PART I

A. EFFECT OF DIFFERENT DOSES OF X-RAYS ON THE MITOTIC CHROMOSOMES OF MALE NYMPHS OF LYgaeus hospes

So far as the present author is aware no work on the effects of X-rays on the chromosomes of heteropteran nymphs has been carried out. The present study was, therefore, aimed at to assess the effect on the chromosomes of X-irradiated nymphs of L. hospes. Since the mitotic activity in the nymphs was likely to be more than that of old adult males, the present study might enable us to see the effect of X-rays on mitotic chromosomes after several cell generations. It has been mentioned before that the effect of X-rays on homopteran chromosomes has mainly been studied, in species scale insects having parthenogenetic development (Hughes Schrader and Ris 1941) where mitotic process of division was predominant. This study was directed to verify the centromeric constitution of the chromosomes while later the work of Brown and Nelson-Rees (1961) on same insects was directed to verify the role of paternal chromosomes in X irradiated Lecanoid system of chromosome behaviour. It has also been mentioned that Seshachar et al (1959) made a claim that homopterans may have chromosomes with localised centromere from their study carried out on chromosomes of X-irradiated testes of a homopteran species while Halkka (1966) worked on a homopteran species belonging to the genus Limotettix
where the males were irradiated with X-rays and their testes contained variable number of chromosome fragments after successive cell generations. On the other hand so far only one species of Heteroptera, viz. Q. fasciatus has been studied with regard to the effects of X-rays on the meiotic chromosomes of male individuals only (LaChance and Degrugillier 1969, LaChance and Richard 1937). The study was however directed more on the sterility test although the effect on the chromosomes was also mentioned. None of these workers studied in details the effect of X-rays on the chromosomes at different stages of spermatocytes as has been done in the present study.
MATERIAL AND METHODS

Adult male and female plant bugs, *Lygaeus hesperus* (family Lygaeidae: Hemiptera) were collected from their host plants, *Solanum chineae* (Solanaceae), *Euphorbia hirta* (Euphorbiaceae) and *Vernonia cinerea* (Asteraceae) growing in and around Kalyani, West Bengal. They were kept in the laboratory in insect cages provided with twigs of the host plants as their food for mating. After mating the females laid their eggs in the lower side of the leaf of the host plants in clusters which were allowed to grow till the time of emergence of the first instar nymph. The leaves along with the eggs were kept in petridish moist with a wet filter paper. In the life-history of this plant bug, the development of the egg to adult was seen to be completed within three weeks of which the emergence of the 1st instar nymph from the egg took place in about 3-4 days. The time taken for the development of 2nd instar from 1st instar was about 4-5 days and then 2nd to 3rd instar in about 4-5 days, 3rd to 4th instar in about 3-4 days and the 4th instar to adult in about 48 hours. The 1st instar nymphs after their emergence from the eggs in the petridish were reared in the insect cages provided with food plants. The 3rd and the 4th instar nymphs used in the present study were thus obtained from the stocks reared in the laboratory.
(a) Irradiation of 3rd Instar Nymphs:

Two sets of 3rd instar nymphs kept under the cover of a muslin bag were irradiated from the X-ray machine installed at the Department of Zoology, Kalyani University with a dose of 200 r and after a week of radiation when they already emerged as adults were sacrificed to fix the testes of the males and the ovaries of the females in acetic-alcohol for the cytological studies. Another set of 3rd instar nymphs in a muslin bag were also X-irradiated with a dose of 400r from the same machine and on the 4th day after irradiation when they already emerged as young adult were sacrificed to fix their testes of the male specimens in acetic-alcohol for cytological preparations. In another set the 3rd instar nymphs in the same way were irradiated with the same X-ray dose of 400r and on the 6th day after radiation when they already emerged as adults were sacrificed to fix their testes. The irradiated 3rd instar nymphs were allowed to develop into adult stage firstly because the sexing was difficult at 3rd instar stage and secondly by allowing time to grow after irradiation it could be known if the dose was lethal or not and the affected chromosomes might have completed few cell generations.

Untreated 3rd instar nymphs were reared in the same condition and on becoming adult their testes were fixed at the same time of the treated one which served as the controls.
(b) Irradiation of 4th Instar Nymphs:

A set of 4th instar male nymphs were irradiated by the same X-ray machine with a dose of 400 r and they were sacrificed at six intervals, 5 min, 30 min, 1 hr, 4 hr, 8 hr and 24 hr in that nymphal condition to fix their testes. Untreated 4th instar nymphs were also kept in the similar condition and their testes were also fixed at the corresponding intervals with that of the treated ones mentioned above.

The testes of each of the control and the treated specimens were fixed separately in acetic acid-alcohol mixture (1:3) for variable time from 5 minutes onwards. If the tissues were kept longer in the fixative, they were treated with 45% acetic acid for a minute or two before squashing to make them soft otherwise they were squashed directly. The testes of each individual were squashed on albuminised slides (clean slides rubbed with Myer's albumin and dried) in a small drop of 45% acetic acid with the care that materials did not move out of the limit of the cover glass. The tissue after squashing was feebly warmed up at intervals to dry up gently the excess fluid and to strike the cells to the surface of the slide. After about 30 minutes to one hour of the process of alternate feeble heating and cooling the slides were immersed in a jar containing 50% alcohol and were generally left in it overnight. By this time the cover glass mostly detached automatically into the 50% alcohol jar, if not it was lifted by pushing a sharp
edge of a knife between it and the slide. The squashed material struck to the slide after the removal of the cover glass. The slides with the squashed material were passed through down grades of alcohol to water and were stained mostly in Iron-alum-haematoxylin. Before putting slides into the stain they were mordanted in 3% Iron-alum solution for about an hour. After mordanting the slides were rinsed in water and put into mature 1% haematoxylin stain for about 5-10 minutes. The stained material in the slides were differentiated in saturated aqueous solution of picric acid under a microscope and were thoroughly washed in running water for about an hour. The slides were then passed through different upgrades of alcohol and after complete dehydration in absolute alcohol were transferred to xylol with two changes and finally mounted in Canada balsam or DPX. Since Iron-alum haematoxylin stain was very much dependable and last for any length of time, it was used extremely but as a check very rarely Feulgen stain was used.

Sometimes the irradiated 3rd instar nymphs emerged as adult females, the ovaries of which were fixed in acetic-alcohol and were subjected to temporary acetic-orcein and acetic-carmine squash preparation to study the oogonial chromosomes.

In the testes of the irradiated specimens there were different types of chromosomes aberrations induced by X-rays. In presenting the data all of them were not shown separately so as to minimize the space of the table. Some of them such as gaps and constrictions have been grouped together though
the frequency of constrictions was much higher than that of gaps. Further on rare occasion it was difficult to demarcate the exact nature of the aberration. In very few instances confusion arose about some aberrations for considering them as a true break or a constriction when the broken part was lying very close to the point of origin. Anyhow, unless I was very sure, I have not included any break into the said category. Further, out of good number of translocations encountered, in 3 or 4 instances confusion arose about their true nature whether they were true translocations and firm terminal associations of two bivalents. We have, therefore, put them into the category of translocation. If they were not, the frequency would be little less than what has been scored. It should also be noted that in spite of our best effort very limited number of first and second division anaphases could be detected and sometimes because of their unfavourable dispositions they could not very critically studied. Therefore, for scoring the abnormalities at anaphases more attention was given to detect if there was any bridge or laggard or not. In scoring the data of the aberrant frequency in other stages like spermatogonial metaphases, diplotene-diakinesis, metaphase I and metaphase II cells with clear disposition of chromosomes were taken into consideration. Thus if any cell had too much sticky effect or otherwise unfavourable for study, they were discarded. If they were accounted, the frequency determined would have been more on the
higher side than what was presented. Lastly attempts were made to obtain the same number of 300 cells in each of dividing stage at all intervals but because of the inherent difference in the frequency at different divisional stages, it could not be materialized. Spermatogonial metaphases were rare in occurrence while amphases I and II were still more rare. Moreover in the treated series, it appeared that due to the effect of radiation there were some amount of degeneration and/or inhibition of dividing cells for which at some interval adequate number could not be obtained.

All the aberrations encountered were drawn by camera lucida for detailed analysis while photomicrographs of some of them were also taken.
OBSERVATION

Effect of X-rays on Meiotic Chromosomes of Nymph to Young Adult

Control Series:

As a check on the frequency of chromosome aberrations induced by X-rays in nymphs, chromosomes in almost the same number of cells as that of treated series, at different stages of the division from the testes of the control specimens were examined (Table 1). There were negligible number of aberrations and they were only of physiological type in the form of constriction and were not true breaks. Since the meiosis in male nymphs has not been worked out before, this was also studied in order to verify if the structure and behaviour of chromosomes as described in adult by Hanna (1951) was the same. The diploid number determined from the oogonial (Figs. 1 and 2) and spermatogonial prometaphase and metaphase (Figs. 3 and 4) was 14 chromosomes. The size-grouping of chromosomes was not very conspicuous, though in the oogonial complement the smallest pair of chromosomes, could be recognised which revealed on comparison with the spermatogonial complement to be the pair of X-chromosomes. Besides the smaller pair of X-chromosome, a pair of largest chromosome could also be recognised but it was not very conspicuous. In the spermatogonial complement out of the 14 chromosomes a small heteromorphic pair could be recognised from
other larger homomorphic pair of chromosomes. Out of the small heteromorphic pair the smallest element, since it was present only in spermatogonial complement and absent in oogonial complement, should be the Y. It formed the most conspicuous marker chromosome not only for its smallest size but also sometimes due to its differential staining behaviour. The size of the X-chromosome was close to that of the smaller pair of autosomes but it could be demarcated/critical analysis. The size-grouping of chromosomes could more conveniently be done when the chromosomes at metaphase I were analysed. In such analysis the X and the Y appeared to be conspicuously small and one bivalent demarcably large while the remaining bivalents were of different sizes (Figs. 7 and 8). Manna (1951) on the basis of the metrical study of the volumes of chromosomes at metaphase I determined the chromosome formula for L. hesperus as $1L_1 + 2L_2 + 2M_1 + 1M_2 + 1S_1 + 1S_4$ which corresponded to the chromosomes at metaphase I in the nymphs.

The early primary spermatocyte prophase is represented by compact thread-like chromosomes within the nucleus with one or two deeply stained mass representing perhaps the sex chromatin. This stage appeared to be leptotene-zygotene which was followed by a growth period. Thus the chromosomes at the zygotene-pachytene nuclei appeared diffusely stained as they took up very little stain but the sex chromosomes either forming a single mass or two separate unequal elements appeared as
positively heteropyonotic bodies. The bivalent structure of autosomes could be followed from the diplotene stage and onward. In the diplotene stage since the stainability was still poor the bivalents appeared to be very thin thread-like structure, mostly with one, rarely with two chiasmata but the two positively heteropyonotic univalent sex chromosomes mostly lying separate could be demarcated (Fig. 5). The bivalents at this stage having normally one chiasma either at the terminal or interstitial region while sometimes the longest bivalent had more than one chiasma. By the time the diakinesis stage was reached, the staining difference between the autosomal bivalents and the sex chromosomes obliterated. But the sex elements even in this situation could be identified by their univalent nature and having a chromatid split. Since the number of bivalents and the sex chromosome could more accurately be studied from late diplotene onwards, in scoring the data the effect on the diplotene and the diakinesis stages have been taken together (Table 3). The arrangement of the bivalents at primary spermatocytic metaphases was typical for Lygaeidae with $2n = 14$ chromosomes, where the $X$ and the $Y$ as univalents were situated at the centre of spindle surrounded by 6 autosomal bivalents. This arrangement was also very typical for Pentatomid bug (Manna 1962).

By the time metaphase I stage was reached, chiasma in all the bivalent was terminalised and the bivalent mostly appeared as
durable-shaped structure. First division anaphase as typical for Heteroptera was equational for the sex chromosomes as a result of which each daughter half received 8 elements, 6 autosomes and the X, the Y (Fig. 9). It was interesting to note that the longer autosomes showed two hanging arms with a conical part directed towards the pole which recalled the anaphase movement of chromosome with the localised centromere. The only difference being that the two were not extending from a definite centromeric point. This was also recorded in some other heteropteran species studied by Manna (1961). The second division metaphase arrangement was characterized by the presence of 6 autosomes surrounding the centrally situated pseudobivalent formed by the X and the Y (Figs. 10 and 11). The individual autosome appeared as a rod-like structure with split in the middle much like a bivalent but the configuration supporting the typical heteropteran meiotic axial orientation of chromosomes (White 1973). The X and the Y were associated to form a pseudobivalent structure or else they were having no physical connection. The size of the Y in comparison to the X was much smaller. Second division metaphase was followed by the second division anaphase. It was quite clear in properly oriented anaphase plate (Fig. 12) the reduction division of the sex chromosomes as they moved in the opposite poles along with 6 autosomes. The study of the meiosis in the normal nymph clearly showed that the structure and behaviour of chromosomes was in no way different from what has been reported for the adult (Manna 1961).
The study of chromosomes in the testes of the control specimens showed that the frequency of aberrations was very low and it appeared to be spontaneous in occurrence. The type of aberration encountered was only some constriction (Table 1).

An analysis of the data of the control series studied against materials treated with 200r in the 1st lot of specimens fixed on the 7th day, revealed no aberration in 20 spermatogonial metaphases, 50 diplotenes, 300 diakinesis, 80 anaphases I and 10 anaphases II while in 300 primary spermatocytic metaphases and in 300 metaphase II only one bivalent showed constriction in each stage (Table 1). Similarly in the 2nd lot there was no aberration in 25 spermatogonial metaphases, 15 diplotenes, 300 metaphases I, 30 anaphases I, 300 metaphases II and 5 anaphases II while only one constriction in a bivalent was found in 300 diakinesis nuclei examined. Thus in the control specimens out of the total 2050 (Table 1) cells in different stages of division examined only 3 constrictions were observed and the frequency of aberration was, therefore, meagerly about 0.1% which was more or less same as the spontaneous frequency found in different insects. The frequency of aberrations in the different stages of meiosis appeared to be different. However, the occurrence of diakinesis, metaphase I, and metaphase II appeared more frequent than other stages but it was to be accepted with reservation as the spermatogonial metaphases were infrequent, so also anaphase I and anaphase II. Since the study was carried
out in squash preparation I could not put undue weight on the occurrence of different stages in different frequency, but as this study was carried out quite extensively, there was every possibility that the duration of the different stages was not the same for which the different frequency was observed.

Treated Series:

The 3rd instar nymphs were irradiated with a dose of 200r in two occasions and in both the cases the specimens were sacrificed on the 7th day after irradiation. By this time the nymphs emerged as young adults after molting through 4th instar stage.

Three female nymphs were also irradiated and on the 7th day after irradiation, temporary aceto-carmin squash preparations were made of their ovary. Only twelve clear oogonial metaphase complements were obtained in the whole material among which three were affected. Of the 3 affected complements one complement had an extra small fragment of unknown origin (Fig. 13) which could be easily demarcated by its difference in size from the regular number of the oogonial complement. The origin of the fragment was however, not indicated by the structure of other chromosome because the regular 14 chromosomes could be homologized into seven homologous pairs. Therefore, it was possible that the
fragment survived while the paternal chromosome either got it repaired or lost in previous cell generation. In the second oogonial metaphase complement (Fig. 14) regular chromosomes were affected, one showing a constriction, while the other had a constriction at one end and a break at the other. That none of the affected chromosomes was the X could be said by examining the size of the chromosome. The third oogonial metaphase complement (Fig. 15) had a break in the mid-region of a medium sized chromosome, with two fragmented parts lying close to each other in its normal position. The distribution of the breaks in the two chromosomes were quite different, one was in the terminal region and the other was at the middle. As the oocyte divisions were not suitable for staining because of their restricted appearance they have not been studied extensively.

It is well known that the dividing cells can very commonly be seen in the testes for which detailed analysis of the chromosome aberrations induced by X-rays has been attempted in the male. Most of the spermatogonial metaphase complements were not affected but the affected plates showed different types of aberrations. Out of 25 spermatogonial metaphase complements encountered, 10 in the 1st lot and 15 in the 2nd lot (Table 1) only 5 aberrations were found. They were in the form of constriction and break in chromosomes which were in the same way as found in the oogonial complements. Further, the X or
the Y chromosome were not seen to be affected in the limited number of plates available for the study.

The effect of X-rays in the spermatocytic chromosomes could be studied from late diplotene onwards. In the late primary spermatocyte prophase stages, the aberrations were mainly in the form of gaps (P.M. 6) and breaks (Fig. 27). The effect appeared to be milder than what was seen in 400r. The broken fragment in case of chromosome break was situated at a distance from point of break while in case of the gap it appeared as an unstained line across the chromosome. In the diplotene-diakinesis stage no aberration was involved in the sex chromosome but as frequency in general was low it was not unexpected. The sticky effect was not present which could be due to the fact that the effect was studied after a long time when the expected physiological effect was over. Very few primary spermatocytic metaphases were seen to be affected for irradiating specimens at a dose of 200r. There was a plate containing a translocation or else the fusion of two chromosomes of two bivalents (Fig. 28) because the configuration was not very convincing for claiming it a true translocation. Chromosomes of anaphase I were very rarely affected, but individual chromosomes always assessable as they were found mostly in crowded condition. Only one chromatid bridge was found at this stage. The types of aberration found in metaphase II were constrictions, breaks and fragment of unknown origin. Sometimes the chromosomes showed sticky effect with disorganized orientation (P.M. 21).
No aberration was found at anaphase II though the number of plates available was very meagre. An analysis of the data of the chromosome aberrations induced by 20r studied in two lots (Table 1) revealed that the effect was not very strikingly high. In a total of 2307 cells examined there were only 32 aberrations in the two lots combined together. Thus the average frequency was 1.4% against 0.1% in the control (3 out of 2036 cells studied). If the data of the different stages were compared the effect would appear to be very negligible as the frequency was very low. However, it was relatively high so far as the spermatogonial metaphases were concerned. Out of the total 25 metaphases altogether 5 aberrations were encountered making a frequency as high as 20% while in the control series there was not a single aberration in a total of 45 spermatogonial metaphases examined. Further it was interesting to note that the frequency of aberrations in the spermatogonial metaphases in the 1st lot and in the 2nd lot was the same e.g. 20% since in the 1st lot the number was 2 in 10 and in the second lot 3 in 15 (Table 1).

The frequency of aberrations in the late spermatocytic prophase stages (diplotene and diakinesis) was nill in the 1st lot while in the 2nd lot it was 0.7% (2 in 272 diakinesis) against 0.3% in the control (1 in 300). If both the data were examined the aberrations frequency would be only 2 out of 615 diplotene-diakinesis nuclei examined and that too was occurred
only in one nucleus. Similarly in the control series there was only 1 aberration in 665 diplotene-diakinesis nuclei examined. On the whole the effect was very negligible and none was involved in the sex chromosomes. Further, it was already pointed out that the aberrations were only 1 gap and 1 break.

In the primary spermatocyte metaphases, the frequency of aberrations was also low but it was little higher than that of prophase I. There were 7(1.5%) aberrations out of 455 metaphases I in 1st lot and 3(0.9%) in 327 metaphases I examined in the second lot (Table 1). Thus out of the total 782 metaphases I, there were only 10 aberrations and the average was 1.2% against the control frequency of 1 in 600 (about 0.1%). Therefore, there was some amount of effect of ionizing radiation on chromosome of metaphases I though it was very mild. The frequency of aberration in anaphase I was 1 in 106 plates (0.9%) against nil in the control series. In comparison to the effect of the X-rays in the primary spermatocyte metaphase chromosomes, it was higher in the metaphases II. There were 8 in 460 (1.7%) plates in 1st lot and 6 in 300 metaphases II in the 2nd lot (2.0%). Thus on an average the frequency was 1.8% (14 aberrations in 760 metaphases II) in the treated series, against the frequency of 0.1% in the control series indicating an increase of 1.7% aberrations due to radiations. No effect was found at anaphase II but the number of plates available for study was too meagre to expect any aberration.
Result and Comment

The effect of 200r on the 3rd instar nymph was very mild as it revealed from the analysis of frequency data. However, that there was some amount of effect which was revealed when the control and the treated data were compared. Since the time of fixation was after a week after the radiation, the types of aberrations encountered could possibly be due to the effect of radiation on the structural integrity of the chromosomes. However, some of the aberrations like gap and constriction were considered by some workers as physiological effects which has been discussed elsewhere. The frequency of aberrations in spermatogonial metaphases was undoubtedly quite high though the number of plates available for study was very limited. Further, the presence of relatively more breaks clearly indicated that the gonial chromosomes were more sensitive than the spermatocytic chromosomes. In the absence of extra fragment in the limited number of affected spermatogonial metaphases it could not be decided if the fragment could survive through several cell cycles as was expected for fragments originating in holokinetic chromosomes. Within a span of 7 days the affected cell could have undergone one or two division cycles and if the fragment was produced, it could have survived as well. The same situation was not seen in oogonial metaphase because out of 3 metaphases having aberrations, one had an extra fragment.
Effect of 400r on Meiotic Chromosomes of Nymph to Young Adult

Control Series:

Nymphs at the 4th instar were reared in the laboratory condition and after 4th day and 6th day the young adults were sacrificed to assess the chromosome aberrations if any in the spermatogonial and spermatocytic stages as a check against the males treated with a dose of 400r of X-rays. An examination of a total 25 spermatogonial metaphase complements, 15 from 4th day specimens and 10 from 6th day specimens revealed no chromosome aberration (Table 2). The morphology of chromosomes were normal as described earlier. During the spermatocytic divisions, a study of 40 diplotene nuclei in 4th day sample and 20 in the 6th day sample also did not reveal any aberration while out of 300 diakinesis nuclei examined each in 4th day and 6th day samples only 1 gap type aberration was observed. Thus out of the total 660 late spermatocytic prophase stages (diplotene-diakinesis) examined, only 1 aberration was noticed. The frequency was, therefore, meagrely 0.1%. An examination of 300 metaphases I each at two intervals did not revealed any aberration. Similarly no aberration was observed in a total of 40 anaphases I (20 + 20) and 25 anaphases II (5 + 20), while out of a total 600 (300 + 300) metaphases II only 1 aberration (a constriction) was observed. Therefore, in the control series out of the total 1960 dividing cells examined there were only 2 aberrations, 1 constriction and 1 gap were found and that too were considered as physiological
The average frequency were 0.1% and it was same as obtained in the control series of the 3rd instar nymph to adult individual (Table 1). Further in both the control series the aberrations were only gaps and constrictions which could also be found in the normal individuals in such a low frequency. In other word the aberration frequency found in the control series was that of spontaneous one.

Treated Series:

The treatment of 4th instar male nymphs with the X-ray dose of 400r and fixing them as young adult on their emergence after 4th and 6th day of irradiation yielded good amount of chromosome aberrations (Table 2) as compared to 200r irradiated series described before (Table 1). Qualitatively since the effects at both the fixation intervals were the same, they have been described together. The spermatogonial metaphases were affected more than that of the spermatocytic cells and the aberrations were in the form of constrictions, breaks and fragments of unknown origin. Out of 15 spermatogonial metaphases examined, 5 aberrations were encountered in specimens fixed at 4th day and also 5 aberrations in 12 metaphases examined in the sample of 6th day (Table 2). In the control series of both samples not a single aberration of any kind was encountered. The average frequency of aberrations in the spermatogonial metaphases in 2 samples taken together was 10 in 27 metaphases.
This high average frequency should be considered with reservation as the number of metaphases available for study was very limited. However, there was no doubt that the frequency was relatively higher than the aberration frequency found in the spermatocytes (Table 2). Among the different types of aberrations encountered in spermatogonial metaphases the frequency of breaks and constrictions was relatively higher than the other types of which the breaks were highest.

The frequency in late spermatocytic prophase stages (diplotene and diakinesis) was 1.0% (8 in 300) in diakinesis at 4th day and 0.67% (2 in 300) on 6th day sample. The aberrations were in the form of constrictions and breaks (Fig. 29). Further though there was no aberration in 40 diplotene nuclei on the 4th day but on 6th day out of 15 diplotene nuclei examined 2 had chromosome breaks. When the data of the diplotene and diakinesis stages were combined, out of the total 650 nuclei there were 7 aberrations (about 1.0%), while in the control series it was only 0.1%. The frequency of aberrations at metaphases I was 0.67% in the 4th day and 1.67% in the 6th day sample, the average frequency was 1.1%. The aberrations were in the form of breaks, translocations, fragments of unknown origin (Fig. 36; P.N. 14). There was no aberration at anaphase I in both samples though only a total of 50 plates (15 + 35)were examined. The frequency of aberrations at metaphases II was 1.3% in the former and 0.67% in the latter.
Only 28 (3 ± 25) anaphase II plates were available which did not contain a single aberration.

A comparison of the data (Table 2) of the chromosome aberrations obtained in the specimens fixed on the 4th day with that of the 6th day would reveal that the average frequency was 1.4% in the 4th day sample (14 aberrations in the total of 968 dividing cells) and 1.6% (16 in 987 cells) in 6th day sample. Thus although the frequency in some individual type for example in spermatogonial metaphases and in diplotene showed glaring difference between the two samples but that was just due to small number because in the average the difference in the frequency was very negligible. If the two data was taken together the average frequency was 1.5% (30 aberrations in 1955 cells).

The present data when compared with that of 200r (Table 1), it would be seen that the average frequency of 1.6% aberration at 400r was not least strikingly different from 1.4% found in 200r. Further if the frequency of individual type was taken into consideration, no significant difference would be seen except in those cases where the sample was inadequate. Thus the present study at least did not show what may be known as dose-dependent aberration effect of radiation.
Effect of 400r in the Chromosome of 4th Instar at Different Intervals.

With a view to finding out the effect of X-rays at different close intervals in the meiotic chromosome of male 4th instar nymphs, they were irradiated with a dose of 400r and the effect was studied at 6 different intervals within a day (Table 3). Qualitatively the effect was more or less same as described before with some additional ones. Since the aberration types at various intervals was more or less same they have been described together irrespective of their occurrence at various intervals.

The effect in the spermatogonial chromosomes was studied at prometaphases and metaphases. In the spermatogonial prometaphases some fragments of unknown origin, breaks, constrictions were found (Figs. 16 and 17). Even the same plate contained more than one type of aberrations. Similarly in the spermatogonial metaphases (Figs. 18 to 25) aberrations like breaks, constrictions and fragments of unknown origin were found. In the present study some spermatogonial metaphases were seen where some of the chromosomes were fused to form a chromatin block (Fig. 26; P.M. 1 to 3) which possibly originated by the breakage-reunion as a result of radiation or else, they were having differential sticky effect. Since in the present series I obtained more clear evidences of translocation at
meiotic stages I believed that these clumped elements were in reality translocated chromosomes. But because of the clumping effect, their garniture could not be clearly followed. It was interesting to note that some chromosomes showed constriction at about the mid-region as if indicating the primary constriction (Figs. 16 and 15). It seemed that the same fragments could survive through successive cell division because there were metaphases which contained more than one fragment of similar size (Figs. 22 to 23). The number of extra elements found was not more than two and the size of the fragments was also variable in some plates (Figs. 22 to 25; P.M. 2a). Sometimes some plates appeared to be polyploidy in nature (P.M. 3). As the present study was carried out from early hour, there were some amount of sticky effects which was manifested both in spermatogonial and spermatocytic stages. The primary spermatocytic late prophase stage (Diplotene and Diakinesis Figs. 29 to 32; P.M. 4 to 9) contained various types of chromosome aberrations. There were breaks (Figs. 29 and 30; P.M. 5 and 7), gaps, constrictions (P.M. 5 and 6) interlocking (P.M. 9) etc. Besides them some translocations and/or association were also encountered (Figs. 31 and 32; P.M. 4 and 8). There were number of plates showing interbivalent connections and some disorganised chromosome structure (P.M. 10 to 13). In latter cases the stage of division appeared to be prometaphase I but the outline of the chromosome was sometimes not very clear because of their sticky
EXPLANATION OF FIGURES

Camera lucida drawing X Ca 2500 of chromosomes of irradiated nymphs with a dose of 400r.

Fig. 21. Spermatogonial metaphase with a minute extra fragment, smaller than the Y.

Fig. 22. Spermatogonial metaphase with 2 extra unequal small elements of unknown origin, one which is about the size of the Y.

Fig. 23. Spermatogonial metaphase with 2 fragments of unknown origin.

Fig. 24. Spermatogonial metaphase with 2 unequal extra elements of which one is identifiable for its smallest size while the other is about the size of small autosome.

Fig. 25. Spermatogonial metaphase with 16 elements of which two chromosomes showing a break in each, another with a constriction and two extra elements of unknown origin, one of which is identifiable for its small size like Y.

Fig. 26. Spermatogonial metaphase with 18 elements consisting of a minute fragment, 10 normal chromosomes while 4 chromosome seemed to have fused to form a large chromatin mass.

Fig. 27. Diakinesis showing two bivalents, one with a chromosome break and another with a gap.

Fig. 28. Prometaphase I with two bivalents showing terminal association/translocation between them.

Fig. 29. Diakinesis showing one bivalent with a break and another with a constriction.

Fig. 30. Diakinesis showing sticky bivalents among which two bivalents with a break in each. The X and the Y remain free.

Fig. 31. Diakinesis showing X-autosome fusion and some extra elements attached to a bivalent by thin connection.

Fig. 32. Diakinesis showing X and autosome association/fusion.

Fig. 33. First division metaphase showing a constriction in the terminal region of a bivalent.

Fig. 34. First division metaphase showing hump-like projection two fused bivalents.
Photomicrographs of meiotic chromosome aberrations induced by X-irradiated the nymphs.

**P.M. 1.** Three spermatogonial metaphases, two are very sticky, while the third one showing fusion in some long-sized chromosomes and there are two minute fragments determined from their conspicuously small size (400r).

**P.M. 2.** Four spermatogonial metaphases, each showing fusion in longer chromosomes. One plate (2a) showing about 18 elements which includes four minute fragments (400r).

**P.M. 3.** Spermatogonial metaphases. One at the centre is polypliod while the other at their lower side contained some extra fragments (400r).

**P.M. 4.** Late spermatocytic prophase showing one quadrivalent and the terminal association of two bivalents (400r).

**P.M. 5.** Late diplotene with a break in a bivalent and two other bivalents each with a constriction (400r).

**P.M. 6.** Late diplotene showing a break with the displaced fragment in one arm and the other arm with a chromatin break. Another bivalent with a faint gap at the terminal region (400r).

**P.M. 7.** Late diplotene showing two bivalents each with a distinct break (400r).

**P.M. 8.** Late diplotene with a multivalent configuration (400r).
Plate 2

Photomicrographs of meiotic chromosomes aberrations in X-irradiated L. homoea.

F.M. 9. Diakinesis showing three associated ring-bivalents of which two seemed to be interlocked (400r).

F.M. 10-13. Prometaphase I showing sticky bivalents and some aberrations.

F.M. 13. Some sticky bivalents forming a group one bivalent showing constriction (400r).

F.M. 11. Chain-like association of some bivalents while the X and the Y remained separate (400r).

F.M. 12. Some bivalents with thread-like connection and one bivalent with a deep constriction (200r).

F.M. 13. Highly sticky prometaphase with several bivalents clumped together (400r).

F.M. 14. Metaphase I, showing the X and one bivalent fused end to end (400r).

F.M. 15. Metaphase I, showing chromatic break in the X and a multivalent structure.

F.M. 16. Metaphase I, showing one bivalent with deep constriction, another with a hump-like outgrowth and a minute fragment of unknown origin (400r).
EXPLANATION OF TOMICROGRAPHS

PLATE 3

Photomicrographs of meiotic chromosomes aberrations in X-irradiated L. bossea.

PM. 17. Metaphase I, two bivalents seemed to be fused at one end (200r).

PM. 18. Anaphase I, showing a fragment of the equatorial region (400r).

PM. 19. Anaphase I, several separating chromosomes showing a stout sticky bridge (400r).

PM. 20. Anaphase I with a long chromatin bridge (400r).

PM. 21. Metaphase II with disorganized and sticky chromosomes (200r).

PM. 22. Sticky metaphase II, some chromosomes appeared to be precociously separated.

PM. 23. Metaphase II, one chromosome with a gap and an association of the sex-pseudobivalent with a chromosome (400r).

PM. 24. Metaphase II with several extra elements.
association. Even in such case some fragments of unknown origin and constriction could be demarcated, in these bivalents which were lying free. The inter-bivalent connections (Figs. 30 and 32; P.M. 11) was quite conspicuous in this series. In metaphase I aberrations of various types were encountered (Figs. 33 to 36; P.M. 14 to 16). There were constrictions (Fig. 33), gap, break etc. besides some multivalent configurations which were possibly formed due to translocation (Fig. 34; P.M. 15 to 17) but the other possibility that some of them could have been formed due to close interbivalent association or fusion could not be ruled out. The X chromosome aberration was observed very rarely (Fig. 35; P.M. 15) though in some plates all the elements could not be studied individually due to excessive stickiness some of which could have masked the sex chromosome aberrations. In the present study a number of anaphase I plates was encountered which showed bridges of different nature (P.M. 19 and 20) and laggards (P.M. 18). Second division metaphases also contained different type aberrations (Figs. 37 to 40; P.M. 21 to 24). In good number of cases the orientation of chromosome was disturbed and the chromosome seemed to have developed a tendency of stickiness (P.M. 21 to 24). In spite of this some plate showed clear translocation between chromosomes.

The frequency of aberrations at different time intervals was variable (Table 3). At 5 minutes out of only 5 spermatogonial
metaphases available for the study, 2 had chromatid breaks and 1 had a fragment of unknown origin. The frequency was, therefore, very high (60%) but as the number of plates examined was meagre, no importance was attached on the frequency. However, that the treated series had high frequency of breaks at spermatogonial metaphases was apparent when similar high frequency of aberrations was found at other intervals as well (Table 3). No aberration in the spermatogonial stages was found in the control series.

In the spermatocytic division no aberration was encountered in the diplotene and diakinesis stages 100 nuclei in the control and 88 nuclei in the treated series examined. Metaphase I also had very few aberrations. There was not much difference in the frequency of aberrations between the control and the treated series because in the control series there was only one constriction in 100 metaphases and the same number in 119 metaphase I of the treated series. No aberration, both in the control and the treated series was encountered in meagrely 5 and 2 anaphase I and 5 and 4 in anaphase II examined respectively while at metaphase II there was no aberrations in 100 plates in the control series but the treated series had only 1 extra fragment of unknown origin in 128 plates examined. Thus in the testes fixed at 5 minutes there was only 1 aberration in 320 spermatocytic cells in the control series (0.3%) against 5 aberrations in 346 cells in the treated series (1.4%). Thus it was apparent that the spermatogonial metaphase chromosomes were
sensitive to radiation since within this short time a number of aberrations were observed in only 5 plates available for study while in 343 cells examined at different stages of meiosis had negligible number of aberrations.

The number of aberrations at 30 minutes was 4 in 7 spermatogonial metaphases (57.1%) which was very high but this frequency could not be relied fully as the number of plates available was very inadequate. In 10 metaphases complement in control series there was, however, no aberration. The types of aberration found in the spermatogonial metaphases were constriction breaks and fragments of unknown origin.

The frequency of aberrations at the diplotene and diakinesis was little higher than that of the control series because there were 3 aberrations in 206 nuclei in the treated series against 1 in 200 nuclei of the control series. The aberration types found were gap, constriction and breaks. At metaphase I no aberration was seen in 200 plates in the control series against 2 aberrations in 283 in the treated series. These aberrations were break and fragment of unknown origin. Only very few plates of anaphase I were available for the study which contained no aberrations both in the control and treated series. In metaphase II no aberration was encountered both in the control and the treated series though only very few plates were available for study. Therefore, in 30 minutes the frequency of total aberrations in the spermatocyte chromosomes
was 0.3% (2 in 630) in the control series against 1.5% (9 in 612) in the treated series. The data showed that the average frequency in the control series as well as in the treated series had no change between 5 and 30 minutes. The 4th instar nymphs irradiated with 400r and their testes fixed at one hour yielded the frequency of aberrations in spermatogonial metaphase complement 37.5% (9 in 24 plates) and the aberration types were like that of 30 minutes which consisted of 2 gaps, 5 chromosome breaks and 2 fragments of unknown origin in 24 metaphases examined. On the other hand in the control series there was no aberration in 10 complements available for the study. Since the high frequencies of spermatogonial chromosome aberrations was determined from very limited number of plates, I could not attach undue importance to it but as the same trend was seen at 5 min and 30 min in the treated series against no aberration in the control series, it would be reasonable to accept that the spermatogonial chromosomes were highly susceptible to X-ray damages though the exact frequency could be relied upon only when the data could be scored from good number of spermatogonial complements. Further, the spermatocyte chromosomes were relatively resistant as compared to the damages inflicted by X-rays on spermatogonial chromosomes. The frequency of aberrations in the spermatocytic stages of division was very negligible. It was only 1 constriction in 121 (0.8%) diplotene and diakinesis nuclei against none in 150 nuclei of the control
series and 1 break at metaphase I out of 127 plates examined against no aberration in 150 metaphase I of the control series. Only very few plates were found in anaphase I, anaphase II both in the control and the treated series but none of them had any form of aberration but 150 of the control and 159 of treated metaphase II had in same state of no aberration. The frequency of total aberrations at 1 hour was 11 in 441 dividing cells (2.5%) against none in 480 cells in the control series. The higher average frequency was mainly due to the presence of more aberrations in spermatogonial chromosomes. If only average frequency of aberrations at the spermatocytic stages was considered it was only 0.4% (2 in 417).

At 4 hours the number of aberrations in the spermatogonial metaphases was 14 in 18 plates examined (77.7%) in the irradiated series against none in 15 plates of control series. Thus the frequency at 4 hr was higher than in the earlier hours. The aberration types were however same as that of 30 min and 1 hr having breaks, constrictions and fragments of unknown origin. The spermatocytic chromosomes unlike earlier intervals were also considerably affected at this interval because out of 21 diplotene and diakinesis nuclei examined there were 9 gaps and constrictions, 8 breaks, 1 fragment of unknown origin and 1 terminal association or translocation making a total of 19 aberrations and the frequency was (90.5%). This frequency was remarkably high in comparison to what was observed at 5 minutes.
30 minutes (1.4%) and 1 hour (0.8%). On the other hand in the control series just like early intervals no aberration was found at this stage in 50 nuclei. The frequency of aberrations at metaphase I was also remarkably high, because in 124 metaphases examined there were 8 gaps and constrictions, 9 breaks, 1 fragment of unknown origin, 2 translocations and 7 unclassified aberrations of which some were possibly translocations making a total of 27 aberrations and the frequency was 21.7%. At metaphase I the frequency was also much higher than that of early intervals as it was 0.8% at 5 minutes, 0.7% at 30 minutes and 0.8% at 1 hour. It was also very striking to note that out of 17 first division anaphases examined, 13 plates had bridges and some had laggards (76.4%). Unfortunately this at 4 hr sample the number of 2nd division metaphases available for study was only 2 which had 1 gap, 1 break and 1 fragment of unknown origin for which it would be futile to calculate the frequency, while in 150 metaphases II of control series not a single aberration was seen. Anaphase II there was no chromosomal aberration though the number of plates examined was only 8 in the treated series and 10 in the control series. In a total of 190 cells examined in the treated series 76 aberrations were found making the average of 40% against meagrely 0.2% in the control series. The average of 40% aberrations at 4 hrs was very high in comparison to that of 1.4% at 5 min, 1.5% at 30 min and 2.5% at 1 hr. It seemed that there was also some
degenerating effect for which adequate number of spermatocytic divisional stages were obtained, specially at primary spermatocytic prophase and at metaphase II. Lastly the average frequency of aberration was 32.5\% (62 in 172) in spermatocytic chromosomes against 77.7\% in spermatogonial chromosomes. Therefore, though the spermatogonial chromosomes had more aberrations like earlier data but spermatocytic chromosomes had abrupt high incidence of aberrations than what was found at early intervals.

At 8 hr the frequency of aberration in the spermatogonial metaphase chromosomes was 85\% because out of 20 plates examined there were 6 gaps and constrictions, 9 breaks and 2 fragments of unknown origin, making a total of 17 aberrations. The frequency was thus even little higher than that of 4 hrs (77.7\%). There is no aberration in 17 spermatogonial plates of the control series. As stated earlier the chromosome of spermatogonial metaphase were more susceptible to X-rays but since the frequency had to be determined from inadequate number of plates because of their limited occurrence I had determined the frequency value and put the caution. As the same trend was seen at all intervals there was less doubt that the spermatogonial metaphase chromosomes had higher susceptibility which increased with the lapse of time after irradiation. In the spermatocytic division at the diplotene and diakinesis stages there were 5 gaps and constrictions, 7 breaks, 1 fragment of unknown origin,
2 translocations and 1 miscellaneous type in 60 nuclei examined making a total of 16 aberrations (26.6%) against 1 in 100 control nuclei at the same stage (Table 3). The frequency was however lower than 90.5% of 4 hrs, but in that case the frequency was determined from insufficient number. The frequency of aberration at metaphase I was also very high. Out of 109 plates examined there were 9 gaps and constrictions, 9 breaks, 2 fragments of unknown origin and 7 miscellaneous types which seemed to include few translocations. The frequency was therefore, 24.7% which was little higher than that of 4 hrs (21.7%). In the control series no aberration was found in 150 plates. At anaphase I only 5 plates were available of which 2 had bridges which made the frequency 40%. The frequency was found to be lower than that of 4 hrs (76.4%) but in both the cases, since very few plates were available for the study, the frequency determined from them had great limitation. At metaphase II out of 77 plates examined there were 4 constrictions and gaps, 9 breaks, 1 fragment of unknown origin and 1 miscellaneous type made the frequency as 19.4% which was lower than that of 4 hrs but in that hour only 2 plates were observed. In the control series there was only 1 aberration in 100 cells. No aberration was found at anaphase II in 10 plates of control and in 3 plates of treated series. The average frequency at 8 hours was 28.1% (77 out of 274 cells) against 0.6% (2 in 386) in the control series. Thus the average frequency at 8 hr was lower than that of 4 hr as it was 40% but it was much higher
than that of still early intervals. Further, the frequency of aberrations in spermatocytic chromosomes was 23.6% against 86% of the spermatogonial chromosomes. Therefore the higher radiation sensitivity of the latter chromosomes was shown as found at all other intervals though the degree was variable. At 24 hour, out of 12 spermatogonial metaphase plates examined there were 7 breaks and 8 translocated elements against none in 10 plates at control. Thus in the X-irradiated series all the plates were affected and the frequency was 126% as more than one chromosome is affected in one plate. At diplotene-diakinesis stage only 15 cells were available for study and out of them 4 had gap, constriction, break and fragment of unknown origin making a frequency of 26.6% against 1 constriction in 50 nuclei examined in the control series. The frequency of aberrations was also very high at metaphase I, and also there was some amount of degenerating effect for which no adequate number of plates could be studied. Out of 55 plates examined there were 7 gaps and constrictions, 3 breaks, 2 fragments of unknown origin and 1 unclassified type making a frequency of 23.6%. The frequency was very close to that of 8 hr (24.7%) but higher than that of 4 hrs (21.7%) and much higher than that of early hours. If the degenerating plates were accounted, the frequency would have been still higher. In the control series no aberration was encountered in 100 plates. At anaphase I, one bridge out of 8 plates examined was found while none was found in 10 plates in the control series. The
frequency of aberration at anaphase I at this hour was much lower than that of 8 hr and 4 hr though I should not attach much importance on the frequency as in all cases the number of plates available for study was extremely low. At this 24 hour material 2nd metaphase plates were very rarely encountered which could be due to some degenerating effect. After a thorough search only 3 plates were available for study where no aberration was found. There was also no aberration in 50 plates in the control series. No aberration was also found in both control and treated series in anaphase II however, the cells were very few.

The data of all intervals taken together (Table 3) the frequency of chromosome aberration in the spermatogonial metaphase complements was nil in 70 plates in the control series against 72% (62 in 86 plates) in the X-irradiated samples. Among the stages of spermatocytic division the frequency of aberration in the diplotene-diakinesis stage was 0.4% in the control series (3 in 650 nuclei) against 8.4% of the treated series (43 in 511 nuclei). In the metaphase I the aberration frequency was 0.2% (2 in 850 nuclei) in the control series against 8.9% (71 in 792 nuclei) in the treated series. The frequency of aberration in anaphase I was nil in 55 plates in the control series against 35.5% (16 in 45) in the treated series. In Metaphase II the aberration frequency was 0.2% (2 in 750) in the control series against 3.8% (19 in 496) in
in the treated series. However, no aberration was observed in anaphase II in 55 plates of the control and 29 plates in the treated series. That the frequency of spermatogonial chromosome aberration was much higher than that of the spermatocytic stages would be clear since the frequency of chromosome aberration in the spermatogonial chromosomes was 72.0% against 7.9% (149 in 1875 dividing cells), aberrations in the spermatocytic divisional stages all taken together. The present data also showed that the effect was much accelerated at about 4 hr which was the maximum. But in subsequent intervals though the frequency was lower than the peak of 40%, but it was quite high in comparison to the data taken at early intervals. At present we have no data in between 48 hr and 4th day of the nymph treated with 400r, though within this interval the fourth instar nymph will mostly emerged as young adult. The frequency of total aberration on the 4th day was 1.4% (14 in 968 cells) taking all stages together in the 4th instar nymph treated with 400r against the total aberration of 10.7% (211 in 1961 dividing cells) obtained in 24 hr with the nymphal testes fixed at various intervals. Even if only the data of 24 hr was compared the frequency in the 4th day (1.4%) of the previous series would be much lower than that of 24 hr itself (33.6%). The analysis made above would show that with the lapse of time the frequency of aberration dropped down considerably on the 4th day but on successive days it did not vary much because in the present
study the average frequency was 1.5% which was close to that of the 4th day.

**General Comment on the Effects of X-rays on Nymphs:**

The cytogenetical effect of X-rays in the developing embryos have been studied by the different workers using different materials. Fox (1966a, b, 1967a, b) made a series of studies on the effects of X-rays on chromosome of locust embryos with regard to the early response on chromatid interchanges, aberration types, dose-effect etc. He found that there was apparently an insensitive stage which was followed by stickiness due to general physiological effect, and side-arm bridges due to localised exchanges of discreet subunits of chromatids. There was also an inhibitory effect. He also suggested that most chromatid interchanges were induced between polymerized chromosomes, though the occurrence of loops to the lesser extent of terminal overlapping might subsequently modify the appearance of those aberration at metaphase (Fox 1966b). His study also (Fox 1967a, b) supported Revell's (1959) exchange hypothesis because that would cover up whole spectrum of aberration induced by X-rays. It was also found by him that there was marked variation in yield of aberration types depending on the stage of chromosome in the interphase nucleus. The effect of radiation on the
chromosomes, if irradiated at late S phase and G2 phase of DNA synthesis results were more variable. Thus Fox made his studies on the mitotic chromosomes of the embryonic cells of locust which were most suitable for various reasons. However, the material of the study was not equally convenient for two main reasons, firstly the chromosomes were without localised centromere and secondly they were relatively small in size.

In spite of these facts it was still used for the present study for some other purpose as mentioned elsewhere to reinvestigate the centromeric problem in Heteroptera and the behaviour of holocentric meiotic chromosomes for the irradiation with X-rays. The radiosensitivity of the meiotic stages in the eggs of silk worm was studied by Murakami (1971) who irradiated the oocytes at various meiotic stages of the silk worm and the radiosensitivity was determined by the hatchability of irradiated eggs against control. The study revealed that cells in first division were more resistant than those in the second meiotic stage. The difference in the radiosensitivity was suggested to be related to the DNA synthesis and chromosome duplication. Though the study was not directed to the same line and irradiated meiotic cells of males, it appeared that the chromosomes at second division meiosis were less sensitive than that of first meiotic division. Whiting (1945a, b) like Murakami (1971) also studied the problem of hatchability and effect on chromosomes of ionizing radiation on 1st division
The studies on the effect of X-rays on eggs and embryos of coccids were, however, directed mainly to verify if the chromosomes were holokinetic or not (Hughes-Schrader and Rix 1941). Later work on coccids was to inactivate a set of paternal or maternal chromosomes to verify which set was responsible to undergo heterochromatization in lecanid system (Brown and Nelson-Rees 1951).

The chromosomes of most higher organisms have the localised centromere. The aberrations in them were basically distinguished according to the unit of breakage of exchange involving both chromatids of a chromosome as chromosome break or isochromatid breaks, only one chromatid broken as chromatid break and the third category as subchromatid break. They could be located in the successive cell cycle. With these basic forms the structural changes can occur between and within chromosomes and chromatids as deletion leading toacentric fragments as well as exchanges which have been described by various authors (Evans 1962, Kihlen 1966, White 1973). Unfortunately the present material was not very suitable to analyse the breakage data in the same light of X-ray induced chromosome aberrations carried out in the material with localised centromeres as in Vicia, Tradescantia, grasshopper etc. However, some of the types of aberrations I found in the present material were not always clearly distinguishable e.g., chromatid and subchromatid breaks, as well as the exchange type.
aberrations. Other types of aberrations clearly demarcated were chromosome breaks, constrictions, fragments of unknown origin and few translocations. Sometimes the terminal association or the chromosome fusion could not be claimed with absolute certainty. Another interesting finding of the present study was the process of restitution. It has generally been accepted induced breaks by ionizing radiations are initially more but with the lapse of time some are restituted while others retain structural changes originated due to breaks by the X-rays. Thus Manna and Mazumdar (1962, 1967a) showed that the X-ray induced breaks in the X chromosome of grasshopper were to some extent restituted because the frequency was much higher at the earlier fixation hours than that at late hours. The present study did not lead to a conclusive position because the breaks in the spermatogonial and oogonial chromosomes possibly survived but as the number of fragment did not increase with the lapse of time. Further the number of breaks in a chromosome was mostly one unlike the X chromosome of grasshoppers (Manna and Mazumdar 1962, 1967a) for which it could not decisively be said that there was some restitution of breaks with the lapse of time. The number of breaks rather increased with time. Halkka (1966) in his study of X-ray induced changes in the chromosomes of a homopteran species, Limotettix atricola found anaphase bridges and laggards and variable number of chromosome elements in gonial stages survived over a period of
7 days; this he argued would favour that the chromosomes were with diffuse centromeric activity or holokinetic nature. He also showed that post-reductional meiosis was not a pre-requisite of holokinetic organisation of chromosomes, as claimed by other worker (Nordenskiold 1963), since Limotettix having pre-reductional meiosis had holokinetic chromosomes. It was also argued by other worker (Nordenskiold 1963) that bridges and laggards were practically absent in 1st and 2nd anaphases in Luzula which attributed to the situation of the post-reductional mode of meiosis particularly in the repairing of homologous chromosome before the 2nd division. But bridge formation could be possible by radiation induced translocation. In the present study the frequency of anaphase bridges or laggards at anaphase I was very low or absent in most of the intervals. Only in the material fixed from 4 hour to 24 hour showed some amount of bridges and they were possibly due to some structural changes which was supported from the fact that the meiotic abnormalities were quite common at those hours. However, since the anaphase bridges were rather uncommon in the material fixed at 4th and 6th day after x-irradiation it was possible that the chromosomes with the structural changes did not survive. On the other hand the occurrence of breaks in spermatogonial chromosome was somewhat regular and the presence of limited number of fragments at various intervals would possibly support the existence of holokinetic chromosomes in the present species. Though
characterization of the presence or absence of some particular type of aberration could not be generalized as advocated by some workers. In other words the present study and that of Halkka (1965) indicated that the aberration types induced in the holokinetic chromosome did not show many characteristic differences with that of chromosomes with localized centromeres. Only the survivality of fragments through successive cell cycle would be the most important support in distinguishing holokinetic chromosomes from chromosome with localized centromere, as acentric fragment does not survive during cell division.

It is generally accepted that X-ray effects are dose dependent which have been tested using various parameters like inactivation of viruses, induction of dominant lethals, chromatid breaks, X chromosome breaks and so on (Lea 1962, Manna and Mazumder 1962, 1967a). In the present study, however, this was not indicated; for example the frequency of total aberrations was 1.4% in the material irradiated with 200r and scored on 7th day while material irradiated with 400r had the total frequency of 1.5% on the 6th day after irradiation. The difference was very negligible. Therefore there was no dose-dependent effect on chromosomes for the use of two doses. It was also expected that the dose dependent effect would not be shown when all sorts of aberrations were included specially in mixed occurrence of different types of aberrations and very
likely in the present study it was not shown because we took into account of all sorts of aberrations. So far no study on the dose-dependent effect has been carried out in hemipteran materials. This was mainly due to the fact that it was not known whether the aberrations encountered in them originated due to single hit or multiple ones and fate of this aberrations through cell cycles were not thoroughly studied.

Differential radiosensitivity has been tested using various parameters and different stages of divisions. Whitting (1945a, b) observed that meiotic metaphases were most sensitive for the production of dominant lethal in the irradiated oocytes of the wasp, Habrobracon. The study of the salivary gland chromosomes of the irradiated females of Sciara (Bozeman and Metz 1949) indicated that anaphase I was most sensitive while in mice it has been claimed that (Oakberg and Diminno 1960) diakinesis and metaphase I were most sensitive stage for breakage. Data on the relative sensitivity of the stages of mitosis are very limited that the available ones indicated that metaphases were most sensitive in both plants (Conger 1947, Bishop 1950) and animal species (Belyaeva and Pokrovskaya 1959). In the present study the analysis of data of 400r of the material fixed at different interval between 5 minutes to 24 hours (Table 3), the spermatogonial metaphases were found to be most sensitive than the meiotic stages. Though the data of spermatogonial metaphases was not very adequate but even with
this limitation there could be no doubt that it was most
sensitive since the frequency of aberrations were very high
in comparison to any other stages. So far as the sensitivity
of the different spermatocyte stages was concerned anaphase I
appeared to be most sensitive since the frequency of aberrations
was 35.5% while in the other stages it was 8.4% in diplotene-
diakinesis, 8.9% at metaphase I and 3.8% in metaphase II.
Since the data of anaphase I was not adequate the conclusion
suffered great limitation. The data showed that the meiotic
chromosomes of late prophase and metaphase I were relatively
more sensitive than the metaphase II. According to some
workers the frequency of aberrations at spermatogonial metaphase
and metaphase II were almost the same which in the present study
was not confirmed but as already mentioned, chromosomes of
spermatogonial metaphases were much more sensitive than that
of metaphase II.
Third instar nymphs of the Lygaeid bug *L. hesperus* were irradiated with the X-ray dose of 200r. The frequency of chromosome aberrations in the meiotic cells in the control and x-irradiated specimens on the 7th day by the time they emerged as adults, was assessed. The experiment was repeated once. In the control series the frequency was very negligible. In a total of 2035 dividing cells examined at various stages of division, only 3 constrictions were encountered. The frequency of aberration was therefore 0.1%. In the irradiated specimens an examination of 2037 dividing cells of various stages of division revealed 5 gaps and constrictions, 18 breaks, 1 bridge and 5 fragments of unknown origin and 3 unclassified ones making the average frequency of 1.4%. The average frequency in the 1st and the 2nd lot was also not much different.

In the 200r X-irradiated specimens spermatogonial metaphases appeared to be most sensitive as it contained relatively high frequency of aberrations (20% or 5 in 25 plates) though the number of plates analysed was only 25 as compared to 2262 spermatocytic stages available for the analysis. It contained only 27 aberrations and the frequency was about 1.2%. The frequency of aberrations in the different stages of meiosis was very low and in some stages even not a single aberration was observed.
Fourth instar nymphs were also irradiated with the X-ray dose of 400r and the testes of the specimens were fixed on 4th and 6th day after radiation, when the specimens turned to be adult. The frequency of aberrations on the 4th day was 33.3% in spermatogonial metaphase, none in diplotene, 1.0% in diakinesis, 0.67% in metaphase I, nil in anaphase I and II and 1.3% for metaphase II. The average frequency was 1.4%. The chromosomes of spermatogonial metaphases appeared to be most sensitive while the frequency of aberrations in different stages of meiosis was very low but higher than the corresponding control frequencies.

The frequency of aberrations in the testes fixed on the 6th day after irradiation with the dose of 400r was 41.6% for spermatogonial metaphase, 13.3% for diplotene, 0.67% for diakinesis, 1.67% for metaphase I, nil for anaphase I and anaphase II and 0.67% for metaphase II. The average frequency was 1.5% which as compared to that of 200r, the difference was negligible. The frequency of spermatogonial chromosome aberrations in the two doses was however, strikingly different.

Fourth instar nymphs were irradiated with the same dose of 400r and the testes of the control and treated specimens were fixed at 6 different intervals between 5 minute and 24 hour. The frequency of aberrations in the treated series were 1.4% at 5 min, 1.5% at 30 min, 2.5% at 1 hr 40.0% at 4 hr, 28.1% at
8 hr and 33.6% at 24 hr. On the other hand the frequency of aberration in the control series was 0.3 at 5 min and at 30 min, nil at 1 hr, 0.2% at 4 hr 0.5% at 8 hr and 0.4% at 24 hr. The frequency of aberrations at different stages of division taken together in the X-irradiated series was 72% at spermatogonial metaphases, 8.4% at diplotene-diacinesis, 8.9% at metaphase I, 35.5% at anaphase I, 3.8% at metaphase II and nil at anaphase II. The average frequency was 10.7%. The data on analysis revealed that spermatogonial metaphase chromosomes were most sensitive, the next one was first division meiotic stages.

The types of aberrations encountered in the irradiated materials were mostly constrictions, breaks, fragments of unknown origin and few translocations and some unclassified ones. Among the different types of aberrations the frequency of breaks was higher than any other types. The result have been discussed with regard to aberration type, dose dependent aberration effect, stage sensitivity etc. It has been suggested that the data did not show dose-dependent effect. Spermatogonial metaphase stage was most sensitive and the chromosomes appeared to be holokinetic but the aberration types encountered did not show significant difference from those of chromosomes with localized centromere.