GAMETOGENESIS AND RADIATION EFFECTS ON THE REPRODUCTIVE TISSUES OF OULEMA MELANOPA (L.)

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ABSTRACT

GAMETOGENESIS AND RADIATION EFFECTS ON
THE REPRODUCTIVE TISSUES OF
OULEMA MELANOPA (L.)

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The normal development of gonads, some histochemical aspects of the ovary, radiation response, and histopathological effects of radiation on adult cereal leaf beetle, Oulema melanopa (L.) were studied. Tetrahydrofuran-parlodion double embedding techniques were used for most of the histological preparations and Feulgen - Fast green stains were employed to trace the development of gonads. Dosage levels of 500r to 20,000r X-ray radiation were used to study the effects on reproductive tissues.

It was found that the male beetles attained sexual maturity earlier than females of the same species. The ovary was in an underdeveloped state in most of the diapausing females and developed after cessation of diapause. Observations indicated that the ovarioles of this beetle were of the acrotrophic type and each beetle had 14 ovarioles, 7 on each side. Follicular epithelial cells also appeared to have a nutritive function, particularly in the latter stages of oocyte development.

Radiation at levels of 5,000r and above caused over 90 percent mortality within two weeks. Sperm motility was not

affected by radiation up to 20,000r. Dosage of 2,500r and above affected oviposition drastically and 5,000r irradiation reduced viability of the eggs nearly 100 percent. Radiation treatment of 7,500r and above resulted in considerable inhibition in the development of the ovary. The histopathological effects caused by radiation were degeneration of germarium, malformation, abnormal, and slow development of the oocytes. The degenerating germarium of the irradiated beetles showed loss of DNA positive material in the nuclei of the cells of the germarium.

GAMETOGENESIS AND RADIATION EFFECTS ON THE REPRODUCTIVE TISSUES OF *OULEMA MELANOPA* (L.)

By

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INTRODUCTION

The cereal leaf beetle, Oulema melanopa (L.), an introduced pest in this country, has assumed serious proportions in some parts of Michigan and has caused impressive losses particularly on small grains.

The purpose of this study was to consider the possibility of controlling the cereal leaf beetle, Oulema melanopa (L.) by releasing irradiated males with dominant lethal genes, which is usually referred as the sterile-male-release technique of insect control. Current interest in the sterile male method of controlling the cereal leaf beetle stems from the successful elimination of the screw worm fly, Cochliomyia hompivorax (Coq.) in Curacoa and Florida (Bushland & Hopkins, 1953, Baumhover et al., 1955), where this insect disappeared after repeated releases of sterile individuals.

The present work reports a brief study of the testes and ovaries of the cereal leaf beetle, conducted to determine the sequence of events in normal spermatogenesis and oogenesis, in order to establish a basis for evaluating the effects of radiation on the growth of the reproductive organs, oviposition, and eclosion. In addition, the present work deals with the dosages of X-rays required to induce sterility and also provide information on the effects of radiation on the ovary. However, the present experiments were not designed for detail cytological study of such effects.

IIA. REVIEW OF LITERATURE ON GAMETOGENESIS

The first research on insect gonads was initiated in 1847 by Stein (according to Bonhag, 1958) with his histological study of the female reproductive organs of beetles. The next study that concerned reproduction in beetles was in 1900 when Bordas divided Coleoptera into two main groups, with regard to the types of testes, those with simple, tubular testes and those with complex testes. In the first group with simple tubular testes belong the Carabidae, Cicindellidae, Dytiscidae, etc., whose whole male genetal system exhibit a primitive condition. The beetles with complex testes Bordas further divided into those with fascicular testes and those with cluster or grape like testes.

Saling (1907) in his investigation of the gonads of Tenebrio molitor L. was primarily concerned with tracing the development of these organs and therefore presented only a cursory survey of the histological elements in the adult ovary. And Gardiner (1934) used the ovaries of T. molitor L., in addition to other tissues, in her study of the nucleolus but did not give an extensive account of the other structures in the ovariole.

The general morphology of the female reproductive organs is given in Snodgrass (1935). He described a typical ovariole as consisting of three parts: a terminal filament, an egg tube, and a pedicel. In describing the various types of egg tubes, Snodgrass used the generally accepted classification

of Berlese (1909), which divides them into panoistic, polytrophic, and acrotrophic or telotrophic types. In the panoistic type there are no special nutritive cells differentiated from the egg cells. This type occurs in the Apterygota, Ephemeroptera, Odonata, Orthoptera, and Siphonaptera. The polytrophic type contains an alternating succession of oocytes and trophocytes and is characteristic of the Anoplura, Neuroptera, Coleoptera; Adephaga, Lepidoptera, Hymenoptera, and Diptera. A few insects, the Hemiptera and the Coleoptera; Polyphaga possess the acrotrophic type of egg tube. Here the oogonia give rise to nurse cells and oocytes. The former remain in the upper part of the egg tube, while the oocytes separate from them as a series of egg cells, increase in size in the vitellarium. There are protoplasmic connnections between the two types of cells and by means of these strands the oocytes in the egg tube continue to receive the yolk forming material from the nurse cells.

There is some confusion as to which of the Coleoptera have telotrophic ovarioles. Stein (1847), Gross (1903), and Imms (1948) have stated that the Adephaga have polytrophic ovarioles and the rest of the beetles contain telotrophic ovarioles. Weber (1922) and Wigglesworth (1950), on the other hand, have characterized the Adephaga as having telotrophic ovarioles and the Polyphaga as having the polytrophic type. Numerous other papers on the Coleopteran ovary support the original view of Stein. Bryan (1954) stated that the nutritive cords of telotrophic beetles are not usually as conspicuous as those found in Hemiptera and may even be absent in

some. Bonhag (1958) points out that the criterian for distinguishing between panoistic and telotrophic ovarioles is the presence of trophocytes in the germarium. According to him, in the absence of nutritive cords, a detailed study of oogenesis is often required to determine whether the apical cells of the germarium are cogonia or trophocytes.

The relationship between nurse cells and oocytes has been investigated by many workers, i.e., the papers of Giardina, Korschelt, Nussbaum-Hilarowicz (reviewed by Wislon, 1928), Shaffer (1920) and others. Such investigations suggested that nurse cells were abortive ova specialized for the formation of nutritive materials to supply the needs of the developing egg. This problem was reinvestigated in the Hemiptera by Schrader and Leuchtenberger (1952) using cytochemical procedures. Their findings showed that the nurse cells did contribute material to the egg cytoplasm and furthermore that this nutritive substance was in part derived from the desoxyribose nucleic acid (DNA) of the nurse cell nuclei. Bryan (1954) made a cytological and cytochemical study of oogenesis in Popilus disjunctus Ill. (Coleoptera; Polyphaga) and reported that the ovarioles of this beetle exhibit a modified telotrophic condition. His cytological and cytochemical study of the oogenesis of P. disjunctus also suggests large amounts of PAS positive substance in the apex of the germarium and also the trophic function of this region. His study did not show the presence of plasmatic strands connecting the apical region with the developing oocytes.

Krause (1946, 1947) studied the structure of the gonads and their development in the wood eating beetle, Passalus

cornutus Fabricius. He noted that in the long germarium the tightly packed cells appeared to be all alike when young, and the spiral like tip of the germarium had relatively few nuclei and great amounts of brightly staining material (especially with Orange G and Eosin). Though he did not make a detailed analysis of this material he concluded that because of its apical position and characteristic staining it is likely that this region corresponds to the typical feeding chamber of the acrotrophic type. However, he did not find definite and continucus strands connecting these with the spiral tip of the germarium. He noted only that the smaller developing egg cells possess plasmic strands and large globules of bright staining material in the germarium proper. This led him to conclude that the smaller oocytes receive nourishment from the apical feeding chamber via the plasmic strands until they acquire a follicle cell envelope of their own. In order to provide a detailed histological basis for further work on the postembryonic development and histochemistry of the ovary of T. molitor L., Schlottman and Bonhag (1956) made an extensive and detailed study on the ovary of this insect. They interpreted the localizations of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) in the ovary and also determined whether DNA was discharged from the apical trophocyte nuclei as had been reported in the telotrophic ovariles of certain Heteroptera (Schrader & Leuchtenberger, 1952; Bonhag, 1955 a & b). In Tenebrio they noted the nutritive cords originate at the posterior boundry of the trophic tissue and extend to the developing oocytes in the vitellarium. So they concluded

that in contrast to the strongly developed cords usually found in the telotrophic ovarioles of hemipterous insects, the nourishing strands in T. molitor L. are of much more delicate nature and may be quite easily overlooked.

IIB. REVIEW OF LITERATURE ON RADIATION EFFECTS TO INSECTS

Since a remarkable and extensive body of literature is available concerning the relation of X-rays and gamma rays to physiological and pathological processes on one hand, and with genetic change and its subsequent consequence on insects on the other hand, the topic under review demands restraint in the selection of published work. It was found necessary to by-pass certain papers simply because they did not bear a direct relationship to the subject selected here, or did not concern the order Coleoptera.

In his classic communications Muller (1927, 1928) mentioned that untreated female Drosophila flies mated to heavily treated males laid eggs that failed to hatch. He interpreted the mortality of eggs fertilized by irradiated sperm as resulting from chromosomal changes which he described as a "dominant lethal" effect. There are numerous other publications in the fields of genetics and cytology dealing with the effects of radiation. The various papers have been reviewed and interpreted in comprehensive publications by Muller (1940, 1941), Lea (1947) and Catcheside (1948).

There is some early work with radiation effects on the Coleoptera. For example, Runner (1916) studied the effects of roentgen rays on the tobacco or cigarette beetle, Lasioderma sericorne (F.), and reported that infertile eggs were deposited after exposure. He also reported that the treatment had the effect of stopping the activity and development of larvae and causing death of the treated larva before reaching pupal stage. Davey (1928) showed that the life of Tribolium confusum Jacquelin duVal may be prolonged by the use of X-rays given in a series of small daily doses and the prolongation of life is greater than that of larger doses given all at once. He emphasized that by merely varying the quantity of the dose, a purely physical agent, X-ray, may be made to produce at will a stimulation, a destructive effect which occurs only after a latent interval, and an instantly destructive effect.

The successful application of the sterile-male technique in the control and eradication of screw-worm flies, (Bushland & Hopkins 1951, 1953; Lindquest, 1955; Baumhover, et al., 1955) has stimulated interest in the possibility of using this method to control other insect species. Their laboratory observations showed that it was most efficient to irradiate pupae within two days of emergence and a dosage of 2,500r sterilized the males and twice that dosage sterilized the females. In addition their laboratory studies also indicated that sterilized males competed about equally well with normal males for mates. Bushland and Hopkins (1953) also made a comparative study of the effects of X-ray and gamma rays and investigated the effects

of gamma radiation on longevity, fertility and fecundity of adult screw-worm flies. Their comparative study on the effects of X-rays and gamma rays indicated no difference between the two kinds of radiation. Knipling (1955, 1959) and LaChance and Knipling (1962) formalized the approach which involves sustained release of sterile males in numbers exceeding those in a normal population and also discussed insect population control on an area wide basis. They stressed the need for investigations to determine the feasibility of applying the sterile male technique for the control of other insect pests.

In a paper on radiation control of insects, Grosch (1956) reported that irradiated Braconid wasps, with large single doses of X-rays, under starvation diet, induced lethargy in the animals and at a certain level increased the life span. Jaynes & Godwin (1957) made a preliminary study of sterilization of the white pine weevil, Pissodes strobi (Peck) with gamma radiation. Their study indicated that an exposure of 5,000r to 10,000r to be the best for this weevil because longevity of the weevils was not reduced and egg sterility was at an acceptable level. However, they pointed out that diminished feeding and oviposition rates suggested the possibility that other activities such as frequency of flight may also be altered or reduced. Cork (1957) from his study on gamma radiation and longevity of the flour beetles reported that life span of a given number of flour beetles may be extended by several percent by radiation with gamma rays and beetles that survive a single large dose of gamma rays appear to have a survival rate superior to those that receive no

radiation. According to him, this may be a single dose of 3,000r or chronic daily doses of 100r. He also pointed out that 20 percent of the animals receiving 100r daily dosage lived more than 450 days and in that time they received 45,000r. This was more than twice the amount that would have produced complete annihilation in a single irradiation. However, his conclusion was based on a single experiment, though done under ideal conditions over a period of two years.

Park, et al. (1958) studied the relation of X-radiation to the fecundity (number of eggs laid) and fertility (egg hatched) of two species of flour beetles, Tribolium confusum Duval and Tribolium castaneum Herbst. They tried four dosages of X-rays (2,000r to 5,000r) in their experiment, and advanced conclusions that there were differences between the two species with respect to fecundity and fertility. They noticed fertility was more affected by practically all components of treatment than was fecundity. They also concluded that the relation of increase in dosage to decrease in reproduction was essentially linear rather than curvilinear and irradiation at 2,000r to 5,000r levels did not reduce adult longevity.

Rogers and Hichey (1960) studied the post irradiation feeding activity of T. castaneum and arrived at the conclusion that the nutritional state of beetles after radiation affects their life expectancy. Dennis (1961) reported that an exposure of 100,800r gamma rays killed all of the confused flour beetle adults and larvae, saw toothed grain beetle adults, lesser grain borer adults, granary weevil adults, and rice weevil adults. He pointed out that confused flour beetles

exposed to 8,400r did not reproduce during a two month period after exposure. Bletchly (1962) studied the effects of a single X irradiation at 4,000r on the larvae of powder post beetles, Lyctus brunneus (Steph.) and suggested that irradiation of larvae at a dosage too low to prevent completion of development would be of little value as a practical method of control, because of recovery in the population level of subsequent generations of the treated strain. Hoover, Floyd & Richardson (1963) subjected all developmental stages of rice weevil, Sitophilus oryzae (L.) to radiation by X-rays and found resistance to the effects from radiation exhibited by this insect increased as development increased. In addition, they reported that the LD_{50} and the point of complete sterility for the adult stage was between 7,500r and 10,000r.

THE TEST INSECTS

The cereal leaf beetle has an adult diapause during winter. Preliminary histological study of the pre-diapause and diapausing insect indicated that most of the male beetles had fully matured sperm. But in case of female beetles, it appeared that the insect was not sexually mature at this period as indicated by ovarian development.

In order to find out how sexual development takes place in the adult beetles, a brief study of gametogenesis was undertaken as it is one of the important prerequisites for controlling the insect by sterile-male release.

Most of the insects used in this study were summer adults, collected in the field by the Department of Entomology, Michigan State University.

DEVELOPMENT OF GONADS IN THE

ADULT CEREAL LEAF BEETLE

Materials and Methods. The beetles used for studying the development of gonads had the following treatments: They were collected from the field on July 25, 1962, and maintained in a bioclimatic chamber on living plants until July 31, 1963. They were then placed in one pint paper cartons at a constant temperature of 43° F on August 8th until September 16th. From September 16th to September 17th they were maintained at 25° F for 15 hours. In the bioclimatic chamber they were given 15½ hour daylight. The temperature at day was 82° F and at night 77° F. The cold treatment apparently was necessary to break diapause. Three hundred and fifty beetles were taken out from the cold storage on September 17th, and put into cages (50 each) made of a glass chimney and having oat seedlings.

Samples were taken each day, except the first sample which was taken after 18 hours, and fixed in KAAD (Peterson, 1948) immediately. (KAAD = Kerosine, Acetic acid, 95% Alcohol and Dioxane in ratio of 1:2:7:1.) Samples were taken up to 14 days. When the beetles started feeding voraciously, they were changed to new cages containing fresh oat seedlings. Each day about 20-25 insects were sampled. Before fixing in KAAD, the heads of the beetles were cut off with a sharp razor blade and the legs and wings were carefully removed. This enabled a better penetration of the fixative. The vials containing the

beetles in the fixative were kept under vacuum to remove air bubbles. The beetles were left in the fixative for a couple of days and then dehydrated in tetrahydrofuran for two hours and double embedded as per method suggested by Salthouse (1958), in tetrahydrofuran containing 1% and 2% parlodion for 24 hours and 36 hours respectively. Some were embedded with tetrahydrofuran containing 3% parlodion. In that case the beetle was double embedded in tetrahydrofuran containing 1% and 2% parlodion for 24 hours each and in tetrahydrofuran containing 3% parlodion for 36 hours. After this treatment, the beetles were given a quick rinse in tetrahydrofuran and embedded in tissuemat (61° C) for 3 to 4 hours, making 2 changes. The blocks were then sectioned on a microtome at 10 microns thickenss. In addition, a few laboratory reared larvae were allowed to pupate and the freshly emerged adult beetles were fixed in KAAD, within 3 days after emergence. These were also sectioned, stained and studied.

Staining. Most of the slides were stained with Feulgen Stain, made as reported in Stain Technology (1951), and counter stained with fast green. The length of acid hydrolysis varied between 6-7 minutes at 60° C. The slides were treated with Feulgen Stain for 45 minutes and with fast green for about 1½ to 2 minutes.

Results. The sections from beetles fixed after 18 hours to 3 days after removal from the cooler show a suspensory ligament, 6-7 ovarioles on each side of the ovary and the stalk bearing them. The ovary on each side is composed entirely of

the germarium and the slender stalk or pedicel bearing it. The germarium is composed of densely packed, extermely Feulgen positive, more or less spherical cells (Fig. 1). The cells in the germarium all look alike at this time. (In many of the sections 6 ovarioles and in some 7 ovarioles on each side of the ovary were observed. However, this depends on the plane of sectioning, but the maximum number of ovarioles observed on each side of the ovary was 7.) The stalk and the lateral oviducts are slender during the early period. No young oocytes are visible at this time, and the ovary appears to be immature.

After 3 days or so, the vitellarium, where the egg cells grow, develops gradually. The cells in the immature, densely packed, germarium contain large nuclei and are almost devoid of any cytoplasm. But when oocyte development and enlargement begin the cells in the germarium appear to clearly have cytoplasm, as is evidenced by the presence of the counter stain, fast green, around the large nuclei.

After oocyte development starts, the posterior end of the germarium shows a diminution of Feulgen positive material, and the cells appear granular at this time. The young oocytes, which are irregularly located, are intermixed with slightly elongate pre-follicular cells (Fig. 2). Very rarely, disintegration or pycnosis of the brightly staining, Feulgen positive cells in the germarium is noted.

The young oocytes in the posterior portion of germarium appear to have filamentous chromosomes. As the development progresses, the young oocytes appear tobe situated more or less side by side among the pre-follicular cells. When the oocytes

grow larger, they change from a spherical to an oval shape and become oriented in a line. Various sizes of oocytes are seen in a developing ovariole. The first stage small oocyte and the second stage oocyte appear to have columnar follicular epithelium (Fig. 3a, 3b). The second stage oocyte is larger in size than the first and in both the nuclei have Feulgen positive chromatin strands. The follicles are quite characteristic in the vitellarium in that they have a different kind of follicular epithelium. The third stage oocyte has a cuboidal follicular epithelium and the individual cuboidal follicular cells appear to be horizontally elongate (Fig. 4). The follicular cells at the third stage have a well defined cytoplasm. The nucleus of the oocyte at this stage appears faint and clear Feulgen positive chromatin material is not visible. However, it is evident that the nucleus undergoes a gradual transformation and develops into the germinal vesicle. The transformation that takes place in the oocyte nucleus during the development of the germinal vesicle is too complicated and is not handled here in detail. The fourth stage oocyte is quite large and also has a cuboidal follicular epithelial cells, but these appear slightly elongate vertically. The cuboidal follicular epithelial cells at this stage also have a well defined cytoplasm and a brightly staining Feulgen positive nucleus. The cuboidal follicular cell nucleus appears to be little larger than in the previous stage. Yolk synthesis seems to proceed here. The fifth stage is quite characteristic in that the follicular epithelial cells have attained a squamous condition (Fig. 5). A great amount of yolk synthesis has taken place by this time (Fig. 6). The chorion

is not laid down until yolk formation has been completed. Since the formation of follicles is a continuous process, the exact number present at any specific time is subject to variation. However, it appears that 3 to 5 follicles, frequently 3, appear in the vitellarium before the largest, most posterior oocyte is released into the lateral oviduct.

When oocytes are found in the vitellarium, the cells in the germarium appear to be less densely packed and each cell appears to have a well defined cytoplasm (Fig. 7).

The interfollicular tissue (the mass of cells in between two successive oocytes) seems to be composed of small follicular cells which have accompanied the oocytes during their growth and have consequently become wedged in between the successive follicles of the vitellarium.

Beyond the last egg chamber a mass of follicular cells form the plug, which closes the proximal portion of the egg tube. At the time of ovulation the epithelial plug appears to break down, and the preceeding follicle then takes the terminal position and the accompanying interfollicular tissue becomes the functional plug.

The pedicel (Fig. 8) or stalk, is a duct that connects the vitellarium with the lateral oviduct. The wall of the pedicel is composed of simple columnar cells covered exteriorly by a continuation of the outer epithelial sheath of the ovariole. The cells of the stalk are also brightly Feulgen positive. Before the development of the oocytes, the stalk of the oviducts are comparatively slender, and the cells in this region appear to be densely packed. Also the Feulgen

positive material is prominent because of the compactness of the columnar cells. When oogenesis commences the stalk and the oviduct becomes distended.

In the beetles which have started oviposition, the oviducts are characterized by the presence of remains of the degenerating egg follicles at the base of the vitellarium. In beetles which are actively forming eggs, these bodies are difficult to distinguish.

It is important to note that the nutritive cord or plasmatic strands connecting the trophocytes in the germarium with the developing oocytes were not observed in this beetle during the entire course of this work. Not even a slightest trace of this cord was found. (c.f. Schlottman and Bonhag, 1958)

The testes of the adult male cereal leaf beetle consists of 2 pair of lobuler testes, one pair on each side of the body in the abdominal region. They appear pale white with a yellowish tinge. They are moderately big for this small beetle. Each lobe of the testes is more or less bean shaped and in between the bean shaped lobes there appears a duct in which fully matured spermatoza are seen. Around the testes, there is an investing coat composed of a single sheet of cells.

Sections from the few laboratory raised adult male beetles showed at least a few fully matured spermatoza within 3 days after emergence from the pupal stage. Mostly, sex cells in different stages of development and a few mature spermatoza in bundles were seen at this period (Fig. 9a). In the case of females, the development of the oocytes were never noticed at this period. This indicates that the males become sexually mature earlier than females.

Near the outer periphery of the lobular testis spermatocytes in different stages of meiosis are visible (Fig. 9b & 10). Towards the inner side, spermatids and transformation of spermatids into flagellated spermatozoa can be observed (Fig. 11). The mature spermatozoa appear in bundles towards the central duct. The spermatids are at first compressed and round and then they become elongate and tappering on both the sides giving an appearance of a spindle (Fig. 12a, 12b). Later they are dissolved and released.

It appeared that when the beetles entered diapause, the males had fully matured spermatozoa and also sex cells in different stages of development. Many sections from diapausing males showed large amounts of matured spermatozoa, and also the males that emerged from diapause showed large amounts of matured spermatozoa.

FIGURE 1

LONGITUDINAL SECTION OF THE OVARIOLE OF OULEMA MELANOPA (L.)

Fixed in KAAD and Stained in Feulgen and Fast green (265x).

Showing suspensory ligament, ovariole sheath, bright staining germarial cells, prefollicular oocytes and prefollicular cells.

FIGURE 2

HORIZONTAL SECTION OF THE OVARIOLES OF OULEMA MELANOPA (L.)

Magnification 540x.

Showing the germarium and three successive developing oocytes in the vitellarium.

FIGURE 3a

HORIZONTAL SECTION OF THE OVARIOLE OF OULEMA MELANOPA (L.)

A germarial stalk oocyte and the second stage oocyte with Columnar follicular epithelial cells. 1100x

FIGURE 3b

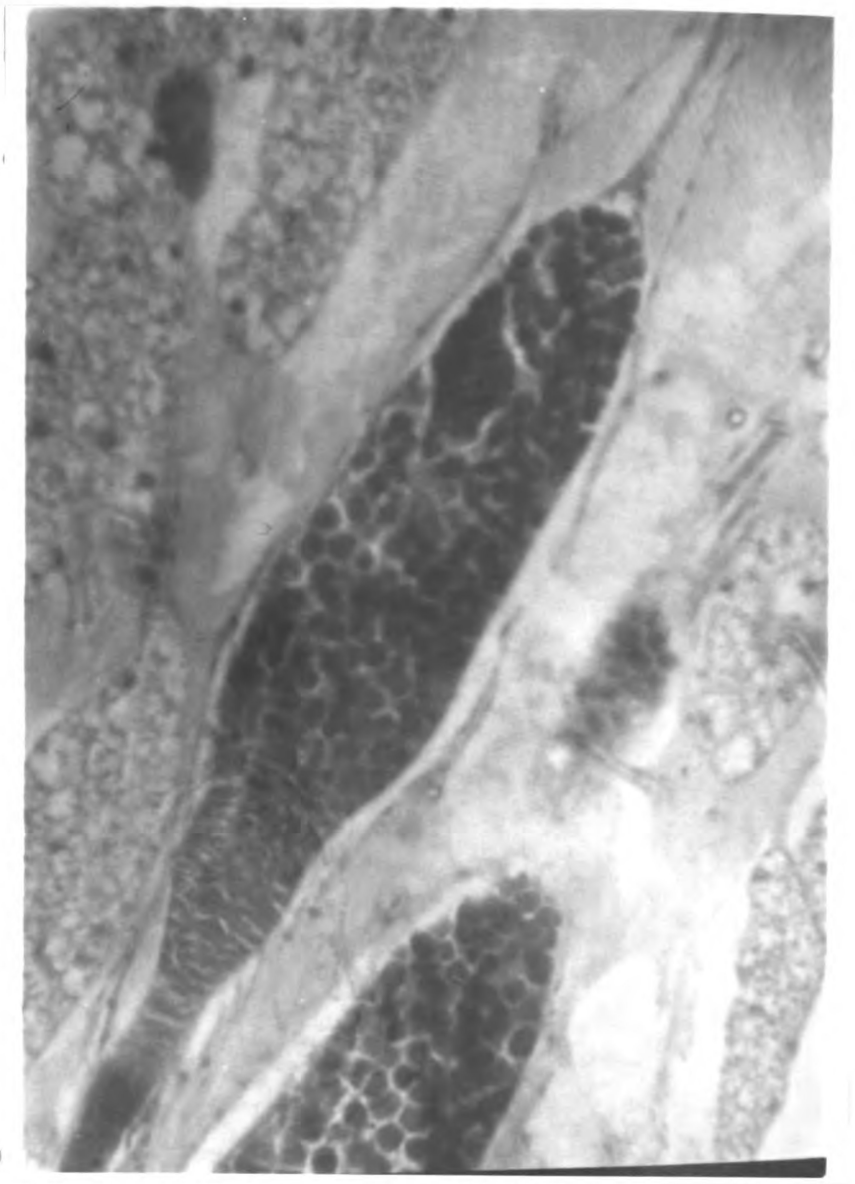
CROSS SECTION OF THE OVARIOLES OF OULEMA MELANOPA (L.)

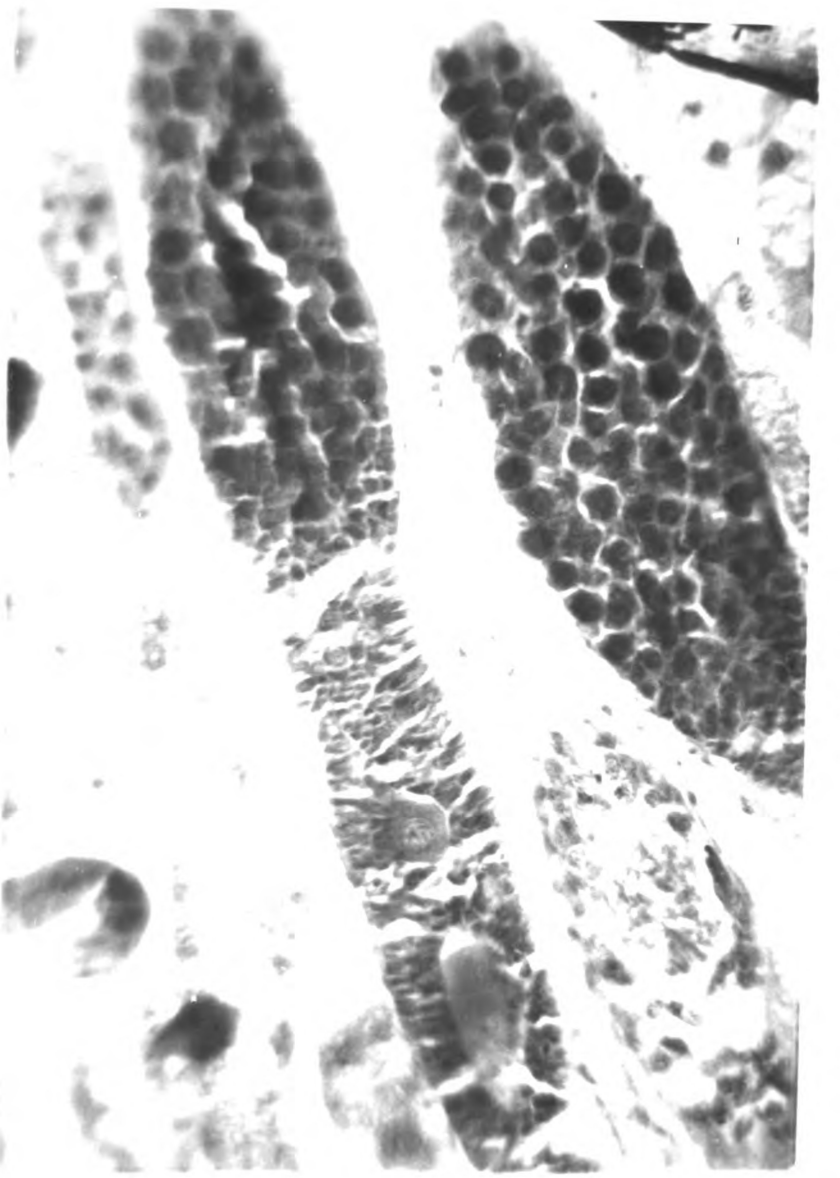
First stage oocyte of an ovariole and cuboidal follicular epithelium of a third stage oocyte of a neighbouring ovariole. 540x

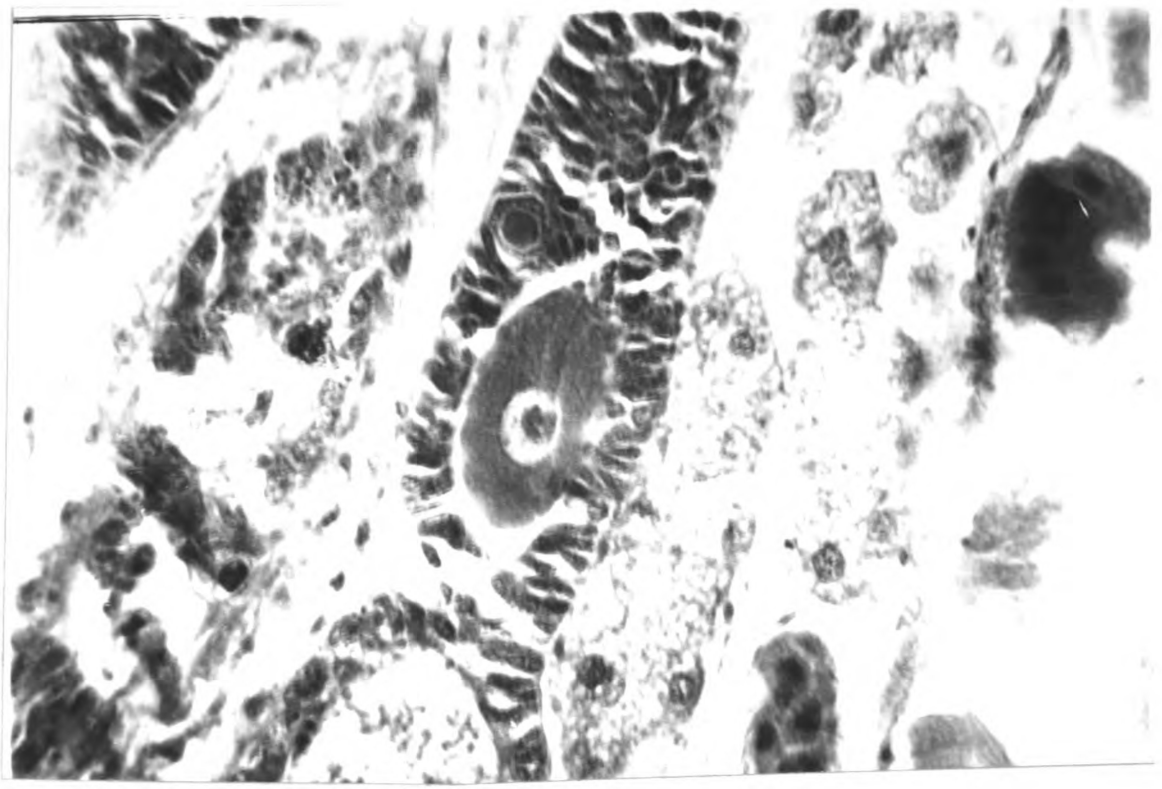
FIGURE 4

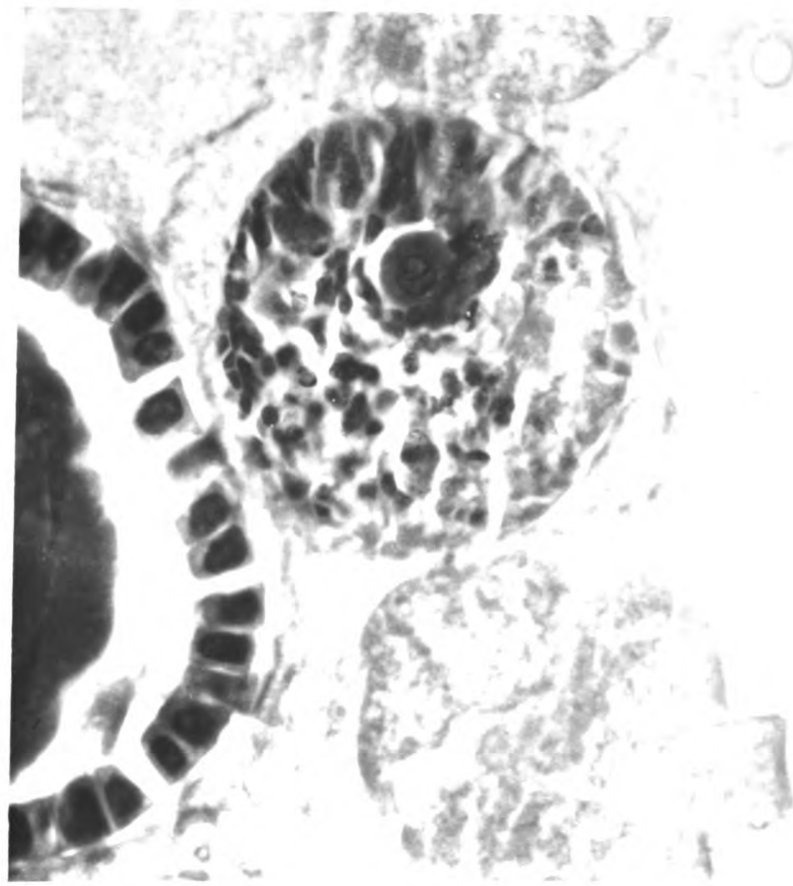
HORIZONTAL SECTION OF THE CUBOIDAL FOLLICULAR EPITHELIAL CELLS OF THIRD STAGE OOCYTE OF OULEMA MELANOPA (L.)

Mangification 2,700x.









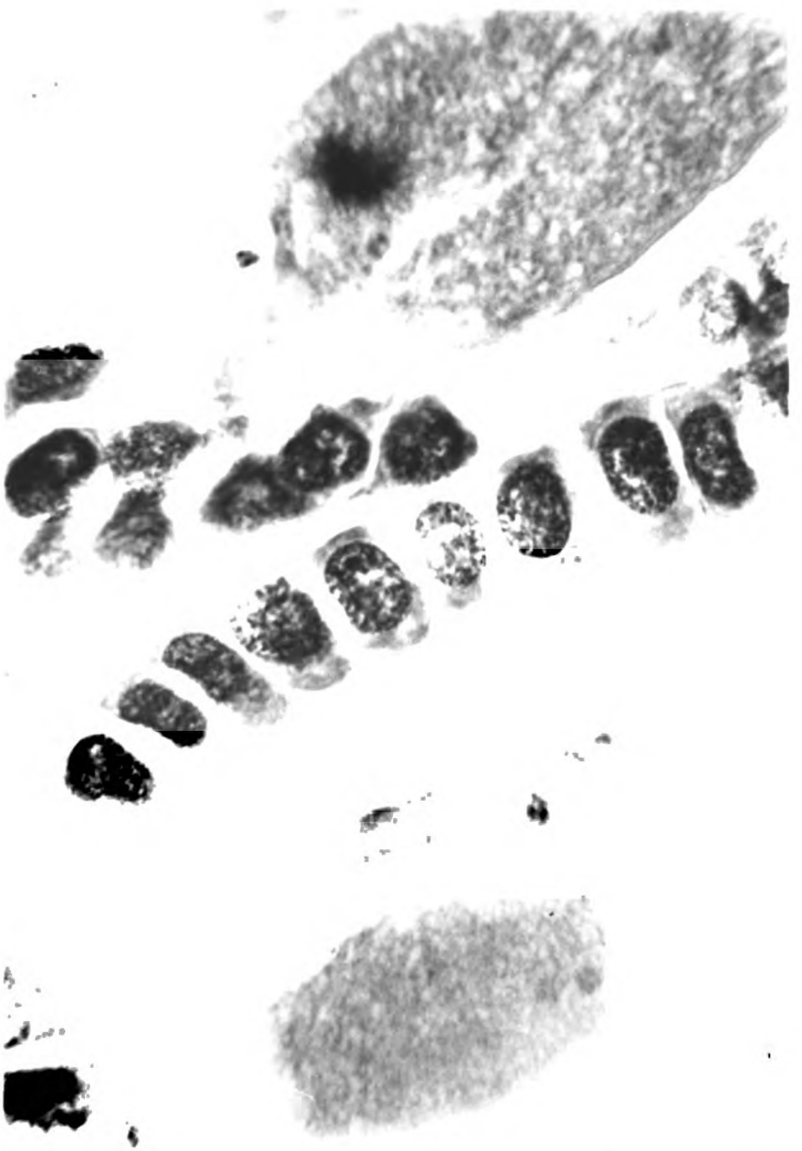


FIGURE 5

HORIZONTAL SECTION OF THE SQUAMOUS FOLLICULAR EPITHELIAL CELLS OF THE FIFTH STAGE OOCYTE OF OULEMA MELANOPA (L.)

Magnification 1080x.

FIGURE 6

HORIZONTAL SECTION OF THE FIFTH STAGE OOCYTE OF OULEMA MELANOPA (L.)

Showing the squamous follicular epithelial cells and abundance of yolk. 540x

FIGURE 7

HORIZONTAL SECTION OF THE GERMARIUM OF A DEVELOPING OVARIOLE OF OULEMA MELANOPA (L.)

Cells in the germarium are less densely packed and cytoplasm is well defined. 540x

FIGURE 8

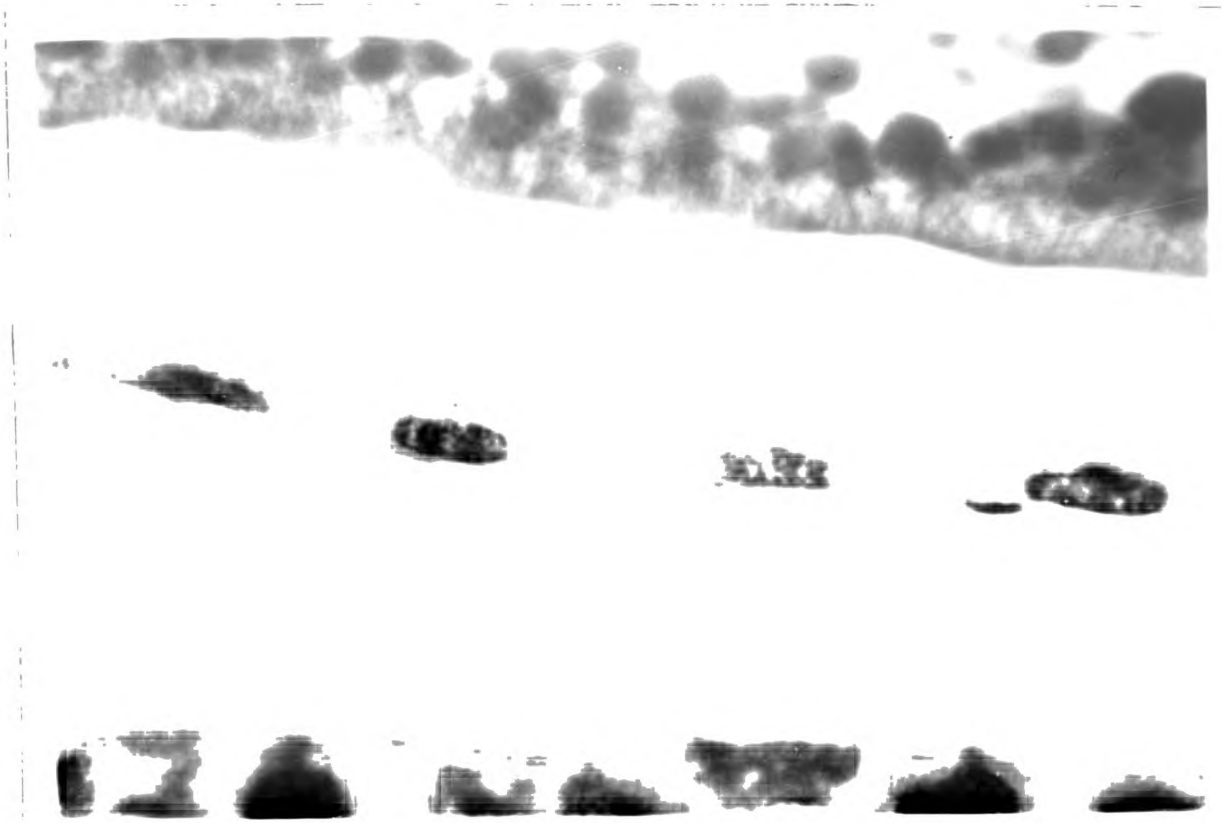
HORIZONTAL SECTION OF THE FIFTH STAGE OOCYTE OF OULEMA MELANOPA (L.) ON THE PEDICEL

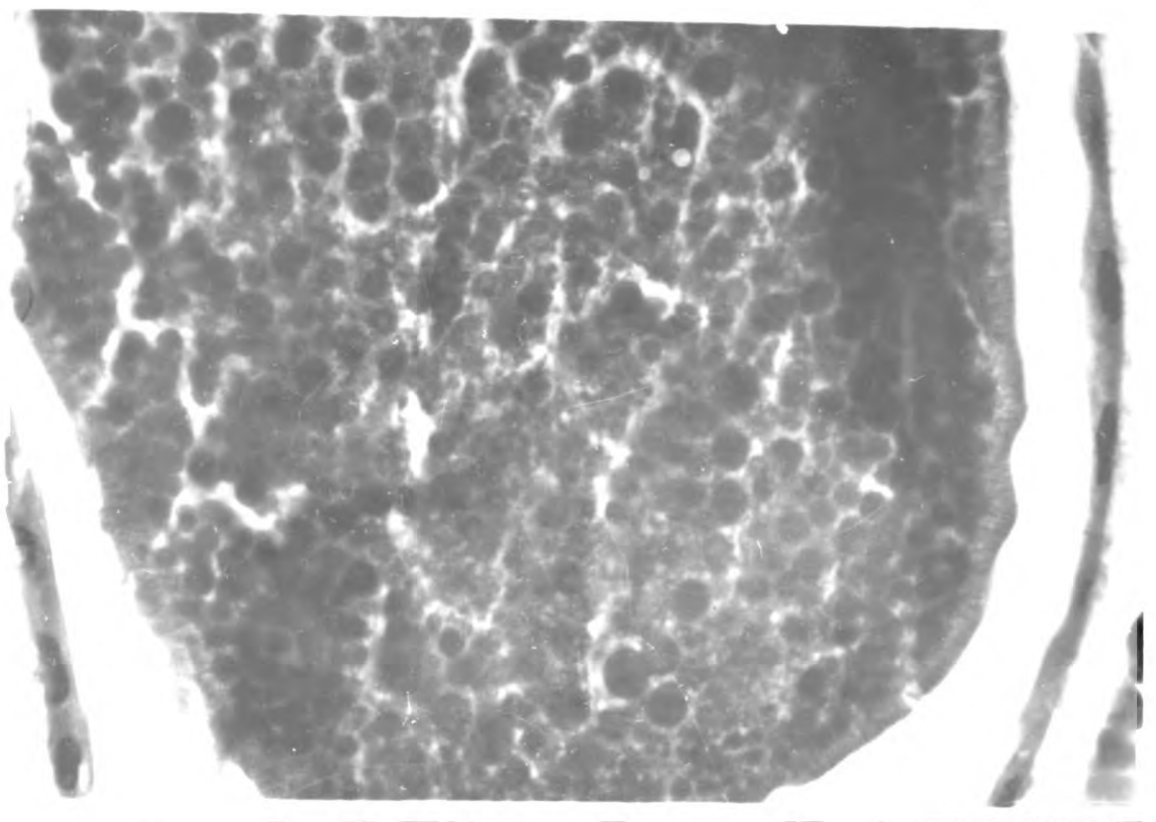
Portion below the oocyte is the plug. 265x

FIGURE 9a

LONGITUDINAL SECTION OF THE TESTIS OF A YOUNG ADULT

Showing mostly dividing cells, a few spermatids and spermatozoa. Arrow indicates a single bundle of spermatozoa. 265x





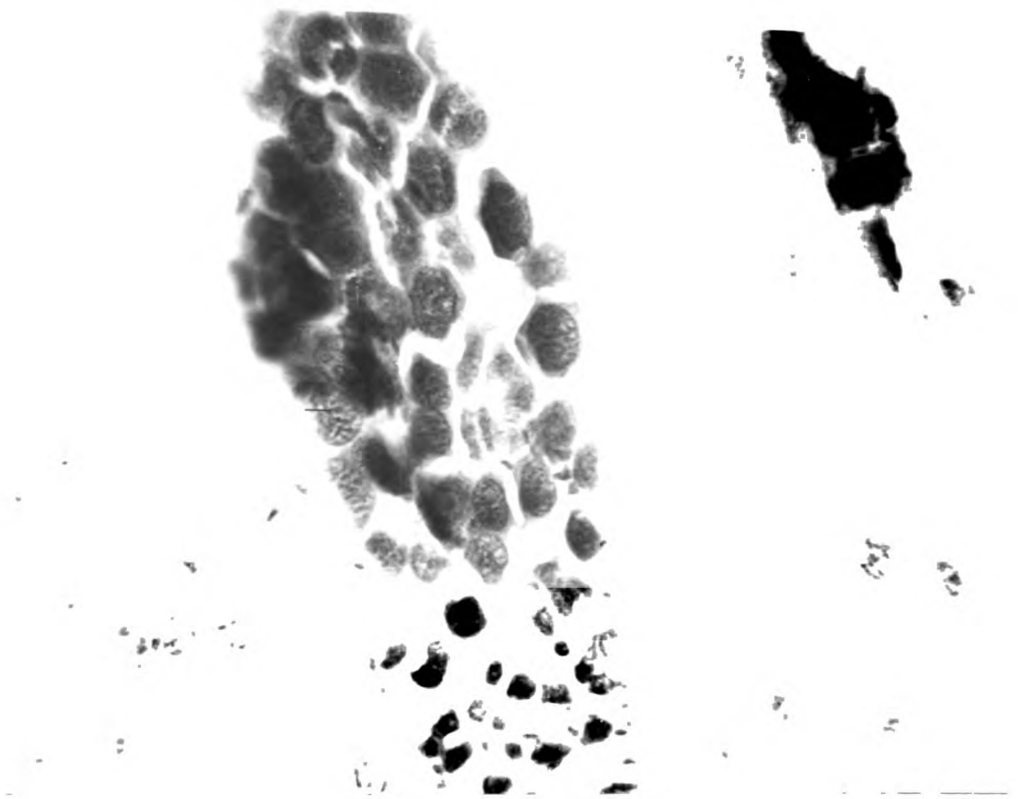


FIGURE 9a

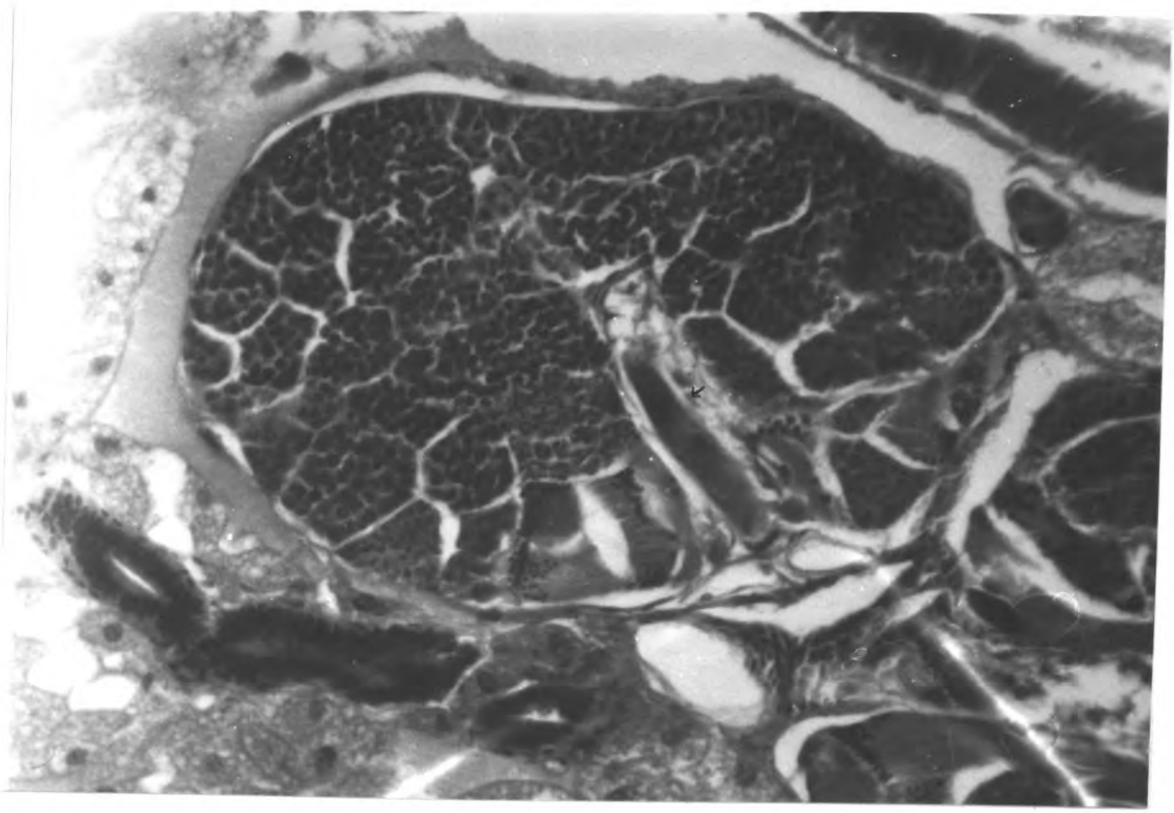


FIGURE 9b

SPERMATOCYTES, SPERMATIDS AND THE TRANSFORMATION OF SPERMATIDS INTO SPERMATOZOA IN THE TESTIS OF OULEMA MELANOPA (L.) THREE DAYS AFTER EMERGENCE FROM PUPA.

1080x

FIGURE 10

CROSS SECTION OF ONE OF THE LOBULAR TESTES OF OULEMA MELANOPA (L.)

Showing different stages of spermatogenesis near the periphery and abundant spermatozoa in bundles towards the inner side. 540x

FIGURE 11

TESTIS OF OULEMA MELANOPA (L.) SHOWING EARLY SPERMATIDS AND FLAGELLATED SPERMATOZOA IN BUNDLES

1080x

FIGURE 12a

SPERMATIDS IN THE TESTIS OF OULEMA MELANOPA (L.) IN THE PROCESS OF TRANSFORMATION INTO SPERMATOZOA

Magnification 2,700x

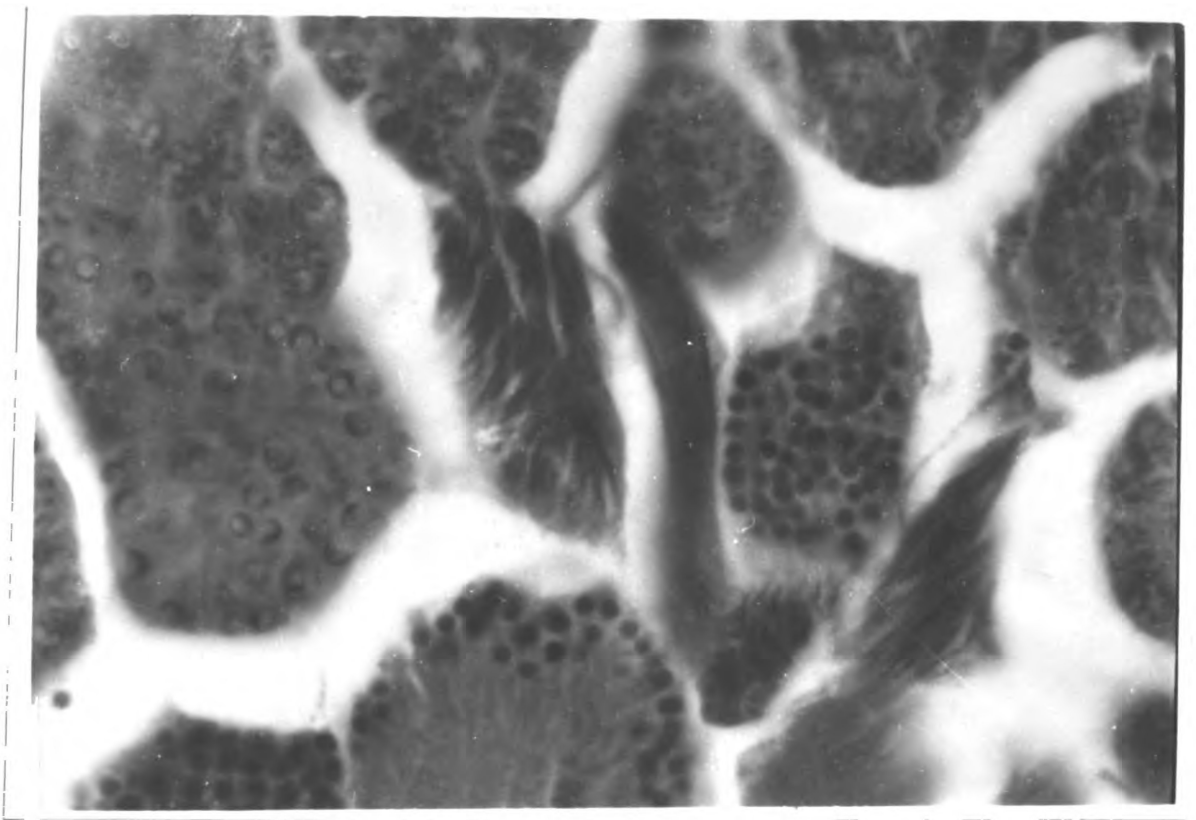
FIGURE 12b

CROSS SECTION OF TESTIS OF OULEMA MELANOPA (L.) SHOWING SPERMATIDS IN GROUPS BEING TRANSFORMED INTO SPERMATOZOA IN BUNDLES

1080x

FIGURE 10

FIG. 11



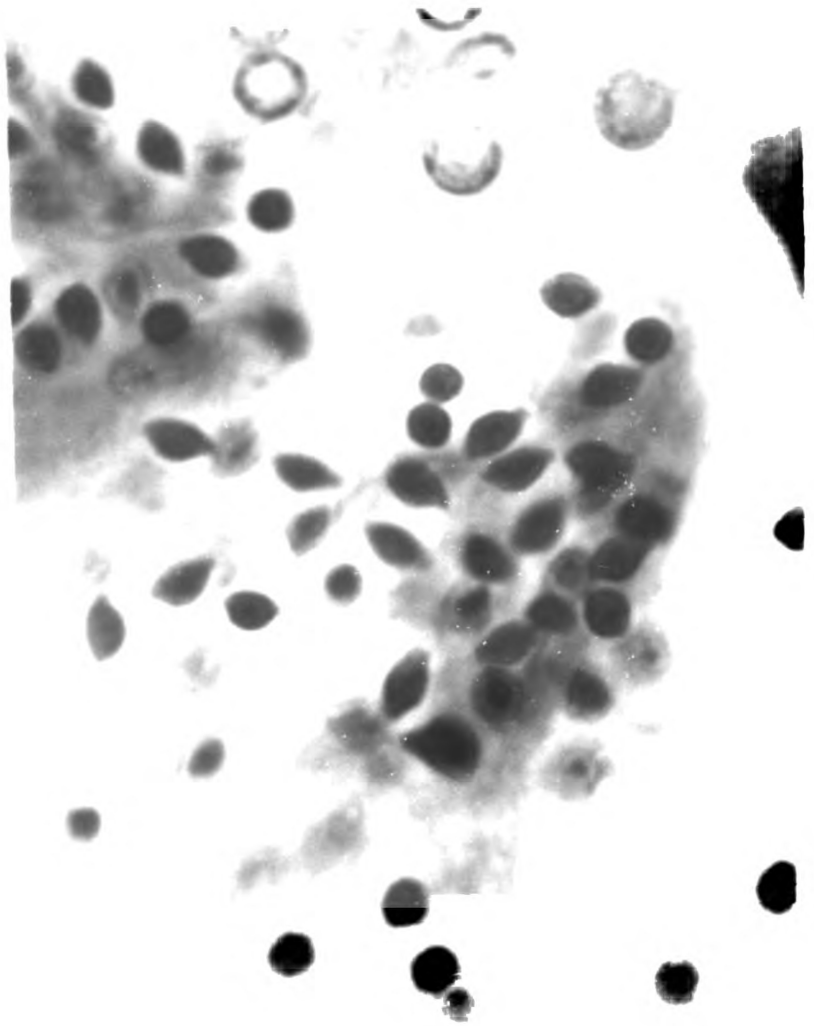
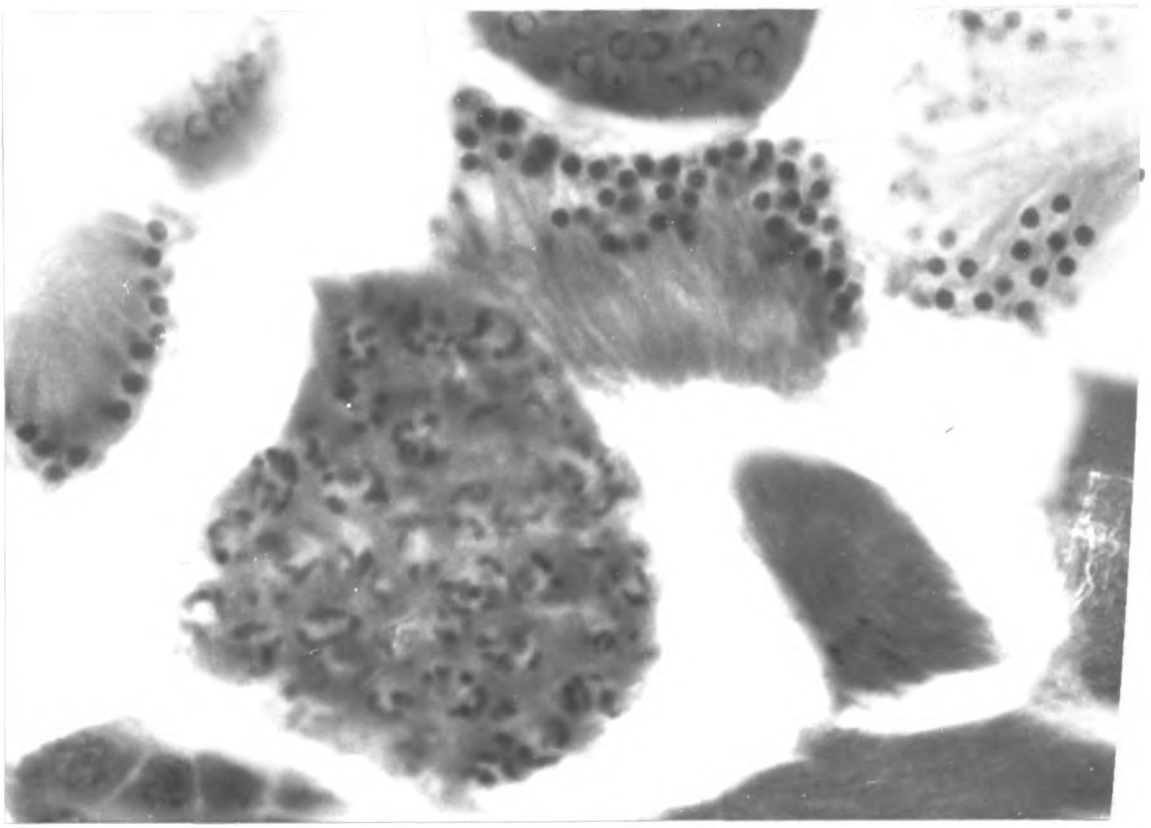


FIGURE 12b.



HISTOCHEMICAL STUDIES ON THE OVARY OF CEREAL LEAF BEETLE

The germarium of the cereal leaf beetle showed bright staining cells when stained with Feulgen Stain, or Pyronin Y and methyl green. Both Feulgen and methyl green are specific for nuclear DNA, and Pyronin Y for RNA.

To confirm whether the germarium is rich in DNA and RNA, some of the histological preparations obtained from the female cereal leaf beetles were treated with DNAase and some with RNAase (Pearse, 1961) and then subject to the usual staining procedures.

Material and Methods. The histological preparations made by the tetrahydrofuran and parlodion double embedding process were treated with DNAase after the usual dehydration process in graded alcohol series and rinsing finally in glass distilled water. Then the slides were treated for 30 hours at room temperature with 3,000 Dornase units of DNAase per milliliter in 0.025 M. vernol buffer at pH 7.5 containing 0.003 M. $MgSO_4$. The enzyme solution was renewed after 15 hours. At the end of 30 hours the slides were rinsed in water and the standard Feulgen reaction was performed (The DNAase used in this experiment was supplied by Nutritional Biochemical Corp. containing 30,000 Dornase units per mg.). The controls were not treated in the enzyme preparation.

The Ribonucleic acid extraction was done as follows: the slides were dehydrated in graded alcohol series in the usual manner and rinsed in glass distilled water and incubated for 1-1½ hours at 37° F in a solution of RNAase (1mg/ml.) in glass distilled water. (RNA'se was supplied by Nutritional Biochemical Corp.) After this treatment, the histological preparations were washed in distilled water and stained with Pyronin Y and methyl green. The controls were also stained with Pyronin Y and methyl green without treating them in the enzyme preparation.

Results. The histological sections treated with DNA'se were Feulgen negative (Fig. 13) and the controls Feulgen positive (Fig. 14). This indicated that the germarium was very rich in DNA.

The sections treated with RNA'se and stained with Pyronin Y and methyl green showed absence of RNA in the germarium. It appears that the germarium of the cereal leaf beetle is rich in both DNA and RNA. This result is suggestive of the trophic function of the germarium in the cereal leaf beetles although no nutritive cord was observed to connect the developing oocyte with the germarium. The nucleic acid contents of the follicular epithelial cells was also removed by the DNAase and RNAase treatments, particularly in the later stages of development.

FIGURE 13

GERMARIUM OF OULEMA MELANOPA (L.) TREATED WITH DNAase AND STAINED WITH FEULGEN AND FAST GREEN

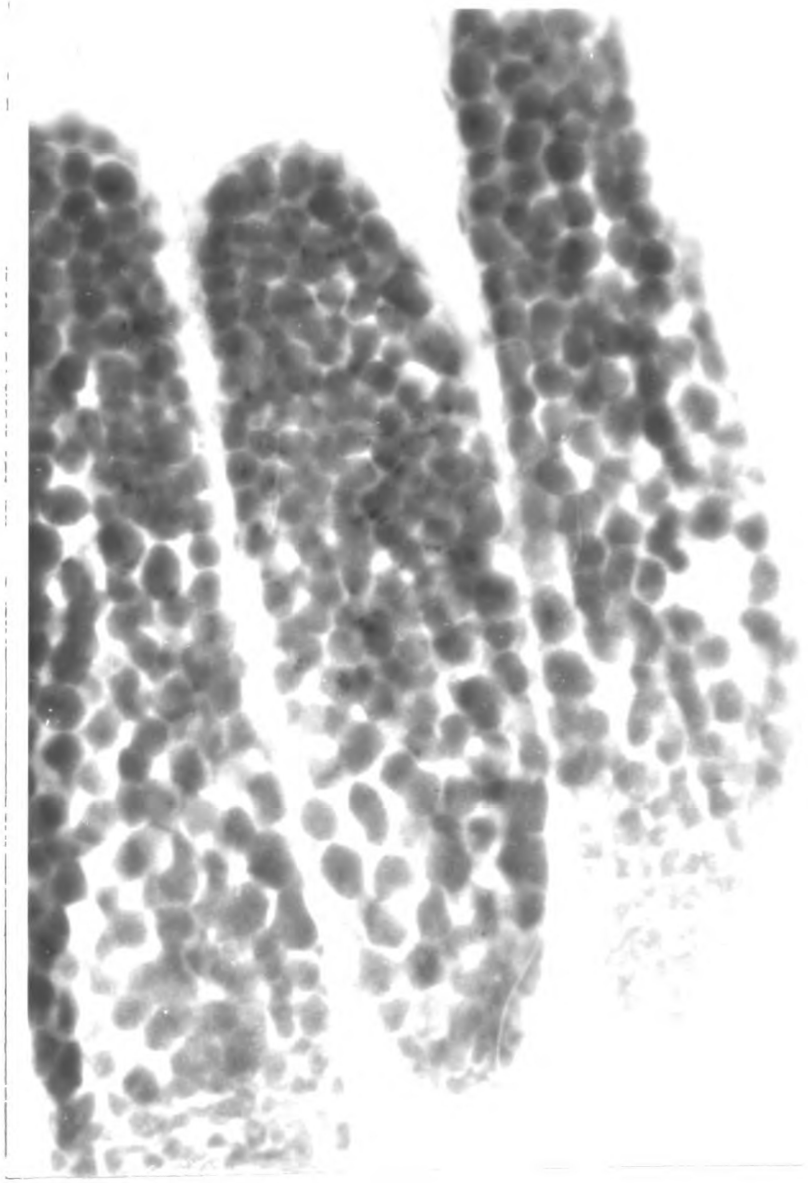
Showing loss of DNA positive material. 540x

FIGURE 14

GERMARIUM OF OULEMA MELANOPA (L.) STAINED WITH FEULGEN AND FAST GREEN

Showing abundance of DNA positive material in the nuclei. 265x

FIGURE 19



EFFECTS OF RADIATION ON BEETLES

TREATED WITH X-RAYS

Materials and Methods. Two trials were conducted at different periods. The beetles used in the first trial were collected from field on July 26, 1963 by the Department of Entomology. The beetles had the following treatments before they were used in this experiment: The beetles were placed in a bioclimatic chamber on July 31, 1963, until they were moved to cold storage at 43° F on August 6, 1963. Five hundred and sixty beetles were removed from the cooler on October 14, 1963, and divided into lots of 80 beetles each. Except the control, the lots of 80 beetles were irradiated with the following dosages: 198r/min., no filter, ISMA, FSD50 CM.

Dosage	Time Required
1,000 Roentgens	5.5 min.
2,500r	12.6 min.
5,000r	25.2 min.
7,500r	37.8 min.
10,000r	50.4 min.
20,000r	100.8 min.

The beetles were not sexed. Characters for sexing the living beetles were not available. Many attempts were made to sex them but met with failure.

Ten beetles from the control, and from each treated sample, were taken for study of their sperm motility immediately

after treatment and the rest were left in cages containing oat seedlings. The beetles were changed to new cages containing fresh oat seedlings when they started actively feeding. This was done more or less every day. At the start of oviposition the beetles were transferred to new cages containing fresh seedlings every day in order to take an egg count each day and to keep the eggs laid in a day for viability counts.

The sperm motility was observed in the following manner: The insects were dissected carefully and the testes were removed and placed on a slide containing two drops of Yeager's Saline (Yeager 1939) or housefly saline (Brebbia and Ludwig, 1962) and squashed with a glass rod having a bulbous tip. Then the squashed preparation was taken on a cover glass and observed by the hanging drop method under a phase microscope.

Visual observation on the feeding habits of the normal beetles and the irradiated beetles were also made.

The insects used in the second trial of irradiation were summer adults, collected on June 9, 1963. These beetles were put in bioclimatic chambers on July 15, 1963, and became inactive on July 21, 1963. They were transferred to the cooler (43° F) on July 23, 1963.

The beetles were taken out of the cooler on February 7, 1964, and divided into lots consisting of 100 each. Then they were treated at different dosage levels of radiation using X-rays. The following dosages were used in the second trial: 1) 500 Roentgens, 2) 1,000r, 3) 3,000r, 4) 5,000r, 5) 0. After irradiation, they were put into chimney cages containing oat seedlings, for feeding and oviposition. Approximately 50

beetles were left in each cage. Some of them appeared to be injured or damaged while handling and soon died (See Table III for detail). When the beetles started feeding actively, they were transferred to new cages containing oat seedlings every two days. Mortality records and egg counts were made at this time. The eggs laid in two days were kept for studying the effect of X-rays on eclosion.

Results.

TABLE I

Percent Mortality of Cereal Leaf Beetles Irradiated with X-Rays -- First Trial.

Treatment	No. of Beetles left in cage	No. of Beetles alive after 15 days	% Mortality in 15 days
Control	61	40	34.4
1,000r	70	43	47.1
2,500r	70	34	51.4
5,000r	70	2	97.0
7,500r	70	3	90.0
10,000r	70	6	91.4
20,000r	70	20	71.4

TABLE II

Observation on Oviposition and Eclosion of Cereal Leaf Beetles Irradiated with X-Rays -- First Trial.

Treatment	No. of Beetles left for egg laying	Eggs laid in 1 day	No. of eggs hatched	% Eclosion
Control				
24-25th Oct.	43	49	28	57.1
25-26th Oct.	43	99	63	63.6
27-28th Oct.	41	157	50	31.8
28-29th Oct.	40	149	49	32.8
	Total	454	Total 190 Ave.	41.8
1,000r				
24-25th Oct.	43	72	14	19.4
25-26th Oct.	43	64	18	28.1
27-28th Oct.	36	81	none hatched	0
28-29th Oct.	33	63	none hatched	0
	Total	280	Total 32 Ave.	11.4
2,500r*				
28-29th Oct.	34	29	2 hatched	6.9
29-30th Oct.	34	12	none hatched	0
2- 3rd Nov.	20	8	none hatched	0
	Total	49	Total 2 Ave.	4.1
5,000r	10	40 in 3 days	nil	0
5,000r (29-30)	2	7 in 1 day	nil	0
		47 Total		

*Oviposition was considerably delayed in beetles treated with 2,500r and 5,000r

Table II (Continued)

7,500r	did not lay any eggs	-
10,000r	did not lay any eggs	-
20,000r	did not lay any eggs	-

There was motility of the spermatoza in all treatments immediately after irradiation and thus they were not immediately affected by the radiation. Some of the histological preparations from the irradiated female beetles also showed spermatozoa in the spermatheca up to 20,000r radiation. This indicates the sperm motility is not affected by X-ray radiation, particularly the first few days after irradiation

Feeding habits of the irradiated beetles. The beetles irradiated with 10,000r and 20,000r,did not feed as normally as the control. The 10,000r irradiated beetles during the first week after radiation were feeding on the leaves to a lesser extent than the control, but during the second week they stopped feeding to a considerable extent. The 20,000r irradiated beetles did not feed actively either during the first week or the second week after irradiation. In other treatments, the difference in feeding habits were not pronounced.

TABLE III

Percent Mortality of Cereal Leaf Beetles Irradiated with X-Rays -- Second Trial.

Treatment	Initial No.	No. Alive After 7 days	No. Alive After 10 days	% Mortality in 10 days
Control	91	60	55	39.7
500r	96	50	47	51.0
1,000r	96	43	28	70.8
3,000r	100	42	11	89.0
5,000r	100	40	8	92.0

(In the second trial, first eggs were noticed on February 11th.)

TABLE IV

Observations on Oviposition and Eclosion of the Cereal Leaf Beetles Irradiated with X-Rays -- Second Trial.

Treatment	No. of Insects	Eggs Laid	Eggs Hatched	% Eclosion
11th-13th, Feb.				
Control	71	240	126	52.5
500r	65	144	17	11.8
1,000r	74	2	nil	0
3,000r	85	nil	-	0
5,000r	82	-	-	0
13th-15th, Feb.				
Control	61	508	180	35.4

TABLE IV (Continued)

500r	56	287	24	8.3
1,000r	56	18	7	38.8
3,000r	75	nil	nil	0
5,000r	69	nil	nil	0
15th-17th, Feb.				
Control	60	500	132	26.4
500r	50	344	29	8.4
1,000r	40	35	5	14.2
3,000r	33	nil	-	0
5,000r	32	nil	-	0
17th-19th, Feb.				
Control	55	463	185	39.9
500r	47	294	25	8.5
1,000r	28	23	2	8.7
3,000r	11	5	2	40
5,000r	8	nil	nil	0
19th-21st, Feb.				
Control	44	263	119	45.2
500r	40	184	26	14.1
1,000r	9	12	1	8.3
3,000r	Many had died by this time and the rest were used for histological work	---	---	---
5,000r		---	---	---

TABLE V

Percent Eclosion from the Total Number of Eggs from Each Treatment of Radiation -- Second Trial.

Treatment	No. of Eggs Laid	No. Hatched	% Eclosion
Control	1,974	742	37.6
500r	1,253	121	9.6
1,000r	90	15	16.6*
3,000r	5	2	40
5,000r	nil	---	0

* The total number of eggs laid in this treatment was far less than the control and 500r, but the percent eclosion was higher in the small number of eggs.

Mortality of the beetles was very high in beetles receiving X-rays over 2,500r in the first trial (Table I). Some of the beetles receiving 1,000r lived up to one month. Observation was not made beyond this period. Some beetles receiving 20,000r were observed alive at the end of 15 days (Table I). However, they died subsequently. The percent mortality in control was 34.4 in the first trial, whereas the percent mortality of beetles irradiated with 5,000r, 7,500r, and 10,000r was over 90 percent; this was in 15 days. In the case of 20,000r irradiated beetles the percent mortality in 15 days was 71.4 percent (Table I) and the 20,000r treated ones did not feed actively.

From the results obtained in the first trial, it appears that radiation affects egg production. There was considerable reduction in the number of eggs laid by the irradiated beetles (Table II). Beetles receiving over 7,500r did not lay any eggs at all during the period they were under observation. In the case of beetles receiving 2,500r and 5,000r radiation, the egg production was extremely low when compared with the control. Also oviposition was delayed by 3-4 days in these two treatments as compared to the control and the 1,000r irradiated beetles (Table II).

There was also considerable difference between the eclosion of the control and the irradiated beetles. Percent eclosion in the first trial, in the control was 41.8; 11.4 percent in 1,000r; and 4.1 percent in 2,500r treated beetles. The eggs of 5,000r irradiated beetles did not hatch at all (Table II). The beetles treated with 2,500r and 5,000r laid only 49 and 47 eggs respectively during the period they were under observation. Out of this only 2 eggs of 2,500r irradiated beetles hatched (Table II).

Usually the eggs of normal beetles take 5 to 6 days for hatching. However, in the present study the eggs were observed for eclosion till the 12th day of their oviposition.

Mortality in the control was 39.7 percent in 10 days in the second trial (Table III) whereas it was 34.4 percent in the first trial in 15 days (Table I). Mortality of 1,000r irradiated beetles was 47.1 percent in 15 days in the first trial and 70.8 percent in the second trial. The 5,000r treated beetles had

a mortality of 97 percent in 15 days in the first trial and 92 percent in 10 days in the second trial (Table I and III).

In general, mortality in the second trial was higher than in the first trial (Table I and III). This may be due to the fact that the insects used in the second trial were older than the insects used in the first trial. The insects used in the second trial were collected from the field on June 9, 1963, and remained in the cooler from July 23, 1963 to February 7, 1964, whereas the insects used in the first trial were collected from the field on July 26, 1963 and given cold treatment from August 6, 1963 to October 14, 1963. This difference in the pretreatment may also account for the disparity and higher mortality in the second trial.

The total number of eggs laid by control and 500r treated beetles were 1,974 and 1,253 respectively from February 11 to February 21. However, only 37.6 percent of eggs from control and 9.6 percent of eggs from 500r treated beetles hatched (Table V). It appears from the data obtained in this experiment that as low a dosage of radiation as 500r affected oviposition to some extent, and eclosion considerably (Table V). 1,000r irradiated beetles in the second trial also oviposited fewer eggs than the control. Out of the 90 eggs oviposited by 1,000r treated beetles, only 15 hatched in the second trial, giving percent eclosion of 16.6. Though the total percent eclosion in 1,000r treated beetles in the second trial appeared to be higher than 500r treated ones the total number of eggs laid by 500r irradiated beetles was much higher than the 1,000r irradiated beetles (Table V).

Beetles irradiated with 3,000r laid only 5 eggs during the period they were under observation. Out of this 5, only 2 hatched, giving a percent eclosion of 40, though the total number of eggs oviposited was far less than the control and 500r treated beetles. 5,000r irradiated beetles in the second trial did not lay any eggs during the period they were under study (Table V). However, in the first trial a total number of 47 eggs was obtained in this treatment, out of which none hatched (Table II). The data obtained in the present study suggests that X-ray radiation over 7,500r brings total sterility as far as the female beetles are concerned. Beetles treated with 7,500r to 20,000r did not oviposit at all during the period they were under vigilant observation (Table II). The data obtained in the present study also indicates that 5,000r irradiation of the adult beetles caused total egg sterility in the few eggs oviposited (Table II).

HISTOPATHOLOGY OF THE IRRADIATED GONADS

Since there was considerable diminution in the fertility, of the irradiated beetles, it was thought worthwhile to investigate briefly the effect of X-ray radiation on the development of gonads.

Material and Methods. Beetles used in the experiment on oviposition effects of X-rays were sampled for this experiment. The beetles were removed from the cage at certain periods and fixed in KAAD and histological sections of 10 Microns thickness were made in the same manner as mentioned earlier. Feulgen and fast green were used for staining the histological preparations.

Results. It was observed that in beetles receiving higher dosage of radiation, e.g., 10,000r and 20,000r the development of ovary was at a standstill. The ovary was in the same stage as it was found in the diapausing beetle. Different stages of oocyte development were not at all noticed in the small samples (about 6 to 12 beetles per sample). Beetles receiving 5,000r also showed no development or a slow development of the ovary (Fig. 15). In the case of male beetles, the testes did not show immaturity or absence of spermatozoa. It is important to note that the males had fully matured spermatozoa at the time of irradiation.

In case of beetles sampled from the second irradiation trail, beetles receiving 500r showed oocytes in different

stages of development and also fully matured eggs (Fig. 16a). But frequently malformations and distortions in the oocytes were observed (Fig. 16b, 16c). These kinds of abnormalities were observed in beetles sampled 15 days after irradiation. In addition degeneration of the germarium was also noticed (Fig. 17). In some cases oocytes in different stages of development were noticed just below the degenerating germarium. Many cells in the degenerating germarium did not indicate any Feulgen positive material (Fig. 18). Instead, they took the counter-stain, fast green. Some cells in the germarium showed complete absence of the nucleus and in some cases only traces of Feulgen positive material was observed. Cells which showed traces of Feulgen positive material seemed to be in the process of degeneration. In some instances, the degeneration and disappearance of the nuclei were observed in patches in the germarium (Fig. 18). A larger number of partially developed oocytes were observed on the pedicel than in the control (Fig. 16a). In these cases the germarium above them was in varying degrees of degeneration. However, in some cases the development of the ovary was normal and no germarial degeneration or abnormal development of the oocyte was observed. In beetles receiving 1,000r, 3,000r and 5,000r degeneration of the germarium was also observed in some beetles along with a normal germarium in other beetles. Beetles receiving 3,000r and 5,000r X-rays showed frequently only a few stages in development and many did not show advanced stages of oocytes, particularly the 5,000r irradiated beetles. The development of the oocytes in these beetles appeared to be rather slow when compared with the control.

Male beetles, some which received higher dosages, e.g., 3,000r and 5,000r, showed some reduction in the amount of spermatozoa after 10 days of irradiation. It seems likely that after they exhaust the mature spermatozoa the production of new is affected. Some of the beetles which died after 3,000r and 5,000r radiation were also studied within few hours of their death. These histological preparations also indicated a reduction in the amount of mature sperm. However, it is not known whether it is due to radiation or exhaustion of the sperm supply. This study on histopathological effects of radiation is rather indicative more than conclusive because of the small number of insects sampled.

FIGURE 15

ARRESTED DEVELOPMENT, MALFORMATION AND DEGENERATION OF THE OVARIOLES OF OULEMA MELANOPA (L.) TREATED WITH 5,000r X-RAYS. 265x

FIGURE 16a

TWO PARTIALLY DEVELOPED OOCYTES AND ONE FULLY DEVELOPED OOCYTE ON THE PEDICEL OF THE OVARIOLES OF OULEMA MELANOPA (L.) TREATED WITH 500r X-RAYS. 265x

FIGURE 16b

MALFORMATION AND DISINTEGRATION OF A FOURTH STAGE OOCYTE OF OULEMA MELANOPA (L.) TREATED WITH 500r X-RAYS. 540x

FIGURE 16c

DISINTEGRATION OF YOLK IN A FOURTH STAGE OOCYTE OF OULEMA MELANOPA (L.) TREATED WITH 500r X-RAYS. 540x

FIGURE 17

DEGENERATING GERMARIUM AND A MALFORMED POST GERMARIUM OF A NEIGHBOURING OVARIOLE OF A 500r IRRADIATED OULEMA MELANOPA (L.) 540x.

FIGURE 18

DEGENERATION OF THE GERMARIUM OF A 5,000r IRRADIATED OULEMA MELANOPA (L.) SHOWING PROGRESSIVE LOSS OF NUCLEAR DNA. 1080x

FIG. 15

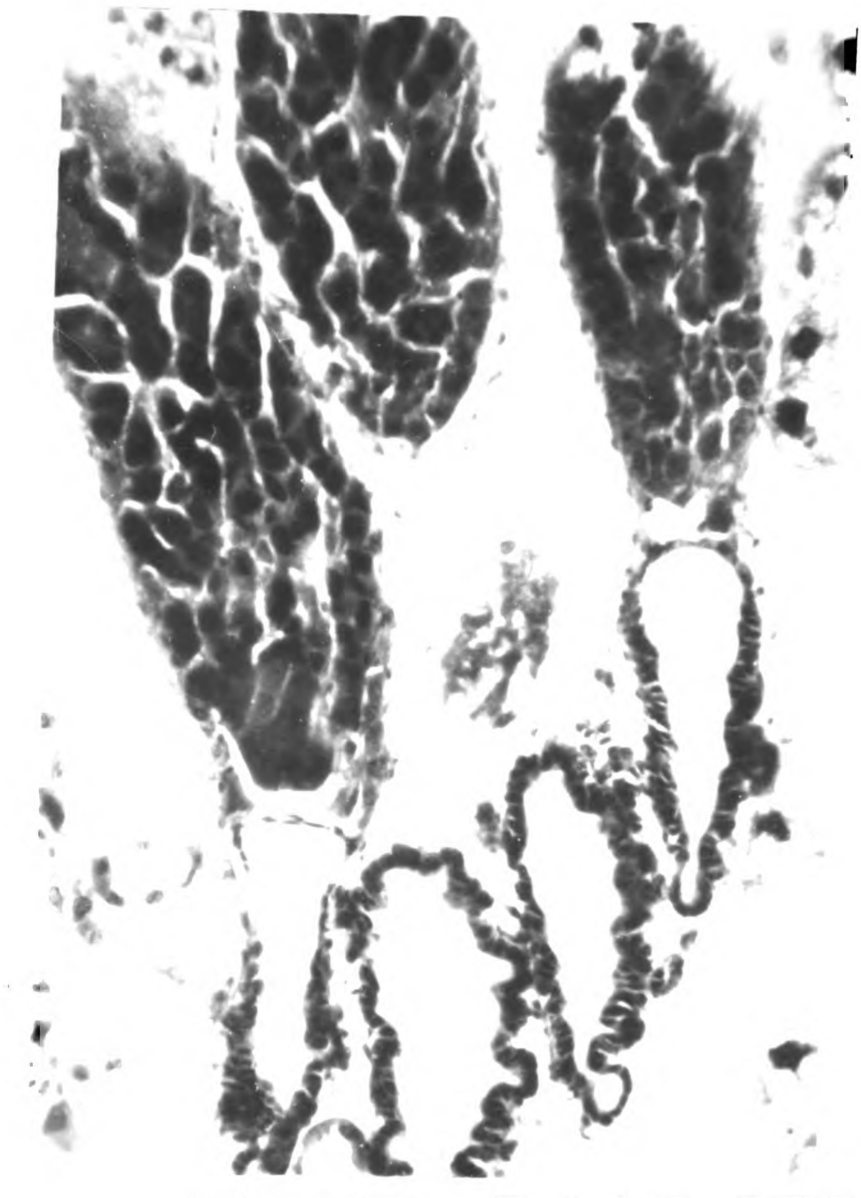


FIGURE 16 a

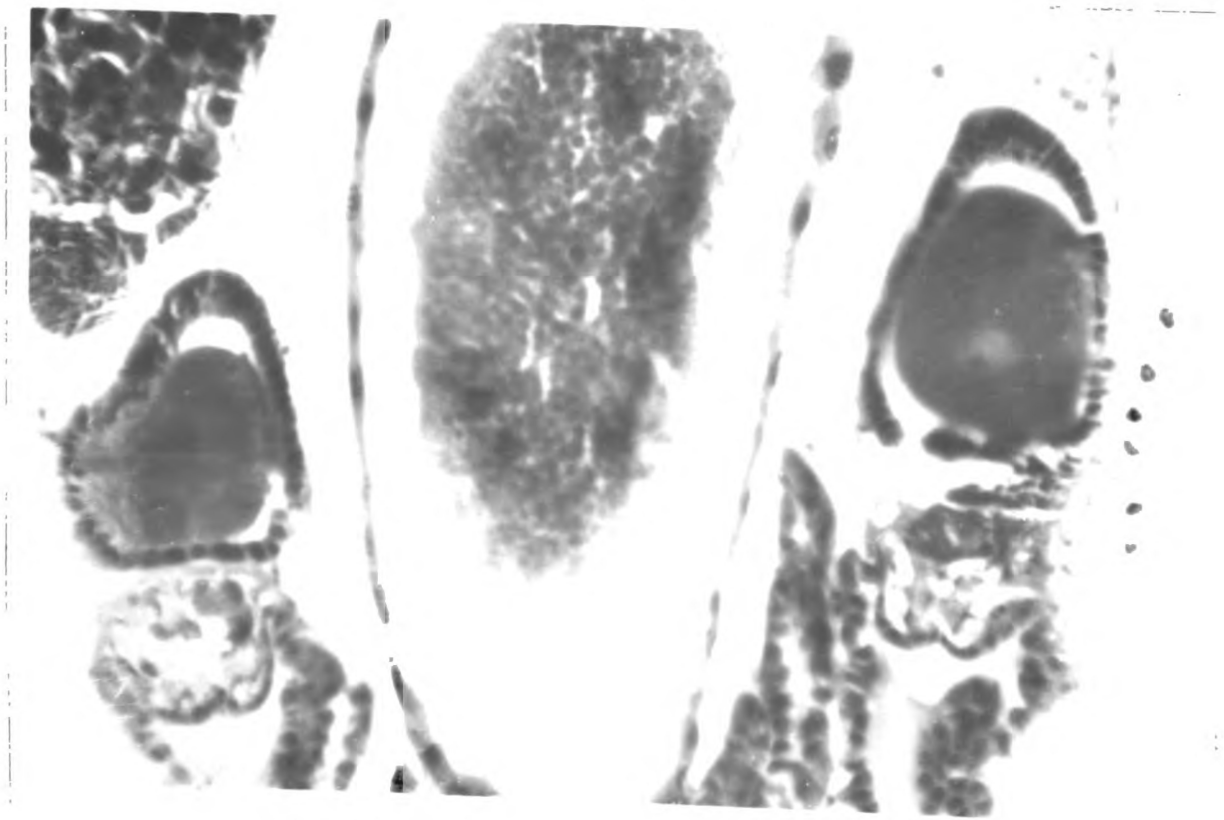
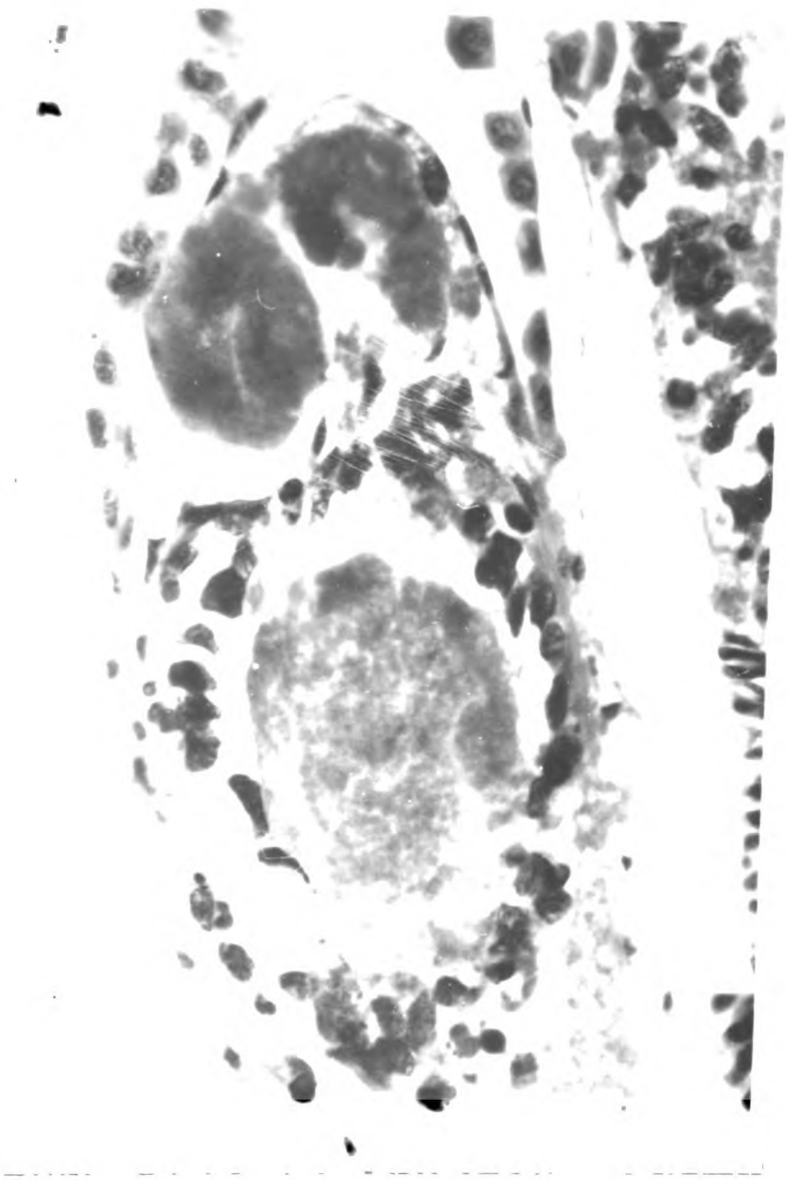
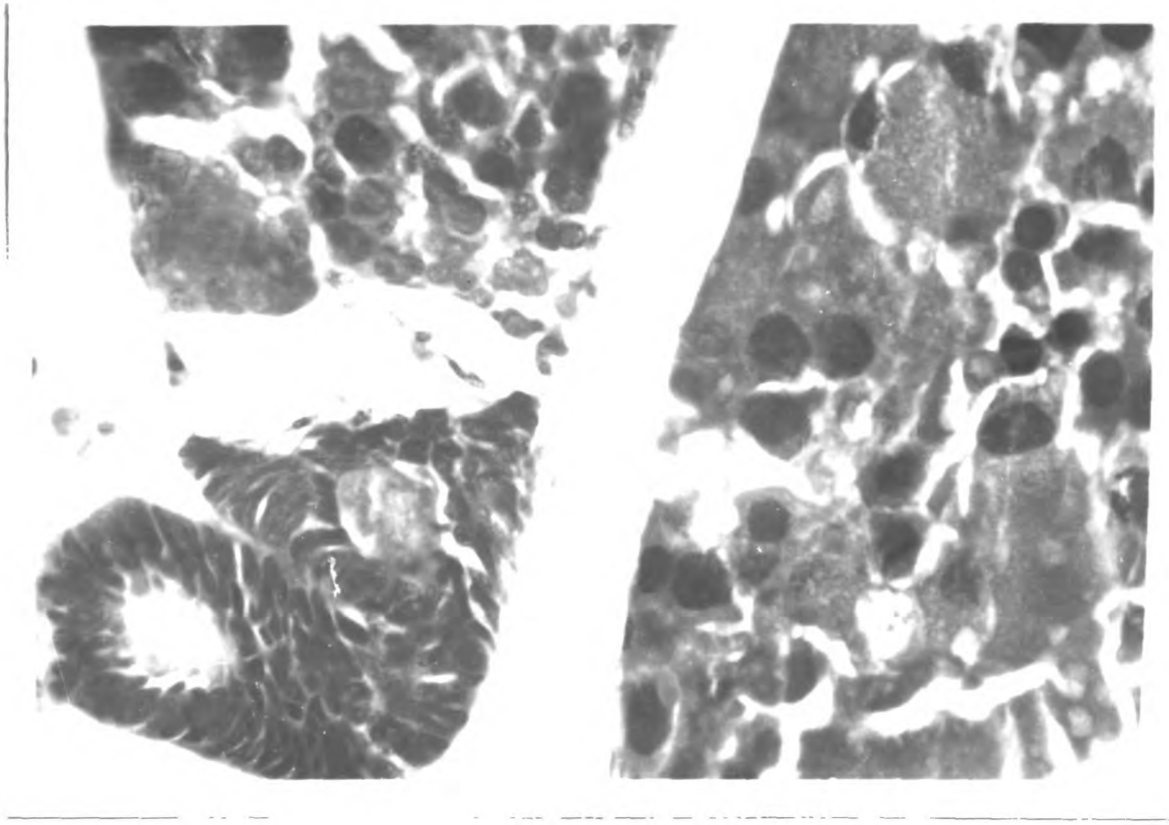
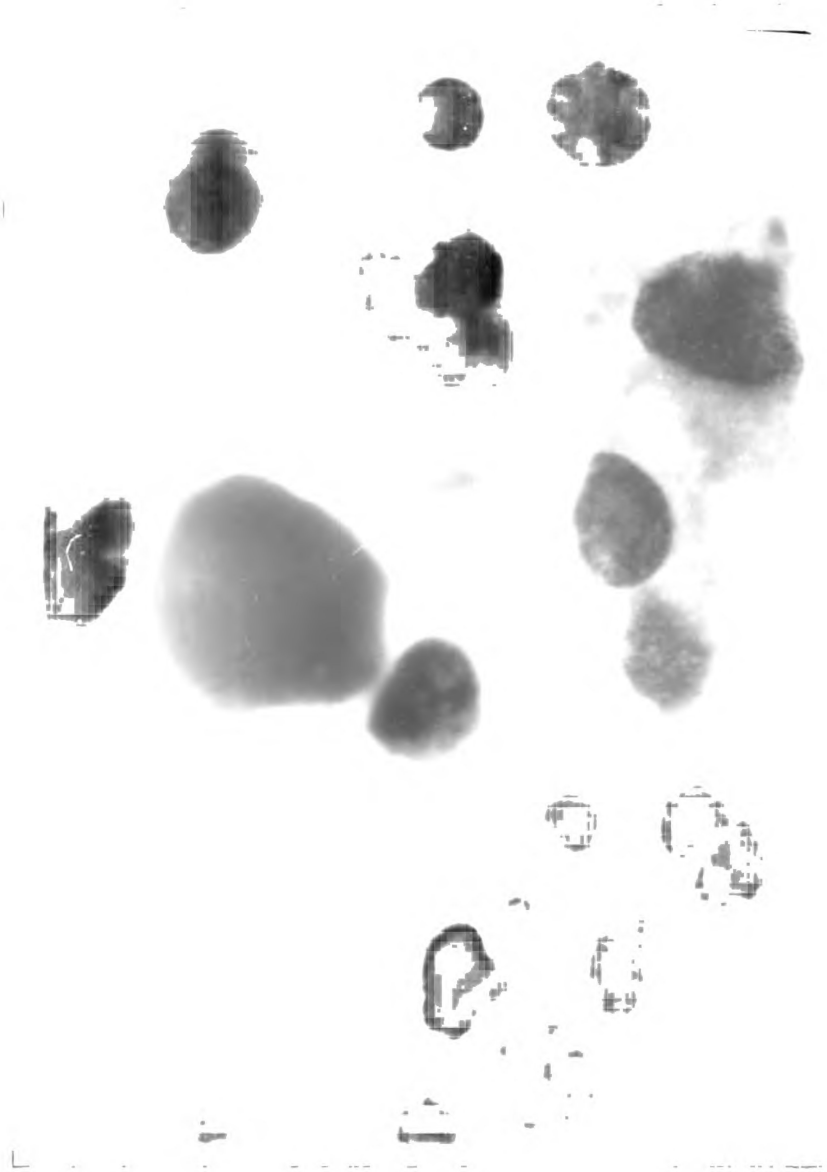


FIG. 16







DISCUSSION

It appears from this investigation that the females of the cereal leaf beetles attain sexual maturity later than the males. Histological preparations made of female beetles within 3-4 days of emergence, pre-diapause beetles and diapause beetles showed arrested development or immaturity of the ovary. Whereas many of the histological preparations made of males 3-4 days after emergence, pre-diapause and diapause beetles showed spermatocytes, spermatids and some mature spermatoza. The female beetles seem to require at least a short period for the full development of the ovary immediately after diapause. Most of the female beetles sectioned after they came out of diapause showed gradual development of the ovary after 2-3 days. Beetles sectioned in the pre-diapause, diapause, and post-diapause state showed bright staining DNA and RNA positive cells in the germarium with very little cytoplasm during the underdeveloped period of the ovary. Although nutritive cords, connecting the bright staining cells in the germarium with the developing oocytes, were never observed during this investigation, it seems quite likely that the bright staining cells in the germarium do perform some sort of nutritive function. The findings of Krause (1946) on Passalus, and Schlottman and Bonhag on Tenebrio molitor L. also support this view. Krause (1946) noted bright staining material at the tip of the ovariole of Passalus and the smaller developing egg cells possess plasmatic strands. However, he could not demonstrate direct and

definite protoplasmic connections between oocytes and the distal tip of the ovariole,but presented evidence that some sort of protoplasmic connection does exist. Schlottman and Bonhag (1956) noted definite protoplasmic strands unite the folliculate oocytes with the nutritive cells in the germarium and suggested that in contrast to the strongly developed cords usually found in the telotrophic ovarioles of hemipterous insects, the nourishing strands in Tenebrio molitor L. are of a much more delicate nature and may be quite easily overlooked. Wilde, et al. (1959) working on Colorado potato beetle also reported that they could not find cytoplasmic connections from the inconspicuous nutritive cells with the oocytes after being released into the vitellarium. In the light of these previous reports on other beetles and the present observation of apical and dense bright staining nature of the germarium, it is suggested that the ovarioles of cereal leaf beetle, Oulema melanopa (L.) are of acrotrophic type.

Although the oocytes grow and enlarge in the vitellarium, the presence of yolk is not clear until the oocytes reach the stage when the surrounding follicular cells become cuboidal in shape. This finding is quite in agreement with that of Schlottman and Bonhag (1956) on Tenebrio molitor L.

The presence of bright staining Feulgen positive (DNA) and RNA positive substances in the follicular cells, particularly in the later stages, suggests that the follicular epithelium in the cereal leaf beetle may also play at least some part in the nutritive function of the developing oocyte along with the DNA and RNA rich germarium. It may be probable that the

increasing nutritive demands of the developing oocytes require additional supply of this kind of material which is not solely met by the germarium during this period. This view is also supported by the fact that bright staining, large and cuboidal follicular cells rich in nucleic acids are present in the third and fourth stages of the oocytes. However, investigators in this field, particularly Schlottman and Bonhag (1956), attribute the alteration in the size and shape of follicular cells to changes in volume of the growing oocyte. According to them, as the oocyte within the follicle grows larger, it exerts an ever increasing pressure upon the cells of the follicular epithelium. This pressure is alleviated in the incipient follicles chiefly by cellular multiplication and growth of the daughter cells. Since they noted that the mitotic ability is apparently lost by the epithelial cells of the more advanced follicles, they concluded that accomodation to changes in volume of the oocyte in Tenebrio molitor L. occurs through an alteration in the size and shape of the follicular cell. The oocyte growth and continuous change in shape of the surrounding follicular epithelial cells by the gradual transition of the columnar epithelium into a cuboidal condition and finally into a squamous type was also observed in the present study on the ovary of cereal leaf beetles. However, Schlottman and Bonhag (1956) agree that there is evidence that at least in some telotrophic ovarioles nutritive substances are passed from both the follicular epithelium and the apical trophocytes to the developing oocytes. Also their cytological observations indicated that nucleic acids (DNA and RNA) or their derivatives

may be contributed to the enlarging oocytes by the follicular epithelium. The observation of bright staining DNA and RNA positive follicular epithelium, particularly in the later stages of the oocytes of the cereal leaf beetle also supports the view of Wilde, et al. (1959) and Schlottman and Bonhag (1956) that follicular epithelium also makes a nutritive contribution to the developing oocytes in some telotrophic ovarioles.

The present study on the effects of radiation on the reproductive aspects of Oulema melanopa (L.) was designed to form the basis of experiments more directly related to the practical problem of controlling this insect by the sterile-male technique. Radiation, even as low as 500r, appeared to affect the normal development of the ovary of a fair number of susceptible adult female beetles used in this experiment. However, radiation up to 20,000r did not seem to affect much the motility of the spermatozoa particularly in the first few days after radiation. This conclusion is supported by the fact that in some of the 20,000r treated beetles, the females sectioned after about two weeks showed spermatozoa in the seminal receptacle. At this point, it is important to point out that dominant lethality does not mean the cessation of the motility of the sperm. "Dominant lethality is the change caused by radiation in genetic constitution of a sperm which may not prevent fertilization of an egg, but may make the zygote incapable of developing to maturity, usually causing death in the embryonic stage" (Bushland and Hopkins, 1953).

The histological preparations made from the irradiated females showed lack of development of the ovary above a radiation dosage of 5,000r. Many of the 3,000r and 5,000r treated females also showed a kind of inhibition of the development of the ovary. Oviposition was drastically reduced in beetles treated with X-rays 2,500r and over, and mortality was greatly increased in beetles receiving over 2,500r radiation. In general, the cereal leaf beetle appeared to be radio sensitive above the dosage of 2,500r.

The sterile-male method may not be feasible with certain species of insect because of the damaging effects of radiation in the vigor of the insect. As LaChance and Knipling (1962) pointed out, one of the most important requirements that must be met in applying the sterile-male technique successfully is that the sterilized males must not be seriously affected by the sterilization procedures so that they will be reasonably competitive with normal males. The present study showed that in general radiation increased mortality, particularly the dosages over 2,500r. This finding is contrary to one of the important requirements of sterile-male technique of controlling insects. Secondly, the dosage of 2,500r and 3,000r appeared to allow some eggs to develop in these treatments and also seemed to affect the normal development of the ovary in many. Higher dosages of radiation also appeared to affect the feeding habits, particularly 10,000r-20,000r. Since at 5,000r treatment, the females oviposited a few eggs out of which none hatched, it is probable that 100 percent dominant lethals in male is induced at this level of radiation. As the irradiation

of the beetles was done without sexing, it is not definitely known whether the failure of egg hatch is due only to dominant lethals in the spermatozoa or some other defects caused by radiation in the females. However, the confirmation of 5,000r inducing dominant lethals in males is not possible unless sexing of the beetle is done. Sexing the beetles is highly essential becuase the definite, conclusive evidence can be obtained only when the eggs obtained from normal females mated with the 5,000r treated males do not hatch. Even if it is confirmed that 5,000r induces total dominant lethals in male, the likelihood of using 5,000r for sterilizing the male is also precluded by the fact that this dosage causes a mortality of over 90 percent as evidenced by both the first and the second trial.

Female beetles receiving above the dosage of 5,000r, did not lay any eggs during the period they were under study. Histological preparations made from these beetles showed lack of development of the ovary comparable to the immature state of the ovary. It appeared that the development was very much inhibited by radiation. In the case of adult males, histological preparations from testes showed all stages of spermatogenesis, from the early stage to spermatozoa, before they were irradiated. So it was not possible to find out whether the production of spermatozoa was affected by radiation or not. However, there was a little indication that when the supply of mature spermatozoa was exhausted, the production of spermatozoa may be affected by high dosage radiation.

The ovaries did not have well developed oocytes at the time of radiation; they had only the bright staining cells in

the germarium and the very young, prefollicular oocytes. These prefollicular oocytes appeared to be more sensitive to radiation above the dosage of 5,000r and this factor was probably more responsible for the absence of oviposition in beetles treated with X-rays over 5,000r. Considering the fact that the males become sexually mature quite soon after emergence from pupal stage, it may be feasible to use radiation to induce dominant lethals in either larval or pupal stage to a better advantage. Further work is necessary on this line. However, the practical problem of obtaining required number of pupae and handling them also exists as this beetle pupates in the soil.

In the larval or pupal stage the gonads may consist of primordial or younger germ cells at the time of irradiation. It may be possible to induce dominant lethals effectively in a very early stage of spermatogenesis. This view is also supported by the results obtained by Bushland and Hopkins (1951) on screw worm flies in that it is most efficient to irradiate the pupal stage.

Histological preparations of irradiated beetles showed that irradiation at levels of 7,500r and over, interferred with the ability of the ovary to undergo further growth. Beetles receiving 500r irradiation showed oocytes in different stages of development and fully matured eggs. But frequently degeneration of the germarium, malformation and distortion of the developing oocytes were observed in beetles sampled 15 days after irradiation. At 3,000r and 5,000r, these abnormal conditions were also noticed, but oocyte development was far less, and advanced stages of oocytes were noticed only in few cases.

Mostly a degeneration of the germarium occured. Many such similar conditions were noted by LaChance and Bruns (1963) working on oogenesis and radiosensitivity in Cochliomyia hominivorax (Coq.). King (1957) working on Drosophila melanogaster irradiated with gamma rays also reported damaged oocytes undergoing pycnotic degeneration and ascribed many surviving oocytes showing abnormalities to radiation induced disturbances of cell growth, division, migration and differentiation. The main kind of abnormalities noticed in the present study were germarial degeneration and abnormal development and malformation of the oocytes. In rare instances disintegration of the oocytes were also noticed. The observation on the degenerating germarium indicated that the Feulgen positive material was gradually lost in many cells in the germarium, thus forming patches of this kind of degenerating cells. This typical condition was never noticed in the control. Only very rarely a pycnotic nucleus was observed in the control. Schlottman and Bonhag (1956) working on the histology of the ovary of Tenebrio molitor L. also noted instances of cellular degeneration and pycnotic nuclei which they attributed to pathological conditions brought about by the action of certain protozoan parasites. However, no parasite was noticed in the degenerating germarium of the ovary of the irradiated beetles or the normal germarium of the control. Just below the degenerating germarium oocytes with abnormal development were noticed in beetles receiving lower dosage of radiation. In cases where the germarium was drastically damaged, the development of the oocytes appeared to be in a standstill and no signs of oviposition having

occured were observed. However, both normal germarium and normal development of the oocyte and degenerated germarium and malformed oocytes were observed side by side in the ovarioles of the same ovary of the 500r treated beetles.

SUMMARY

Studies on the normal development of gonads, some histochemical aspects of the ovary, radiation response, and histopathological effects of radiation on the adult cereal leaf beetle, Oulema melanopa (L.) were undertaken. Among a number of conclusions advanced in the present work the following merit brief summary here.

The total number of ovarioles in the cereal leaf beetle appears to be 14, 7 in each of the paired ovaries. Evidence to support the acrotrophic nature of the ovariole in this beetle is presented. The germarium and the latter stages of the follicular epithelium are rich in nucleic acids and appear to provide nutrition to the developing oocytes. Male beetles attain sexual maturity earlier than females. The ovaries of most of the diapausing females are immature and development of oocytes begin after cessation of diapause.

Radiation in general increases the mortality of this beetle and radiation dosage of 5,000r and above causes over 90 percent mortality in two weeks. Motility of the sperm is not affected by 20,000r radiation. Oviposition is drastically reduced above a dosage of 2,500r and 5,000r radiation causes total egg sterility. Radiation treatment of 7,500r and above appears to cause considerable inhibition in the development of the ovary affecting oviposition. 5,000r radiation is indicative rather than conclusive of causing dominant lethals in male

spermatozoa. Radiation as low as 500r causes degeneration of germarium, malformation and abnormal development of oocytes in the more susceptible beetles. The main histopathological effect of radiation seems to be on the DNA content of the nuclei of the germarial cells.

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