a higher dose was disproportional. The frequency reached the maximum at 24 hour in 25 μg in treated series.

Discussions have been made on the mechanism of inhibition of cell division and chromosome aberrations induced by AMD.
The action of AMD might cause alteration of the properties of DNA protein complex. It is known to affect the DNA dependent protein synthesis at lower concentration and at higher concentration even affect DNA synthesis. The action of AMD on the chromosome in the present study has been compared with other data. The origin of the different types of aberration has also been discussed. Some weaker regions of the chromatids have been suspected to be broken to give rise to the localized form of breaks.

3. Na-Novobiocin (NB)

1.25 mg of NB was injected intraperitoneally in each mice. The specimens were sacrificed after 4, 8, 24 and 48 hour for the cytological preparations of the bone marrow cells.

The chemical was found to have inhibitory action on cell division. The inhibitory effect on the mitotic index was different from that of AMD. But the mitotic index was higher at 4 hour but it came down to minimum at 24 hour. At 48 hour the frequency was approaching close to the frequency of the control series.

The treatment of NB produced mainly the chromatid type breaks. The breaks were simple and not more than one was present in a chromatid. They were occurring in the localised regions, mostly in the middle and the distal regions. Chromosome type and exchange type aberrations were absent. The fragment
of the chromatid was found to be situated in various positions. The frequency of chromatid aberrations was determined from 750 metaphases and it was higher at 24 hour (7.5%) than at 4 hour (2.4%) or at 48 hour (2.8%). A comparison of the chromosomal effects produced by the treatment of NB has been made with that of AMD.

Discussions on the inhibitory action and mutagenic effects on bone marrow cells have been made. Inhibitory action has been suspected as due to its effect on nucleic acid synthesis. Along with it other possible mechanisms have also been considered.

The mechanisms of chromosome aberrations induced by NB as presented by different workers has been considered. Their views were different. The effect of NB in the production of chromosome aberration was considered to be mainly dependent on DNA. It had no marked effect on protein framework of the chromosome. The breaks produced by NB have also been suspected for its action on metal ions. According to some authors NB inhibited certain magnesium dependent enzyme system in DNA polymerisation. This was supported by the reduction in the frequency observed for the exogenous Mg++ supply. Besides the mechanisms, the nature of aberration, the question of localisation etc have also been considered.

4. Dihydrosreptomycin (DHSM)

2.5 mg and 7.5 mg of DHSM were injected into two sets
of mice and the specimens were sacrificed at 4, 12, 24 and 48 hour after the treatment. The effect of DHSM on mitotic index and on chromosome has been studied.

The inhibitory action of DHSM was less pronounced as compared to that of actinomycin D. The mitotic frequency reached the minimum at 24 hour (1.8%). At 48 hour it regained almost the normal frequency (2.7%). At 4 hour to some extent the inhibitory effect was also present (2.4%). The results of the inhibitory effect of three antibiotics AMD, NB and DHSM have been compared.

The effect of DHSM on the chromosomes were of two types - sticky effect and the chromatid type breaks. No chromosome type of exchange type aberration was encountered. Chromatid breaks were localized mostly in the distal and the middle regions of the affected chromatids. The fragments were found to be variously situated. 400 metaphase complements were analysed for the treatment of 2.5 mg and 7.5 mg DHSM. The percentage of chromatid aberrations did not vary much in two concentrations. They were 6% in 2.5 mg and 7.5% in 7.5 mg DHSM injected specimens.

Discussions have been made on the action of DHSM in causing inhibition of cell division as well as the chromatid aberration. Its various properties like inhibition of protein synthesis, precipitating action on nucleic acids, damaging the cell membrane, and break down of RNA etc. have been considered
to explain its inhibitory action on cell division and the chromosome breaking property expressed in the bone marrow cells. Attention has also been paid on its effect on respiration and oxidative phosphorylation in explaining the chromosome aberration. Its association with ribosome has also been taken into consideration. The chromosome breaking activity has been thought to be due to its indirect action via ribosome. The effect possibly took place at $G_2$ phase. The present result has indicated that DHSM has some effect on nucleoprotein of the chromosome structure. The origin of chromatid aberration, their localization, etc. have also been considered.

A general comment has also been made on the comparative data of the aberrations induced by three antibiotics under present study. The three antibiotics having different mechanisms of action produced some common types of effects in the bone marrow cells of mice.

5. 2,4-Dinitrophenol (DNP)

0.25 ml., 2.5 ml. and 1 ml of saturated solution of DNP were injected in different sets of mice and they were sacrificed at 24 hour after the treatment. The inhibitory action of cell division and chromosome breaking activity of DNP on bone marrow cells have been studied.

The inhibitory action of DNP was quite striking and it was maximum at 24 hour. The data have been compared with the
antibiotic treated one.

The effect of DNP on chromosomes was manifested mainly in the form of chromatid breaks and a few exchange type aberration. The effect was more severe than that of antibiotics. Chromatids with only one break were most common but sometimes chromatids with two or more breaks were also encountered. The breaking points in the chromatids have been analysed and they were localized in the middle and distal region. The quantitative data on the frequency of chromatid breaks were scored from 770 metaphase complements. The frequency was found to increase disproportionately with the increase of the doses. The chromatid breaks were 6.9%, 14.5%, 17.2% for the treatment of 0.25 ml, 0.5 ml and 1 ml. saturated solution of DNP respectively. The break per chromosome was also increased. It was 0.0018, 0.0048 and 0.0057 respectively for the use of three doses.

The inhibitory effect of DNP on cell division is thought to be due to its action on the oxidative phosphorylation. Various biochemical processes have been considered to explain the possible mode of inhibitory action of DNP. The mechanism of inhibition has been compared with those of antibiotics. Similarly the different biochemical changes likely to be involved in the production of chromosomal aberration due to the treatment of DNP have been considered. The effect was antimetabolitic in nature. The action of DNP was no doubt an indirect one.
Discussions have also been made on the production of different types of aberrations, localized chromatid breaks etc. The effect has been compared with the chemical and it was found to be very severe.

6. **p-aminophenol**

0.01M and 0.02M of freshly prepared aqueous solution of p-aminophenol was injected into mice and they were sacrificed at 24 hour after the treatment.

The treatment of p-aminophenol produced mainly the chromatid breaks. The effect was not very severe. The breaks were found in some of the localized regions of the chromatids and fragments were situated in various conditions. In 970 metaphases, chromatid breaks were 3.50% and 7.11% for injection of 0.25 ml and 0.5 ml of 0.01M solution respectively. It was 8.75% for the injection of 0.5 ml of 0.02M solution.

Discussions have been made on the oxidizable property of the chemical as the source for inducing the chromosome aberration. Since this compound is readily oxidizable the chromosome breaking activity would be more common after the treatment. The result of the present study has been compared with DNP. The nature and location of chromatid break have also been considered with that of other chemicals.
7. *Pyrogallol*

0.25 ml of each of 0.01, 0.02 and 0.03M aqueous solution was injected into the mice and the specimens were sacrificed at 24 hour after the treatment.

Qualitatively the effects of pyrogallol on the chromosomes were manifested in the form of stickiness and chromati breaks. Translocations and the ring chromosomes were very rarely present. The distribution of breaking points in the different regions of the affected chromatids has been analysed. The fragments were found to be situated in various positions. A total of 450 metaphases was analysed. The frequencies of the chromatid breaks did not differ much for the treatment of different concentrations of pyrogallol. They were 4.7% and 6.6% in 0.02M and 0.03M respectively. No chromatid breaks was encountered for the treatment of 0.01M solution.

Different mechanisms in the production of chromosome has been explained as due to the depolymerisation of the nucleoprotein of the chromosome structure. The chromosome breaking activity of the chemical has been considered as due to its oxidizable nature. Some of the oxidizable products of pyrogallol like CO, CO₂, acetic acid, oxalic acid, etc. with other things could be responsible for the induction of chromosome breaks. Discussion has also been made as to the localization and the nature of breaks and their origin.
8. **Gallic Acid**

0.25 ml of 0.02, 0.03, 0.04 and 0.05M solution were injected into different individuals and the specimens were sacrificed at 24 hour after the treatment.

Qualitatively the effect of gallic acid on the chromosome of bone marrow cells was in the form of stickiness, good amount of chromatid breaks and few subchromatid breaks. No chromosome type breaks and the true translocation were encountered. The chromatid breaks were located mainly in the middle and the distal part of the chromosomes. One fourth of the total breaks was present in the longer chromosome. 2000 metaphases were examined and the frequencies of chromatid breaks were 4.5%, 15.0%, 5.2%, 2.8% due to the treatment of 0.02, 0.03, 0.04 and 0.05M solution respectively. The maximum effect was encountered for the treatment of 0.03M solution.

The mechanism of chromosome aberration was discussed in the light of its powerful reducing property. It has been assumed that this property of the chemical produced some effect on the redox potential in cellular metabolism. Therefore, the chromosome breaking activity of this chemical was possibly due to its antimetabolitic action. The frequency of breaks at different concentrations, the localization of breaks etc. have also been discussed. The maximum amount of chromatid breaks was found at 0.03M as compared to other lower or higher concentration. Some suggestion has been made on the result.
A general consideration on the phenol induced chromosome breaks has been made. The effect has been generalized as inhibition of cell division, chromosome poison and spindle poison. The effects in relation to DNA synthesis have been considered. The chromosome aberration produced by the three phenolic compounds have been compared and their similarities have been mentioned. The localized breaks produced by them have been explained as due to weaker spots in the chromosome.

9. Hydroxylamine (HA)

$10^{-3}$, $10^{-2}$ and $10^{-1}$M solutions at the rate of 0.25 ml were injected separately into different specimens and they were sacrificed at 24 hour, 48 hour and 72 hour after the treatment.

The effect of HA on the chromosomes were mainly in the form of chromatid type breaks and centromeric dissociation. Some multiple configuration was also observed. No chromosome or isochromatid break was encountered, but the same chromatid was sometimes broken in two places. Some metaphases contained more than one aberration. The breaks were localized mostly in the middle and then in the distal region of the affected chromatids. The fragments were placed in various positions. 450 metaphases were analysed for the aberration frequency study. It was evident that the centromeric dissociation type of aberration was more than the chromatid break type aberration. The frequencies of the aberrations were found to be higher in the
higher doses but the increase was disproportionate. Further, the aberration frequency was more at 48 hour than that of 24 and 72 hour.

Discussions have been made on the centromeric dissociation in the light of the claims made by other workers. It has been argued that the centromeric dissociation was not the centromeric break and it was early separation of the structure. The mechanisms of action of HA have been critically evaluated. The action of HA on cytosine moieties of DNA has been considered to be less attractive than on the main chain seission on DNA. No specific action of HA in the centromeric region could be substantiated in the present study. It was not applicable to explain the chromatid breaks localized more in the mid-region of the affected chromatid. It has been considered that the localized breaks were not due to the specific chemical reaction with HA but due to some other mechanical reason.

10. Phenyl hydrazine (PH)

0.25 ml of 0.01M solution of PH was injected into mice and they were sacrificed at 24 and 48 hour after the treatment. The effect of PH on the chromosome was mainly the chromatid type breaks and some centromeric dissociation. The effect was less severe than HA. The breaking points of the chromatids were analysed and they were more in the mid-region. In 400 metaphases the frequencies of chromatid breaks were 3% and 2% and the centromeric dissociations were 4.5% and 2.5% at 24 and 48 hour respectively.
The mechanism of chromosome aberration produced by PH has been discussed in the light that it has a specific action on uracil. It perhaps acted indirectly by impairing the RNA synthesis which ultimately caused the chromosome aberrations. The action of PH has been compared with HA. Discussions have also been made on the origin of localized breaks in the chromatids.

11. Semicarbazide (SC)

0.25 ml of 0.01M solution of SC was injected into mice and they were sacrificed at 24 and 48 hour after the treatment. The effect of SC on chromosome was mainly the chromatid type breaks. The breaks were simple and localised more in the mid-region of the affected chromatid. The fragments were variously situated. No chromosome or exchange type aberration was found. 400 metaphases were analysed and the frequency of the chromatid break was very low. It was 4% and 2.5% at 24 and 48 hour respectively.

The mechanism of action of SC in chromosome breakage has not been worked out by previous workers. Since the chemical nature of SC is like that of two substituted amides like HA and PH, the possibility of its similar mode of action has been considered. But the action of SC on DNA base is not known at present. Some possible mechanism on the localized chromatid aberration has also been considered.
12. Caffeine

0.25 ml of 0.1% and 1% solution of caffeine were injected into different specimens. They were sacrificed at 4 and 24 hour after the treatment. The effect of caffeine on chromosomes was mainly the chromatid type breaks as found with other chemicals. The breaks were localized mostly in the mid-region of the affected chromatids. The fragments were situated at various positions. Not more than one break was found in a chromatid. No chromosome or exchange type aberration was observed. The aberration frequency was studied from 800 metaphase plates. In this study the frequency of the chromatid breaks was also not very high. The frequencies of breaks for the treatment of 0.1% solution were 3% at 4 hour and 3.5% at 24 hour and with the treatment of 1% solution they were 3.5% and 5% at 4 and 24 hour respectively.

The mechanism of production of chromosome aberration by caffeine has been considered. Since caffeine is a purine compound it probably formed a complex with DNA in causing the aberrations. Other possibilities have also been discussed. The solubilizing property of this chemical which might be responsible for induction of chromosome aberration has been examined. Besides this aspect, discussions have been made with regard to the localized breaks caused by the chemicals.
13. **Carbon tetrachloride**

0.25 ml of concentrated carbon tetrachloride was injected into mice and they were sacrificed at 4 hour after the treatment. The effect of this chemical on chromosomes was mainly the chromatid type breaks. Analysis of the breaking point and the position of the fragments have been accounted. No multiple break in a chromatid or isochromatid break was found. An examination of 200 metaphase plates revealed that the frequency of chromatid breaks was only 4.5%.

It has been suggested that as this chemical has very high lipid dissolving property, it could cause some injury to the membrane of the cytoplasmic organelle. It might have damaged the lysosomal membrane thereby releasing DNase. The enzyme DNase might have induced the chromatid break acting upon DNA strands of chromosome. Other possibilities have also been discussed. But the actual mechanism of chromatid breaks has not been clearly understood at present. Discussions have also been made with regard to the localization and the nature of break.

14. **Lithium Chloride**

0.25 ml of 10^{-3} and 5x10^{-1}M solution was separately injected into different mice and they were sacrificed at 24 hour after the treatment. An examination of 400 metaphase complements did not reveal any type of chromosomal aberration.
The reason for obtaining the negative result has been considered.

15. **Effect of X-rays on chromosome**

3 to 4 months old mice were given a X-ray dose of 200 r in 7 seconds. They were sacrificed at 4 and 24 hour after the irradiation. Non-irradiated specimens injected with 0.04% colchicine one hour before fixation served as controls. The bone marrow tissues of control and treated specimens were obtained for cytological preparation. 1000 metaphase complements were examined from the control material, which revealed only 2 breaks in the chromosomes. The effect on the chromosomes due to whole body irradiation was mainly chromatid break, few translocations, fragment of unknown origin etc. Not a single clear chromosome type break was encountered in 250 metaphase complements examined. Out of 43 simple chromatid breaks, 21 were in the middle, 15 in the distal and 7 in the proximal region. Again out of 43 chromatid breaks, 10 were in the longer chromosome. It was evident from the quantitative data that at 4 hour the breaks were more frequent than at 24 hour.

The frequency of chromosomal aberration was much higher in the X-ray series in comparison to the chemically treated series. A comparative study of the X-ray and chemically induced aberration has been made. The indirect action of the X-rays in the product of chromosome aberration has been considered. In the chemical
treated series the effects were produced due to their action on nucleic acid, nucleoproteins or linkers, of the chromosome structure which reached there directly or indirectly in various ways. The nature of the effects of some chemicals on chromosome has been critically compared with that of X-ray.

It was very surprising that in both the treatments only the chromatid breaks were most prevalent. Because of this fact it was assumed that the breaks were affected after the duplication of the chromosomes. The production of different types of aberration by the physical and chemical agents has been compared. Special emphasis were given in the analysis of the localized chromatid breaks since both the agents produced more or less similar effect. The breaks were predominantly produced in the middle and in the distal regions of the affected chromatid due to X-ray and chemical treatment. This has been taken as a supportive evidence in assuming some weaker spots in the chromosomes which were frequently broken than others.