CHAPTER V

HISTOCHEMICAL OBSERVATIONS IN THE OVARIAN SYSTEM OF

Periplaneta americana

AND

Poekilocerus pictus
(A) HISTOCHEMICAL OBSERVATIONS IN THE OVARIAN SYSTEM OF Periplaneta americana, Linn. (Orthoptera Acrididae)

Histochemical observations are expressed on the following aspects:

(I) HISTOCHEMICAL OBSERVATIONS ON THE OVARIAN SYSTEM AND PESTICIDE TREATED SERIES:

The ovaries of Periplaneta americana are paroistic type in comparison to the normal insects. The ovaries of control series are more or less similar in histochemical aspects but in pesticide treated female insects, showed depletion, destruction and degeneration of all types of metabolites viz. protein, glycogen and lipid etc. which are histochemically confirmed by different histochemical techniques e.g. Mercuric Bromophenol Blue (Hg. B.B. for protein), Methyl green pyronin (P/MG, for DNA and RNA), Periodic Acid Schiff's reaction (PAS for carbohydrate) and Sudan Black 'B' (for lipid) were applied to detect the fate of various metabolites during pesticide treatment, on the following aspects:

(1) THE GERMARIIUM: In control series of Periplaneta americana, the germarium showed the high intensity of protein-positive, RNA-positive and PAS-positive materials in the developing oocytes but with very less lipid contents as observed in the germarium of normal insects but in pesticide treated insects the slow rate (in STTFI)
or nil (in LTTFF) resulted into very low intensity of protein-positive, PAS-positive and RNA-positive materials, while sudanophilic material is not observed. The decline in the intensity of different metabolites in germarium is correlated with the pesticide toxicity.

(ii) THE VITELLARIUM: The vitellarium of control series of *Periplaneta americana* showed the arrangement of oocytes in successive manner within the ovariole as observed in normal insects. The immature oocytes are located at the anterior end of vitellarium i.e. in between germarium and mature yolky oocytes, while mature oocytes are situated posterior to immature oocytes. The terminal oocyte is the lower most (first formed) oocyte of the ovariole is bigger than other oocytes, is megalolecithal type. A penultimate oocyte is situated above the terminal oocyte in each ovariole. Both terminal (ultimate) and penultimate oocytes are nearly similar in size, shape and in their yolk contents in control series. The previtellogenic and vitellogenic phases are similar in comparison to normally developed oocytes, but a few oocytes showed some adverse histochemical symptoms in the ovarian architecture, while in pesticide treated insects, not only the immature but also the mature oocytes showed the depletion of histochemical materials as follows:

(a) IMMATURE OOCYTES:

(i) CONTROL SERIES: In *Periplaneta americana*, immature oocytes are encircled with regular follicular epithelium
which is protein-positive, RNA-positive and PAS-positive with less sudanophilic nature. The oocyte nucleus is protein-positive, RNA-positive and PAS-positive in nature. The oocyte nucleus is uniformly stained structure. At the earlier stages of growth period, small homogenous and deeply stained granules are seen in the phase of vitellogenesis as observed in normal insects. These granules are actually made up of protein-carbohydrate complex, highly protein-positive and PAS-positive in nature and afterwards there is an increase in the synthesis and deposition of PYP, yolk bodies, yolk spherules within the ooplasm but in immature oocytes some lipid spherules are visible.

(ii) PESTICIDE TREATED SERIES: The follicular epithelium of immature oocytes of Periplaneta americana is irregular in outline and these oocytes are smaller in size in comparison to that found in normal and control insects. The follicular epithelium showed the less intensity of protein-positive, RNA-positive and PAS-positive material in comparison to that present in control and normal insects. The oocytes does not show the presence of any type of granules, spherules or platelets etc. in the ooplasm which are uniformly distributed within the irregular and immature oocytes. The ooplasm is with very light protein-positive, RNA-positive, PAS-positive or sudanophilic material. The lightly stained oocyte nucleus is irregular with lacunae and without nucleolus which is generally PAS-negative in nature and initiate nucleus degeneration. The tunica propria is intensely protein-
positive, RNA-positive and PAS-positive in mature cells.

(b) MATURE OOCYTES:

(i) CONTROL SERIES: The follicular epithelium of mature oocytes of Periplaneta americana with highly Hg.B.B.-positive (protein-positive) and RNA-positive cytoplasm in which darkly protein-positive, DNA-positive nuclei, then intensity increases steadily towards the terminal oocytes which are with the follicular epithelium of high DNA-positive intensity. The small sized protein-positive and PAS-positive granules clumped together to form large sized protein-positive bodies, spherules, protein platelets, PYP bodies and yolk bodies which are abundant in number in penultimate and terminal oocytes as observed in normal insects. The nucleus of the penultimate and terminal nucleus is not situated in the centre of oocyte but it somewhat migrate towards the proximal end and is mostly protein-positive, RNA-positive and PAS-negative in nature. It does not show the presence of sudanophilic nature, while in ooplasm, adequate amount of PAS-positive, protein-positive PYP, yolk bodies', RNA-positive bodies, sudanophilic granules and $L_1$, $L_2$ and $L_3$ lipid bodies etc. are abundant as observed in normally grown ultimate and terminal oocytes. The tunica propria is with highly protein-positive, RNA-positive, PAS-positive in nature with intense sudanophilic material.

(ii) PESTICIDE TREATED SERIES: The pathogenic and subpathogenic oocytes of Periplaneta americana showed the presence of necrotic follicular epithelium which becomes stratified and irregular in outline with adequate amount
of protein-positive material while it's RNA-positive intensity gradually decreases in LTTFI. The DNA-positive intensity of follicular nuclei is very high in Malathion and Endosulfan exposed insects in comparison to the control and normal ones. The PAS-positive intensity of follicular epithelium of pesticide treated insects is very low in comparison to control and normal insects. High degree of vacuolization is observed in resorbed ooplasm, showed the active resorption due to pesticide intoxication in Periplaneta americana. These vacuoles are protein-negative, RNA or DNA-negative and also PAS-negative and does not show the presence of sudanophilic material within them. The oocyte nucleus which is less RNA-positive is sometimes protein-negative and PAS-negative in nature in STTFI, becomes disintegrated, in LTTFI and in severely damaged pathogenic oocytes. The oocyte nucleus is totally resorbed. Vacuolization in follicular epithelium is the specific feature of all pathogenic oocytes. The reduction in the size of the pathogenic and subpathogenic oocytes is correlated or directly proportional to the amount of protein-positive, RNA-positive, PAS-positive materials from these oocytes. In some oocytes, the entire ooplasm obliterated by the presence of large number of protein-positive, DNA and RNA-positive and PAS-positive lecitholytic cells which are proliferated from the follicular epithelium. The size of the severely damaged oocytes reduced due to shrunken of ooplasm by the invaded follicular epithelium. The tunica propria which is intensely protein-positive, RNA-
positive in nature becomes steadily increases in the intensity of all these histochemical materials. The oocyte nucleus showed low intensity of sudanophilic material. The ooplasm of STTFI is with adequate amount of $L_1$, $L_2$ and $L_3$ lipid bodies, sudanophilic granules and spherules shows the gradual decrease in MTTFI to LTTFI and in LTTFI, there are few indications of the resorption of lipid bodies by necrotic follicular epithelium. The tunica propria is sudanophilic in nature while follicular epithelium of pathogenic oocytes in LTTFI is more sudanophilic than found in STTFI, indicates that the follicular epithelium also takes active part in the resorption of the sudanophilic material from the disintegrated ooplasm.

HISTOCHEMICAL OBSERVATIONS ON THE CORPUS LUTEUM OF Periplaneta americana, Linn.

(1) PROTEIN:

(a) CONTROL SERIES: In Periplaneta americana the protein-positive intensity in the corpus luteum of control females is very high as seen in normal insects. The pycnotic nuclei and their chromatin material is highly protein-positive and scattered in the intensely protein-positive cytoplasmic syncytium. An empty space which is created due to oocyte ovulation from the respective follicle is totally protein-negative, while it contains some intensely protein-positive material. The much folded tunica propria is less intensely protein-positive. The size of the corpus luteum is bigger in comparison to Poekilocerus pictus.
(b) PESTICIDE TREATED SERIES: In pesticide treated females, the protein-positive intensity of corpus luteum decreases from short term to long term exposure of pesticides while the vacuolization in cytoplasmic syncytium presumably due to the resorption of protein-positive material from the site of vacuolization. Vacuoles are totally protein-negative. The protein-positive intensity of cytoplasmic syncytia gradually decreases from short term to long term treated insects. The number and protein-positive intensity of pycnotic nuclei steadily reduce in the cytoplasmic syncytium. In some pycnotic nuclei the chromatin material converted into chromatin granules, which are highly protein-positive in STTFI. Some of the pycnotic nuclei burst and liberate their chromatin granules in the cytoplasmic syncytium. These granules are highly protein-positive and scattered in the cytoplasmic syncytium. In long term exposure of pesticide, the protein-positive intensity of tunica propria, gradually increases while the inner lining of the tunica propria which is in direct contact with the corpus luteum and support the folds of tunica propria showed the same protein-positive intensity in short term as well as in long term exposure of pesticide.

(2) NUCLEIC ACIDS:

(a) CONTROL SERIES: In Periplaneta americana the DNA and RNA-positive intensity of corpus luteum is greater in comparison to the normal insects, the central empty space observed with RNA-positive material. The intensely DNA-positive nuclei which are large in number, scattered in
the less RNA-positive cytoplasmic syncytium. The pycnotic nuclei are smaller in size in comparison to normal insects. DNA-positive granules are scattered in the intensely RNA-positive cytoplasmic syncytium. The tunica propria is less RNA-positive in comparison to the normal insects.

(b) PESTICIDE TREATED SERIES: In pesticide treated females of Periplaneta americana, the RNA-positive intensity of corpus luteum decreases while DNA-positive intensity increases from short term to long term exposure of pesticides. The vacuolization in cytoplasmic syncytium is presumably due to the resorption of RNA-positive material from the site of vacuolization; vacuoles are of variable sizes and are DNA and RNA-negative. The number of pycnotic nuclei steadily decreases; they are DNA-positive showing high intensity. A large number of DNA-positive granules found scattered in the RNA-positive cytoplasmic syncytium. The RNA-positive intensity of tunica propria steadily increases from short term to long term exposure of pesticides.

(3) PERIODIC ACID SCHIFF'S REACTION (PAS) FOR CARBOHYDRATE DETECTION:

(a) CONTROL SERIES: The PAS-positive intensity in the corpus luteum of control laid female is very high as seen in normal insects. The big sized pycnotic nuclei and its chromatin material is highly PAS-positive and scattered in the intensely PAS-positive cytoplasmic syncytium. The central empty space is totally PAS-negative and contains some PAS-positive material. A
number of PAS-positive granules have been observed scattered in the cytoplasmic syncytium. The tunica propria is also much folded, showed PAS-positive intensity.

(b) PESTICIDE TREATED FEMALES: In *Periplaneta americana* due to pesticide intoxication, the PAS-positive intensity steadily decreases from short term to long term exposure. The PAS-positive intensity of cytoplasmic syncytium gradually decrease, is dose dependent as observed in *Poekilocerus pictus*.

The PAS-positive cytoplasmic syncytium is also decreased in amount and consequently reduced corpus luteum. Vacuolization is exhibited in the cytoplasmic syncytium, vacuoles are totally PAS-negative, presumably due to the resorption of PAS-positive material at the site of vacuolization. The number and the PAS-positive intensity of pyknotic nuclei steadily decreases. The intensely PAS-positive granules which are more in number in short term administration of pesticides gradually decreases in number. The PAS-positive intensity of tunica propria steadily increases, presumably due to the resorption of PAS-positive material from diminishing cytoplasmic syncytium as observed in *Poekilocerus pictus*.

(4) LIPID CONTENTS:

(a) CONTROL SERIES: In *Periplaneta americana* the corpus luteum is big sized as observed in normally laid females of *Poekilocerus pictus*. The cytoplasmic syncytium is less sudanophilic, while the pyknotic nuclei showed the high intensity of sudanophilic material. Though
vacuolization is seen in cytoplasmic syncytium, but the vacuoles are neither sudanophilic nor lipoid in nature. The tunica propria is intensely sudanophilic in nature as observed in Poekilocerus pictus.

(b) PESTICIDE TREATED SERIES: In pesticide treated females the intensity of sudanophilic material is gradually decreases from short term to long term treated insects. The pyknotic nuclei are intensely sudanophilic in nature. The cytoplasmic syncytium with decreased amount of sudanophilic material. Some lipoid granules are found scattered in the cytoplasmic syncytium but exact lipid bodies e.g. L₁, L₂ and L₃ bodies have not been observed in any stage of corpus luteum resorption. The sudanophilic material in tunica propria gradually decreases from short term to long term treated females.

HISTOCHEMICAL OBSERVATIONS ON THE RESORPTIVE BODIES OF Periplaneta americana:

(1) PROTEIN:

(a) CONTROL SERIES: Generally in control series a few resorbed oocytes are found along with the normal developing oocytes in the ovarian architecture. The nuclei of follicular epithelium are highly protein-positive while the cytoplasm is intensely protein-positive. The ooplasm showed the presence of large number of PYP, yolk bodies, yolk platelets and yolk granules, which are highly protein-positive. The oocyte nucleus is intensely protein-positive but it’s nuclear membrane and nucleolus are highly protein-positive. The tunica propria which is firmly attached with the entire
follicular epithelium is intensely protein-positive.

(b) PESTICIDE TREATED FEMALES: In pesticide treated females the protein-positive intensity of resorbed oocyte is gradually decreases. The reduction in the amount of protein-positive material is correlated with the rate of resorption. The follicular epithelium (which is stratified in long term treatment) showed decrease in the protein-positive intensity. The pycnotic nuclei of follicular epithelium which are intensely protein-positive in earlier stages of resorption, steadily loses their protein-positive intensity. The lecitholytic cells which are highly protein-positive showed decrease in their protein-positive intensity. The vacuolization has been first observed on the periphery of ooplasm which then progresses towards the medullary region. The vacuoles are of variable sizes and protein-negative. The high vacuolization in vicinity of follicular epithelium is probably the indication of resorption of the PYD, yolk bodies, yolk platelets and spherules from the site of the ooplasm by the lecitholytic cells of follicular epithelium. The protein-positive intensity of oocyte nucleus and tunica propria becomes gradually decreases from short term to long term treated insects of Periplaneta americana.

(2) NUCLEIC ACIDS:

(a) CONTROL SERIES: In Periplaneta americana, the resorbed oocytes, the DNA-positive intensity of follicular epithelium, nuclei steadily increases. The pycnotic nuclei showed very high intensity of DNA, the
RNA-positive intensity of the entire follicular epithelium, indicates the presence of highly RNA-positive cytoplasm. The oocyte nucleus is RNA-positive. It is accenitic in position. Ooplasm is highly RNA-positive and the large number of oocytes does not show any sign of resorption. The tunica propria is intensely RNA-positive.

(b) PESTICIDE TREATED SERIES: In pesticide treated females, most of the resorbed oocytes showed the presence of high percentage of RNA-positive nature of ooplasm in short term exposure, while in prolong treatment, large amount of RNA-positive ooplasm is resorbed by the lecitholytic cells of follicular epithelium. The oocyte nucleus is less RNA-positive while its nucleolus is highly RNA-positive. In prolong treatment the oocyte nucleus becomes totally resorbed. The follicular epithelium is lecitholytic and stratified in nature and their nuclei are DNA-positive. The DNA-positive intensity of follicular epithelium is moderate in mid-term treated insects while it is lesser in prolong treatment. The RNA-positive intensity of follicular epithelium gradually decreases. The tunica propria is RNA-positive.

(3) PERIODIC ACID SCHIFF'S REACTION (PAS FOR CARBOHYDRATE DETECTION):

(a) CONTROL SERIES: The pathogenic oocytes are a few in number, in comparison to the normally developed oocytes in ovarian architecture; their PAS-positive intensity is high in comparison to the pesticide treated insects. The vacuoles within the ooplasm are a few and showed PAS-negative nature. The higher PAS-positive
intensity of ooplasm showed the presence of large amount of glycogen within the oocytes, while the oocyte nucleus is less PAS-positive in comparison to the ooplasm. The oocyte nucleolus is highly PAS-positive. The follicular epithelium showed intensely PAS-positive nature. The tunica propria is highly PAS-positive.

(b) PESTICIDE TREATED SERIES: The Pesticide intoxication effects the resorption of PAS-positive ooplasm by the lecitholytic cells. The amount of PAS-positive ooplasm of resorbed oocytes showed decrease in long term exposure of pesticides. The rate of vacuolization in ooplasm is higher in severely damaged oocytes, showed the resorption of PAS-positive material from the site of vacuolization. These vacuoles are of variable sizes and totally PAS-negative. The increased PAS-positive intensity of follicular epithelium and tunica propria indicates the resorbed PAS-positive material from the ooplasm.

(4) LIPID CONTENTS:

(a) CONTROL SERIES: Resorbed oocytes are a few in number, in comparison to the normally developed oocytes in the ovarian architecture. The pathogenic oocytes are with intense sudanophilic follicular epithelium. The oocytes are with eccentric nuclei which are less sudanophilic in nature. Large number of L₁, L₂ and L₃ lipid bodies are abundant in ooplasm. The tunica propria is with less sudanophilic material.

(b) PESTICIDE TREATED SERIES: The pathogenic oocytes showed gradual decrease in the number of lipid bodies. In
short term exposure the resorptive bodies showed the large number of $L_1$, $L_2$ and $L_3$ lipid bodies while in mid term treated insects, the pathogenic oocytes showed decline in the number of $L_3$ lipid bodies while $L_2$ lipid bodies are more in number, but in prolong treatment, the $L_3$, $L_2$ and $L_1$ lipid bodies are decline in number. In long term treated insects, the large number of sudanophilic granules have been present, these lipid granules are formed by the break down of $L_3$, $L_2$ and $L_1$ lipid bodies. In severely damaged oocytes, the sudanophilic granules have been observed declined in number. The ooplasm showed the negative lipid nature. The follicular epithelium becomes stratified and in some pathogenic oocytes, the presence of inter follicular lipid presumably due to the resorption of lipid by the lecitholytic follicular epithelium. The nuclei of follicular epithelium becomes less sudanophilic while tunica propria showed the presence of intensely sudanophilic material.
(B). HISTOCHEMICAL OBSERVATIONS IN THE OVARIAN SYSTEM OF

*Psillocerus pictus*, Fabr. (Orthoptera Acrididae)

Histochemical observations are expressed on the following aspects:

(I) HISTOCHEMICAL OBSERVATIONS ON THE OVARIAN SYSTEMS AND PESTICIDE TREATED SERIES:

The ovaries of *Psillocerus pictus* are paedomorphic type in comparison to the normal insects. The ovaries of control series are more or less similar in histochemistry, but in pesticide treated female insects showed depletion, destruction and degeneration of different metabolites e.g. protein, glycogen and lipid etc. which are histochemically distinguished by different staining techniques e.g. Mercuric Bromophenol Blue (Hg. B.B. for protein), Methyl green pyronin (P/MG, for DNA and RNA), Periodic Acid Schiff’s reaction (PAS for carbohydrate) and Sudan Black ‘B’ (for lipid) were applied to detect the fate of various metabolites during pesticide treatment.

(i) THE GERMARIUM: In control series of *Psillocerus pictus*, the germarium showed the high intensity of protein-positive, PAS-positive and RNA-positive materials in the developing oocytes but with very less lipid contents as observed in the germarium of normal insects but in pesticide treated insects, the slow rate (in short term treatment) or nil (in prolonged exposure to pesticides) of oogenesis resulted into very low intensity of protein-positive, PAS-positive and RNA-positive materials, while sudanophilic material did not observe.
The decline in the intensity of different metabolites in germarium correlated with the pesticide toxicity.

(ii) THE VITELLARIUM: The vitellarium of control series of Poekilocerus pictus showed the arrangement of oocytes in successive manner within the ovariole as observed in normal insects. The immature oocytes are located at the anterior end of vitellarium i.e. in between germarium and mature yolky oocytes, while mature oocytes are situated posterior to immature oocytes. The terminal oocyte is the lower most (first formed) oocyte of the ovariole is bigger than other oocytes, is megalecithal type. A penultimate oocyte is situated above the terminal oocyte in each ovariole. Both terminal (ultimate) and penultimate oocytes are nearly similar in size, shape and in their yolk contents in control series. The previtellogenic and vitellogenic phases are similar in comparison to normally developed oocytes, but a few oocytes showed some adverse histochemical symptoms in the ovarian architecture, while in pesticide treated insects not only the immature but also the mature oocytes showed the depletion of histochemical materials as follows:

(a) IMMATURE OOCYTES:

(i) CONTROL SERIES: In Poekilocerus pictus, immature oocytes are encircled with regular cuboidal follicular epithelium which is protein-positive, RNA-positive and PAS-positive with less sudanophilic nature. The oocyte nucleus is centric with single nucleolus. The nucleus is highly protein-positive, RNA-positive but not showed PAS-
positive or sudanophilic material. The deposition of protein-positive and PAS-positive densely stained granules around the oocyte nucleus and in the ooplasm in vicinity of follicular epithelium as observed in the immature oocytes of normal insects.

(ii) PESTICIDE TREATED SERIES: The follicular epithelium becomes irregular in outline and immature oocytes are smaller in size in comparison to that found in normal and control insects. The follicular epithelium showed the less intensity of protein-positive, RNA-positive and PAS-positive material in comparison to the control and normal insects. The ooplasm does not show the presence of any type of granules, spherules or platelets etc. but they are uniformly distributed within the ooplasm of irregular and immature oocytes. The ooplasm is with very light protein-positive, RNA-positive, PAS-positive or sudanophilic material. The lightly stained oocyte nucleus is irregular with lacunae indicates degeneration.

(b) MATURE OOGENES:

(i) CONTROL SERIES: The follicular epithelium of mature oocytes of Poekilocerus pictus with Hg.B.B.-positive (protein-positive), RNA-positive nuclei, which are smaller in size and show a bit of DNA-positive intensity which steadily increases the terminal oocytes which are with the follicular epithelium of high DNA-positive intensity. The small sized protein-positive and PAS-positive granules clumped together to form large sized protein spherules, protein platelets, PYP bodies
and yolk bodies, which are abundant in number in penultimate and terminal oocytes as observed in normal insects. The nucleus is not situated in the centre of oocyte but shift towards the proximal end and is mostly protein-positive, RNA-positive but PAS-negative in nature. It does not show the sudanophilic nature, while in ooplasm, large number of PAS-positive bodies, protein-positive PYP, yolk bodies, sudanophilic granules, $L_1$, $L_2$ and $L_3$ lipid bodies etc. are abundant, mostly in penultimate and terminal oocytes as observed in normal insects. The tunica propria is highly protein-positive, RNA-positive and PAS-positive in nature.

(11) PESTICIDE TREATED SERIES: The pathogenic oocytes showed the necrotic follicular epithelium which becomes stratified and irregular in outline. It is intensely protein-positive, while RNA-positive intensity gradually decreases in LTTFI. The DNA-positive intensity of follicular nuclei is very high in Malathion treated insects in comparison to the control and normal ones. The PAS-positive intensity of follicular epithelium is very low in pesticide treated insects in comparison to that of control and normal insects. Hyperactivity of ooplasm presumably due to increase in the vacuolization of ooplasm. These vacuoles are protein-negative, RNA and DNA-negative and also PAS-negative, does not show the presence of sudanophilic material within them. The oocyte nucleus which is less RNA-positive is sometimes protein-negative and PAS-negative in STTFI, becomes disintegrated in LTTFI and in severely damaged pathogenic oocytes. The
oocyte nucleus is totally resorbed. Vacuolization in follicular cells is obvious in MTTFI, which increased steadily in LTTFI. In LTTFI, the reduced size of the pathogenic oocyte is correlated with the resorption of the amount of protein-positive, RNA-positive and PAS-positive materials from these resorbed oocytes. In some oocytes the entire ooplasm becomes obliterated by the presence of large number of protein-positive, DNA and RNA-positive and PAS-positive lecitholytic cells. The size of the severely damaged oocytes reduced due to the active resorption of ooplasm by the invaded follicular epithelium and also by the islands of lecitholytic cells within the ooplasm. The tunica propria which is intensely protein-positive, RNA-positive and PAS-positive in nature becomes increases in the intensity of these metabolites.

The tunica propria is sudanophilic in nature while follicular epithelium of pathogenic oocytes in LTTFI is more sudanophilic than found in STTFI. The oocyte nucleus showed low intensity of sudanophilic material. The ooplasm of STTFI is with large number of $L_1$, $L_2$ and $L_3$ lipid bodies, sudanophilic granules and spherules but their number gradually decreases in MTTFI to LTTFI; in LTTFI indicates the resorption of lipid bodies by necrotic follicular epithelium.

HISTOCHEMICAL OBSERVATIONS ON THE CORPUS LUTEUM OF *Poekilocerus pictus*, Fabr.

(1) PROTEIN:

(a) CONTROL SERIES: The protein-positive intensity in the corpus luteum of control females is very high as seen
in normal insects. The pycnotic nuclei and their chromatin material is highly protein-positive and scattered in the intensely protein-positive cytoplasmic syncytium. An empty space which is created due to oocyte ovulation from the respective follicle is totally protein-negative, while it contains some intensely protein-positive material. The much folded tunica propria is less intensely protein-positive.

(b) PESTICIDE TREATED SERIES: In pesticide treated females, the protein-positive intensity of corpus luteum decreases from short term to long term exposure of pesticides while the vacuolization in cytoplasmic syncytium presumably due to the resorption of protein-positive material from the site of vacuolization. Vacuoles are totally protein-negative. The protein-positive intensity of cytoplasmic syncytia gradually decreases from short term to long term treated insects. The number and protein-positive intensity of pycnotic nuclei steadily reduce in the cytoplasmic syncytium. In some pycnotic nuclei the chromatin material converted into chromatin granules, which are highly protein-positive in STTFI. Some of the pycnotic nuclei burst and liberate their chromatin granules in the cytoplasmic syncytium. These granules are highly protein-positive and scattered in the cytoplasmic syncytium. In long term exposure of pesticide, the protein-positive intensity of tunica propria, gradually increases while the inner lining of the tunica propria which is in direct contact with the corpus luteum and support the folds of tunica
propria showed the same protein-positive intensity in short term as well as in long term exposure of pesticide.

(2) NUCLEIC ACIDS:

(a) CONTROL SERIES: The DNA and RNA-positive intensity of corpus luteum is greater in comparison to the normal insects, the central empty space observed with RNA-positive material. The intensely DNA-positive nuclei which are large in number, scattered in the less RNA-positive cytoplasmic syncytium. The pycnotic nuclei are smaller in size in comparison to normal. DNA-positive granules are scattered in the intensely RNA-positive cytoplasmic syncytium. The tunica propria is less RNA-positive in comparison to the normal.

(b) PESTICIDE TREATED SERIES: In pesticide treated females, the RNA-positive intensity of corpus luteum decreases while DNA-positive intensity increases from short term to long term exposure of pesticides. The vacuolization in cytoplasmic syncytium is presumably due to the resorption of RNA-positive material from the site of vacuolization; vacuoles are of variable sizes and are DNA and RNA-negative. The number of pycnotic nuclei steadily decreases; they are DNA-positive showing high intensity. A large number of DNA-positive granules found scattered in the RNA-positive cytoplasmic syncytium. The RNA-positive intensity of tunica propria steadily increases from short term to long term exposure of pesticides.
(3) PERIODIC ACID SCHIFF'S REACTION (PAS) FOR CARBOHYDRATE DETECTION:

(a) CONTROL SERIES: The PAS-positive intensity in the corpus luteum of control laid female is very high as seen in normal insects. The big sized pycnotic nuclei and its chromatin material is highly PAS-positive and scattered in the intensely PAS-positive cytoplasmic syncytium. The central empty space is totally PAS-negative and contains some PAS-positive material. A number of PAS-positive granules have been observed scattered in the cytoplasmic syncytium. The tunica propria is also much folded, showed PAS-positive intensity.

(b) PESTICIDE TREATED FEMALES: Due to pesticide intoxication, the PAS-positive intensity steadily decreases from short term to long term exposure. The PAS-positive intensity of cytoplasmic syncytium gradually decrease, is dose dependent.

The PAS-positive cytoplasmic syncytium is also decreased in amount and consequently reduced corpus luteum. Vacuolization is exhibited in the cytoplasmic syncytium, vacuoles are totally PAS-negative, presumably due to the resorption of PAS-positive material at the site of vacuolization. The number and the PAS-positive intensity of pycnotic nuclei steadily decreases. The intensely PAS-positive granules which are more in number in short term administration of pesticides gradually decreases in number. The PAS-positive intensity of tunica propria steadily increases, presumably due to the
resorption of PAS-positive material from diminishing cytoplasmic syncytium.

(4) LIPID CONTENTS:

(a) CONTROL SERIES: The corpus luteum is big sized as observed in normally laid females. The cytoplasmic syncytium is less sudanophilic, while the pycnotic nuclei showed the high intensity of sudanophilic material. Though vacuolization is seen in cytoplasmic syncytium, but the vacuoles are neither sudanophilic nor lipoid in nature. The tunica propria is intensely sudanophilic in nature.

(b) PESTICIDE TREATED SERIES: In pesticide treated females the intensity of sudanophilic material is gradually decreases from short term to long term treated insects. The pycnotic nuclei are intensely sudanophilic in nature. The cytoplasmic syncytium with decreased amount of sudanophilic material. Some lipoid granules are found scattered in the cytoplasmic syncytium but exact lipid bodies e.g. L₁, L₂ and L₃ bodies have not been observed in any stage of corpus luteum resorption. The sudanophilic material in tunica propria gradually decreases from short term to long term treated females.

HISTOCHEMICAL OBSERVATIONS ON THE RESORPTIVE BODIES OF Poekilocerus pictus:

(1) PROTEIN:

(a) CONTROL SERIES: Generally in control series a few resorbed oocytes are found along with the normal developing oocytes in the ovarian architecture. The nuclei of follicular epithelium are highly protein-
positive while the cytoplasm is intensely protein-positive. The ooplasm showed the presence of large number of PYP, yolk bodies, yolk platelets and yolk granules, which are highly protein-positive. The oocyte nucleus is intensely protein-positive but its nuclear membrane and nucleolus are highly protein-positive. The tunica propria which is firmly attached with the entire follicular epithelium is intensely protein-positive.

(b) PESTICIDE TREATED FEMALES: In pesticide treated females the protein-positive intensity of resorbed oocyte is gradually decreases. The reduction in the amount of protein-positive material is correlated with the rate of resorption. The follicular epithelium (which is stratified in long term treatment) showed decrease in the protein-positive intensity. The pyknotic nuclei of follicular epithelium which are intensely protein-positive in earlier stages of resorption, steadily loses their protein-positive intensity. The lecitholytic cells which are highly protein-positive showed decrease in their protein-positive intensity. The vacuolization has been first observed on the periphery of ooplasm which then progress towards the medullary region. The vacuoles are of variable sizes and protein-negative. The high vacuolization in vicinity of follicular epithelium is probably the indication of resorption of the PYP, yolk bodies, yolk platelets and spherules from the site of the ooplasm by the lecitholytic cells of follicular epithelium. The protein-positive intensity of oocyte
nucleus and tunica propria becomes gradually decreases from short term to long term treated insects.

(2) NUCLEIC ACIDS:

(a) CONTROL SERIES: In the resorbed oocytes, the DNA-positive intensity of follicular epithelium, nuclei steadily increases. The pycnotic nuclei showed very high intensity of DNA, the RNA-positive intensity of the entire follicular epithelium, indicates the presence of highly RNA-positive cytoplasm. The oocyte nucleus is RNA-positive. It is eccentric in position. Ooplasm is highly RNA-positive and the large number of oocytes does not show any sign of resorption. The tunica propria is intensely RNA-positive.

(b) PESTICIDE TREATED SERIES: In pesticide treated females, most of the resorbed oocytes showed the presence of high percentage of RNA-positive nature of ooplasm in short term exposure, while in prolong treatment, large amount of RNA-positive ooplasm is resorbed by the lecitholytic cells of follicular epithelium. The oocyte nucleus is less RNA-positive while its nucleolus is highly RNA-positive. In prolong treatment the oocyte nucleus becomes totally resorbed. The follicular epithelium is lecitholytic and stratified in nature and their nuclei are DNA-positive. The DNA-positive intensity of follicular epithelium is moderate in mid-term treated insects while it is lesser in prolong treatment. The RNA-positive intensity of follicular epithelium gradually decreases. The tunica propria is RNA-positive.
(3) PERIODIC ACID SCHIFF'S REACTION (PAS FOR CARBOHYDRATE DETECTION):

(a) CONTROL SERIES: The pathogenic oocytes are a few in number, in comparison to the normally developed oocytes in ovarian architecture; their PAS-positive intensity is high in comparison to the pesticide treated insects. The vacuoles within the ooplasm are a few and showed PAS-negative nature. The higher PAS-positive intensity of ooplasm showed the presence of large amount of glycogen within the oocytes, while the oocyte nucleus is less PAS-positive in comparison to the ooplasm. The oocyte nucleolus is highly PAS-positive. The follicular epithelium showed intensely PAS-positive nature. The tunica propria is highly PAS-positive.

(b) PESTICIDE TREATED SERIES: The Pesticide intoxication effects the resorption of PAS-positive ooplasm by the lecitholytic cells. The amount of PAS-positive ooplasm of resorbed oocytes showed decrease in long term exposure of pesticides. The rate of vacuolization in ooplasm is higher in severely damaged oocytes, showed the resorption of PAS-positive material from the site of vacuolization. These vacuoles are of variable sizes and totally PAS-negative. The increased PAS-positive intensity of follicular epithelium and tunica propria indicates the resorbed PAS-positive material from the ooplasm.

(4) LIPID CONTENTS:

(a) CONTROL SERIES: Resorbed oocytes are a few in number, in comparison to the normally developed oocytes
in the ovarian architecture. The pathogenic oocytes are with intense sudanophilic follicular epithelium. The oocytes are with eccentric nuclei which are less sudanophilic in nature. Large number of $L_1$, $L_2$ and $L_3$ lipid bodies are abundant in ooplasm. The tunica propria is with less sudanophilic material.

(b) PESTICIDE TREATED SERIES: The pathogenic oocytes showed gradual decrease in the number of lipid bodies. In short term exposure the resorptive bodies showed the large number of $L_1$, $L_2$ and $L_3$ lipid bodies while in mid term treated insects, the pathogenic oocytes showed decline in the number of $L_3$ lipid bodies while $L_2$ lipid bodies are more in number, but in prolong treatment, the $L_3$, $L_2$ and $L_1$ lipid bodies are decline in number. In long term treated insects, the large number of sudanophilic granules have been present, these lipid granules are formed by the break down of $L_3$, $L_2$ and $L_1$ lipid bodies. In severely damaged oocytes, the sudanophilic granules have been observed declined in number. The ooplasm showed the negative lipid nature. The follicular epithelium becomes stratified and in some pathogenic oocytes, the presence of inter follicular lipid presumably due to the resorption of lipid by the lecitholytic follicular epithelium. The nuclei of follicular epithelium becomes less sudanophilic while tunica propria showed the presence of intensely sudanophilic material.
DISCUSSION

The literature on histochemical aspects of the panoistic type of ovary was studied in *Periplaneta americana* by Nath and Mohan (1929), Gresson (1931), Ranade (1933), Kugier et al. (1956), Nath et al. (1958), Bonhag (1959), Nath (1968) in *Schistocerca gregaria* by Lusis (1963), Highnam (1964), Phipps, (1966), Uvarov, (1966), in *Poekilocerus pictus* Sahai (1978, 1984) described that development and resorption of oocyte is related with the availability and depletion of metabolites in haemolymph respectively but no worker yet paid any attention on the histochemical aspects of the ovarian system in pesticide treated insects of Orthoptera or any other orders, so the findings are original and cannot be compared with other insects.

In the present investigations histochemical studies have been done on the ovaries of *Periplaneta americana* and *Poekilocerus pictus* on the following aspects viz germarium, vitellarium, immature and mature oocytes, resorptive bodies (resorbed oocyte) and corpus luteum etc. suggested that the pesticide create gradual changes in the histochemistry of the ovaries.

In *Schistocerca gregaria*, Highnam (1964), Lusis (1963), Phipps, (1966), Uvarov (1966), suggested that yolk forming material was obtained from the haemolymph in *Oxya Velox* (Verma and Das, 1974), Chrotogonus trachypenthus (Verma and Mohanty, 1979) by way of follicular epithelium while Telfer (1960 and 1961) showed that in saturnoid moth *Oxya Velox* (Verma and Das, 1974) the yolk protein could be
absorbed directly from haemolymph reaching the peripheral oocyte cytoplasm (ooplasm) by an intercellular route as observed in Musca (Bier, 1963), Schistocerca gregaria (Lusis, 1963), Panorpa communis (Ramamurthy 1964) and in Poekilocerus pictus (Sahai, 1978, 1984). The activity of the follicular epithelium during vitellogenesis indicated that these cells might control the synthesis of protein and carbohydrate but in present investigation the pesticide intoxication is resulted into destruction of germarium by inhibiting the process of oogenesis hence the germarium in Periplaneta americana and Poekilocerus pictus showed the low intensity of protein-positive, RNA-positive, PAS-positive and sudanophilic material as observed in Periplaneta americana (Bhide, 1986) after Y BHC exposure and in Poekilocerus pictus (Ahl, 1986) after Endosulfan treatment.

Anderson (1964) has described pinocytosis in the newly formed oocytes enhanced by a layer of follicle cells in Periplaneta americana. According to Anderson (1964) the material of the yolk sphere is a carbohydrate-protein complex and he has concluded that "Yolk formation" is an independent process of any cytoplasmic organelles system of the oocyte and the precursors of the deutoplasmic substances are manufactured outside the ovary and are engulfed by the process of pinocytosis, so the follicular epithelium plays a very crucial function during vitellogenesis. Various chemical components from the fat body or the haemolymph have to cross the ovariolo sheath and the tunica propria before they can reach the ooplasm. According to Quattropani and Anderson (1964), follicular epithelial cells have a variety of
functions. Koepple et al., (1980) suggested both intracellular and extracellular changes occur in the tissue.

In Periplaneta americana and Poekilocerus pictus in the vitellarium the immature oocytes showed the presence of active nucleus which is intensely RNA-positive and more or less ellipical or oval in shape in control and normal insects as observed in Poekilocerus pictus (Sahai, 1978, 1984). The peripheral ooplasm or oocyte cortex showed the presence of gradual increase of protein-positive and PAS-positive granules or globules and suddenly in older oocytes, they grow and invade the entire ooplasm as observed in cockroach ray (Kugler et al., 1956) but their globules are sudanophobic, Anderson (1964) has shown with electron microscopy and histochemical study that the yolk in cockroach is carbohydrate-Portein in nature; the protein moiety is contributed by RNA rich particles from the germinal vesicle which migrate into the ooplasm and the polysacaharide moiety by the follicular epithelium but after pesticide exposure, the oocyte nucleus showed variable degree of degeneration. Oocyte showed irregular protein-positive, RNA-positive, DNA and PAS-negative follicular epithelium with less sudanophilic material as observed in Nasonia vitropennis (Hopkins et al., 1964 and Kingetred 1968) showed its uniform appearance throughout the duration of treatment and does not show depletion of PAS-positive or protein-positive granules as observed in Poekilocerus pictus (Jain and Bhide, 1990).

In pesticide treated insects the mature oocytes showed the presence of stratified, irregular follicular epithelium invade inside the ooplasm as observed in
Aulacophora foveicollis (Saini, 1967). It is necrotic in nature and interrupts the passage of nutrients thus affecting the maturity of oocytes. Cells detached and migrate inside the ooplasm and act as lecitholytic cells in Periplaneta americana (Bhide, 1986), while in control insects it is regular and composed of columnar epithelial cells as observed in Poekilocerus pictus (Sahai, 1978). The protein-positive, RNA-positive and DNA-positive intensity of follicular epithelium, steadily decrease in the mature oocyte of treated insects and the highly protein-positive, PAS-positive, PYP bodies, Yolk bodies yolk spheres, yolk spherules and yolk platelets etc. are abundant and in adequate amount, prior to treatment becomes disintegrated, destroyed and are broken down and resorbed by necrosis and lecitholytic cells as observed in Schistocerca gregaria, Lusis (1963). The protein-positive and PAS-positive material present inside the resorbed oocytes get reduced and the rate of reduction is directly related to the rate of oosorption, so the depletion of their metabolites inhibits the maturation of resorbed oocytes while in control, the normally developed oocytes reached to the normal stage of the maturity which is required for the successful ovulation in Periplaneta americana and Poekilocerus pictus as observed in Locusta migratoria and Schistocerca gregaria (Singh, 1958; Lusis, 1963).

The vacuolization of ooplasm is the peculiar feature of all resorbed oocytes and the rate of vacuolization is also dependent. These vacuoles are totally protein-positive, PAS-positive, RNA-positive and DNA-positive and not showed the deposition of any sudanophilic material as
observed in the resorptive bodies of *Dysdercus Similis* (Bhide, 1986) and in *Sphaerodema rusticum* (Bhide, 1987).

Nath et al., (1958) noticed that the lipid bodies in the panoistic ovary of *Periplaneta americana* are of three types i.e. L1, L2 and L3 as observed in *Poekilocerus pictus* (Nath, 1963). The L1, L2 and L3 lipid bodies are abundant in STTFI while in MTTFI they are less in number and scattered in the ooplasm. The lipids are resorbed but the pigment dissolved becomes more concentrated then crystallized and deposited at the neck of the ovariole. The same phenomena reported in *Schistocerca gregaria* (Lusis, 1963) follicle in *Periplaneta americana* and *Poekilocerus pictus* resembles with the resorption of the corpus luteum in *Schistocerca gregaria* and *Locusta migratoria* (Singh, 1958), *Schistocerca gregaria* (Lusis 1963, 1981) *Periplaneta americana* (Sahai 1978, 1981-84, Bhide, 1978, 1982, 1985), but in present investigation the oosorption is the common feature in ovaries of *Periplaneta americana* and *Poekilocerus pictus* by pesticide intoxication.

In the later stages of treatment when the pathogenic and subpathogenic oocytes are about to be completely resorbed, they are encircled by a large number of lecitholytic cells (which are highly protein-positive, RNA-positive & sudanophilic in nature) and a few protein-positive yolk precursors. According to Highnam et al., (1963) in *Schistocerca gregaria* oocyte resorption is correlated with the amino acids level in the haemolymph and if this level falls below a certain point the developing oocytes in the ovarioles are resorbed and form resorptive bodies. In
present investigation the pesticide intoxication is one of the cause of resorption as observed in Periplaneta americana (Bhide, 1986) after BHC treatment. According to Phipps (1966) in Acridiae observed that the food supply effects ovulation. The short food supply reduce the rate of ovulation and increase the rate of oocyte resorption but in present investigation the depletion of metabolites in the haemolymph and ooplasm is the main cause of oosorption.

In corpus luteum the protein-positive, PAS-positive and RNA-positive intensity gradually reduces in short term to long term treated pesticides. The pycnotic nuclei which are largely protein-positive, DNA-positive and intensely PAS-positive in nature steadily decreases in number and size and are scattered in the syncytium. The increase in the vacuolization of cytoplasmic syncytium is the sign of degeneration of corpus luteum. The protein-positive, RNA-positive, PAS-positive intensity and sudanophilic nature of tunica propria is steadily increases from short term to long term treated insects but no worker has paid any attention regarding the histochemistry of corpus luteum. The toxicity of pesticides is correlated with the number and the size of the corpus luteum. In pesticide treated Periplaneta americana and Poekilocerus pictus, the number and size of the corpus luteum is always small in comparison to the normal or control insects. The size of the corpus luteum in Periplaneta americana is always bigger than Poekilocerus pictus.

In Namoria vitripennis (Hopkins et al.,1964) observed that the amount of RNA in the central region of the resorbed oocyte decreases gradually while there is a marked
increase in the peripheral region of the ooplasm. There is high concentration of RNA during the later stage of resorption.

It is therefore evident that the oocyte which is unable to be laid down, instead of decaying, it becomes resorbed to form a pigmented "Resorptive body". It is suggested that the oocyte has an inherent potential for resorption. The realization of which depends upon various factors within the body as reported by Lusis (1963) in Schistocerca gregaria. An abnormal oocyte may cease to grow, leaves its yolk and can be resorbed at any time. The process of resorption is not affected by the age of an individual insect.

Food supply also effects ovulation. Poor supply of food reduces the rate of ovulation and leads to resorption of egg within a fairly short period as observed in many species of grasshopper (Phipps, 1966), he observed that darker pigmented resorptive bodies are produced by resorption of an egg and pale pigmented bodies are formed by continuous lipid resorption in a follicle undergoing resorption.
FIG. 25  Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with BHC showing intensely protein-positive ooplasm, follicular epithelium and protein-negative vacuoles (X100).

FIG. 26  Section of the degenerate oocyte of LTTFI, *Periplaneta americana* treated with DDT showing highly protein-positive nuclei and intensely protein-positive ooplasm (X100).

FIG. 27  Section of the degenerated oocyte of MTTFI, *Periplaneta americana* treated with Endosulfan showing highly protein-positive lecitholytic cells within ooplasm (X200).

FIG. 28  Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with Endosulfan showing highly protein-positive nature of necrotic cells (X200).
FIG. 29  Section of the corpus luteum of LTTF1, *Periplaneta americana* treated with Endosulfan showing large number of protein-positive nuclei and highly protein-positive tunica propria (X100).

FIG. 30  Section of the resorbed oocyte of LTTF1, *Periplaneta americana* treated with Malathion showing highly protein-positive tunica propria, intensely protein-positive lecitholytic cells (X200).

FIG. 31  Section of the germarium of MTTF1, *Periplaneta americana* treated with Sevin showing high rate of vacuolization, highly protein-positive nuclei (X100).

FIG. 32  Section of the resorbed oocyte of MTTF1, *Periplaneta americana* treated with Sevin showing highly protein-positive follicular epithelium and ooplasm, less protein-positive oocyte nucleus and protein-negative vacuoles (X200).
FIG.33  Section of the developing oocyte of *Poekilocerus pictus* showing highly protein-positive tunica propria and highly protein positive precursors in vicinity of follicular epithelium (X100).

FIG.34  Section of the immature and mature oocytes LTTFI *Poekilocerus pictus* treated with BHC showing irregular highly protein-positive tunica propria (X100).

FIG.35  Section of the degenerated oocytes of LTTFI, *Poekilocerus pictus* treated with DDT showing the intensely protein-positive nature (X100).

FIG.36  Section of the degenerated oocytes of LTTFI, *Poekilocerus pictus* treated with Endosulfan showing small sized, irregular oocyte and highly protein-positive lecitholytic cells (X100).
FIG. 37  Section of the resorbed oocyte of LTFI, *Poekilocerus pictus* treated with Malathion showing less protein-positive pycnotic nuclei and highly protein-positive ooplasm (X200).

FIG. 38  Section of the corpus luteum and resorbed oocytes of *Poekilocerus pictus* treated with Malathion showing highly protein-positive tunica propria and highly protein positive pycnotic nuclei (X200).

FIG. 39  Section of the resorbed oocytes of *Poekilocerus pictus* treated with Sevin showing highly protein-positive nuclei and intensely protein-positive ooplasm (X100).

FIG. 40  Section of the corpus luteum and degenerated oocytes of *Poekilocerus pictus* treated with Sevin showing highly protein-positive follicular epithelium and less protein-positive ooplasm (X100).
FIG. 41  Section of the immature and mature oocytes of LTTFI *Periplaneta americana* treated with BHC showing intensely DNA-positive follicular epithelium and smooth RNA-positive ooplasm in immature oocytes (X100).

FIG. 42  Section of the immature and mature oocytes of LTTFI, *Periplaneta americana* treated with DDT showing RNA-positive intensity (X100).

FIG. 43  Section of the immature and mature oocytes of *Periplaneta americana* treated with Endosulfan showing active mitosis and highly DNA-positive follicular nuclei (X450).

FIG. 44  Section of the immature oocytes of *Periplaneta americana* treated with Malathion showing DNA-positive follicular epithelium and intense RNA-positive ooplasm (X100).
FIG. 45  Section of the ovariole of LTTFI, *Periplaneta americana* treated with Sevin showing RNA-positive immature oocytes (X100).

FIG. 46  Section of the degenerated oocyte of LTTFI, *Periplaneta americana* treated with Sevin showing highly DNA-positive follicular epithelium and RNA-positive degenerated oocyte nucleus (X100).

FIG. 47  Section of the corpus luteum and resorbed oocyte of *Periplaneta americana* showing RNA-positive tunica propria and DNA-positive pycnotic nuclei (X100).
FIG. 48  Section of the resorbed oocyte of MTTFI, *Poekilocerus pictus* treated with BHC showing intensely RNA-positive tunica propria and ooplasm and DNA-positive follicular epithelium (X200).

FIG. 49  Section of the resorbed oocyte of LTTFI, *Poekilocerus pictus* treated with DDT, showing highly DNA-positive nuclei of lecitholytic cells (X200).

FIG. 50  Section of the resorbed oocyte of LTTFI, *Poekilocerus pictus* treated with Endosulfan showing highly DNA-positive follicular epithelial nuclei (X100).

FIG. 51  Section of the resorbed oocytes of LTTFI, *Poekilocerus pictus* treated with Malathion showing highly DNA-positive nuclei of lecitholytic cells (X200).
PERIODIC ACID (SHIFFS REAGENT)

FIG.52 Section of the immature and mature oocytes of *Periplaneta americana* treated with Endosulfan showing the intensely PAS-positive material in vicinity of follicular epithelium, tunica propria and ooplasm while follicular epithelium is less PAS-positive (X100).

FIG.53 Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with DDT showing highly PAS-positive tunica propria and less PAS-positive follicular cells (X450).

FIG.54 Section of the resorbed oocyte of MTTFI, *Periplaneta americana* treated with BHC showing highly PAS-positive tunica propria, ooplasm intensely PAS-positive follicular epithelium and PAS-negative oocyte nucleus (X450).

FIG.55 Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with Malathion showing highly PAS-positive tunica propria and less PAS-positive follicular cells (X450).
FIG. 56  Section of the corpus luteum and resorbed oocytes of *M. T. T. F. I.*, *Periplaneta americana* treated with Sevin showing highly PAS-positive ooplasm, tunica propria, less PAS-positive cytoplasmic syncytium and follicular epithelium (X100).

FIG. 57  Section of the resorbed oocyte of *L. T. T. F. I.*, *Periplaneta americana* treated with Sevin showing highly PAS-positive tunica propria (X200).
FIG. 58  Section of the corpus luteum and resorbed oocytes of LTT FI, *Poekilocerus pictus* treated with BHC showing highly PAS-positive intensity of ooplasm and highly folded tunica propria of corpus luteum (X100).

FIG. 59  Section of the immature and mature degenerated oocytes of LTT FI, *Poekilocerus pictus* treated with DDT showing less PAS-positive intensity of multilayered follicular epithelium (X100).

FIG. 60  Section of the corpus luteum and resorbed oocytes of LTT FI, *Poekilocerus pictus* treated with Endosulfan showing less intensity of PAS-positive material (X100).

FIG. 61  Section of the resorbed oocytes of LTT FI, *Poekilocerus pictus* treated with Malathion showing vacuolated highly PAS-positive material (X100).
SUDAN BLACK 'B'

FIG. 62 Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with BHC showing highly sudanophilic L₁, L₂ and L₃ lipid bodies in ooplasm and also showing the presence of interfollicular lipid with the follicular epithelium (X200).

FIG. 63 Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with DDT showing less number of L₁ and L₂ lipid bodies interfollicular lipid has also seen (X200).

FIG. 64 Section of the resorbed oocyte of LTTFI, *Periplaneta americana* treated with Endosulfan showing less number of lipid bodies (X200).

FIG. 65 Section of the degenerated resorbed oocytes of LTTFI, *Periplaneta americana* treated with Malathion showing intense sudanophilic follicular epithelium and less lipid bodies (X200).
FIG. 66 Section of the LTTFI, Poekilocerus pictus treated with BHC showing highly sudanophilic tunica propria, intensely sudanophilic follicular epithelium and less number of L₁, L₂ and L₃ lipid bodies (X200).

FIG. 67 Section of the LTTFI, Poekilocerus pictus treated with DDT showing the disintegration of lipid bodies (X200).

FIG. 68 Section of the resorbed oocyte of LTTFI, Poekilocerus pictus treated with Endosulfan showing highly sudanophilic inner boundaries of follicular epithelium, tunica propria, less number of L₁ lipid bodies and lipid granules in vicinity of follicular epithelium, intense sudan Black 'B' material in follicular epithelium and less sudanophilic central ooplasm (X100).

FIG. 69 Section of the degenerated oocytes of LTTFI, Poekilocerus pictus treated with Malathion showing less intensity of sudanophilic material (X100).