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Effects of Metepa on Gametogenesis and Embryogenesis in the Large Milkweed Bug Oncopeltus Fasciatus (Dallas)

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Donald E. Lawson
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Effects of Metepa on Gametogenesis and Embryogenesis in the Large Milkweed Bug, *Oncopeltus fasciatus* (Dallas)

Donald E. Lawson and Harold J. Ball

**SUMMARY**

Applications of an alkylating agent, metepa (tris [1-(2 methyl) aziridinyl] phosphine oxide), were made to the large milkweed bug (*Oncopeltus fasciatus*) at various rates and under several conditions. Data were obtained with regard to certain responses to treatment:

*Hatch of eggs* from treated females or hatch of eggs from untreated females mated to treated males.

*Number of eggs* laid per female per day.

*Mortality* of treated insects.

Histological sections were taken from the reproductive tissues of both treated and untreated insects and from eggs obtained from both treated and untreated sources.

Metepa treatment of male or female *Oncopeltus* is effective in reducing the percent hatch of eggs from such sources. There is some indication that sperm vigor may be affected to a degree dependent on dosage. The degree of response in terms of reduction of percent egg hatch is dependent on the dosage of metepa applied and the relationship is directly proportional.

Metepa is preferential in its choice of reactive sites. The most preferred site appears to be the nuclear components of mature sperm and oocytes.

Successively less favored targets are attacked to the degree that metepa molecules are available for reaction. Somatic tissues are affected only when the amount of available metepa exceeds the reactive potential of the reproductive tissues.

Examination of histological sections shows that sterilization of mature gametes can be accomplished without histologically visible effects. However, dosages which just sterilize mature sperm or oocytes do not greatly affect germinal tissue and these unaffected tissues can subsequently produce viable gametes.

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1 Instructor in Entomology and Professor of Entomology, respectively.
Dosage rates which do affect future viability of gametes produce histologically visible effects in the form of chromosomal abnormalities and necrosis of tissues.

The effects of metepa treatment on the gametes is ultimately expressed in the egg in different ways. In general the higher dosages cause dominant lethal mutations and subsequent death of the zygote at an early stage. The lower dosage rates may produce a variety of responses:

- **Zygote death** prior to cleavage of the fusion nucleus or death in early cleavage.
- **Death** subsequent to formation of a definitive embryo but prior to hatch.
- **Survival** of the embryo through eclosion and subsequent nymphal forms to the imago with delays in developmental events.

The basic mechanism whereby metepa exerts its effects was not determined in this study. However, this investigation did show that the nuclear components of the reproductive systems are the favored target for alkylation by metepa.

It is likely that the effects of alkylation were in the form of translocations or losses of small sections of chromosomes. This is indicated by the fact that chromosomal DNA, which is affected by metepa, is able to replicate but may not transmit the correct information for proper embryonic development.

**INTRODUCTION**

Considerable research is currently being directed toward discovering means of controlling insects other than by applying insecticides.

There are several promising concepts of control which minimize the use of insecticides and one of these is the use of sterilizing techniques. Basically, such techniques involve the use of an agent to sterilize the male insect prior to release into the environment or the use of agents in baits or attractants to be contacted by the wild population already in the environment.

The efficacy of such measures depends on the degree to which sterile males compete with normal males both in numbers and mating vigor.

The most notable successes have been achieved by sterilizing large numbers of male insects with gamma irradiation and releasing them in quarantined or isolated situations to compete with wild males.

Promising results have also been attained by exposing wild insect populations to chemosterilants where it was possible to present them in a bait form which was highly attractive to the subject insects.

The sterile-male release method has been the most effective, but its success depends on rather specific circumstances of isolation and quarantine which are difficult to attain with most insects.
The method requires the rearing of very large numbers of male insects, a difficult undertaking for most economically important species, especially when conducted on a large scale.

In view of these and other considerations, some emphasis is currently being placed on the development of chemosterilant techniques. Many of the studies have been concerned with the screening of potentially active chemicals, whereas other research has dealt with the efficacy of field application. The more basic work has been accomplished through the participation of several disciplines.

The purpose of this study was to characterize the effects and mode of action of a particular chemosterilant, metepa (tris [1-(2 methyl) aziridinyl] phosphine oxide) on the large milkweed bug, *Oncopeltus fasciatus* (Dallas).

Attention was given to determination of the locus of attack and the relationship of dosage to effects on tissue from both embryonic and reproductive sources. The effects of dosage level on egg viability and female fecundity were also studied.

**LITERATURE REVIEW**

The development of sterilizing agents which inhibit or prevent normal reproduction in insects parallels closely the development of cancer therapeutic agents. The requirements for a mode of action are similar in both cases. The cells of neoplastic tissue are similar to germinative cells of the reproductive organs, in that the rate of cell division is greater than that ordinarily found in somatic tissue.

The criterion for judging the efficiency of a compound for either purpose would be based on the degree of interference as contrasted to or with proper cellular division.

The most successful groups of compounds for both purposes are the alkylating agents and antimetabolites.

The earliest reference to the unique biological properties of materials subsequently used as anti-cancer agents was made by Paul Ehrlich (1898) who reported the action of ethylenimine and ethylene oxide on animal tissue. These two compounds are closely related alkylating agents and possess biological activity similar to other chemicals of this group.

A monograph published in 1958 by the New York Academy of Sciences presents a comprehensive picture of research accomplished up to that time on the clinical and biological effects of alkylating agents.

Schmidt (1958) observed that about 45 years had elapsed before interest was shown in the biological properties of alkylating agents as first described by Ehrlich. Following the onset of World War II, considerable effort was placed on studying the effects of nitrogen mustard derivatives on biological systems.
These efforts were directed primarily towards possible usage in chemical warfare, although other aspects of these compounds were also investigated.

In 1942, Goodman and Gilman noted the effects of alkylating chemicals on lymphoid tissue and rapidly dividing cells as subsequently reported by Gilman and Philips (1946). These latter authors pointed out that a large family of related chemicals remained to be evaluated for their effects on proliferative cells. Subsequently such evaluations were made, and many new compounds were synthesized for this purpose.

The phenomenon of induced insect sterilization was first reported by Runner (1916) who observed that cigarette beetles, Lasioderma serricorne (Fabricius) produced infertile eggs after exposure to roentgen rays. Later, Muller (1927) noted that mutations could be induced in Drosophila melanogaster through exposure to radiation.

In 1938, Knipling proposed the idea of introducing sterile males into a natural population of screwworm flies to achieve control. After several years of preliminary research, such a program was initiated by Baumhover (1954) on the island of Curacao. The program was remarkably successful and resulted in eradication of the screwworm fly on the island.

The details of this venture have been well described by Lindquist (1959) and Knipling (1960).

The success of this method on Curacao and later in a number of our southern states gave rise to interest in the use of chemicals to induce insect sterility. In 1959, Knipling advanced a theoretical model involving the use of chemical sterilizing agents in a natural population. At that time, investigations were being carried out to determine what chemicals could be used for sterilization.

In 1960 and 1961, La Brecque, et al. reported that some alkylating agents (aziridinyl derivatives) induced sterility in house flies. The damage done by these chemicals to the affected tissue was of a type similar to that caused by radiation, thus giving rise to the designation of the aziridinyls as "radiomimetic" compounds.

Other types of compounds were also investigated by La Brecque and all were grouped under the generic term "chemosterilant."

After structure-activity relationships became known, many compounds were assayed to determine their value as chemosterilants. Hundreds were found to have some degree of sterilizing activity, although relatively few were really promising.

Chemosterilants can be grouped into three different categories according to their mode of action:

- Antimetabolites.
- Miscellaneous compounds.
- Biological alkylating agents.
The antimetabolites are primarily female sterilants, although some will also sterilize males. Antimetabolites act by blocking synthesis or activity of nucleic acids.

The miscellaneous compounds form an uncertain group which includes several very promising chemicals.

The most important seem to be a series of structurally related amides. The amides possess good sterilizing properties but are not alkylating chemicals.

Börkovec (1964) reported that these compounds apparently did not have mutagenic properties. However, subsequent investigation by Palmquist and LaChance (1965) showed that they do possess mutagenic qualities and thus must be considered as hazardous as the alkylating agents insofar as handling is concerned.

Also, they have longer residual properties which could present problems in field dissemination.

The term "alkylating agent" denotes a compound that is capable of replacing a hydrogen atom with an alkyl radical.

The first agent thoroughly studied for its alkylating effects was nitrogen mustard. The activity of nitrogen mustard depends on the splitting off of a chlorine atom in solution, leaving a positively reacting intermediate. The action of the other mustards is the same and theoretically all react readily with carboxyl groups of proteins and phosphate groups of nucleic acids. The general scheme for the reaction is shown in Figure 1.

Many other chemicals having alkylating properties were tested and all were found to be similarly reactive. Since these chemicals were alike in no other respects, their action was concluded to be due to their alkylating abilities in biological systems. These substances include:

*Epoxides.*
*Ethylenimines.*
*Methane sulfonates.*

---

![Figure 1. General reaction scheme of mustards with proteins and nucleic acids.](image-url)
As previously mentioned, the ethylenimines and epoxides had already been cited by Paul Ehrlich for their unusual properties. Subsequent investigations of these compounds and their many derivatives by Ross (1958), Stacey, et al. (1958), Alexander (1960) and others have shown them to be the most reactive of the known alkylating agents.

An important property of alkylating agents is the number of reactive groups contained in each molecule. The original materials tested (the mustards) have two reactive groups and such compounds proved to be much more active than compounds with only one reactive group.

The alkylating agents therefore are described as mono-functional, bi-functional or poly-functional according to the number of reacting groups.

The general alkylating scheme is shown in Figure 2 (Ross, 1958). Other data by Ross, as presented in Figures 3, 4 and 5, indicate that the radiomimetic action of the aziridines might be explained by the reactions with the available groups of the nucleic acids. Since there are many reactive sites, it would seem possible that the attachment of an alkylating agent to a nucleic acid molecule would effect its ability to properly function as genetic material.

The status of knowledge on the use of ionizing radiation and chemicals for the induction of dominant lethal mutations in insects was reviewed by LaChance (1967), who pointed out that sterility in an insect may occur for a variety of reasons.

The phenomena of aspermia and sperm inactivation are common in males; infecundity and inability to mate take place in females, whereas dominant lethal mutations occur in both sexes.

Any of these conditions can be induced by both ionizing radiation and chemicals, and probably a combination of both results under some treatment conditions.

On the basis of earlier work (LaChance and Riemann, 1964) it was thought that the most important causative factor for sterility in insects was the induction of dominant lethal mutations.
### Proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>pKa</th>
<th>Fraction of groups in reactive form</th>
</tr>
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<tbody>
<tr>
<td>a-carboxyl</td>
<td>3.0–3.2</td>
<td>0.9999</td>
</tr>
<tr>
<td>Carboxyl (aspartyl)</td>
<td>3.0–4.7</td>
<td>0.9999–0.999</td>
</tr>
<tr>
<td>Carboxyl (glutamyl)</td>
<td>4.4</td>
<td>0.999</td>
</tr>
<tr>
<td>Phenolic hydroxyl (tyr)</td>
<td>10.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Sulfhydryl (terminal cyst)</td>
<td>7.9–8.5</td>
<td>0.100–0.060</td>
</tr>
<tr>
<td>Sulfhydryl (non-term cyst)</td>
<td>10.8</td>
<td>5 x 10⁻⁴</td>
</tr>
<tr>
<td>Imidazolium (histidine)</td>
<td>5.6–7.0</td>
<td>0.990–0.76</td>
</tr>
<tr>
<td>a-ammonium</td>
<td>7.6–8.4</td>
<td>0.440–0.110</td>
</tr>
<tr>
<td>e-ammonium (lysine)</td>
<td>9.4–10.6</td>
<td>10⁻²–10⁻³</td>
</tr>
<tr>
<td>Guanidinium (arginine)</td>
<td>11.6–12.6</td>
<td>10⁻⁴–10⁻⁵</td>
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</table>

### Nucleic acids

<table>
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<tr>
<th>Group</th>
<th>pKa</th>
<th>Fraction of groups in reactive form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary phosphoryl</td>
<td>2.0</td>
<td>0.9999</td>
</tr>
<tr>
<td>Secondary phosphoryl</td>
<td>6.0</td>
<td>0.960</td>
</tr>
<tr>
<td>Aromatic hydroxyl (uracil, thymine)</td>
<td>10.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Aromatic hydroxyl (guanine)</td>
<td>10.1</td>
<td>0.0025</td>
</tr>
<tr>
<td>Sugar hydroxyl</td>
<td>13.0</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>Aromatic amino (guanine)</td>
<td>2.3</td>
<td>0.9999</td>
</tr>
<tr>
<td>Aromatic amino (adenine, cytosine)</td>
<td>3.7–4.2</td>
<td>0.999</td>
</tr>
</tbody>
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### Figure 3. pKa of reactive groups of proteins and nucleic acids at pH 7.5.

Embryos of the screwworm fly were studied following treatment of oocytes and sperm with ionizing radiation and tretamine [2,4,6-tris (1-aziridinyl)-s-triazine]. The effects of irradiation were observed in oocytes during the first two meiotic divisions in the form of chromosome bridges and fragments of chromosomes.

The effects of tretamine were not obvious until the first cleavage divisions occurred or after the treated nuclei had undergone one replication of chromatin material.

The induction of dominant lethal mutations and the resulting death of the embryo as a consequence of chromosomal breakage and bridge formation has been generally accepted as the activity pattern for ionizing radiation. However, the pathway for the induction of dominant lethal mutations produced by chemicals is a matter of some conjecture. As was pointed out earlier, some workers felt that cross-linking of DNA molecules did occur and that this phenomenon led to alteration of the genetic complement.

Hedin, et al. (1967) working with the boll weevil, observed cytological alterations in testicular tissue following treatment of males with tepa [tris (1-aziridinyl) phosphine oxide].

Clumping of chromatin, anaphase bridges, fragmentation and
Figure 4. Hypothetical protein showing possible reactive groups (after Ross, 1958).

Figure 5. Section of a nucleic acid molecule showing possible reactive sites (after Ross, 1958).
general tissue necrosis occurred in most cases. Abnormalities in the conformation of mitotic figures were commonly noted.

Hedin's work compares strikingly with similar observations made by Riemann (1967) concerning the effects of ionizing radiation on the testicular tissue of the screwworm fly. The descriptions of damage were similar to those of Hedin, *et al.* (1967) with regard to the appearance of chromosome bridges and chromatin clumping. Hedin did not note chromosomal breaks or fragmentation as previously reported by Riemann (1967).

Recent work by Mendoza and Peters (1968) demonstrated some of the effects of an aziridinyl alkylating agent, apholate \([2,2,4,4,6,6,\text{-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexa-hydro-1,3,5,2,4,6-triaza-triphosphorine}]\) on the reproductive organs of the southern corn root worm (*Diabrotica undecimpunctata howardii*).

The effects of apholate on alkaline phosphatase activity were investigated particularly because of the relationship of alkaline phosphatase to DNA synthesis. Although apholate injections to adult insects caused a decrease in alkaline phosphatase, and at high levels sperm inactivation and limited mortality, no mention was made as to whether treatment with apholate resulted in sterility.

However, the unpublished portion of Mendoza’s work (Mendoza PhD thesis, 1964) indicated that a degree of sterility was attained even at treatment levels not causing sperm inactivation. Sterility was complete at higher levels.

Several authors have reported that atrophy of testicular and ovarian tissue occurred when relatively high treatment levels of chemosterilants were used (Riemann, 1967; Hedin, *et al.* 1967; Mendoza and Peters, 1968; Smittle, *et al.*, 1966).

Effects on other tissues were not reported but almost all workers reported increased mortality when insects were exposed to high chemosterilant levels.

The use of chemosterilants for insect control on a practical basis has been attempted for a number of insect species. Both the sterile male release method and treatment of natural populations have been tested.

The first field control experiments with insect chemosterilants were carried out in 1961 by LaBrecque, *et al.*, (1962) against the house fly.

An isolated refuse dump in the Florida Keys was baited with corn meal treated with tepa. Several days later, measurements were made of the overall fly population and percent hatch of eggs laid. Observations indicated that both the fly population and percent of egg hatch were markedly reduced.

During the following year LaBrecque, *et al.* (1963) carried out a similar experiment at a poultry farm.

Granular corn meal, granulated sugar and vermiculite containing
0.5 percent metepa were used as baits in this test. The corn meal bait reduced egg hatch to less than 10 percent of normal but the other baits were less satisfactory.

During 1962 and 1963, the chemosterilant-bait experiments were expanded and treatments were made on three islands in the West Indies to ascertain whether it would be possible to eradicate house flies in an isolated situation by the use of chemosterilant baits (Meifert, et al. 1967a). Eradication was not accomplished for several reasons but a good rate of control, ranging from 50 to 90 percent, was achieved.

In 1962 and 1963 large scale releases of tepa-sterilized male Mexican fruit flies (*Anastrepha ludens*) were carried out along the Mexican border near Tijuana (Steiner, 1965) and in mango groves in the interior of Mexico (Shaw, 1965). These releases resulted in a fairly good degree of control, especially in the border area where the treatments were credited with suppression of a potentially serious infestation.

Sterile-male release experiments with apholate-treated fruit flies (*Drosophila melanogaster*) were initiated in 1961 and 1962 in tomato plots by Mason, et al. (1968).

Suppression of fly numbers was achieved in these plots during the period of time the releases were made. The success of these experiments led to further tests in tomato plots in 1963 and 1964 (Mason and Smith, 1968).

Apholate-treated baits were used in this investigation in an attempt to induce sterility into the natural population. A maximum of about 63 percent suppression was obtained as compared with a 93 percent control obtained with Diazinon granules.

A small field experiment was conducted in 1962 by Davich for the purpose of controlling the boll weevil by the release of apholate-sterilized males. This venture produced promising results and similar experiments were subsequently carried out in 1964 (Davich, et al. 1967) on a much larger scale.

Apholate-sterilized males were released in nine cotton fields and a definite reduction in boll weevil damage resulted. Eradication was not accomplished in any of the fields but some control was achieved.

The effectiveness of this experiment was reduced because of mortality and reduced competitiveness among chemosterilant-treated males.

In addition to the papers cited above, there have been a number of other investigations regarding the effects of chemical sterilizing agents on reproduction in insects.

Most of this work has been concerned with determining the degree and form of sterilizing activity produced by various chemicals tested on several different insects. The results of these investigations have been adequately reviewed by Borkevec (1966).

More recently, additional work has been carried out on several aspects of the chemosterilization technique. Considerable effort has

The screening investigations were carried out in USDA facilities for the most part. Three species of flies were used for nearly all this work: house flies, Mexican fruit flies and screwworm flies. About 2,000 chemicals have been tested and the results of these efforts have helped formulate a more rational method of selecting candidate chemicals.


Such work was concerned primarily with the gross effects on reproductive tissues and the subsequent manifestations of such effects in reductions in numbers of progeny. Various degrees of damage to reproductive and somatic tissues were reported in several different insects.

Investigations concerning the physiology and chemistry of chemosterilants and their action on insect tissues have been reported by Chang, et al. 1966a, 1967, Chang and Borkovec 1966b, Hedin, et al. 1967b, Kido and Stafford, 1966, and North, 1967. The results of these studies have been of great value in establishing relationships between the chemical structure of the chemosterilants and their activity in biological systems.


For the most part, these investigations have extended the results of the chemical screening tests to additional species of insects. Considerable contribution has been made by these workers in helping to establish groupings of insects in relation to their response to certain types of chemosterilants.

**MATERIALS AND METHODS**

**Culture Methods**

The milkweed bugs (*Oncopeltus fasciatus* Dallas) used in this study were from a culture which has been continuously maintained
for over 16 years in the University of Nebraska Insectary. The insects, therefore, were quite homogenous in their growth characteristics, size and other attributes.

All individual insects used in this series of experiments were reared under identical conditions so that variations in growth rate, nutrition or other factors would be minimized.

Stock cultures of insects were maintained in wide-mouth quart jars with the opening covered with 80 grade cheese cloth held in place with a rubber band.

The cultures were maintained on a diet of milkweed seed which was provided in small packets made by stapling plastic screen around a thin layer of seeds. This method prevented waste of seed, since it kept the seed away from the bottom of the jar where it could become encrusted with fecal waste. Distilled water was provided in 125 ml. bottles containing a number 3, six-inch dental wick from which the insects could obtain water as necessary.

The stock cultures were kept in a rearing room at about 27° C. Eggs were laid in clusters on the outside surface of the cheesecloth from where they were collected by simply scraping them off with a spatula. Eggs used to provide a continuing supply of test insects were always of a known age so that the resulting adults would be comparable in size and age.

New test cultures were started by placing about 100 eggs of a known age in a wide-mouth quart jar provided with milkweed seed and water. In about 3 weeks, when the test-culture nymphs had reached the 5th instar, they were sexed and separated. Nymphs that had not reached the 5th instar were destroyed, as were those that were abnormal in any way.

Nymphs from the test groups that were not used immediately for experimentation functioned to maintain the stock culture. New stock culture jars were established each week, and in no case was a culture kept longer than 8 weeks.

Insects used for test purposes were selected from the sexed cultures by weighing. Individual insects were weighed to a range of ± 1 mg for males and ± 2 mg for females. The males averaged about 60 ± 1 mg in weight whereas the females weighed approximately 73 ± 2 mg. Much greater variation existed among females and about twice as many were rejected as compared to the males.

**Dosage Preparation and Application**

The metepa used in this work was supplied by American Cyanamid Company as a 92 percent technical material. Metepa is a trade name for tris [1-(2 methyl) aziridinyl] phosphine oxide.

Dilutions of metepa were made in reagent grade methyl alcohol on a volume-volume basis. Six dilutions were made so that each µl of
the resulting solution contained respectively the following amounts of metepa: 100 µg, 50 µg, 7.5 µg, 5.0 µg and 2.5 µg. These solutions were subsequently used to make topical applications to test insects. Variations in the volume of solution applied allowed for application of equivalent dosages to insects of varying weights.

Each treated insect received a volume of metepa solution which corresponded to a dosage rate of 1665.0, 832.5, 250.0, 83.3 or 41.6 µgs of metepa per gram of insect weight. The volume of solution applied to each insect varied from 0.94 µl to 1.1 µl depending on the weight of the insect being treated and the dilution concentration selected. Control insects were treated with 1 µl of methyl alcohol.

Topical applications were made with a model M ISCO micro-applicator, utilizing a ¼-cc syringe coupled to a 27-gauge needle. Insects to be tested were anesthetized with CO₂ and treated by applying the selected dosage to the ventral surface of the abdomen. The insects were then placed in ½-pint holding jars supplied with milkweed seed and water. The water was placed in a 2-dram patent-lip vial into which a 3-inch length of number 2 dental wick was inserted.

A length of 3/16-inch OD plastic tubing inserted into the vial with one end protruding upward through the cheesecloth cover allowed for replenishing the water supply without removing the cover. New watering wicks and fresh milkweed seed were placed in the jars 2 weeks after they were established.

**Experimental Procedures**

The jars containing the treated insects were placed in an incubator maintained at 26° C for the duration of an experiment. Eggs which were laid on the upper surface of the cheesecloth were scraped off daily. Eggs were counted every other day and a sample of 50 eggs from each holding jar was kept at 26° C for observation of percent hatch. The eggs collected on alternate days were discarded.

Mortality of the test insects was noted at the time eggs were counted so that the number of eggs laid per female could be calculated.

When mature male and female milkweed bugs that had been separated since the 5th nymphal instar were confined together, immediate and frequent copulations occurred. Copulation in the holding jars continued throughout the duration of an experiment although the frequency of copulation decreased with time.

Matings by specific pairs of insects were not observed in Experiments 1 through 5 and therefore the chronology of these experiments was based on the day the insects were confined in the holding jar. It was known that the females copulated because the eggs that were subsequently laid showed evidence of cytoplasmic changes which occur in eggs from mated females.
Specific matings were observed in Experiments 6, 7 and 8.

Egg laying by virgin *Oncopeltus fasciatus* females occurs at a low and sporadic rate and therefore such females contribute little to the total numbers of eggs laid. Eggs laid by virgin females were very different in appearance from those laid by fertilized females, being quite shrunken in appearance. Such infertile eggs were very rarely observed in test cultures.

Preliminary studies indicated that treatment at the two highest dosage rates (1665.0 and 832.5 µg/g) resulted in rates of egg hatch which were not within a suitable range for measurement, and therefore these treatments are not considered in detail in this study.

The four remaining dosage rates (250.0, 125.0, 83.3 and 41.6 µg/g) did elicit egg hatch responses within a measureable range and were standard rates for all subsequent experiments.

For each experiment, a particular dosage rate, including the control dosage, was applied to each of 5 individual adult milkweed bugs which were then confined with 5 untreated individuals of the opposite sex.

In one experiment, both sexes were treated prior to being placed together. Each dosage rate consisted of 4 replications of 5 pairs of insects confined in a culture jar.

**Nymphal Treatments**

Sexed, 5th instar nymphs were treated at the 4 dosage rates indicated above, allowed to mature, and then confined with adults of the opposite sex which had developed from untreated nymphs. Observations were made every 2 days of mortality of treated insects and percent hatch of eggs.

**Adult Treatments**

Experiments conducted on adult milkweed bugs were designed to demonstrate the effects of meteopa applied under various conditions. Applications were made with reference to sex, age and fertility. The conditions of these experiments:

Experiment Number 1: Males were treated at the standard dosages and immediately confined with untreated females.

Experiment Number 2: This experiment is the female counterpart of Experiment 1. Females were treated at the standard dosages and immediately confined with untreated males.

Experiment Number 3: Both males and females were treated at the standard rates and immediately confined together.

Experiment Number 4: In this experiment untreated males and females were confined together for 7 days, at which time the males were removed, treated with the standard rates and immediately returned to the test jar.
**Experiment Number 5:** Untreated males and females were confined together in this experiment and after 7 days the females were removed, treated at the standard rates and returned to culture jars with the males.

**Experiment Number 6:** Females were treated at the standard rates and immediately confined with untreated males. Matings were observed and mating pairs were removed as soon as they began copulating and held in individual jars. After each pair had separated, the females were placed back in their respective culture jars and the males were discarded.

**Experiment Number 7:** Untreated males and females were confined together; copulating pairs were removed and held in individual jars. After copulations were completed, the males were discarded and the females were treated at the standard rates.

**Experiment Number 8:** The procedure in this experiment was exactly the same as in Experiment 7 except that the original males were replaced with different untreated 7-day-old males 14 days after the treatment date.

**Experiments Number 9A, 9B and 9C:** This set of experiments was conducted to demonstrate the recovery of sperm viability in treated males without the possible modifying influence of sperm previously introduced into the female.

Males were treated at the standard dosages and immediately confined with untreated females for 7 days. Seven days after the first matings the original treated males were mated again to 7-day-old virgin females and after 7 days of confinement, they were again separated. This process was repeated a third time so that there were 3 sets of females that had been mated to the same group of treated males at approximately 1 week intervals.

The hatching characteristics of eggs from each group of females are shown respectively in Figures 14, 15 and 16. A composite graph, incorporating features from all 3 sets, is shown in Figure 17.

**Histological Studies**

Serial histological sections were taken from ovarian and testicular tissue and from eggs in various stages of embryo-genesis. Testes were dissected from males treated at all metepa dosage levels and at varying times after treatment.

A series of testes from untreated males were sectioned at two days after treatment (of the corresponding treated insects) or at about 9 days after the final molt. Dissections were also made at two weeks and four weeks following treatment.

This same schedule was followed for dissections of males treated at all dosage levels.

A similar schedule was followed in dissecting ovarian tissue.
Eggs to be sectioned were placed in warm Kahle's solution at intervals of 4, 24, 48, 72, 96 and 120 hours following oviposition. This procedure was followed for eggs from untreated pairings and for eggs obtained from cultures of insects treated at all the dosage levels.

All specimens were fixed in Kahle’s solution for 12 hours or longer. Twelve hours after the eggs were placed in Kahle’s solution they were punctured and then fixed for an additional 12 hours.

The specimens were then dehydrated in an alcohol series ranging from 70 percent through absolute and placed in xylene for 15 minutes. The specimens were next placed successively in xylene-paraffin mixtures of 1:1, 1:2 and finally into 100 percent paraplast. The specimens were allowed to remain in each xylene-paraplast mixture for 1 hour and in the 100 percent paraplast for 3 hours or longer.

The specimens were then imbedded in paraffin and 6 micron sections were cut on an International Equipment Company refrigerated microtome and placed on microscope slides coated with fresh egg albumin-glycerine mixture prepared as described on Page 67 in Gray’s “Handbook of Basic Microtechnique.” The slides were dried on a slide warmer for 12 hours, after which they were de-paraffinized in two 15-minute changes of xylene. The slides were hydrated through the alcohol series and stained with hematoxylin.

The hematoxylin method consisted of staining in Fischer’s Delafields hematoxylin for 7 minutes, washing off excess stain for 5 minutes in cold water, rinsing in 50 percent alcohol and then destaining in 70 percent alcohol with 2½ percent HCl for a few seconds. The slides were washed again for a few minutes in tap water and placed in 70 percent alcohol containing a few drops of NH₄OH for 5-10 minutes.

After rinsing briefly in water, the slides were dehydrated through the alcohol series. Some slides were counterstained with eosin at the 80 percent alcohol stage. This procedure consisted of dipping the slides in 5 percent eosin for a few seconds and rinsing in 80 percent alcohol. Following the alcohol dehydration, the slides were placed in two 15-minute changes of xylene and mounted in Fischer’s Permount and covered with a number 2 cover slip.

Some of the eggs were stained in alcoholic carmine-borax prior to being infiltrated and sectioned. The alcoholic carmine-borax stain was prepared as described on Page 19 in Gray’s “Handbook of Basic Microtechnique.”

The specimens were placed in the stain after being fixed and were stained for at least 48 hours. They were then destained for 24-48 hours in the HCl-alcohol mixture previously described.

Following this, they were dehydrated through the alcohol series and cleared in two 15-minute changes of xylene. The specimens were infiltrated, imbedded and sectioned in the same manner as previously
described. The specimens were affixed to slides, dehydrated and mounted in the usual manner without further staining.

All photomicrographs were taken with a 35 mm camera through a Bausch and Lomb flat field microscope, using Panatomic-X film and several combinations of filters.

RESULTS

Preliminary Treatments

Metepa was applied to adult milkweed bugs at rates of 1665.0, 832.5, 250.0, 125.0, 83.3 and 41.6 µg per g of insect weight. Both males and females were treated at these dosages and confined immediately with untreated insects of the opposite sex.

The mortality of both treated males and females was 100 percent 7 days after treatment at the 1665.0 µg/g dosage rate. Treated females did not lay any eggs and only a few eggs were collected from untreated females confined with treated males. None of the eggs collected from such treatments displayed embryonic development.

Mortality was also very high among females treated at the 832.5 µg/g dosage rate and egg laying was severely reduced. A few eggs were laid by such females but again embryonic development was not observed. Mortality of the treated males was lower than for the treated females but was still high. Embryonic development was not evident in the eggs laid by untreated females confined with treated males.

Some hatch occurred in eggs from insects treated at the 4 lowest dosage rates and subsequent experimental applications were standardized to include only 4 dosage rates (250.0, 125.0, 83.3 and 41.6 µg/g).

Nymphal Treatments

Treatment of 5th instar nymphs at the standard dosage rates produced erratic results and therefore additional nymphal treatments were discontinued. Mortality was more than 50 percent at the 250.0 and 125.0 µg/g dosage rates and noticeable mortality occurred at the two lowest dosages.

Survivors of the treatments appeared to be normal and when they became adults, they were confined with untreated members of the opposite sex. The treatment response in terms of percent hatch of eggs laid by the females of such matings fluctuated from day to day and was difficult to characterize.

Data from this experiment were not analyzed because of their inconsistency.
Adult Treatments—Egg Hatch

The percent of egg hatch which resulted from the application of 4 standard dosage rates of metepa (250.0, 125.0, 83.3 and 41.6 µg/g) to adult insects is graphically illustrated in Figures 6 through 17.

The percentage points on the graphs are calculated as a percent of the control which was considered 100. Except for Figures 10, 12 and 13, the points plotted on the graphs represent the average percent hatch for successive 6-day periods after confinement.

In Figures 10 and 12, points for the first 10 days are plotted every 2 days to indicate sudden changes. The points in Figure 13 are plotted every 4 days following confinement.

Significant differences between the means of percent hatch of eggs from treatments at the standard dosage levels was determined by analysis of variance.

Experiment Number 1: When males were treated at dosage rates of 250.0, 125.0, 83.3 and 41.6 µg/g and immediately confined with untreated females, the percent hatch of eggs which resulted from such matings was low. As the time from treatment increased, a gradual increase in percent egg hatch was observed at all except the highest dosage level.

Figure 6 illustrates the percent of egg hatch for the eggs from females mated with treated male insects. Each point represents the average percent hatch of eggs collected every 2 days over a 6-day period. The highest dosage induced complete sterility, whereas the percent egg hatch at other dosage rates occurred in relation to the amount of metepa applied.

Significant correlation existed at the 0.01 level between dosage rates and percent hatch; a significant difference in percent egg hatch between treatments was also evident.

Experiment Number 2: As shown in Figure 7, the pattern of response to treatment of females was much different from that which resulted from male treatment. Percent egg hatch was considerably higher at all dosage rates except the highest.

Although the percent hatch of eggs from treated females was slightly depressed as compared to control insects early in the experiment, the hatch percentage rose midway through the experiment and then decreased steadily to the end of the test period.

This is in contrast to the treated-male response in which there was steady recovery following the very low initial percent egg hatch. At the lowest dosage rate, hatch was not greatly different from the control throughout the first 14 days of the experiment. Hatching percentages began to decline at the mid point of the experiment and steadily decreased to the final sample day.

The mean values of the measurements on the final observation day for comparable dosage rates were somewhat higher in Experiment
Figure 6. Experiment 1. Percent hatch, males treated prior to confinement with untreated females. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control

Figure 7. Experiment 2. Percent egg hatch, females treated prior to confinement with untreated males. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control
2 as compared to Experiment 1. The percent hatch at the highest dosage persisted at a very low and constant level. Significant differences in egg hatch at the 0.01 level existed between all treatments except the 83.3 and 41.6 µg/g rates. The differences in egg hatch between the control and all treatments was also significant.

**Experiment Number 3:** In this experiment, both males and females were treated immediately prior to confinement. The percent egg hatch for the 2 lowest dosage rates was comparable to that obtained from Experiment 1 in which only males were treated.

However, as shown in Figure 8, the effects at the 250.0 and 125.0 µg/g dosage rates were severe with no hatch taking place at the highest rate and only a very low percent hatch occurring at 125.0 µg/g and this during the last 4 days of the experiment.

Percent egg hatch for other treatment rates was low initially and then gradually recovered and approached the point attained when only males were treated.

Correlation of the treatment to hatch response was high and very significant differences in egg hatch existed at the 0.01 level between the 2 higher rates and the 2 lower rates and between the control and all the treatments.

**Experiment Number 4:** This experiment was designed to help assay the effects of successive matings on fertilization of eggs and was accomplished by confining untreated males and females together for 7 days, after which the males were treated and the sexes recombined.

The data in Figure 9 indicate that the hatching percentage of eggs laid on the day following treatment was essentially that of the control.

Soon after treatment the hatch percent began to decrease at all dosage rates and by the end of 13 days after treatment a low point was reached for the 3 lowest rates. Percent hatch of eggs from the highest treatment rate decreased less as time progressed than percent hatch of eggs from the other 3 treatments and continued to decline while egg hatch began to show recovery at the lower rates 19 days following treatment.

No difference in percent egg hatch existed between dosage at the 0.01 level but there was a significant difference between the control and the other treatments.

**Experiment Number 5:** The results of the female counterpart of Experiment 4 were similar to those for the male treatment. The most notable differences in results, as indicated in Figure 10, were the more immediate decrease in percent of egg hatch and a greater degree of recovery for the 2 lowest treatments.

Eggs collected on the day following treatment showed a considerable decrease in percent of hatch at all dosage rates with the maximum effect being reached 3 days after treatment. In contrast, the male
Figure 8. Experiment 3. Percent egg hatch, both males and females treated prior to confinement together. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control

Figure 9. Experiment 4. Percent egg hatch untreated males and females confined together—males treated 7 days after confinement. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control
Figure 10. Experiment 5. Percent egg hatch, untreated males and females confined together—females treated 7 days after confinement. Each point represents the average percent hatch for the successive periods indicated.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control

treatments required 7 days after treatment before the maximum effects were observed.

The highest dosage rate resulted in eggs which were completely sterile whereas only partial sterility resulted when the males were similarly treated. There were significant differences at the 0.01 level between the treatments and between the control and all treatments.

Experiment Number 6: In this experiment, the females were treated prior to mating and were separated from the males as soon as each female had mated. The mean percent of eggs which hatched in Experiment 6 is somewhat lower than that for Experiment 2, where females were also treated prior to confinement with males, but the response pattern is quite similar (Figure 11).

The most conspicuous difference is the lack of recovery of egg hatch percentage following the initial low hatch rate. No statistical difference existed between the percent hatch of eggs from females treated at the 2 high dosages, but significant differences in percent hatch did exist between the other dosage rates and between the control and all the treatments.

Experiments 7 and 8: These two experiments were primarily designed to demonstrate the effects of metepa on sperm stored in the female. In both experiments untreated males and females were allowed to mate and were separated after mating was completed.
Figure 11. Experiment 6. Percent egg hatch, females treated prior to confinement with untreated males—males removed after mating completed. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, • = control

Figure 12. Experiment 7. Percent egg hatch, untreated males and females confined together—males removed and females treated after all pairs had completed mating. Each point represents the average percent hatch for the successive periods indicated.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, • = control (dotted line represents the actual catch percentages for the check.)
Specific matings were observed in these experiments so it was known that all females had copulated. After mating was completed and the males removed, the females were treated at the 4 standard rates. The effect of treatment was immediate and severe, with percent egg hatch dropping off sharply at all dosage levels.

Percent hatch for females treated at the lowest dosage remained above 20 percent in both experiments and was at a measurable level throughout the experiment. Percent egg hatch declined to zero for the 3 higher dosages 3 days following treatment in Experiment 7 and 1 day post treatment in Experiment 8.

In Experiment 7 where females were not allowed to mate again, there was no recovery of egg viability (Figure 12) and the percent hatch for females treated at a rate of 41.6 µg/g declined steadily and was nearly zero for eggs collected on the last sample day.

The actual percent hatch of eggs from the control insects is shown as a dotted line and it can be seen that eggs from control insects showed a decline in percent hatch near the end of both Experiments 7 and 8. In contrast, a definite recovery in hatch was evident for all treatment levels in Experiment 8 where males were placed back with the treated females, as shown in Figure 13. On the last sample day,

![Figure 13. Experiment 8. Percent egg hatch, untreated males and females confined together—males removed and females treated after all pairs had completed mating. New males confined with the original females 14 days after the treatment date. Each point represents the average percent hatch for successive 4-day periods.](image)

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control (dotted line represents the actual catch percentages for the check.)
Figure 14. Experiment 9A. Percent hatch, males treated prior to mating with untreated females. Males removed after 7 days. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ▼ = 41.6, • = control

the percent hatch of eggs from insects treated at all dosages abruptly declined.

The addition of new males had no great effect on percent hatch of eggs obtained from the control females in Experiment 8. There was a very slight increase in hatch percentage after the males were added but the pattern was not appreciably different from that of Experiment 7 in which no males were present.

Significant differences existed between the lowest dosage and the other treatments and between the control and all treatments in both Experiments 7 and 8.

Experiments 9A, 9B and 9C: These 3 experiments are grouped together because the same treated males were successively confined with three different groups of untreated virgin females for a period of 7 days with each group.

Experiment Number 9A: The results as shown in Figure 14 indicate that when treated virgin males were allowed to mate for only 7 days with untreated 7-day-old virgin females, the percent hatch of eggs from this mating was low for all treatment levels.

Experiment Number 9B: Males from Experiment 9A were confined with a second group of virgin females. When this experiment was established the males were then 14 days old and had been treated 7 days previously. Although the percent hatch of eggs from this mating was still low at all dosage levels, an increase was noted at the lowest treatment (Figure 15).
Figure 15. Experiment 9B. Percent hatch, males from Experiment 9A mated to untreated virgin females. Males removed after 7 days. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control

Figure 16. Experiment 9C. Percent hatch, males from Experiment 9B mated to untreated virgin females. Each point represents the average percent hatch for successive 6-day periods.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control
Days after males treated

Egg hatch - percent of control

Figure 17. Experiments 9A, 9B and 9C. Percent hatch, a composite of Experiments 9A, 9B and 9C. Each point represents the average percent hatch of eggs from separate groups of females during the periods after treatment of the males as indicated.

Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, □ = 41.6, ● = control

Experiment Number 9C: Males from Experiment 9B were confined with a third group of virgin females. The males were then 21 days old and had been treated 14 days previously. The percent hatch of eggs obtained from this mating is shown in Figure 16. Compared to the date in Figures 14 and 15, it is obvious that the percent of eggs hatching had increased for all treatment levels.

Egg hatch at the lowest treatment increased to approximately 80 percent while the hatch at the other dosage levels increased to a degree consistent with the amount of metepa applied.

Figure 17 is a composite graph incorporating part of the results obtained in Experiments 9A, 9B and 9C. Each point in this graph represents the mean percent egg hatch for the indicated dosage during the first 7 days of each of the experiments.

The purpose of Figure 17 is to illustrate the ability of sperm from treated males to fertilize eggs from untreated virgin females over a period of time. Since the sperm was transferred to a series of virgin females in each of the experiments, there was no interference in the female of sperm from previous matings.

The data in Figure 17 indicate that there was little increase in percent of egg hatch during the first 14 days of confinement when treated males could mate with different virgin females. However, substantial increases in egg hatch occurred during the period of 14
to 21 days following confinement with females. This result was true only in the 3 lowest dosages, with no hatch at all occurring at the highest dosage.

Figures 18–25. Eggs laid per female per day for 28 days
Dosage in µg/g: □ = 250.0, ○ = 125.0, △ = 83.3, ■ = 41.6, ● = control
Adult Treatments—Eggs Laid/Female/Day

Male Treatment: During the course of this investigation, the number of eggs laid per female per day was recorded for the duration of all experiments. Figures 18 and 19 graphically illustrate such results for each dosage rate for the experiments where only the males were treated. There was no significant difference between the controls and the treatments.

Female Treatment: In all experiments (Figures 20 through 25), treatment of females with metepa at a rate of 250.0 µg/g resulted in a significant reduction in the numbers of eggs laid as compared to the control insects.

In Experiment 3 (Figure 20), the number of eggs laid per female is significantly less than the control for the two highest dosages. Both males and females were treated in this experiment and this may have had an effect on the results.

As time progressed during the 28-day test period, the number of eggs laid per female declined slightly at the high dosage rate. Figure 26 shows the pattern of egg laying when females were treated at the rate of 250.0 µg/g (Experiments 5, 7 and 8).

![Graph](image)

Figure 26. Experiments 5, 7 and 8. Eggs laid per female per day. Each point represents the average number of eggs laid during the period indicated.

Exp’t. 5 = □  Exp’t. 7 = ○  Exp’t. 8 = △

Dosage: 250.0 µg metepa per g insect weight.
It is noteworthy that there was a sudden elevation in numbers of eggs laid in Experiment 8 near the end of the test period. The increase was followed by complete cessation of egg laying. This phenomenon was correlated closely to a corresponding occurrence in egg hatch, in which the percent hatch rose from zero to 7 percent in a period of 4 days.

Positive correlation, significant at the 0.01 level, existed between percent egg hatch and the number of eggs laid per female in all experiments in which the female was treated. There was no correlation between egg hatch and eggs laid per female in the experiments where only males were treated.

**Adult Treatments—Mortality**

Treatments with metepa at the 4 standard dosage levels resulted in no significant effect on mortality of adults in this series of experiments. It is possible that further experimentation might show a relationship between treatment and adult mortality at the dosage levels used, since preliminary studies showed that higher dosages did have definite effects on mortality.

**Histological Studies**

*Male Tissue:* The photomicrographs reproduced on pages 56–77 were selected as typical examples of the histological studies made of the tissues of treated and untreated *Oncopeltus* adults.

Plate I, Figures 1 and 2 are photomicrographs of sectioned testes from an untreated 3-week old adult and are included to show the normal or typical testes and the zones of developing germ cells.

The two higher dosages used in this study (250.0 and 125.0 µg metepa per g of insect weight) induced comparable changes in testicular tissue. Examination of numerous sections from both treatment levels failed to reveal any histological differences between treatments which could be attributed to the application of metepa.

However, since the percent hatch of eggs from the two highest levels is different, it would appear that the histological technique was inadequate to demonstrate such differences.

The gross appearance of the testes at 45X 2 days after treating the males at a rate of 250.0 µg/g (Plate II, Figure 3) is not visibly different from testes from an untreated male (Plate II, Figure 6).

Two weeks after treatment (Plate II, Figure 4), some very drastic changes have taken place. The developing nuclei seen in Figure 3 are no longer present and have been replaced by what appears to be sperm. Four weeks after treatment (Plate II, Figure 5) the testis is greatly atrophied and the only recognizable tissue is the somatic supporting tissue of the testis. Sperm stored in the seminal vesicle appear to be intact while the "sperm" remaining in the testis at the 2-week period (Plate II, Figure 4) has disappeared.
Plate II, Figure 6 is a photomicrograph of a testis from an untreated 4-week old insect; the tissue is normal and no indication of atrophy exists. Testes from insects treated at 41.6 µg/g are not shown because they are similar in appearance to the untreated testes.

A closer look at the spermatocytes in testes from treated insects indicates the presence of functional interference with developing nuclei.

Plate III, Figure 7 (350X) shows an area of a testes from an untreated, 21-day-old adult which includes parts of the zones of spermatogonia, the primary and secondary spermatocytes and spermatids.

In Plate III, Figure 8 (910X), the chromosomes in the primary spermatocytes of an "untreated" testis present a normal appearance with most of the chromosomes being in the metaphase stage. None of the cells in focus in this particular photomicrograph are in the anaphase stage but occasionally such mitotic figures were seen in sections from untreated insects.

Four weeks after treatment (Plate III, Figures 9 and 10) the untreated testes still appear very much as they did at 2 weeks post treatment.

Some alterations in mitotic configurations can be seen in the photomicrographs of testes from males treated at a rate of 83.3 µg/g. Two weeks after treatment the primary spermatocytes (Plate IV, Figure 11, 350X) are similar in appearance to those of the untreated testes (Plate III, Figure 7, 350X). At a greater magnification, however (Plate IV, Figure 12, 910X), definite irregularities can be observed in the appearance of some of the primary spermatocytes. Normally only 9 sets of chromosomes can be counted but in Figure 12 the number is apparently variable. Four weeks after treatment (Figures 13 and 14) much of this irregularity in appearance seems to have disappeared.

Testicular tissue from insects treated with metepa at a rate of 83.3 µg/g was not different in appearance from untreated testes 2 days after treatment but definite alterations existed after 2 days in testes from insects treated at a rate of 125.0 µg/g.

The primary spermatocytes have a somewhat disorganized appearance at 350X (Plate V, Figure 15) and definite abnormalities in mitotic configurations can be seen at a greater magnification (Plate V, Figure 16, 910X).

The primary spermatocytes from treated insects commonly have a number of nuclei with chromosomes in configurations which appear to be in the anaphase stage. Such configurations are occasionally seen in testicular tissue from untreated insects but the number of such figures is comparatively small. Chromosome bridges were noted in many nuclei which were having difficulty in completing the process of division.

Such mitotic figures have also been reported by Hedin, et al.
(1967) in testes from insects treated with gamma radiation and were described as being in an "arrested" state.

At 2 weeks post treatment (Plate V, Figure 17) the spermatogonia and spermatocytes have disappeared in testes from males treated at 125.0 µg/g. It appears that the denser areas in the upper portion of the photomicrograph correspond to the zones of spermatogonia and spermatocytes. Trophocyte cells associated with supporting tissue can still be clearly seen.

Four weeks from the treatment date all the germinal tissue has apparently disappeared as shown in Plate V, Figure 18. There is general necrosis and the only definitive structures remaining are the testicular tubule walls. The concentration of dark staining material may be nuclear remnants from cyst trophocytes and from germinal nuclei.

Female Tissue: Characterizing the effects on ovarian tissue of treatments with metepa proved to be a rather difficult task. Although the dosage rate was a factor in determining the amount of damage observed in the ovaries, such damage was not definitive and a dosage-damage-time relationship could not be seen. The high dosage rate always resulted in severe atrophy of the ovarioles by 3 weeks after treatment.

Plate VI, Figure 21 (110X) and 22 (350X) are photomicrographs of a longitudinal section of an ovariole from a female treated 3 weeks previously at a dosage rate of 250.0 µg/g. This ovariole can be compared to Plate VI, Figures 19 and 20, which are photomicrographs of an ovariole tip from an untreated female. Figure 19 is magnified 48 times and Figure 20 is magnified 350 times.

The treated ovariole is considerably smaller than the untreated one and it is difficult to identify analogous structures. The areas shown in the photomicrographs of treated and untreated tissues are believed to be comparable.

Figure 19 is a section of ovariole showing about ⅔ of the germarium and part of the vitellarium. Several developing oocytes can be seen as well as 1 nutritive cord connecting an oocyte to the trophic core.

Figure 20 includes the posterior portion of the germarium. The trophic core can be clearly seen as well as the trophocyte nuclei associated with this structure. Several primary oocytes can be seen at the posterior end of the germarium.

It appears that the dark-staining structures in Figures 21 and 22 are trophocyte nuclei which have been released from the area of undifferentiated tissue. There is no organization of these nuclei and no trophic core is present.

The anterior end of this structure has evidently atrophied to a considerable extent. There are 3 structures in this area which appear to be multi-nucleate cells. Since such structures are not seen in un-
treated tissues, it is assumed that they developed as a result of the metepa treatment. It is probably significant that the area of damage corresponds to the area where all mitotic events occur in the ovariole.

_Egg and Embryonic Tissues:_ The most profound effects of metepa treatments were observed in the developing egg. At dosage levels which produce no discernible effects in testicular or ovarian tissue, embryogenesis may be greatly altered.

Apparently the treatments which affected sperm did not greatly modify the sperm's ability to carry out the function of fertilization. The ability of sperm to induce fertilization must not have been impaired at any of the dosage levels used since virtually 100 percent of the eggs laid in test cultures exhibited the turgid and glossy appearance of fertilized eggs.

It was not within the scope of this study to characterize the fertilization reaction but it appears that the entry of the sperm into the egg might have effects on the egg structure exclusive of its fusion with the female pro-nucleus.

It was previously pointed out that eggs from virgin females exhibit a shrunken and dull appearance soon after oviposition. When sections were made of such eggs, great difficulty was encountered in sectioning and staining them.

Plate VIII, Figure 27 is a longitudinal section of a 24-hour old unfertilized egg which appears granular and rather structureless. At greater magnification (Plate VIII, Figure 28) there is no indication that structured tissue exists at or near the surface. These photomicrographs can be compared to those of a 24-hour-old egg from an untreated female previously mated to a male treated at a rate of 125.0 µg/g (Plate IX, Figures 29 and 30) in which fertilization has taken place but where there is no cellular layer at the periphery of the egg.

It is obvious that some kind of structure exists at the egg surface and it seems likely that such a structure developed as a result of the sperm entering the egg.

Examination of many sections of eggs fixed within 4 hours after oviposition revealed few observable differences between eggs from treated and untreated insects.

Plate VII, Figure 23 is a photomicrograph of a section of a 4-hour-old egg from an untreated mating at 93X. The egg in this photomicrograph is typical of most of the 4-hour eggs from both treated and untreated matings. The arrow indicates what appears to be the fusion nucleus or structures associated with it.

Plate VII, Figure 24 shows the same area at 910X. This structure was not always visible in sections of eggs from the 2 high treatment levels but was commonly noted in sections of eggs from insects treated at the lower dosages.
After 24 hours of incubation, the developing embryo appears as a complete blastula as shown in Plate VII, Figures 25 and 26.

A single layer of cells then exists at the periphery of the egg and some nuclei are scattered throughout the yolk. These photomicrographs can be contrasted to Plate IX, Figures 29 and 30 which are photomicrographs of 24-hour-old eggs from females mated to males treated at 125.0 µg/g.

In this instance, males only were treated but eggs from treated females had a similar appearance. A blastula does not exist in the 24-hour eggs from treated insects and several large, irregularly shaped nuclei can be seen scattered throughout the yolk.

A closer examination of these nuclei (Plate IX, Figure 31) reveals that the chromatin is aggregated into what seems to be a mitotic configuration. Such configurations were observed in nearly every “treatment” egg in which early cleavage occurred but in which development did not progress to the blastula stage. Plate IX, Figure 32 is a photomicrograph of a 48-hour egg from the same treatment as Figures 29, 30 and 31 showing additional cleavage nuclei.

Plate X, Figures 33, 34, 35 and 36 are photomicrographs of sections of eggs from untreated matings showing the normal progression of embryonic development at 48, 72, 96 and 120 hours respectively after oviposition. The first 3 are sagittal sections while the last is a frontal section. The usual hatching time under the conditions of these experiments was between 120 and 144 hours.

The appearance of eggs that failed to hatch was highly variable and many degrees of embryonic development were observed. Only a few eggs from insects at the 2 high dosage levels showed visible signs of tissue development. It was common to observe the formation of pigmented tissues in eggs from insects treated at the lower dosages and embryos of quite normal appearance often developed but did not hatch.

Many of the eggs which did not develop pigmented tissues showed a clearing of yolk at the anterior end, usually involving about 1/3 of the egg. Microscopic examination of sections from such eggs revealed that a small area of tissue growth usually existed at the interface of the cleared area and the yolk. However, eggs which exhibited no external changes whatsoever always showed a cleared area in the yolk which corresponded to the usual position of the fusion nucleus.

Plate XI, Figure 37 is a photomicrograph of a section from a 144-hour-old egg from a female mated to a male treated at 125.0 µg/g. The arrow indicates the cleared area and what appears to be the remnant of a cytoplasmic structure.

Closer examination (Plate XI, Figure 38) does not clarify the characteristics of this area except to show that some of the yolk globules have been dissolved.

Plate XI, Figures 39 and 40 are photomicrographs of typical sec-
tions of “treatment” eggs which show tissue formation. Both eggs were from females mated to males treated at 125.0 µg/g and both were 120 hours old. The “embryos” present in these eggs cannot really be characterized except to say that they are abnormal. Apparently there is some organization since there are structures which appear to be the anlagen of appendages.

Observations of the embryological development of many eggs from treated insects revealed a wide variety of expression to the presence of metepa. One such expression was the extension of the incubation period for many of the eggs that ultimately hatched. No records were kept of this phenomenon but it was quite common for eggs from treated insects to hatch as much as 48 hours later than eggs from control insects. The nymphs from these late-hatching eggs appeared to be normal and mortality was not excessively high.

Some late-hatching nymphs were reared to maturity and mated. Observations were made of the hatching characteristics of eggs from the mated insects. Although complete records were not kept of those observations, enough data were obtained to indicate that egg development was erratic.

Further, the observations appear to indicate that some latent effect is genetically induced as a result of metepa treatment of one of the parents.

**SUMMARY OF TREATMENTS AND EGG-HATCH RESULTS**

To recapitulate, metepa was applied to 7-day-old virgin insects in all experiments at one of the following rates: 250.0, 125.0, 83.3, 41.6 µg metepa per g insect weight and control.

* Experiment 1: Males treated and immediately confined with untreated females.

* Results: Initial egg hatch 4 days following confinement was low for all treatment dosages.

  Subsequent percent egg hatch for each dosage was inversely proportional to dosage with no hatch occurring at 250.0 µg/g.

* Experiment 2: Females were treated and immediately confined with untreated males.

* Results: Initial egg hatch 4 days following confinement was about 90 percent of the control at the lowest dosage and approached zero for the highest dosage.

  Subsequent percent egg hatch increased during the first 14 days following confinement at all dosages and then decreased slightly to the end of the experiment.

* Experiment 3: Males and females were both treated and immediately confined together.

* Results: Initial egg hatch 4 days following confinement was about 3 percent for the lowest dosage and zero for all other dosages.
Subsequent percent egg hatch increased for the two lowest dosage rates to the approximate level attained in Experiment 1. The highest dosage resulted in zero percent egg hatch and the next highest dosage resulted in a very low percent egg hatch which occurred during the last few days of the experiment.

Experiment 4: Males and females were confined together. Seven days after confinement, the males were removed, treated and immediately returned to confinement.

Results: Initial percent egg hatch 4 to 8 days following confinement was equal to the control.

Percent of egg hatch decreased during the period from 8 days to 20 days following confinement and then increased for all dosage rates, except the highest, to the end of the experiment.

Experiment 5: Males and females were confined together for 7 days at which time the females were removed, treated and reunited with the males.

Results: Initial percent egg hatch 4 to 6 days after confinement was equal to the control.

Subsequent percent egg hatch decreased markedly at all levels to about 50 percent of the control egg hatch for the lowest dosage and decreased to about 15 percent or less for eggs from the other dosages. Percent egg hatch for eggs from females treated at the highest dosage decreased to zero 10 days after confinement, while percent hatch of eggs from females treated at the other dosages showed increases according to the rate of metepa applied.

Experiment 6: Females were treated and immediately confined with untreated males. When all females had mated, the males were removed and destroyed.

Results: Initial egg hatch 4 days following mating was about 65 percent of the control at the lowest dosage and ranged down to about 5 percent of the control at the highest dosage.

Subsequent percent egg hatch remained at approximately the same level through the 20-day period following mating, after which a slight decrease in percent hatch occurred.

Experiment 7: Males and females were confined together until all females had mated, after which the males were removed. The females were treated on the 7th day after confinement.

Results: Initial percent egg hatch 4 to 6 days following confinement was about the same as the control.

Subsequent percent egg hatch decreased to about 20 percent of the control for the lowest dosage and to zero for the other dosages by the 10th day after confinement. Percent hatch at the lowest dosage slowly decreased to near zero by the end of the experiment and remained at zero for the other dosages.

Experiment 8: Males and females were confined together until all females had mated. On the 7th day after confinement, the males
were removed and the females were treated. Twenty-one days after confinement, new males were confined with the females.

Results: Initial percent egg hatch 4 to 6 days following confinement was about the same as the control. Subsequent egg hatch decreased to about 20 percent of the control for the lowest dosage and to zero for the other dosages 10 days after confinement, remaining at about that level up to the 22nd day following confinement. After the 22nd day, percent egg hatch increased for all dosages to the 26th day and then abruptly decreased again on the 30th day after confinement.

Experiment 9A: Males were treated and immediately confined with untreated females. Males were removed after 7 days and retained.

Results: Hatch occurred only in eggs from the lowest dosage treatments and showed no increase in percent hatch with time.

Experiment 9B: The males removed from Experiment 9A were immediately confined with 7-day-old virgin females. The males were removed after 7 days and retained.

Results: Percent egg hatch for eggs from the lowest dosage treatment was initially low, rose to about 28 percent by the 14th day after confinement and then decreased. There was no hatch at the other dosages.

Experiment 9C: The males removed from Experiment 9C were immediately confined with 7-day-old virgin females.

Results: Initial egg hatch ranged from 50 to 73 percent of the control for the 3 lowest dosages, with no hatch occurring at the highest dosage. Subsequent percent hatch increased steadily at the lowest dosage and decreased for the other dosages.

DISCUSSION AND CONCLUSIONS

Preliminary Treatments

The results of treating both male and female adult Oncopeltus fasciatus with metepa at dosages of 1665.0 and 832.5 µg/g indicate that vital body functions are impaired, as shown by the high mortality which resulted from treatment at such dosage rates.

Mortality of adult insects treated at the 4 standard dosages was not significantly different from the control for any of the treatments, although none of the experiments were primarily designed to measure mortality. The nature of the lethal effects induced by metepa was not investigated in this study.

Nymphal Treatments

Treatment of 5th instar nymphs was done on a preliminary basis and the results of such studies of the effects of metepa were not con-
clusive. A high percent of the treated nymphs died and such death was correlated with initiation of molting.

Such a phenomenon may indicate that metepa preferentially alkylates some groups (perhaps amino acid or protein end groups) that are essential precursors in the synthesis of materials necessary to the process of molting. Gametogenesis is minimal in 5th instar nymphs but reproductive tissue is well developed.

Further study of the effects of metepa on nymphal tissue was outside the scope of this study.

**Adult Treatments**

The normal course of events in the reproductive cycle of the large milkweed bug should be briefly outlined to provide a background for comparison to the effects resulting from treatment with metepa.

Adult milkweed bugs usually begin mating about 6 or 7 days after completing the imaginal molt. The period of time from the imaginal molt to first copulation is apparently dependent on maturation of the female since males only 2 to 3 days old will copulate with older females.

Copulation is frequent among week-old adults and appears to be practically continuous for a few days after first copulation. Oviposition begins a day or two after mating and continues at a fairly constant rate for about 21 days and then begins to decrease.

As time passes, copulation becomes less frequent and occurs only occasionally after about 35 days following the first mating. At about this time, mortality among females first becomes noticeable and egg laying has decreased to a very low rate.

If 35-day-old males that have been confined continuously with females are placed with virgin females, they will copulate readily and successfully.

Virgin females that are prevented from mating will lay a small number of eggs, all of which are shrunken and wrinkled. Females isolated from males for 35 days or more and then allowed to mate, lay fertile eggs in numbers that approach the total that could be expected if the same females had mated at the age of 7 days. Noticeable mortality of such females begins about 25 to 30 days after first mating and subsequent mortality is high.

Males which are isolated from females live for long periods of time with minimal mortality. One group of males was kept for 180 days before they were allowed to mate. Mortality during that time was about 20 percent.

After 180 days, the males were confined with 7-day-old virgin females and mating began immediately. Mortality among these males was extremely high during the following 21 days and reached 100 percent about 30 days after they were first confined with females.
It appears that longevity of both males and females is determined principally by the extent of their reproductive activities, although males live longer than females under any circumstances. It is also apparent from the observations made of virgin females that copulation must occur before a substantial oviposition ensues.

The ovaries in the large milkweed bug are telotrophic, each ovary being composed of 7 ovarioles. Each ovariole consists of a terminal filament, germarium, vitellarium and pedicel. At the apex of the germarium, there is a group of undifferentiated cells which divide to produce trophic cells, which migrate posteriorly towards the trophic core.

As the trophic cells approach the trophic core, their boundaries disappear and the nuclei of the cells cluster together around the trophic core. These nuclei move into the trophic core where they disintegrate and are used to nourish developing oocytes. The primary oocytes arise from oogonia at the base of the germarium and are attached to the trophic core by nutritive cords. As the oocytes mature they move out of the germarium into the vitellarium.

In the male milkweed bug, spermatogenesis is initiated by a group of cells, the apical complex, located at the apex of the testes. These cells divide mitotically and produce spermatogonia which are clustered together in a cyst. The cysts maintain their identity through subsequent divisions and stages of development of the sperm.

The primary spermatogonia divide several times by mitosis to produce secondary spermatogonia, which in turn divide by mitosis to produce primary spermatocytes. The diploid primary spermatocytes divide meiotically to form haploid secondary spermatocytes.

Mitotic division of the secondary spermatocytes results in spermatids which differentiate into sperm. The mature sperm are stored in the seminal vesicle and are transferred directly to the female spermatheca during copulation.

Continued copulations did not appear to deplete the supply of sperm within the 30-day period that observations were made. The histological studies indicate that sperm is still being actively produced at this advanced age.

Before the eggs are laid, they are fertilized by the sperm stored in the spermatheca. The exact mechanism of entry of the sperm into the egg is uncertain but it is thought that entry is made through one of the micropylar openings.

The presence of a spermatozoan within the egg produces a cytoplasmic reaction which results in the formation of a structure at the surface of the yolk. It is possible that initiation of this cytoplasmic response depends on fusion of the pronuclei but there is evidence that the response may occur independently of this event.

Many of the sections of eggs from females of treated pairs showed
no evidence of a fusion nucleus or cleavage nuclei and yet the cytoplasmic reaction had occurred.

According to Butt (1947), fusion of the pronuclei, or syngamy, occurs within ½ hour after the egg is laid.

The structure indicated in Plate III, Figures 23 and 24 is identical to the one illustrated and identified by Butt as the fusion nucleus. In this study, the fusion nucleus could not be located 1 hour following oviposition but was readily observed at 4 hours.

Eggs from virgin females were wrinkled and shrunken almost immediately after oviposition, whereas fertilized eggs were turgid and smooth. This seems to indicate that the cytoplasmic response induced in the egg was a result of sperm entry and not syngamy.

In *Oncopeltus*, as in other insects, after the sperm enters the egg, the sperm nucleus migrates to the center of the egg. By this time the primary oocyte nucleus has migrated to the surface of the egg and has divided by meiosis, producing 3 polar bodies. The now haploid secondary oocyte nucleus, known as the pronucleus, migrates back near the center of the egg and fusion of the male and female pronuclei occurs.

Cleavage of the now diploid zygote nucleus begins and the resulting cleavage nuclei migrate to the periphery of the egg. Some cleavage nuclei migrate back from the periphery to remain in the yolk and are regarded as trophocyte cells or vitellophags which probably function in some of the synthetic activities which occur in the yolk (Counce, 1961).

In *Oncopeltus*, 24 hours after oviposition at 26° C, a single layer of cells, the blastoderm, exists at the periphery. To this point there has been no evidence of cellular differentiation except for the vitellophags. Such cellular differentiation is now initiated with the beginning of gastrulation.

This process and subsequent organogenesis has been well described by Butt (1947) and will not be reviewed in this discussion.

Superficially speaking, an embryo can be seen in the egg approximately 72 hours after oviposition and hatching begins about 130 hours following oviposition.

The ultimate criterion of treatment effects for the purposes of this study was the percent hatch of eggs that were laid as a result of mating between pairs of insects in which one or both sexes had been treated.

It is known that copulation is frequent during the first few days following confinement of males and females and that sperm is transferred from the male to the female during confinement.

Copulation continues throughout the life of the insect, although frequency declines with age. The possibility that each spermatozoan stored in a female might have equal opportunity to inseminate eggs currently being laid was a point that had to be considered in evaluat-
ing some of the results, and it was hypothesized that this does indeed occur.

The results of Experiment 1 indicated a substantial recovery of sperm viability as time from treatment increased, especially for the lower dosage rates. It was not known, however, to what degree the sperm from earlier matings were modifying the percent of egg hatch near the end of the experiment.

Experiments 9A, 9B and 9C were designed to give information which could clarify this problem. The results of Experiment 9C, as shown in Figure 25, indicate that the ultimate recovery of sperm viability was quite high at the 41.6 µg/g dosage rate. The effects of other dosage rates used also show some evidence of increasing sperm viability to a degree which appears comparable to the relative amount of metepa applied.

The percent hatch of eggs from females mated to males treated at all dosage rates is substantially higher in Experiment 9C than the equivalent points in Experiment 1.

The results of Experiments 1 and 9C cannot be directly compared but it is possible that the lower percent egg hatch at the end of Experiment 1 as compared to Experiment 9C, was caused by sperm dilution in Experiment 1. Such dilution could be a result of sperm from copulations which occurred immediately after treatment being mixed with sperm from more recent copulations. Dilution could not occur in Experiment 9C since only recent copulations had taken place.

The males, however, had been confined with other females prior to being used in Experiment 9C, and it was assumed that they had copulated several times.

The fact that sperm dilution is a factor in modifying the percent of egg hatch is substantiated in an inverse fashion by the results obtained in Experiment 4, in which pairs were confined together for several days before the males were treated. Hatch of eggs from the lowest dosage treatment was reduced slowly to a fairly low percent following treatment but recovered rather well by the end of the experiment.

The fact that the percent egg hatch decreased slowly indicates that the sperm from treated males can effectively fertilize eggs but that the chances of this occurring are considerably lessened by the presence of "untreated" sperm (transferred prior to treatment) in the female.

If only the most recently transferred sperm were active in fertilization, then the reduction in percent hatch could be expected to occur at a much greater rate.

The effect of successive copulations in causing diluted sperm is emphasized by comparing the results obtained from Experiments 5 and 7. Untreated pairs of insects were confined together for 7 days
in Experiment 5 whereas in Experiment 7 all females were allowed to mate once.

Seven days after confinement, the females were treated in both experiments. The males were removed from Experiment 7 at this time but the males in Experiment 5 were not removed. By the next day in both experimental situations there was a reduction in the percent of egg hatch at the lowest dosage rate to about 55 percent of the original level.

In Experiment 5 there was practically no further reduction of percent egg hatch because new “untreated” sperm were added to the “sperm pool” available for fertilization. The percent hatch rose steadily toward the end of the sampling period, finally reaching about 95 percent of the original hatch level.

In Experiment 7, the percent hatch declined rapidly for two more days, then more slowly until it was nearly zero by the end of the experiment. It could be concluded from these results that the percent egg hatch remained low in the absence of new “untreated” sperm.

The continued decline in percent egg hatch as time passed indicates that metepa may also have some latent effect on the sperm. The possibility also exists that effects of metepa on the female may be manifested by a continuous, slow decrease in hatch of eggs even though the percent egg hatch is much lower than could be expected from treatment of the female prior to mating.

If untreated males and females are mated, and the females treated subsequent to the mating, as in Experiment 7, then the sperm present in the female at the time of treatment is considered to be treated. This supposition is substantiated by comparing the percent hatch of eggs from two groups of females that are allowed to mate only once and in which one group is treated prior to mating (Experiment 6) and the other group is treated subsequent to mating (Experiment 7).

The average percent hatch of eggs from females treated before mating was substantially higher than was the case with females treated after mating. This fact strongly suggests that the sperm contained in the spermatheca at the time a female was treated was affected by the application of metepa to the female. Apparently, mature sperm or the nuclear components of mature sperm have a great affinity for alkylating groups. The possible basis for this affinity will be examined in more detail in subsequent discussion.

In seeking explanations for increases in viability of sperm produced by treated males, several possibilities were considered. Such an increase could result from the return of viability to the mature “treated” sperm contained in the spermatheca of females or in the seminal vesicles of treated males. If such phenomena did occur it would indicate that the alklylation of the susceptible components of the sperm was a transitory reaction. However there is quite strong
evidence that such a “de-alkylation” does not occur as shown in the experiments in which “treated” sperm in the spermatheca of a female is not diluted by subsequent mating (Figures 12 and 14). In these 2 experiments, the females were treated after mating and were allowed no further copulations. Percent egg hatch was initially low at all dosage rates and did not increase for the duration of the experiment, thus indicating that alkylation of the susceptible components of sperm was permanent.

The most likely explanation for increases in sperm viability is that the mature “treated” sperm contained in the seminal vesicle of a treated male is depleted through continued copulation and is replaced by non-alkylated sperm.

The ability of testes in metepa-treated males to produce viable sperm may be a result of incomplete alkylation of potential receptors in the testes or it may be a result of metabolic elimination of the alkylated products. The nature of the alkylation reaction will be reviewed in more detail in a subsequent discussion.

Apparently the degree of recovery of sperm viability is dosage dependent. Increases in percent of egg hatch were substantial at the 41.6 µg/g dosage rate but extremely low at the 250.0 µg/g rate. Intermediate dosages gave clearcut intermediate effects. The percent hatch of eggs laid by check insects was maintained at a high rate throughout the experiments in which males were removed. Non-hatching eggs from all experiments were fertilized, thus indicating that sperm were actually present in the female.

**Effects of Treatment on Males:** The treatment of adult male milkweed bugs with metepa resulted in good response to variations in dosage rates as measured by the percent hatch of eggs laid by females mated to treated males. The differences in response are highly significant from a statistical standpoint and certainly indicate that metepa is rather specific in its mode of action in a biological system.

The effects of metepa on mature sperm have already been discussed and it has been shown that such effects are permanent for individual sperm exposed to metepa. Results from several experiments also indicate that spermatogenesis is affected by the application of metepa and that the effects may be transitory or permanent depending on dosage.

The evidence seems to be quite strong that the effects of the lowest dosage level are confined almost entirely to the mature sperm. This possibility is indicated by the results of Experiments 1 and 9C in which the recovery of sperm viability was quite high.

Exactly how much time is required for sperm to be discharged and replaced by new sperm under conditions of active copulation is not known but apparently spermatogenesis occurs throughout the life of the male; there was no evidence of sperm depletion even in 5-week-old males that had been mated frequently as adults. Addition-
ally, production of spermatogonia and subsequent stages in spermatogenesis were still active processes in 5-week-old males.

The results of treating males with high dosage levels of metepa were different in several respects from those obtained using low dosages. In Experiments 1 and 3, where the male treatments were applied prior to mating, complete sterility resulted and no eggs hatched for the entire experimental period.

In Experiment 4, the results were modified by the fact that the males were not treated until seven days after their confinement with untreated females. The fact that percent of egg hatch at the highest dosage remained higher than at the other dosage rates for about 14 days following treatment was an unexpected result, since in all the other experiments egg hatch was much lower at this treatment rate.

It appears likely that this result was caused by greater effect of sperm already present in the females prior to treatment of the males. Apparently sperm transferred to the females subsequent to treatment was not competing equally with pre-treatment sperm.

If it is assumed that “treated” sperm were indeed sterile (as should have been the case as indicated in all other experiments), than some factor in addition to sterility must have been in effect in sperm from males treated at the highest dosage.

It is possible that such sperm were less vigorous or less motile or perhaps a lesser number of sperm were available as a result of treatment.

It is apparent from examining the data that the two highest dosage levels have effects on gametogenesis in addition to effects on viability of the mature sperm. This is evidenced by the fact that no viable sperm was produced after treating the males at the 250.0 µg/g level (Experiment 1). Even at the 125.0 µg/g dosage the degree of recovery was relatively small.

Apparent effects on spermatogenesis were also noted in Experiment 3 but because the females were also treated it would be difficult to assess the meaning of the results in this case. It does appear that some egg hatch might be expected if the female treatment was considered as the limiting factor.

This possibility is shown in Experiments 2 and 6 in which females only were treated prior to mating. The percent of egg hatch may be reduced even more by compounding effects when both sexes are treated. This can be deduced from Experiment 3 in which the percent egg hatch was reduced in the 125.0 µg/g treatment considerably more than it was in any of the experiments where only one sex was treated.

The effects noted above on testicular tissue lead one to the conclusion that metepa is selective in a biological system in choosing chemical moieties to attack or substrates on which to act. When applied at a low dosage to a male, metepa appears to preferentially act upon
the sperm. There is little observable reaction with other tissues although it is probable that other reactions do occur.

The reasons for the vulnerability of sperm to metepa in comparison to other tissues is not readily apparent. One clue may be the physical condition in which the genetic material exists in the systems being considered. In the sperm, the chromosomes are de-condensed and thus may be more vulnerable to the alkylating action of metepa. The situation in the spermatocytes is reversed in that the chromatin is highly condensed and perhaps not so favorably exposed to attack.

An even more likely explanation for the affinity of metepa for sperm is that substrate conditions are more favorable in the sperm because of the relatively small amount of other components as compared to zones of development in the testes. The spermatids could possibly be the next most favored target since they would be second in the progression by ratio of non-nuclear receptor sites to metepa.

This hypothesis would satisfactorily explain the apparent selectivity of target tissues at the different dosage levels of metepa. If metepa existed in excess to the amount required to alkylate the susceptible sites of sperm, then some metepa would be available to react in the next most favorable environment such as the spermatids.

A continuation of this progression would mean that successively less favored targets would be affected by successively greater amounts of alkylating agent until enough tissues were affected to interrupt vital body functions. This degree of interaction between metepa and susceptible target tissue should eventually cause death, which it indeed does at very high dosage rates.

Effects of Treatment on Females: The affect of metepa on the female reproductive process is much more difficult to characterize than it is for males.

It is obvious that equivalent dosages on a weight-weight basis affect females less than males. Perhaps this is logical when the relative mass of the reproductive tissues in the sexes are compared. However, the total number of critical mitotic events is far greater in the male.

In view of this situation, it could be assumed that much of the metepa applied to female insects alkylates susceptible groups other than the nuclei of oocytes or oogonia.

In comparing the results of treatments made prior to mating in terms of percent of egg hatch, especially when treated at the low dosage levels the females produced eggs which originally increased and then decreased in percent hatch until the experiment was terminated. Percent hatch at the end of the experiment, however, was not much different from the original level.

By contrast, percent hatch of eggs from untreated females mated to treated males was originally low and gradually increased to an end point which was comparable to that observed for the treated females.
The reason for the lack of increase in percent egg hatch from females treated before mating is not particularly evident. It is possible that metepa does not act directly on mature oocytes as it does on spermatozoa and therefore initial percent egg hatch was comparatively lower for female treatment. Perhaps the effects of metepa on germinal tissue are not expressed at first and only become more evident as time progresses.

The effects of metepa treatment on the total number of eggs laid was most evident at the highest dosage rates. There was a significant reduction in the number of eggs laid per female per day in each instance where the females were treated at a rate of 250.0 µg/g.

There was also a significant reduction in the number of eggs laid at the 125.0 µg/g dosage rate in some of the experiments. Examination of the ovaries from females treated at the highest dosage revealed that considerable necrotic tissue resulted from metepa treatment. The extent of such necrosis varied even within the same ovary, with different ovarioles exhibiting various degrees of damage following treatment.

This apparent variation is a probable explanation for the ability of females treated at the highest dosage to continue to lay eggs at a reduced rate, since some of the ovarioles may not have been completely incapacitated by the damage they sustained.

The result obtained in Experiment 8 is interesting in several respects. The simultaneous increase in the numbers of eggs laid and the percent hatch of eggs appeared to result from the addition of untreated virgin males to the culture of three-week-old treated females and may indicate a relationship between sperm viability and sperm activity.

Perhaps the treated sperm stored in the spermathecae of the females lacked some essential quality which contributes to the process of oviposition. This quality may simply be vitality or motility or it may be a less obvious attribute.

**Histological Studies**

*Male Treatments:* Evidence of damage could not be seen in sections of testes of adult insects treated at 41.6 µg/g. It is certainly possible that some of the developing stages of spermatozoa were affected but this could not be determined by the techniques employed in this study.

The absence of visible morphological effects of metepa on testicular tissue from insects treated at the lowest dosage rate adds credence to the conclusion that effects of treatment at such levels were confined primarily to the mature sperm.

Examination of sections made from testes of insects treated at the two highest dosages showed that the morphologically evident effects of metepa were about equal at these rates.
However, the percent egg hatch data indicate that differences in effects between the two dosage levels actually do exist. Apparently such differences are of a nature too subtle to be seen by ordinary microscopy.

It was pointed out earlier that the reduction in percent egg hatch resulting from metepa treatment at the highest level was evident almost immediately after application.

The presence of mitotic configurations in a state of arrested development 2 days after treatment is shown in Plate V, Figure 16. It seems doubtful that such nuclei would mature in a normal manner although they do continue to develop.

Sections taken from testes removed 2 weeks after treatment (Plate II, Figure 4 and Plate V, Figure 17) show that all the developing germ cells have disappeared and the zones of development formerly occupied by them contain what appear to be differentiated spermatozoa.

This transformation is certainly not within the normal sphere of activity and the basis for such developments must be embodied in the alkylation reactions of metepa within the susceptible sites in the testes.

It could be speculated that the normal developmental activities in the germinal area of the testes maintains the spatial relationship between the various zones. The divisions that occur in the germarium continuously produce spermatogonia which divide and mature and move posteriorly into the zone of spermatocytes.

The cells in this latter zone divide and mature and in turn replace spermatids which are differentiating into spermatozoa. If all this divisional activity were to cease, then perhaps the result would be a cessation of the progression.

However, it is difficult to visualize how spermatogonia or even primary spermatocytes could develop into spermatozoa without further division.

Perhaps it could be speculated further that if the basic divisions occurring in the apical complex ceased as a result of the metepa treatment, no further impetus for movement of the cells would exist.

Examination of testes 4 weeks after treating adult males with metepa at a rate of 250.0 μg/g indicates that necrosis of the germ cell complex had taken place. It was difficult to characterize the testes but it appears that the only testicular structures remaining were the tubule walls and some dark-staining aggregates which may have been trophocyte nuclei.

Although the testes appeared necrotic 4 weeks post treatment, the seminal vesicle was still packed with sperm.

This could indicate that sperm in the testes had moved into the seminal vesicle to replace sperm transferred to females during earlier copulation.
It also could mean that a combination of a low rate of sperm transfer from the seminal vesicle and dissolution and resorption of testicular contents had occurred.

However, it was not determined how much of the sperm present in the seminal vesicle at the time of treatment was subsequently transferred during copulation. Copulations continued through 4 weeks between females and the males treated at 250.0 µg/g and fertilized eggs were laid.

It was shown that sperm were actually transferred as long as 3 weeks after treatment by the results of Experiment 9C.

In this experiment males treated 3 weeks previously were confined with virgin females, and these females subsequently laid eggs which were fertilized. This is only evidence that some sperm were transferred, however, and does not indicate the numbers involved.

Good evidence exists concerning the intermediate effects of metepa on testicular tissue when males were treated at a dosage of 83.3 µg/g. The metepa either does not affect all the germinative cells or its effects are transitory in nature. This conclusion was borne out by the degree of recovery in percent egg hatch in the experiments in which the males were treated. Although some of the primary spermatocytes appeared to be affected 2 weeks following treatment (Plate IV, Figure 12) the spermatocytes produced at the end of 4 weeks seem to be normal. The chromosomal configurations noted 2 weeks after treatment appear abnormal primarily because of the number of visible extra chromosomes. It could be speculated that these unusual aggregations of chromatin occurred as a result of faulty separation of chromatids during meiosis. It is also possible that the configurations represent incomplete attempts at mitotic division. Such configurations were not observed in any of the sections from testes fixed 4 weeks after treatment, although it is possible that some existed and were not observed.

**Female Treatments:** Sections of ovarioles from females treated at the lowest dosage rate were comparable to sections from untreated specimens. The differences noted in percent of egg hatch may have occurred as a result of a limited number of reactions which metepa might have with susceptible tissues.

The number of eggs laid by females treated at a rate of 41.6 µg/g is not different from the number of eggs oviposited by the check insects, which seems to indicate that the effects on percent egg hatch are quite specific at this treatment rate. The metepa probably reacts with receptor sites in the developing oocytes but not to a degree which inhibits maturity.

The appearance of ovaries from insects treated at the highest dosage level is quite indicative of the effects as expressed in terms of numbers of eggs laid and percent of egg hatch. Most of the ovarioles
were severely reduced in size and it appeared that the contents had been resorbed.

The more mature oocytes in such ovaries appeared to escape the necrotic effects described above and would probably have been subsequently oviposited. Egg numbers were greatly reduced and the percent of egg hatch was extremely low.

Oviposition persisted in some of the test insects until the end of the experimental period, indicating that the oocytes that had matured to some degree accumulated enough essential substances to complete their development to mature ova.

Morgan and LeBrecque (1962 and 1964) studied the effects of metepa and several other alkylating agents on the ovarian tissue of house flies and found that the first target of attack by alkylating agents such as metepa was likely to be the developing oocytes and secondarily the trophic cell nuclei. It appears that a similar type of selectivity for certain target tissues occurs in the milkweed bug, and the observations made here support the findings of Labrecque and Morgan.

**Mode of Action of Metepa**

Determination of the specific site of action of metepa, or other alkylating agents, has been a difficult problem for investigators. Several workers have investigated this phenomenon with some success but without obtaining completely definitive results.

Studies made by Ross (1958), as presented in Figure 2 through 5, show that many molecular sites within the testes or ovaries are potentially receptive to an alkylating agent. The fact that numerous potential sites for alkylation existed made it difficult for Stacey (1958) to explain the specific action of alkylating agents in preventing DNA and/or RNA from performing its proper function.

It has been shown (Price, 1958) that differences in availability of reactive sites exist in a biological system because of differences in dissociation constants but a significant number of such sites remain available.

With such information in mind, it would appear that a relatively large amount of alkylating agent would be required to exert its effects on a specific site of a particular molecule in the cell. However, Stacey, et al. (1958) pointed out that this supposition is not necessarily true. In actuality, a very low ratio of alkylating agent to cell component is necessary to induce an alteration of nuclear function.

In fact, the concentrations necessary to induce important changes in the cell are so low that most of the possible receptor molecules must be untouched.

In investigating the phenomenon of receptor sites, Ogston (1948) found that there was a wide variation in the rate at which even the most reactive forms of the various chemical end groups in cells com-
bined with alkylating agents. His study showed that the aromatic amino groups and the phosphoryl groups ranked rather high in relative reactivity with the alkylating agents, and indicates that there are few sites where extensive reaction takes place. Such a system still provides a fairly high number of possible reactive centers.

Clarification of this phenomenon was accomplished by Stacey (1958) who reported that under *in vitro* conditions, when an excessive number of sites was available for alkylation as compared to the amount of reagent, the most characteristic reactions were the esterification of an anion such as the carboxyl group in proteins and the phosphate groups in nucleic acids. The mere formation of such esters, however, was not considered to be an important reaction in producing radiomimetic effects. This belief was deduced from the fact that mono-functional agents can form such esters without exhibiting much biological activity.

Stacey, *et al.* (1958) investigated the effects of rupture of the DNA chain caused by alkylating agents and compared such results to the effects of ionizing radiation. Although both types of treatment can cause breaks in the main DNA chain, it was concluded that some additional phenomenon was involved.

The number of such breaks was observed to be relatively small and moreover the radiomimetic effects could be observed prior to DNA chain rupture.

At the present time, the precise mode of action by which the alkylating agents exert their effects is unresolved. Stacey, *et al.* (1958) proposed that the poly-functional character of the most active aziridine derivatives enabled them to produce crosslinks either between different groups in the same molecule or between molecules. In either case, the result could be an alteration of the character of the macro-molecule which could prevent it from exercising its normal function.

Some experimental evidence was presented by Stacey (1958) to support the DNA crosslinking theory. Treatment of herring sperm heads with bi-functional alkylating agents resulted in internal crosslinking of DNA molecules as demonstrated by light-scattering measurements. The molecular weight was unchanged but the average volume of the molecules was reduced, indicating a "coiling up" of the DNA polymers. The change was also revealed by electron micrographs which showed that untreated DNA molecules assumed rather straight configurations whereas the treated molecules were clumped into more compact aggregations.

Evidence of crosslinking between DNA molecules was demonstrated by the formation of gels when higher concentrations of alkylating agent were used. By measuring the protamine concentration before and after centrifugation, it was proved that this gel did not involve crosslinking with protamine.
Further evidence to support the crosslinking theory was presented by Brookes and Lawley (1961) through reacting certain mono-functional and di-functional alkylating agents with nucleic acids. Brookes and Lawley theorized that the site of alkylation is preferentially the N-7 moiety of guanine. This finding was later substantiated by Lett, et al. (1962).

If the findings of Brookes and Lawley, and Lett, et al. are correct and crosslinkages do occur between paired DNA strands as a result of reactions with alkylating agents, the effects of metepa treatment observed in this study might be explained on that basis.

If crosslinking of DNA strands is induced by metepa in the sperm, the phenomenon might be expressed following entry of the sperm into the egg.

The exact mechanism of fusion of the male and female pronuclei in the large milkweed bug has not been elucidated but fusion does take place prior to cleavage. It is assumed that there must be a restoration of the diploid condition in the fusion nuclei and that replication of chromosomes must occur prior to cleavage.

The existence of crosslinkages within DNA strands perhaps would not interfere with fusion of the chromosomes of the pronuclei. It is also possible that linkages between separate strands or between different points of the same strand would not interfere with the formation of the zygote nucleus.

Once fusion is complete, however, and the resulting diploid nucleus begins the process of replication of genetic materials, the probability of interference by crosslinked DNA molecules is greater.

If crosslinkages were numerous, it would be unlikely that sufficient replication could be accomplished to allow mitotic division to proceed.

On the other hand, if only a small number of such crosslinkages occur, perhaps replication could be accomplished and mitosis could proceed.

Failure of the zygote to initiate or complete mitotic division would certainly result in cessation of embryonic development, which would be the ultimate expression of a dominant lethal mutation. Such a chain of events would account for the observation that most eggs from the highest dosage treatments showed no embryonic development beyond the possible fusion of the pronuclei.

It seems likely that the extent of embryological development could be correlated with the extent of alkylation with metepa, although such a correlation would be difficult to measure. In general, advanced stages of embryogenesis were reached only in the eggs oviposited by females from the lower dosage treatments.

It is possible that mutations in the milkweed bug arise as a result of lost chromosome parts or translocations of chromosome parts. This possibility is apparently diminished by the fact that hemipteran
chromosomes have diffuse centromeres but it remains nevertheless an attractive hypothesis to explain some of the effects of metepa treatment (Virkki, 1965).

The avenues by which the chromosomal mutations might exert their effects must be multitudinous. Some of the routes are direct and apparently obvious in the cases where the fusion nucleus fails to divide or where the chromosomes of cleavage nuclei have difficulty in separating.

Beyond the effects on cleavage nuclei, however, it is impossible to define cause and effect relationships. Once the cleavage nuclei reach the periphery of the egg and begin to establish cell membrane boundaries and synthesize the various inclusions necessary to greater cellular activity, they are more vulnerable to expressions of chromosomal damage.

Up to this point in embryogenesis, the activities of the presumptive cells have been minimal and concerned primarily with DNA replication and sufficient metabolic activity to provide energy. Once a layer of complete cells is established, the production of the many proteinaceous compounds required for differentiation and subsequent embryonic development is necessary.

If the proper "genetic message" is lacking for the synthesis of a single enzyme, a whole series of events necessary for initiation of a given stage in embryogenesis could be effectively halted.

The fact that the effects of many mutations are not expressed until the later stages of embryological development suggests that the alkylation of some DNA occurs in a way that allows for continued replication.

It would be highly speculative to consider how replication could continue if crosslinkages existed between the complementary pairs of DNA strands. In order for replication to occur, it is presumed that the strands must separate so that each DNA strand of the pair can act as a template for production of a new complementary strand. It would conceivably be possible for such separation to occur successfully except for the point or points where the metepa molecule formed a linkage between the DNA strands.

Crosslinkage between paired strands could certainly result in the failure of fusion nuclei to divide if such crosslinkages existed in mature sperm. This occurrence could account for the very low percent egg hatch for eggs fertilized by sperm that was mature at the time of treatment.

Crosslinking may not, however, be a logical explanation for the results observed in matings in which it was known that the sperm were not mature at the time of treatment and that subsequent developmental divisions had occurred in the testes. The evidence is fairly convincing that divisions of developing germ cells do continue to
occur in testes following treatment of males with metepa but the possibility still exists that divisions do not occur in cells in which DNA strands are alkylated.

If alkylated DNA strands do not divide or replicate, then the sperm produced in the testes in treated insects must develop because by chance its preceding stages were not alkylated.

It is more likely, however, that sperm from “treated” testes arise from germ cells in which the character of the DNA has been altered. This probability is indicated by the fact that most successful hatches of eggs from treated insects were delayed for up to 48 hours longer than eggs from check insects with the only variable being metepa treatment.

This delay would indicate a reaction with metepa which is non-lethal in nature. For replication and division of the strands to occur, the proposed metepa–DNA linkages must either occur between points on the same strand or the cross-linkages must be of a nature which allows for separation of paired strands.

The separation of such crosslinked strands could conceivably result in deletions of parts of DNA molecules by breakage and recombination or by loss of the ends of some strands. This kind of damage could be hidden for many nuclear divisions until such time as the information embodied in the missing areas was required for particular developmental activities.

The normal course of events in replication and division of genetic material is still not well elucidated on a molecular basis. Many of the assumptions regarding DNA and RNA synthesis are largely hypothetical, especially with respect to the chemical interactions that occur during the process of nuclear division.

It has been observed that the effects of alkylating agents in causing chromosome breakages sometimes are not seen until several cell divisions following such alkylation (Borkovec, 1966). Because of these observations and because some nuclei continue to divide after being treated, it seems probable that a mode of action exists for metepa in the systems under consideration here other than, or in addition to, crosslinkage of DNA strands.

The common observation of delayed hatching in eggs from “treated” “matings” is perhaps of significance in considering future investigations. This indicates that the metepa caused some effects in the germ tissue which was expressed by delays in embryogenesis, and depressing effects on egg hatch in the next generation.

The assignation of reasons for these sub-lethal effects would be highly speculative since the avenues could be many. Perhaps such effects could stem from a genetic omission as a result of a metepa-induced translocation of small sections of chromosomes.

Such omissions could conceivably result in the lack of synthesis
of essential proteins, thus requiring substitute routes for energy or
synthesis of materials necessary for continued development.

Further speculation about the "delayed hatch" phenomenon could
encompass a great many theoretical situations, some of which could
have practical aspects. It is possible that the reaction causing the delay
phenomenon is somewhat specific. This possibility is indicated by the
fact that such delays occurred in all the individual eggs to about the
same degree for any given treatment on a specific day.

This simultaneous behavior could mean that some specific chem­
ical moiety within the sperm or egg is quite preferentially attacked by
metepa when a limited amount is available. If this speculation is true
then perhaps such specificity could be considered in future research
as an avenue towards insect control.

The effects of parental treatment could be carried over into subse­
quently generations as was suggested by North (1967) who noted aberra­
tions in the F2 generation of cabbage loopers. *Trichoplusia ni*, treated
with gamma radiation. Perhaps similar effects could be expected in
other Lepidoptera and Hemiptera or any other orders with diffuse
centromeres.

It appears that when the results of the various treatments in terms
of egg hatch and the histological observations made of tissues from
treated insects are considered, the action of metepa on the repro­
ductive tissues of the milkweed bug is varied.

A more precise knowledge of the prime reactions of metepa or any
alkylating agent within the systems involved with reproduction will
have to be gained before the effects can be accurately assayed.
Plate I

Figure 1. Anterior end of a testis from a 3-week-old untreated *Oncopeltus* male showing zones of spermatogonia (SPG), spermatocytes (SPC) and spermatids (SPD). The area below the zone of spermatids is filled with maturing spermatozoa. About 110X.

Figure 2. A section of testis from a 3-week-old untreated *Oncopeltus* male showing zones of spermatogonia (SPG) and spermatocytes (SPC). About 350X.
Plate II

Figure 3. Male organs from a 9-day-old adult treated with metepa at 250.0 µg/g, 2 days after treatment, showing the testis (T), zones of maturation including spermatozoa (S), the vasa efferentia (VE) and the seminal vesicle (SV). At this magnification the appearance is similar to that of an untreated testis. About 45X.

Figure 4. Male organs from a 21-day-old adult treated with metepa at 250.0 µg/g, 2 weeks after treatment. The zones of developing spermatogonia, spermatocytes and spermatids can no longer be seen. The cells normally found in these areas have been replaced by what appear to be spermatozoa. About 45X.

Figure 5. Male organs from a 35-day-old adult treated with metepa at 250.0 µg/g, 28 days after treatment. The usual zones of development are completely lacking and the testis has atrophied. The seminal vesicle has a normal appearance. About 45X.

Figure 6. Male organs from a 35-day-old untreated adult from a culture of mixed sexes. There is no discernible difference between testes at this age and those of 9-day-old adults. About 45X.
Plate III

Figure 7. A section of testis from a 21-day-old untreated Oncopeltus adult showing zones of primary (Pr) and secondary (Sec) spermatocytes. About 350X.

Figure 8. A section of testis from a 21-day-old untreated Oncopeltus adult showing a zone of primary spermatocytes. Most chromosomes are in a metaphase configuration and 9 sets of chromosomes can be counted in some of the spermatocytes. About 910X.

Figure 9. A section of testis from a 35-day-old adult showing zones of primary and secondary spermatocytes. No signs of deterioration are evident at this age. About 350X.

Figure 10. A section of testis from a 35-day-old untreated adult showing a zone of primary spermatocytes. About 910X.
Plate IV

Figure 11. A section of testis from a 21-day-old adult sacrificed 14 days after being treated with metepa at a rate of 83.3 µg/g. The appearance of this zone of primary spermatocytes (arrow) is similar to that observed in tissue from untreated testis (figure 7). About 350X.

Figure 12. A section of testis from a 21-day-old adult sacrificed 14 days after being treated with metepa at a rate of 83.3 µg/g showing a zone of primary spermatocytes. The chromosomes appear to be abnormal in configuration; the number of apparent sets is variable. About 910X.

Figure 13. A section of testis from a 35-day-old adult sacrificed 28 days after being treated with metepa at a rate of 83.3 µg/g, focusing on a zone of primary spermatocytes (arrow). This section of testis has a normal appearance, although the number of spermatocytes seems lower than average for a male of this age. About 350X.

Figure 14. Greater magnification of a zone of primary spermatocytes depicted in Figure 13. About 910X.
Plate V

Figure 15. A section of testis from a 9-day-old adult *Oncopeltus* sacrificed 2 days after treatment with metepa at a rate of 125.0 µg/g. The primary spermatocytes (arrow) are somewhat disorganized. About 350X.

Figure 16. A zone of primary spermatocytes from the same testis shown in Figure 15. Several spermatocytes have chromosomes in what appears to be an arrested anaphasic configuration. Chromosome bridges exist between the separated sets. About 910X.

Figure 17. A section of testis from a 21-day-old adult sacrificed 14 days after being treated with metepa at a rate of 125.0 µg/g. The darker areas near the upper portion of the photomicrograph correspond to the location of the zones of spermatocytes and spermatids. A trophocyte nucleus can be seen at the upper left. About 350X.

Figure 18. A section of testis from a 35-day-old adult sacrificed 28 days after treatment with metepa at a rate of 125.0 µg/g. Germinal tissue has apparently disappeared leaving only the tubule walls and other supporting structures. About 350X.
Plate VI

Figure 19. A portion of an ovariole from an untreated 28-day-old Oncopeltus adult. Part of the germarium (G), developing primary oocytes (OOC), and a section of trophic cord (TC) can be seen. About 48X.

Figure 20. An area of the germarium from an untreated 28-day-old Oncopeltus adult. Some of the visible structures are the trophic core (TC'), primary oocytes (POC) and trophocyte nuclei (TN). About 350X.

Figure 21. A distal portion of an ovariole from a 28-day-old adult 21 days after being treated with metepa at a rate of 250.0 µg/g. About 110X.

Figure 22. Greater magnification of the section depicted in Figure 21, showing what appear to be disorganized trophocyte nuclei and 3 multi-nucleate cells. About 350X.
Plate VII

Figure 23. A fertile Oncopeltus egg from an untreated mating fixed 4 hours after oviposition. Near the center of the egg is the fusion nucleus or structures associated with it. About 93X.

Figure 24. Greater magnification of the structure indicated as the fusion nucleus in Figure 23. About 350X.

Figure 25. An egg from an untreated mating fixed 24 hours after oviposition. A single layer of cells is visible at the periphery and several nuclei are scattered throughout the yolk. About 110X.

Figure 26. Greater magnification of a section of the cellular layer at the periphery of the egg shown in Figure 25. It can be seen that these cells have definite boundaries and are apparently complete cells. About 350X.
Plate VIII

Figure 27. An egg from a virgin *Oncopeltus* female fixed 24 hours after oviposition. This egg and all eggs from virgin females have less organized appearance and coarser yolk granules than fertilized eggs. About 120X.

Figure 28. Greater magnification of the egg in Figure 27, showing the lack of structure at the surface of the egg. About 350X.
Plate IX

Figure 29. An *Oncopeltus* egg from an untreated female mated to a male previously treated with metepa at a rate of 125.0 µg/g and fixed 24 hours after oviposition. A cellular layer does not exist at the surface and the nuclei scattered throughout the yolk are quite irregular in shape. About 110X.

Figure 30. An *Oncopeltus* egg from an untreated female previously mated to a male treated with metepa at a rate of 125.0 µg/g and fixed 24 hours after oviposition. Some structure exists at the surface of the yolk but it is not cellular. The nuclei are somewhat irregular in shape (arrows). The chromosomes of migrating cleavage nuclei are usually in anaphase as is the case here. About 350X.

Figure 31. Greater magnification of a different nuclei from those shown in Figure 30. At this magnification, it can be seen that the chromatin material is merely clumped near the center of the nucleus and is not well organized. About 910X.

Figure 32. Additional nuclei in the section shown in Figures 30 and 31. Definite irregularities in shape and arrangement of chromatin material exists in the nuclei visible here. About 350X.
Plate X

Figure 33. Sagittal section of an *Oncopeltus* egg from an untreated female mated to an untreated male showing normal embryonic development at 48 hours after oviposition. About 110X.

Figure 34. Same as Figure 33 at 72 hours. About 110X.

Figure 35. Same as Figure 33 at 96 hours. About 110X.

Figure 36. Same as Figure 33 at 120 hours. About 110X.
Plate XI

Figure 37. An *Oncopeltus* egg from an untreated female previously mated to a male treated with metepa at a rate of 125.0 µg/g. Fixed 144 hours after oviposition. The cleared area near the center corresponds to the area where the fusion nucleus is usually found. About 120X.

Figure 38. Greater magnification of the “structure” indicated by the arrow in Figure 37. This “structure” is difficult to characterize but it most probably represents the remnants of a fusion nucleus. About 350X.

Figure 39. An *Oncopeltus* egg from an untreated female previously mated to a male treated with metepa at a rate of 83.3 µg/g. Fixed 120 hours after oviposition. This partially developed embryo is abnormal and it appears that embryonic development ceased or became aberrant at some stage beyond the time that germ band formation usually occurs. About 110X.

Figure 40. An *Oncopeltus* egg from the same source as shown in Figure 39. Fixed 144 hours after oviposition. Development has proceeded further than that in the embryo shown in Figure 39. Some appendages have been partially formed but otherwise the embryo cannot be characterized. About 110X.
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