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NECROSIS INDUCED BY TEPA IN THE GONADS OF *LOCUSTA MIGRATORIA* (L.)[I]

Vishwa Nath, P.K. Mittal and Chander Sheikher

Department of Zoology, Panjab University, Chandigarh-160014, India

A B S T R A C T

Various doses of the chemosterilant tepa were injected into 5th instar nymphs and adults of *Locusta migratoria* (L.). Tepa causes loosening of the germ cells in the testis. Spermatogonia and primary spermatocytes are the first to be affected, showing pycnosis. The secondary spermatocytes and the spermatid become hypertrophied. The chromatin in the spermatids gets displaced, and fragmentation of chromosomes during meiosis I is observed. In the ovary the follicular cells show abnormal fragmentation of the chromatin material, and their cytoplasm is drawn into the peripheral empty space formed by the contraction of the ooplasm. Yolk formation is inhibited.

Necrosis increases with increase in dose and prolongation of the posttreatment period.

INTRODUCTION

Only few references are available on necrosis caused by tepa in the gonads of orthopteran insects (Burden and Smittle, 1963; Smittle et *al.*, 1966; and Saxena and Aditya, 1969, 1971, 1974). Reviews by Borkovec (1966), LaBrecque and Smith (1968), Campion (1972), and Davidson (1974) refer to studies on the effect of chemosterilants on different animal groups.

This paper reports on a detailed study of necrosis caused by tepa in the gonads of *Locusta migratoria* (L.).

MATERIAL AND METHODS

Specimens of *Locusta migratoria* were collected from the sandy areas of Bikaner (Rajasthan, India) during June and July, 1972, and reared in aluminium cages (volume of 1 cubic foot) under temperature conditions of 28° to 32°C.

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Various doses i.e., 0.01 mg of tepa (always dissolved in distilled water) per 5th instar and per insect, and 0.05 mg/insect, were injected into the locusts' abdominal region. The injected insects were vivisected after various time intervals (1,4,5,7,9 and 15 days). Extraneous material covering the gonads was removed in saline (a solution containing 1 litre of 0.7% NaCl and 2 ml of 10% $CaCl_2$), and the gonads were fixed in Zenker overnight. They were dehydrated, cleared and embedded in paraffin wax (60° to 62°C). Paraffin sections (7μ thick) were cut and stained in Heidenhain's haematoxylin.

RESULTS

Testis: Germ cells in adult testicular follicles reveal a loss of cohesion (Fig. 1) one day after injecting the 0.05 mg dose and seven days after the 0.01 mg dose. When 0.01 mg of tepa is injected into 5th instar nymphs, loosening of germ tissue is observed after four days.

Tepa treatment with these doses results in pycnosis of some spermatogonia, primary spermatocytes and apical cells one day after treatment (Fig. 2). Pycnosis with a subsequent degeneration of germ cells is more pronounced when the post-treatment period is increased to 15 days.

A 0.05 mg dose causes hypertrophy in the nuclei of some secondary spermatocytes (Fig. 3) and spermatids one day following treatment. Hypertrophied cells were also observed four days subsequent to treatment with 0.01 mg. As the post-treatment period is prolonged to 15 days, hypertrophy becomes more pronounced and severe, the testicular tubules containing mostly hypertrophied spermatids and sperm nuclei (Fig. 4).

The cytoplasm of all categories of germ cells shrinks and becomes vacuolated as a result of the tepa treatment. The chromatin in spermatid nuclei is displaced to the periphery in one or more groups instead of being uniformly distributed (Fig. 5).

The chromosomes during meiosis I show abnormal behaviour: they become fragmented. Laggards are also observed at anaphase II (Fig. 6).

Ovary: A dose of 0.01 mg per nymph or adult does not damage the oocytes after one day. After four days, however, the

ooplasm of younger oocytes contracts considerably, and an abnormal fragmentation of the chromatin of the follicular epithelial cell nuclei is observed (Fig. 7). The cytoplasm of some follicular epithelial cells is drawn in the form of prominent projections into the peripheral ooplasm of certain oocytes. Seven days after treatment the ooplasm contracts and the germinal vesicle in some oocytes becomes elliptical. After 15 days of treatment the cytoplasmic membranes of the follicular epithelial cells completely disappear; the ooplasm contracts as in weaker doses; and the cytoplasm of the follicular epithelial cells moves inwards, into the peripheral empty space formed by the ooplasm's contraction (Fig. 8).

One day after treatment with 0.05 mg/insect some pycnotic nuclei may be observed in the follicular epithelium. The number of nucleolar extrusions decrease in the ooplasm. The germinal vesicle becomes elliptical (Fig. 9). After four days more muclei of follicular epithelial cells become pycnotic. An abnormal fragmentation of the chromatin of the follicular epithelial cells is observed. In younger oocytes the nucleolar extrusions decrease in number. In maturing oocytes yolk granules are not observed (Fig. 10). In some oocytes ooplasmic contraction increases. After seven days of treatment the follicular epithelium is reduced in width, and no nuclei are seen in the follicular epithelium. A few pycnotic nuclei migrate into the ooplasm. In mature oocytes the ooplasm is badly damaged inasmuch as it degenerates (Fig. 11). After 15 days the oocytes are very badly damaged, there being no yolk whatsoever.

DISCUSSION

Testis: As described, spermatogonia and primary spermatocytes become pycnotic and gradually degenerate after tepa treatment. Consequently the testicular debris increases. The cytoplasm shrinks and becomes vacuolated. These observations are in agreement with those of various other workers. Saxena and Aditya (1969, 1971) observed pycnotic nuclei and increased testicular debris in *Poecilocerus pictus* F. Schwartz (1965) reported a general necrosis of the germinal epithelium of testis in addition to a loss of testicular weight in the eye gnat (*Hippelates pusio* Loew) after tepa treatment. Hedin *et al.* (1967) noted a decrease in the testicular size in the boll weevil (*Anthonomus grandis* Boheman) 14 days after tepa treatment; and Henneberry *et al.* (1972) observed a decrease in

testis volume after metepa administration in the cabbage looper moth, *Trichoplusia ni* (Hübner).

In male locusts nuclei of the secondary spermatocytes and spermatids become hypertrophied after tepa treatment. Similar observations were made by Saxena and Aditya (1969, 1971) in *P. pictus*. They called these hypertrophied cells giant cells. The hypertrophied sperms suggest a physiological inactivation of the spermatozoon which is otherwise equipped with a well-developed flagellum and acrosome.

A displacement of nucleoprotein material was noted in *Locusta*, which agrees with the observations of Saxena and Aditya (1969, 1971).

From our observations it appears that tepa mainly affects cells during active phases of proliferation, causing either clumping of chromatin material or excessive fragmentation. Prolonging the posttreatment period causes a marked decrease in the spermatogonial and spermatocyte population. Cline (1968) has also observed a marked reduction in spermatogonial generation in house flies after treatment with thiotepa, though the structure of the testis remained unimpaired.

In general, earlier stages of development are more susceptible to radiation injury than later ones (Davidson, 1974). But this may not be the case with alkylating agents. Fahmy and Fahmy (1964) (quoted by Davidson, 1974) observed that TEM was only half as injurious to spermatogonia as to spermatozoa in *Drosophila*, while phenylalinine mustard was four times as injurious to spermatogonia. Taber and Borkovec (1969) observed that the honey-bee queen (*Apis mellifera* L.), when inseminated artificially with preserved drone sperm treated with tepa, laid only non-viable eggs. Dame and Ford (1964) suggested that tepa affects sperms in spermathecae.

Since the earlier stages of development in the locust are severely damaged by tepa, the chances of fertility recovery are slim.

Ovary: In tepa-treated locust ovary the nuclei of the follicular epithelial cells become pycnotic, and, after a prolonged posttreatment period, the epithelium is severely damaged. Such observations were also made by other workers with tepa or its derivatives in other insects. In the tepa-treated panoistic ovary of *P. pictus* the nuclei of the follicular epithelial cells

also become pycnotic, staining deeply with Feulgen's reaction (Saxena and Aditya, 1974). Treatment of *Musca domestica* L. with tepa and metepa (Morgan and LaBrecque, 1964); and thiotepa (Combiesco *et al.*, 1967) caused the nuclei of nurse cells and follicular epithelial cells to condense and become pycnotic.

In locust oocytes treated with tepa the ooplasm contracts considerably; Saxena and Aditya (1974) have also recorded such contraction of ooplasm in *P. pictus*.

In maturing locust oocytes the ooplasm is severely damaged inasmuch as it degenerates. No yolk bodies are found in mature oocytes. Such observations have also been made in regard to other insects. The polytrophic ovary of *Musca domestica* shows growth inhibition of the oocytes after being treated with tepa and metepa (Morgan and LaBrecaue, 1964); and thiotepa (Combiesco *et al.*, 1967). Burden and Smittle (1963), using methiotepa, and Smittle *et al.* (1966) with tepa, found a reduction in the number of distal oocytes in the German cockroach, *Blattella germanica* (L.), as well as the usual ovary disintegration. Reduction in ovary size following tepa treatment was also recorded in *Anopheles labranchiae* Falleroni (D'Alessandro *et al.*, 1966), in *Dysdercus cingulatus* F. (Sukumar and Naidu, 1973), and in the olive fruit fly, *Dacus oleae* Gmelin(Fytizas, 1967; Tzanakakis, 1967).

Campion (1972) reports that in tepa-treated *Diparopsis* the developing oocytes degenerate markedly, and the mature eggs are partially absorbed. Identical observations were earlier made by Bulyginskaya *et al.* (1967) who treated the oocytes of *Cydia pomonella* (L.), *Heliothis armigera* (Hübner) and *Agrotis segetum* (Schiffermüller) with thiotepa and tretamine.

Tepa treatment severly damages the yolk-laden ova of the locust, inasmuch as the yolk globules degenerate. Henneberry *et al.* (1972) found that tepa and metepa-treated ovaries of *Trichoplusia ni* were affected and that ova development was apparently inhibited, except for those in late stages of development. Bertram (1964) treated *Aedes aegypti* (L.) just after emergence with thiotepa and found that oogenesis did not proceed beyond the resting stage (Stage 11). The ovaries of survivors which did not lay eggs lacked an organized structure and showed a cytopathology resembling that caused by antimetabolites. Bing (1966) treated the Oriental house fly (*Musca domestica vicina* Macquart) with thiotepa and

reported degeneration of oogonial cells. Crystal and LaChance (1963), and LaChance and Crystal (1963) treated the screw worm fly, *Callitroga hominivorax* (Coquerel), with thiotepa; greatest inhibition of ovarian growth occurred if the chemosterilants were administered at the stage when nurse cells are in the endomitotic phase.

Necrotic changes were observed by Novak *et al.* (1972) in the telotrophic ovaries of the beetles *Hylobius abietis* (L.), *Acanthoscelides obtectus* (Say) and *Trogoderma granarium* Everts after treatment with tepa. Ondracek and Matolin (1971) reported similar changes in *A. obtectus*, because division of the trophocytes in the germarium's upper zone was suppressed. The descending oocytes were completely devoid of follicular epithelium as the prefollicular cells had earlier been completely destroyed.

No yolk bodies were observed in the mature oocytes of the panoistic ovary of *Locusta migratoria*. Saxena and Aditya (1974) report a similar situation in regard to *P. pictus*.

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EXPLANATION OF FIGURES

Fig. 1. Section of testis showing loosening of germ cells; some pycnotic cells can also be identified; seven days after treatment with a 0.05 mg dose.

Fig. 2. Section of testis showing pycnotic spermatogonia (PSG) and pycnotic apical cells (PA), one day after treatment with 0.05 mg.

Fig. 3. Section of testis showing hypertrophied secondary spermatocytes (arrows), seven days after treatment with 0.05 mg.

Fig. 4. Section of testis showing hypertrophied spermatid (arrow) in maturation phase, 15 days after treatment with 0.01 mg.

Fig. 5. Section of testis showing displaced nucleoprotein (arrows), seven days after treatment with 0.01 mg.

Fig. 6. Section of testis showing laggard at anaphase II (arrow), seven days after treatment with a 0.05 mg. dose.

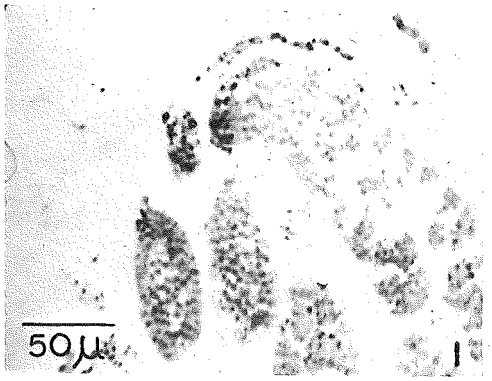
Fig. 7. Section of ovary showing contraction of the ooplasm (O); and abnormal fragmentation of the chromatin of the follicular epithelial cell nuclei (arrow), four days after treatment with 0.01 mg.

Fig. 8. Section of ovary showing the cytoplasmic projections (CP) of the follicular epithelial cells drawn inwards into the peripheral empty space formed by ooplasm contraction, 15 days after treatment with 0.01 mg.

Fig. 9. Section of ovary showing pycnotic nuclei of the follicular epithelial cells (FEC); decrease in the number of nucleolar extrusions (arrow) in the ooplasm; and the elliptical germinal vesicle (GV). One day after treatment with 0.05 mg.

Fig. 10. Section of ovary showing pycnosis and abnormal fragmentation of the chromatin of follicular epithelial cell nuclei (FEC); decrease in number of nucleolar extrusions (NE). Note the absence of yolk precursors in the cortical ooplasm. Four days after treatment with 0.01 mg.

Fig. 11. Section of ovary showing degeneration of follicular epithelium (FE), and damaged yolk (arrow). Seven days after treatment with a 0.05 mg. dose.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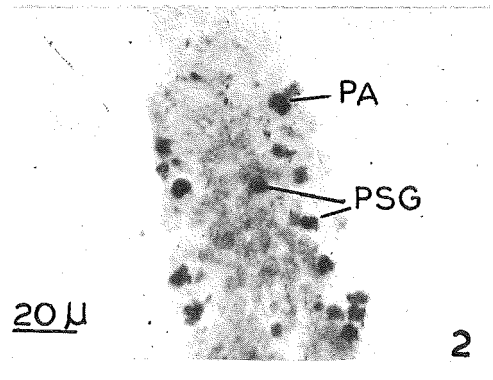
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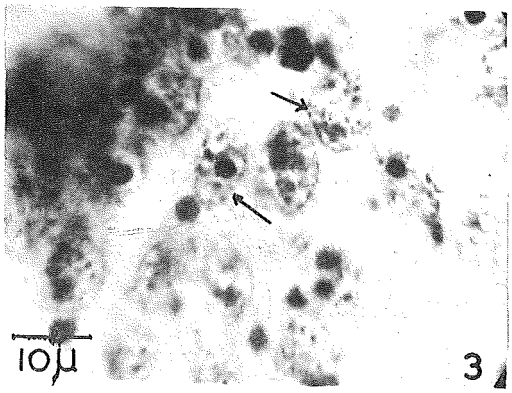
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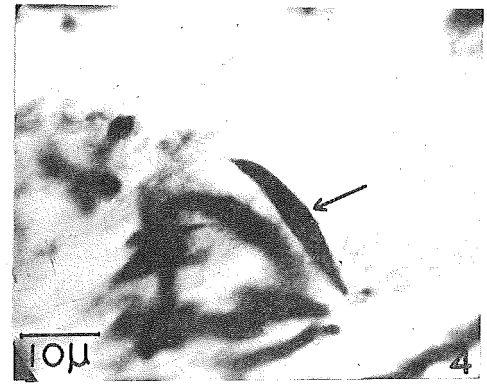
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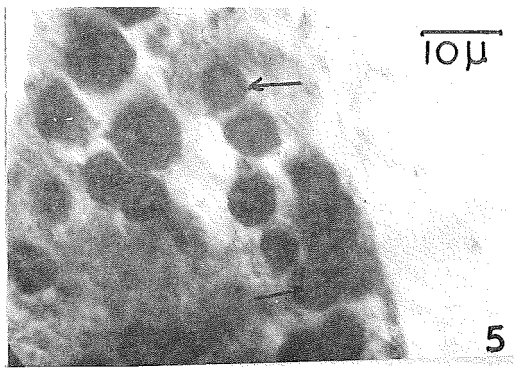
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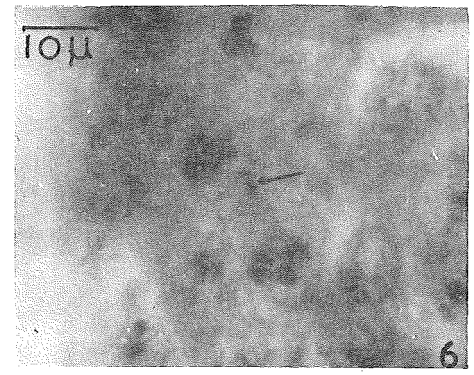
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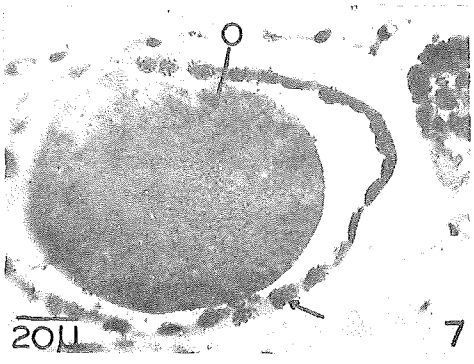
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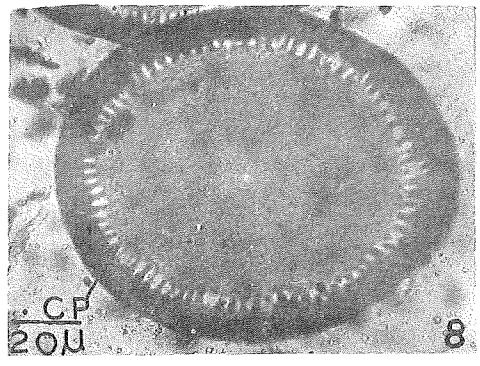
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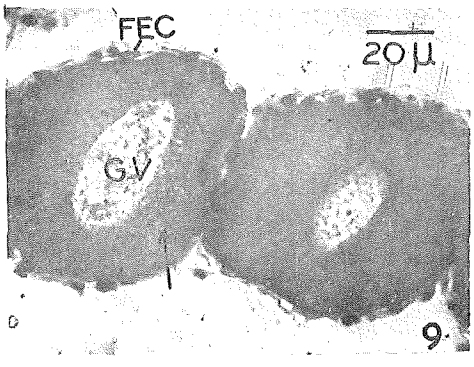
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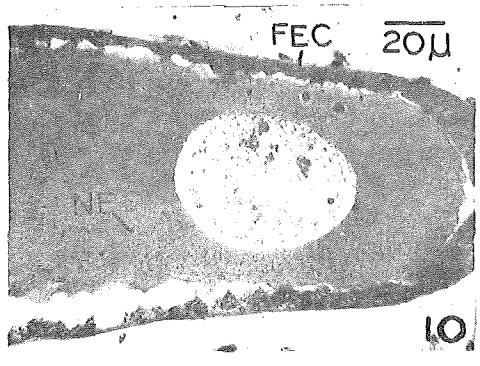
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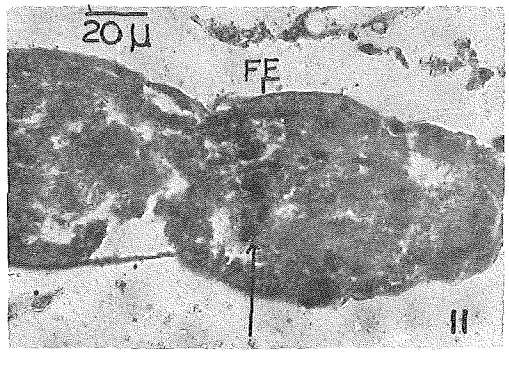
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