RESULTS AND DISCUSSION

The present work deals with the studies on the effect of X-ray doses on the male germinal cells during the process of spermatogenesis of four insects i.e. *Aulacophora foveicollis, Aulacophora intermedia, Corynodes peregrinus* (all coleopterans) and *Dysdercus cyngulatus* (Hemiptera).

The literature reveals that during last five decades a number of eminent scientists studied the effect of X-rays on the male germinal cells of various insects and given their findings on chromosomal disorders, mutations, chiasma frequency, formation of univalent's, bivalent's, their radio resistance, sterile insect techniques etc.

In the present investigation the effect of X-rays at the radiation of 1500r, 3000r and 4000r has been studied and is being discussed on the cytological features during various dividing stages of the spermatogonial, primary spermatocyte and secondary spermatocyte cells including the successive developing stages of spermiohistogenesis.

It may be mentioned here that the field of discussion is mainly focused on the chromosomal studies, nuclear swelling and persistence of the nuclear membranes, nucleolus, nucleolar vacuole, perinucleolar ring, cytoplasmic variations in the meiotic cells of the spermatogonial, primary spermatocyte and the secondary spermatocyte cells. In addition to this during spermiohistogenesis the chromatin behaviour and the formation of tubular vacuoles have been discussed.
5.1 CHROMOSOMAL STUDIES

5.1.1 Chromosomal breakage and fragmentation

The literature reveals that the chromosomal breakage and their fragmentation has been observed by some of the workers such as Raychoudhuri, Ghosh, Nandi and Banerjee (1957) who reported the X-ray induced chromosomal breakage in grasshopper spermatocyte. Kirby-smith and Daniels DS (1958) reported X-ray produce chromosomal breakage in *Tradescantia*. Halkka, O. (1965) in *Limotettix* observed chromosomal fragmentation at the X-ray radiations of 400r and found that irradiations were more effective at anaphase stages and the laggard chromosomes were frequently formed. Lachance, L.E. and DeGrugillier, M. (1969) reported the chromosomal fragments were transmitted through three generations in *Oncopeltus* (Hemiptera). Gururaj and Rajasekarasetty (1970) concluded that the high efficiency of gamma rays are capable of producing chromosomal fragments even at lower doses. Murakami and Imai (1974) reported that in *B. mori* when treated with X-rays and gamma rays produced chromosomal aberrations. Rajasekarasetty, M.R. and Kumaraswamy, K.R. (1979) in *Poekilocerus pictus* (Orthoptera) found that gamma rays induced chromosomal aberrations on short horned grasshopper when exposed to whole body irradiations of nine different doses of gamma rays namely 20r, 40r, 80r, 120r, 160r, 200r, 240r, 280r and 320r form gamma rays. Day and Manna (1983) reported that in the germ cells the x-ray irradiation produces chromosomal fragmentation which is responsible of the sterilization of insects. Carpenter, J.E.(1991) observed the effect of radiation doses on the incidence of visible chromosomal aberrations in *Helicoperva Zea* (Lepidoptera). Sinha and Sinha (1995) has reported that when *B. mori* males were irradiated with 500r,
1000r, 2000r and 5000r the bivalent chain, ring bivalents, uni and multivalent etc. are formed due to chromosomal fragmentation. Laxmikumari, B., Jayaprakash and Ananthanarayan, S.R. (1996) observed that the gamma radiations in *B. mori* induced chromosomal aberrations such as fragmentation, translocation etc. at the exposure of 3000r. Fujiwara, H. and his co workers (2000) in *B. mori* reported that X-ray irradiations produced chromosomal fragmentation. Tothova A marce F. (2001) found that in the male flour moth, *Ephestia Kuchniella* the gamma rays produced chromosomal aberrations. It was also reported that the fragmentations and various translocations were most numerous. At 100 Gy simple translocation occurred, at 150 Gy and 200 Gy multiple translocations occurred further the higher doses increased their number.

As reported by the previous authors cited above, in the present study also, the chromosomal breakage and fragmentation has been observed in the male germinal cells of the insects when irradiated by different doses of X-rays. In this study the occurrence of chromosomal breakage and fragmentation at the X-ray exposures of 1500r, 3000r and 4000r is being discussed in the dividing stages of spermatogonial, primary and secondary spermatocyte cells of all the four insect species.

The spermatogonial cells when exposed to 1500r, and 3000r the chromosomes break into small fragments at the Prophase, and Metaphase stages in all the species i.e. *A. foveicollis, A. intermedia, C. peregrinus* and *D. cyngulatous*. At the Anaphase stage at 1500r the chromosomes become fragmented in *A. foveicollis; A. intermedia* and *C. peregrinus*, but not in *D. cyngulatous*. However, at the radiation of 3000r, such fragments are formed in *A. intermedia* and *D. cyngulatous*. At the exposure of 4000r at telophase stage the chromosomal fragmentation has been found only in *D. cyngulatous*.
The chromosomes of the primary spermatocyte cells, at the exposure of 1500r, 3000r and 4000r also become fragmented at their leptotene, zygotene, pachytene, and diplotene stages, in all the four species. During diakinesis at the radiation of 1500r and 3000r the chromosomes become fragmented in A. intermedia, C.peregrinus and D.cyngulatus and not in A.foveicollis. At metaphase the chromosomes become fragmented in A.intermedia and D.cyngulatus at the exposure of 1500r, 3000r, and 4000r and also in C.peregrinus at 3000r and in A.foveicollis at 4000r.

The secondary spermatocyte cells when irradiated at 1500r, 3000r and 4000r the fragmentation of chromosomes has been observed at prophase in A. intermedia, C.peregrinus, D.cyngulatus and in A.foveicollis. At metaphase stage the chromosomes become fragmented in C.peregrinus at the radiation of 1500r, 3000r and 4000r. This feature also occurs in A.foveicollis and D.cyngulatus at the exposure of 4000r but not in A.intermedia at any radiation. Similarly the chromosomal breakage and fragmentation has been observed during anaphase in C.peregrinus at all the three radiations. In D.cyngulatus it has been seen at the exposure of 3000r and 4000r, and in A.intermedia only at 4000r. Remarkably in A.foveicollis the chromosomal fragments is not seen at all the three radiations.

5.1.2 Chromosomal Condensation and clumping -

Laxmikumari, B., Jayaprakash and Ananthanarayan, S.R. (1996) in Bombyx mori reported that the gamma radiations produce abnormal chromosomal aberrations such as clumping, stickiness, fragmentation and condensation etc. during spermatogenesis.

In the present study also the spermatogonial cells when exposed to the high X-ray radiations of 3000r and 4000r the chromosomes become condensed.
and clumped with each other in *A.foveicollis* at anaphase and telophase stages, in *A. intermedia* and *D. cyngulatus* at their prophase, metaphase and telophase stages, and in *C. peregrinus* at the metaphase only.

In the **primary spermatocyte cells** at the exposure of 4000r the chromosomal condensation and their clumping has been observed in all the dividing stages i.e. leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase, anaphase and telophase stage of all the four species. At the exposure of 3000r it has been observed in *A.foveicollis* at diakinesis in *C. peregrinus* at pachytene and metaphase and in *D. cyngulatus* at diakinesis and anaphase stages. The chromosomal condensation and clumping has also been found even at the exposure of 1500r at the metaphase stage in *A.foveicollis*.

In the **secondary spermatocyte cells** the chromosomal condensation and clumping occurs at the exposure of 4000r at their prophase, metaphase, anaphase and telophase stages in all the four species. It is also visible at the radiation of 3000r in *A.foveicollis* and *D. cyngulatus* only at their telophase stages.

### 5.1.3 Unequal distribution of chromosomes at anaphase and telophase stage-

Only Verma, R.C., Reddy, B.M. and Shevade, A. (1996) reported the stickiness of chromosomes and unequal distribution of chromosomes at anaphase and telophase in *P. dermondii*. The results of the present investigation are also similar to the findings of Verma and Shevade.

It is remarkable that due to the X-ray irradiations the unequal distribution of chromosomes occurs due to mis-saggregation of chromosomes at the anaphase and telophase stages of dividing meiotic cells. This has been seen in the
spermatogonial cells of *D. cyngulatus* at anaphase stage when exposed to 3000r.

In the **primary spermatocyte cells** also the unequal distribution of chromosomes has been found in *A. foveicollis* during telophase at the exposure of 3000r. In *D. cyngulatus* it has been seen at anaphase stage at 1500r and at telophase at the exposure of 3000r and 4000r.

In the **secondary spermatocyte cells** of *A. foveicollis* and *C. peregrinus*, this feature has been observed in telophase when exposed to 3000r and 4000r and in *D. cyngulatus* at the radiation of 3000r. It is difficult to give any explanation for the unequal distribution of chromosomes occurring at anaphase and telophase stages due to X-ray exposures during spermatogenesis. However, it is certain that this sort of behaviour of chromosomes may be a very important event responsible for genetic disbalance of the species.

### 5.1.4 Stickiness of the chromosomes-

When irradiated by X-rays the stickiness of the chromosomes has been observed by previous workers such as John, B., Lewis and Henderson, S.A. (1960). They reported the chromosomal abnormalities in *Chorthippus brunneus* where the stickiness is responsible for failure of pairing. Lim, HC (1972) found that the effects of gamma radiation on the chromosomes of Gryllidae (Orthoptera) encountered only the sticky bridges. Laxmikumari, B., Jayaprakash and Ananthanarayan, S.R. (1996) reported that in *B. mori* the gamma radiations of 3000r induced chromosomal fragmentation, stickiness clumping etc. during spermatogenesis. Verma, Reddy and Shevade (1996) observed stickiness of chromosomes in meiotic studies during radiations in *P. dermondii*. 
As reported by the above mentioned authors, during the present study also the stickiness of the chromosomes has been observed in various dividing cells of spermatogenesis when irradiated by different doses of X-rays.

At the prophase stage the chromosomes of the spermatogonial cells, when irradiated by 3000r and 4000r show stickiness in *A. foveicollis*, *A. intermedia*, *C. peregrinus* and *D. cyngulatus*. This has also been observed at the radiation of 1500r in *A. foveicollis*.

During metaphase stage the chromosomes become sticky and adhere with each other at the exposure of 1500r in *A. foveicollis* and *A. intermedia* and at 3000r in all the four species, and at 4000r in *A. foveicollis*, *C. peregrinus* and *D. cyngulatus*.

At the anaphase stage at the exposure of 3000r the stickiness between the chromosomes has been observed in all the four species. When exposed at 4000r this has been found in *A. intermedia* and *C. peregrinus*. When irradiated at 1500r the stickiness of the chromosomes has been observed only in *D. cyngulatus*.

At telophase stage the chromosomes become sticky when irradiated at 3000r and 4000r in all the four species.

The primary spermatocyte cells of all the four species when irradiated at 1500r, the stickiness has been observed at their pachytene, diakinesis and metaphase stages. It is also found at leptotene in *A. foveicollis* and at diplotene in *A. intermedia*.

When exposed to 3000r and 4000r almost all the chromosomes of leptotene, pachytene, diplotene, diakinesis metaphase, anaphase and telophase stages become sticky in all the four species.
The secondary spermatocyte cells when exposed to 1500r, 3000r and 4000r the chromosomes become sticky at their prophase metaphase, anaphase and telophase stages in all the four species. At the radiation of 3000r this has been found during prophase in *A. foveicollis* and *C. peregrinus* and at anaphase in *A. intermedia* and *C. peregrinus*.

From the present investigation it may be concluded that the chromosome of the spermatogonial as well as the secondary spermatocyte cells are more sensitive to the X-ray radiations at metaphase anaphase and telophase stages and exhibit stickiness. Similarly the primary spermatocyte cells also show stickiness of the chromosomes in almost all the dividing stages except at pachytene in *A. foveicollis* and *D. cyngulatous*.

The stickiness of the chromosomes is produced probably due to the X-ray radiations which may alter the chemical nature of the cytoplasm/nucleoplasm as well as in the chromosomal constituents. The stickiness may also be a cause of mis-saggregation of the chromosomes producing genetic abnormality.

5.2 **NUCLEAR SWELLING AND THE PERSISTANCE OF THE NUCLEAR MEMBRANE** -

Schmid, W. (1961) reported that there exists a relationship between average interphase nuclear volume and cell sensitivity to radiation. Apparently the large nuclear volume has the greater sensitivity (sparrow et. al 1963, casarett 1968). Further he pointed out that this relationship determined in animals (vertebrate) and plants, and used to predict their sensitivity to chronic irradiation may be relevant in insects. Similarly chandana, S. (2004) working on radio - resistance of Lepidopteran insects found that at the exposure of 200 Gy the nuclear size and granularity increases.
The spermatogonial cell, in *A. foveicollis*, at prophase stage the nucleus enlarges when it is irradiated at 1500r, 3000r and 4000r. The size of the nuclear membrane also increases due to the swelling at these radiations and is ultimately lost. In *A. intermedia* also the nucleus enlarges at the radiation of 1500r and 3000r but at 4000r it enlarges abnormally and almost touches the cell membrane. In *C. peregrinus* also the sizes of the nucleus increases at all the radiations i.e. 1500r, 3000r and 4000r. However, it disintegrates at the radiation of 3000r and 4000r. In *D. cyngulatous* the enlargement of the cell nucleus and the breaking of the cell membrane has been noticed in these cells at all the radiations. i.e. 1500r, 3000r and 4000r.

During metaphase and anaphase stages the nuclear membranes don't exist. However during telophase the reformation of the nucleolus as well as the nuclear membrane could be traced only in the daughter cells of *A. foveicollis* and *D. cyngulatous* at the radiation of 1500r. In *A. intermedia* it has been found at 3000r but could not be traced at all in *C. peregrinus*.

The primary spermatocyte cells, of *A. foveicollis*, *A. intermedia* and *D. cyngulatous* at their leptotene stages the nuclear swelling has been observed at the radiation of 1500r, 3000r and 4000r and not in *C. peregrinus*.

At the zygotene stage in *A. intermedia* and *D. cyngulatous* the nucleus swells up when irradiated at 1500r, 3000r and 4000r, but in *C. peregrinus* it is seen when exposed at 3000r and 4000r only. Remarkably the nuclear swelling is not found at all in *A. foveicollis* at all the three radiations. The nuclear membrane disintegrates in *A. foveicollis* at all the three radiation, in *C. peregrinus* and *D. cyngulatous* at 3000r and 4000r and in *A. intermedia* at 4000r only.
At pachytene stage in *A. foveicollis* and *A. intermedia* the nuclear swelling has been found at all the three radiations. In *C. peregrinus* it is seen at the radiation of 3000r and 4000r and in *D. cyngulatus* at 4000r only. The nuclear membranes of *A. foveicollis*, *A. intermedia* and *D. cyngulatus* are lost during all the three exposures, but in *C. peregrinus* it is visible only at the exposure of 1500r.

At the diplotene stage the nuclear swelling has been observed in all the four species at all the three radiations. At this stage the nuclear membrane is lost in *A. foveicollis* during all the three radiations. In *D. cyngulatus* it is present only at the exposure of 1500r. In *A. intermedia*, *C. peregrinus* and *D. cyngulatus* the nuclear membrane disintegrates at the radiations of 3000r and 4000r.

At diakinesis, metaphase and anaphase stages the nuclear swells up but the nuclear membrane is lost at all the three radiations in all the four insect species.

At telophase stages of *A. foveicollis* and *A. intermedia* the nuclear swelling has been observed and the nuclear membrane is lost.

In *C. peregrinus* and *D. cyngulatus* the nuclear membrane is reformed at the radiation of 1500r. In these species the nucleus swells up at all the three radiations.

The secondary spermatocyte cells, at their prophase stage the nucleus swells up and the nuclear membranes are lost in all the four species at all the three radiations. At metaphase and telophase stages the nuclear membranes of the secondary spermatocyte cells are not found but the nucleus swells up at all the three radiations.

At telophase stage the nuclear membrane is visible in *A. intermedia* at the radiation of 3000r and in *D. cyngulatus* at 1500r only. At this stage (i.e. telophase) the nuclear swelling has been noticed at all the three exposures in all the four insect species.
It may be concluded that at the higher radiations of 3000r and 4000r there is a tendency of nuclear swelling as well as the disintegration of the nuclear membrane is one of the remarkable feature.

5.3 NUCLEOLUS -

Only Klasterska, I. and Ramel, C. (1990) during their comparative study of male meiosis in *Drosophila melanogaster* and *D. virilis* reported that in *D. virilis* spermatocytes the nucleolus exhibits changes during the meiotic prophase which may be related to synthetical activities.

In the present study the X-ray effect on the nucleolus of the spermatogonial, primary spermatocyte and secondary spermatocyte cells is being discussed here.

In the spermatogonial cells at prophase stage, the size of the nucleolus measure approximately 1.0μ in its diameter. At the radiation of 1500r it increases a little bit in all the four species i.e. *A. foveicollis, A. intermedia, C.peregrinus* and *D. cyngulatous*. When exposed to 3000r it shrinks and becomes smaller in size in *A. foveicollis* but surprisingly enlarges in *D. cyngulatous*. It is difficult to explain why the nucleolus shrinks in one species and enlarges in another one at the same X-ray exposure. The nucleolus disintegrates at the radiation of 3000r in *A.intermedia* and *C. peregrinus* and at the high exposure of 4000r it disintegrates in *A.foveicollis, A.intermedia* and *C.peregrinus*. It persists only in *D. cyngulatous*.

In the primary spermatocyte cells of all the four species the nucleolus enlarges at the exposure of 1500r during leptotene, zygotene and pachytene stages. At the radiation of 3000r it enlarges in *A.intermedia* during leptotene and in *D. cyngulatous* during leptotene and zygotene stages. Surprisingly at the same
radiation (i.e. at 3000r) it shrinks in *A. foveicollis* and *C. peregrinus* at their leptotene, zygotene and pachytene stages. The nucleolus becomes small due to shrinkage in *A. intermedia* during zygotene and pachytene stages and in *D. cyngulatus* at the pachytene stage. At the high radiation of 4000r it shrinks in *A. foveicollis* and also in *C. peregrinus* at leptotene, zygotene and pachytene stages and in *A. intermedia* and *D. cyngulatus* at their pachytene stages.

It is remarkable to note here that in all the four species at diplotene and diakinesis stages the nucleolus is lost due to the radiation effect of the X-rays.

In the secondary spermatocytes cells, remarkably the nucleolus of the prophase stage is enlarged at the radiation of 1500r, 3000r and 4000r in *A. foveicollis, A. intermedia, C. peregrinus* and *D. cyngulatus*.

It is remarkable to mention here that at a particular X-ray exposure the nucleolus enlarges in one species but shrinks in another one. It is difficult to explain why it is so contradictory.

### 5.4 NUCLEOLAR VACUOLE

The account of the nucleolar vacuole in the cells of spermatogenesis is not available in the existing literature. Presently its presence and persistance at various x-ray exposures is being discussed here.

In the spermatogonial cells the nucleolar vacuole is an unstained small clear space seen within the nucleolus in the prophase stage. These cells when exposed to 1500r it persists only in *A. intermedia* but is absent *A. foveicollis, C. peregrinus* and *D. cyngulatus*. When irradiated at 3000r and 4000r it is not traceable in *A. intermedia* and *C. peregrinus* but is present only in *D. cyngulatus*. However it is present in *A. foveicollis* at the radiation of 3000r.
In the primary spermatocyte cells, remarkably in all the four species i.e. in *A. foveicollis, A. intermedia, C. peregrinus* and *D. cyngulatous* the nucleolar vacuoles are totally absent when irradiated at 1500r, 3000r and 4000r.

In the secondary spermatocyte cells, at the prophase stage the nucleolar vacuole is present in *D. cyngulatous* at the radiation of 1500r and 3000r but at 4000r it is lost due to the high X-ray irradiation. It is remarkable to note here that the nucleolar vacuoles are found absent in the secondary spermatocyte cells of *A. foveicollis, A. intermedia* and *C. peregrinus* at all the radiations.

5.5 PERINUCLEOLAR RING -

In the literature the account of the perinucleolar ring and the effect of radiation on them is not found in the cells of spermatogenesis. This is being described and discussed probably for the first time in the present study.

The perinucleolar ring is a clear unstained ring seen all around the nucleolus in various cells found during spermatogenesis. It may be mentioned here that the persistence of the perinucleolar ring varies in different cells of different species at different doses of the X-ray radiations.

In the spermatogonial cells, the perinucleolar ring is present in the prophase stage and persists even at the radiation of 1500r and 3000r in *A. foveicollis* and *D. cyngulatous* but not in *C. peregrinus*. However, when irradiated at 4000r it becomes disintegrated in all the three species mentioned above. Remarkably in *A. intermedia* the perinucleolar ring is totally absent in resting as well as in the prophase stages. The nucleolus is always absent in the metaphase, anaphase and telophase stage, therefore, the perinucleolar ring also doesn't exist.
5.6 **CYTOPLASMIC VARIATIONS IN THE MEIOTIC CELLS**

The cytoplasm is a general term used for the components of the cell placed outside the nucleus and within the cell membrane. It is translucent, homogenous colloidal fluid and contains all the essential organelles of the cell. This consists of various inorganic as well as organic compounds. It is agranular in appearance when observed under the light microscope.

A review of the literature reveals that the accounts on the cytoplasmic changes and the formation of cytoplasmic blocks have not been given so far by the previous workers. Probably this is the first description being given in the present study.

It has been noticed that during the process of spermatogenesis the cytoplasm of the spermatogonial, primary and secondary spermatocyte cells become more thicker and their stickiness also increases gradually with the increasing doses of X-rays i.e. 1500\(r\), 3000\(r\) and 4000\(r\) respectively.

In the spermatogonial cells, in *A. foveicollis* the cytoplasm becomes gradually thicker and more sticky in their prophase, metaphase, anaphase and telophase stages, at the radiations of 1500\(r\), 3000\(r\), and 4000\(r\). In *A. intermedia* and *C. peregrinus* during prophase and metaphase at the radiation of 1500\(r\) the cytoplasm doesn't become so thick but when irradiated at 3000\(r\) and 4000\(r\) it becomes much thicker and more sticky specially during their anaphase and telophase stages. As at the metaphase stage the nuclear membrane is lost with the result the nucleoplasm and the cytoplasm mix-up with each other. The condensed chromosomes are seen lying in this thick nucleo-cytoplasmic media during all the radiations.
In *C. peregrinus* during anaphase at 1500r and 3000r the cytoplasm becomes little thicker and form the spindle fibers but when exposed to 4000r the cytoplasm becomes much thicker and more sticky therefore, at the anaphase stage the two sets of chromosomes fail to separate from one another at the equatorial plate and very close to each other.

At the radiation of 3000r and 4000r, during telophase the thick cytoplasm is found surrounding the chromosomes of the two newly formed daughter cells. In *D. cyngulatus* at the radiation i.e. 1500r, 3000r and 4000r during prophase, metaphase, anaphase and telophase stage the nucleo-cytoplasm becomes more and more thicker and sticky at every exposure.

In the primary spermatocyte cells, in all the four species i.e. *A. foveicollis, A. intermedia, C. peregrinus* and *D. cyngulatus* the leptotene, zygotene, pachytene and diplotene stages, the thickness and stickiness of the cytoplasm increases gradually when exposed to 1500r, 3000r and 4000r.

In *A. foveicollis* and *A. intermedia* it is remarkable to note that at the exposure of 3000r and 4000r at their anaphase stage a few chromosomes fail to reach their respective poles due to the stickiness of the cytoplasm. At the same exposure i.e. at 3000r and 4000r.

In *C. peregrinus* and *D. cyngulatus* at their anaphase stage the chromosomes even can not separate from the equatorial plate due to the stickiness of the cytoplasm. The spindle fibers are not visible at these exposures.

In *A. foveicollis* and *C. peregrinus* at the high exposures during anaphase stages, the unequal distribution of chromosomes has been observed. It may be due to the obstruction in the movement of the chromosomes through the sticky cytoplasm.
During prophase of the secondary spermatocyte cells in all the four species the stickiness and thickness of the cytoplasm increases according to the increasing doses of X-rays.

During metaphase stage at 1500r the cytoplasm becomes somewhat sticky in all the four species. But at the radiation of 3000r and 4000r the stickiness increases more in *A. intermedia* and *C. peregrinus* as compared to *A. foveicollis* and *D. cyngulatus*.

During anaphase stage in *A. foveicollis* and *A. intermedia* the cytoplasm becomes much sticky at 3000r and 4000r therefore, some of the chromosomes fail to move to their respective poles, through the sticky cytoplasm.

At the telophase stage in *A. foveicollis*, *A. intermedia* and *C. peregrinus* the cytoplasm become much stickier at the radiation of 3000r and 4000r as compared to *D. cyngulatus*. Stickiness of the cytoplasm probably give rise to unequal cytokinesis and the unequal distribution of chromosomes at this stage.

5.7 **FORMATION OF THE CYTOPLASMIC BLOCKS AND THEIR PERSISTANCE** -

Generally the cytoplasm is uniformly distributed all around the nucleus showing no differentiation. But when the different cells of spermatogenesis are irradiated by different X-ray doses, the cytoplasm of some of the cells becomes condensed and form small thick "block" like structures which are seen arranged just below the cell membrane. Interestingly these 'cytoplasmic blocks' have not been reported so far by the previous authors. It is remarkable to note here that these thick cytoplasmic blocks are not formed always by the X-ray irradiations but vary in different cells at different exposures. It is just possible that the physico-chemical nature
of the cytoplasm is changed due to irradiations therefore the thick cytoplasmic blocks are formed just below the cell membrane, however the exact reason for their formation is unknown.

In the spermatogonial cells at the prophase stage 'cytoplasmic blocks' are formed in *A. foveicollis* and *C. peregrinus* at the exposure of 4000r but in *A. intermedia* and *D. cyngulatus* at the exposure of 3000r and 4000r. These are not formed during metaphase, anaphase and telophase stage in all the four species of insects at the radiation of 1500r, 3000r and 4000r.

In the primary spermatocyte cells during leptotene in *A. intermedia* only the cytoplasmic blocks have been found at the exposure of 3000r. Remarkably these are totally absent in *A. foveicollis*, *C. peregrinus* and *D. cyngulatus* in diplotene cells at the radiation of 3000r and 4000r.

During zygotene the cytoplasmic blocks are formed only in *A. intermedia* at all the three radiations but could not be seen in *C. peregrinus* and *D. cyngulatus* at any radiation. However, in *A. foveicollis* these 'blocks' are visible only of the radiation of 3000r in this stage.

During pachytene stage the cytoplasmic blocks are not traceable in any of the species at all the three radiations.

At diplotene stage the cytoplasmic blocks are formed at all the three radiations in *A. foveicollis*, and *C. peregrinus*. In *A. intermedia* these are visible only at the radiation of 1500r, but in *D. cyngulatus* are totally absent during all the three X-ray exposures.

At diakinesis, metaphase and telophase stage of the primary spermatocyte cells the cytoplasmic blocks are not at all formed in all the four species.
at all the three radiations, but during anaphase these are visible only in *D. cyngulatous* at the exposure of 4000r.

In the secondary spermatocyte cells at the prophase stages of *A. foveicollis, A. intermedia* and *D. cyngulatous* the cytoplasmic blocks are formed when irradiated at 1500r. Remarkably at the exposure of 3000r this feature is not formed in all the four species. When exposed to 4000r it is visible only in *A. intermedia*.

During metaphase stage the cytoplasmic blocks are totally absent at all three radiations in all the four species.

At anaphase stage these blocks are seen only in *A. foveicollis* and during telophase stage in *A. foveicollis* at the exposure of 4000r only.

From the above findings it become clear that the cytoplasmic blocks are not formed at the metaphase, anaphase and telophase stages of the spermatogonial cells, and also in the primary spermatocyte cells during pachytene, metaphase, anaphase and telophase stages at all the three X-ray radiations. Further it has been noticed that these blocks are formed more frequently in *A. intermedia* and *D. cyngulatous* in comparison to *A. foveicollis* and *C. peregrinus*.

It may be concluded that *A. foveicollis* and *C. peregrinus* are more X-ray radiation resistant whereas *A. intermedia* and *D. cyngulatous* are more radio sensitive. It is difficult to give any explanation regarding the formation of this structure but one of the reasons may be attributed to the physico-chemical changes which develop in the cytoplasm due to X-ray irradiations.
SPERMIOHISTOGENESIS

During spermiohistogenesis the radiation effect was demonstrated by schlarger (1960) and reported that the existence of sperm precedence in *T. castaneum* are differentially sensitive to X-rays. He found that when males irradiated by 1450r or 2900r x-ray exposures their fertility is depressed. No evidence was found for recovery of the irradiated sperms. Schmid, W. (1961) observed the differential susceptibility of sperm and spermatid to ionizing radiation and found that in *Drosophila* a given X-ray dose cause up to several times more genetic mutations in spermatids then in sperms. During ionization the chromosomal damage was observed. Tahmisian, T.N. and Devine, R.L. (1961) reported that in grasshopper doses of 100 to 600 roentgens were found to retard the differentiation of the nucleus in spermatids. The above doses also induced acrosome, nuclear disorganization as well as the fragmentation. Purdom, C.E. and Meshechy, T.W. (1963) observed that when *Drosophila* was exposed to 800r, mutation frequency was independent of dose rate in each spermatid. Lura, C. Giojalas (1993) reported that ultra structural variations in the spermiogenesis of *Triatoma infestan* induced by temperature changes caused abnormal changes in spermiogenic cells such as lack of spermatid orientation. Dallai, R. (2002) observed that spermatozoa of *A. formicaria* show that the sperm nucleus is characterised by the presence of two lateral grooves which are filled with numerous infoldings of the nuclear envelope.

In the present investigation also the x-ray irradiations produce changes in the spermatids such as swelling of the cell, the nuclear swelling, and chromosomal disorders etc., which are responsible for producing genetic mutations as reported by the previous investigators.
5.8.1 Spermatid swelling and variations in its chromatin -

The spermatids of *A. foveicollis*, *A. intermedia*, *C. peregrinus* and *D. cyngulatous* when irradiated by X-ray exposures of 1500r, 3000r and 4000r the nuclear size increases probably due to the swelling and variable condensations of their chromatin material.

The spermatids of *A. foveicollis* when exposed to 1500r they swells up and their chromatin material is found in diffused form but in *A. intermedia*, *C. peregrinus* and *D. cyngulatous* the chromatin becomes condensed and forms a semi lunar shaped structure at the inner margin of the nuclear membrane.

At the exposure of 3000r the chromatin is gradually dispersed within the nucleus in *A. intermedia* and *C. peregrinus* but in *A. foveicollis* it becomes little condensed. In *D. cyngulatous* it becomes more condensed and fuses to form two to three large granules within the nucleus.

At the high exposure of 4000r in *A. foveicollis* the spermatid nucleus swells up and is enlarged in its size, its chromatin becomes condensed and forms granules. In *A. intermedia*, *C. peregrinus* and *D. cyngulatous* also the chromatin becomes very much condensed and is dispersed within the nucleus.

During the first elongating stage of the spermatid nucleus when irradiated by 1500r, 3000r and 4000r, it has been found that in *A. foveicollis* the chromatin material is extremely condensed and become arranged at the lateral margins of the nucleus. At the exposure of 1500r and 3000r in *A. intermedia* the nuclear membrane swells up and become enlarged, its chromatin becomes granular and is dispersed at the inner surface of the nucleus. At the high exposure of 4000r also the nucleus swells up and the chromatin becomes extremely condensed and aggregates.
at the inner margin of the cell membrane. In *C. peregrinus* the nucleus enlarges in size due to swelling. At the exposure of 1500r the chromatin is dispersed in the nucleus but at 3000r and 4000r it becomes much more condensed and fuses with each other. In *D. cyngulatus* at the radiation of 1500r the chromatin becomes granular and at 3000r and 4000r the spermatid swells up. Its chromatin becomes extremely condensed.

During second elongating stage of the spermatid, in *A. foveicollis* and *A. intermedia* at the radiation of 1500r and 3000r its nucleus becomes oval and swells up, its chromatin becomes condensed. At the high radiation of 4000r the nucleus enlarges and its chromatin show much more condensation. In *C. peregrinus* and *D. cyngulatus* at the radiation of 1500r and 3000r the chromatin is condensed and becomes dispersed at the inner surface of the nucleus. At the high radiation of 4000r the spermatid nucleus swells up and the chromatin becomes extremely condensed and get arranged at its lateral margins.

During third elongation the spermatids of *A. intermedia* and *C. peregrinus* when exposed to 1500r and 3000r the chromatin become condensed, fuse and form few large granules. But in *A. foveicollis* at 1500r and in *D. cyngulatus* at 3000r the chromatin is found arranged at the lateral margin of the elongating sperm nucleus. In *A. foveicollis* the chromatin material fuse to form four to five large granules at the exposure of 3000r. But in *A. foveicollis* and *D. cyngulatus* at 4000r, the nucleus enlarges and the chromatin material is found condensed at the lateral margins of the elongating nucleus.

During fourth and fifth elongating stages of *C. peregrinus* and *A. intermedia*, at the exposure of 1500r, the spermatid nucleus swells and the
condensed chromatin is found arranged at the lateral margins of the nucleus. These at the high exposure of 3000r and 4000r become more condensed and looks like curiled threads (showing coiling). In A. foveicollis and D. cyngulatus the sperm nucleus becomes flat and appears like a 'leaf' or 'trypanosome'. The chromatin material is seen uniformly distributed in the flat nucleus. Further at the radiation of 1500r the chromatin material becomes little condensed and found uniformly dispersed in the flat nucleus. At the high radiation of 3000r and 4000r the lateral margins of the flattened leaf like nucleus roll up and fuse together at the midline, enclosing a longitudinal vacuole within it. Later, the sperm nucleus becomes very much condensed and thick due to swelling and the radiation effect of the x-ray. As a result of this longitudinal vacuole is totally lost.

During sixth elongation the sperm nucleus becomes condensed and contains a vacuole at the radiation of 1500r in all the four species. These when irradiated at 3000r the chromatin of the nucleus becomes condensed and a large vacuole appears within it, in A. foveicollis and C. peregrinus but in A. intermedia the vacuole is lost. At the high radiation of 4000r in A. foveicollis the chromatin becomes condensed and breaks and shrinks. In C. peregrinus the sperm nucleus becomes spirally coiled and in D. cyngulatus the fragments of the chromatin material lie lengthwise, sticking with each other.

5.8.2 Formation of tubular vacuole -

The only reference available in the literature so far is of Khatoon, Shahida, Ghosh, N. and Sharma, Sarita (2006) who observed the variations in the spermiohistogenesis of A. foveicollis and L. quanmaculata (coleopterans) and reported certain remarkable differences regarding the formation of long tubular
vacuoles in both the species. In *A. foveicolis* the elongating spermatid nucleus becomes flat and looks like a 'trypanosome'. Later its margins roll up and fuse together so that a long tubular vacuole is enclosed inside it. But in the case of *L. quadriracetata* the spermatid nucleus doesn't become flat. In this case the nucleus contains 2-3 vacuoles in it and during elongation these increase in number and fuse with each other to form a single long vacuole. The authors did not study the X-ray effect on these insects.

As already mentioned earlier during spermateliosis in *A. foveicolis* and *L. quadriracetata* the spermatid nucleus becomes flattened like a 'leaf' as observed by Khatoon, Shahida, Ghosh, N. and Sharma, Sarita (2006). The lateral margins of the flattened nucleus of *D. cyngulatus* and *A. foveicolis* roll up and become fused together enclosing a longitudinal tubular vacuole within it. At the exposure of 1500r, the chromatin material become more condensed and the tubular vacuole is still visible. At the radiation of 3000r the chromatin become condensed with the result the vacuole is lost. At the high exposure of 4000r the chromatin material of the elongating spermatid become very much condensed and fragmented and are found arranged longitudinally. The tubular vacuole is ultimately lost due to the nuclear condensation.

During early elongating stages of spermatids in *A. intermedia* and *C. peregrinus* the nucleus contains one to two vacuoles, rarely two to three. At the high radiation of 3000r and 4000r the vacuoles become enlarged and fuse with each other and form a longitudinal tubular vacuole. The chromatin material becomes condensed and the tubular space is lost due to radiation effect.

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