Mass feeding of Rhodnius prolixus Stal (Hemiptera: Triatomidae): An experimental approach to the study of drugs in invertebrate systems using a cholesterol biosynthesis inhibitor.

Edward J. McGuire
University of Windsor

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MASS FEEDING OF RHODNIUS PROLIXUS STAL (HEMIPTERA: TRIATOMIDAE) : AN EXPERIMENTAL APPROACH TO THE STUDY OF DRUGS IN INVERTEBRATE SYSTEMS USING A CHOLESTEROL BIOSYNTHESIS INHIBITOR.

A Thesis
Presented to the School of Graduate Studies of The University of Windsor

by Edward J. McGuire

BIOLOGY

In partial fulfillment of requirements for the degree of Master of Science

April, 1971
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ABSTRACT

The cytology and ultrastructure of the fat body cells in *Rhodnius prolixus*, differentiating after feeding on human blood and similar meals with biphenylyl-4-methyl valeric acid (BMVA) additives, are described. When the cells receive nutrient they are induced to an activated state from a period of diapause or reduced metabolism. The animals acquiring both nutrient and a drug concentration illustrate alterations of mitochondrial and rough endoplasmic reticulum structures within the fat body.

Administration of the various concentrations of BMVA in human blood exert an effect on the morphology of the insects. The percentage of mortality after treatment with 21.0 and 42.0 mg of the drug is twice as much as observed in the controls. Only twenty-three percent of the insects ingesting the highest drug concentration moult normally to the fifth instar. This group also demonstrates an accelerated weight loss in the fifteen days following feeding.

The drug, an inhibitor of cholesterol biosynthesis in rats, could possibly interfere with the synthesis of ecdysone which has a similar chemical basic structure to cholesterol. On the basis of this work it is suggested that this drug may have a potential advantage in insect control but further
investigations are necessary.

Also described in this thesis is a mass feeding technique for haemophagus insects, which allows the feeding of human blood and materials suspended in it.
ACKNOWLEDGEMENT

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My thanks also to Dr. W. G. Friend and Miss Edna Cartwright, Ramsay Wright Zoology Laboratories, University of Toronto, for their generous help and assistance in establishing a Rhodnius culture at the University of Windsor.

The compound, biphenylylmethyl valeric acid, supplied by Warner-Lambert Research Institute, Morris Plains, New Jersey, U.S.A. and the human blood supplied by Grace Hospital,
Windsor, Ontario are gratefully acknowledged.

Finally, I would like to thank my parents for their concern and constant encouragement, as well as Miss Erma Sanzosti, who also typed parts of this manuscript.
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INTRODUCTION

Biphenylylmethyl valeric acid (BMVA) has been shown to be an effective inhibitor of cholesterol biosynthesis and also an inductor of liver enlargement (Eades et al., 1966a). This hepatotrophic effect is due to an absolute increase of the liver mass without any change in the number of hepatic cells (de la Iglesia et al., 1969b). Stereologic studies on the hypertrophied liver cells of rats revealed a cytoplasmic volume increase as a result of an increase in the size and population of the liver microbodies (de la Iglesia et al., 1969 c,d).

The specific action of BMVA to inhibit cholesterol biosynthesis and to affect the liver in rodents was the basis to test this drug in an insect, Rhodnius prolixus. Two objectives were posed in this study; one is related to insect development and the other to cellular and subcellular effects in the insect liver or fat body.

Current views on the endocrine control of insect development can be summarized as follows. A hormone produced in the neurosecretory cells of the brain is transported by the axons that end blindly in the corpora cardiaca, from where it is released into the blood (Wigglesworth, 1953). The brain hormone is called thoracotrophic hormone, because of the effects it has on the thoracic glands. The thoracic gland is
stimulated to produce a hormone to cause the epithelial and intersegmental muscle cells to begin the process of the moulting phase. The thoracic gland hormone is called ecdysone. At the same time, at all of the moults except the last, the corpora allata secrete another hormone, the juvenile hormone, also necessary for the development of the larval stages in the life cycle of the insects.

Butenandt and Karlson (1954) extracted 25 mg of crystalline moulting hormone from *Bombyx* pupae. This crystalline extract, which was the hormone ecdysone, induced pupation when as little as 0.0075 µg was injected into blowfly larvae. An accurate chemical analysis was not accomplished on this hormone for some ten years later because of difficulties encountered in crystallizing sufficient quantities. Ecdysone proved to be indisputably a steroid, with a structure shown below (Karlson et al., 1963c).

![Formula for ecdysone](image)

**FIGURE 1: Formula for ecdysone**

Therefore ecdysone has the same chemical basic structure as
cholesterol, illustrated in Figure 2. It was found that when radioactivity labelled cholesterol was administered to Calliphora larvae, great amounts of radioactivity were incorporated into ecdysone (Karlson et al., 1963a). Cholesterol, therefore, seemed to be the precursor of ecdysone in insects. If these findings are correct, the administration of the drug to insect larvae should be detrimental to their development. In the first objective we want to determine whether a drug related effect on insect development can be revealed. Fundamental for the second objective is the consideration that the insect fat body is analogous with the mammalian liver (Kilby, 1963; Orr, 1964). As in mammals, the insect fat body is the site of intermediary metabolism. Further the cell hypertrophy observed in the liver of rats took place in actively functioning cells; it was of interest to test the drug effects in cells in a resting stage or "deactivation", and to analyse effects of cholesterol.

FIGURE 2: Formula for cholesterol

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biosynthesis inhibition when a rapid increase in anabolic activities takes place.

*Rhodnius prolixus*, a blood sucking insect, is a vector of the deadly Chagas disease in South America. This insect has been intensively investigated, not only in areas of morphology and physiology, but also in the mode of feeding employing a feeding apparatus. In this thesis an artificial mass feeding technique is described, which makes *Rhodnius* a valuable adjunct in the study of drugs or other chemicals in biological systems.
MATERIALS AND METHODS

A. Culturing of Rhodnius prolixus

The insect stocks were reared or maintained in incubators at 26°C and about 45 percent humidity. The animals were kept separated in different glass culturing jars according to their stage of the life cycle. Once a month the insects were fed a blood meal using a laboratory rabbit host.

B. Artificial Mass Feeding Technique

(1) Previously Available Artificial Methods

The administration of BMVA to large numbers of Rhodnius prolixus demanded the development of an artificial feeding technique unique from previously available methods. With Harington's apparatus (1960) problems were encountered to fill the vial with blood without trapping air bubbles against the membrane and in maintaining the proper temperature of the diet. Friend (1963) solved these problems and in addition the apparatus had the advantage to feed expensive or radioactive materials. However, a disadvantage of both methods was that only one insect could be fed at any one time and it was also impractical to use human blood because of settling in cellular elements. This separation also applied to additives if present in a suspension in the blood. The apparatus developed here, not only has overcome such deficiencies, but also incorporated some of the ecological
requirements in feeding of Rhodnius.

(2) Environmental Conditions For Successful Feeding

Ecological requirements for feeding include temperature of both environment and nutrient meal, the presence of carbon dioxide, darkness and moisture. The most important factor seemed to be temperature. When holding a jar containing the insects it was noted that they were attracted to the sides at places where the glass was warmed by fingers; therefore, Rhodnius seems to be attracted predominately by warmth. Another precaution for successful feeding was minimal handling of the insect; any disturbance interfered in the feeding response. Further the animals preferred feeding in the dark and were repelled if their legs came in contact with moist surfaces. Finally, initiation and continuation of feeding seemed to be aided by the presence of carbon dioxide.

(3) Description and Success of the Artificial Mass Feeding Technique

One hundred milliliters of human blood were poured into a rubber latex membrane containing a glass vial about the size and shape of an 80 ml beaker and a magnetic stirring bar (Figs. 4 and 5). Then the blood was warmed to 37°C. For feeding the blood container was placed in a water bath in such a position to allow the initiation of stirring at several intervals during the feeding period. The magnetic stirrer, located under the water bath (Figs. 3 and 5), was not operated throughout the entire feeding time because of
liberation of air from the blood; instead the stirrer was
turned on three or four times for a one minute interval
during the total feeding time of thirty minutes.

Immediately prior to feeding, the insects were carefully
transferred to a chilled feeding jar (Figs. 3 and 5). This
feeding jar differed from the incubating container only by
a wider wire mesh top (Fig. 5). Pre-cooling (for thirty
minutes at 4°C) was beneficial to feeding since a thermal
gradient differential was emphasized; the temperature of the
environment for the animals was cooler than the blood (37°C),
and therefore the attraction was more pronounced. To avoid
a thermal shock it was important that the insects did not
chill prior to contact with the feeding membrane. Further,
it was found advisable to place the feeding jar on the
membrane at a slight angle to be certain that trapped air in
the membrane didn't interfere with the feeding of the insects.

Another innovation in this technique was the use of a
stream of carbon dioxide (about 50 ml. per minute) bubbling
from the water of the temperature control bath (Figs. 3 and 5).
Although this was not a strict requirement, it seemed to
contribute to a greater feeding success. To ensure that
light didn't interfere, and to maintain a moist and carbon
dioxide enriched environment, a black cloth covered all of
the feeding assembly.

Several reasons could be advanced when *Rhodnius* failed
to gorge fully over a thirty minute feeding time. Often the
temperature of the blood dropped below 37°C; this was easily
Fig. 3. Complete Feeding Apparatus:
(A) Carbon Dioxide Cylinder
(B) Standard 10 ml Pipette
(C) Feeding Jar
(D) Thermometer
(E) Water Bath
(F) Magnetic Stirrer

Fig. 4. Glass Vial and Stirring Bar.
**LEGEND FOR FIGURE**

Fig. 5. Diagram of apparatus for mass feeding of *Rhodnius*. CO₂, carbon dioxide container; fj, feeding jar; fm, flow meter to regulate delivery of CO₂ at 50 ml./min.; gv, glass vial containing blood; lm, latex membrane; m, stirring bar employed to provide uniformity of suspension during feeding; t, thermometer; tb, thermostatic bath, set at 40±1°C; wmt, wire mesh top (625 openings/sq. in.) placed on membrane covering glass vial.
corrected by rewarming the blood and chilling the feeding jar, while the partly fed animals remained in the 26°C incubator. At times air bubbles created a mechanical obstruction or due to improper handling the insects were too excited to feed.

In our laboratory Rhodnius prolixus has been reared from second instars, through the normal life cycle to the adult stage. Using this artificial technique, it was noted that animals ingesting one times their body weight in human blood, did not moult to the next instar, but those gorging 2.5 times moulted normally; some insects fed to such an extent as to ingest six times their body weight.

These methods of feeding Rhodnius ensure the constant homogeneity of the nutrient meal and the compounds dissolved and/or suspended in it. Mass feeding of up to sixty insects can be easily accomplished using this apparatus. There are also possibilities that insect cultures can be maintained in this manner with the elimination of the laboratory rabbit host. These techniques have wide applications and potentials, not only in the areas of drug testing, but also in research using other blood sucking insects.

C. Experimental Design

(1) Nutrient Meals and Drug Additives

In this study only fourth instar larvae of Rhodnius prolixus were used. De la Iglesia et al. (1969d) administered
30 mg of the drug to rats per 100 g of body weight. Calculations revealed that administration of the same drug concentration to Rhodnius required 10.5 mg of BMVA in 100 ml of human blood based on the finding that fourth instars ingested about 1/20 milliliter of nutrient to moult successfully. However, these calculations of drug concentrations acquired by the insects are somewhat a speculation, because BMVA was fed in suspension. Since there was no way of knowing whether the 10.5 mg concentration would be equally effective in Rhodnius, in the experimental design three other concentrations were tested. Three groups of fourth instar insects received a drug-nutrient meal of 10.5 mg, 21.0 mg and 42.0 mg per 100 ml human blood and are referred to as A, B and C groups respectively. A fourth group served as controls and were fed on blood alone. All blood used in these studies was obtained from a hospital (Grace Hospital, Windsor, Ontario) as outdated whole blood.

(2) Morphology

Larvae in the four experimental groups were examined for changes in weight and morphology. All insects were weighed individually before and after completion of the meal. As cited earlier an insect ingesting at least 2.5 times its body weight in human blood moulted. Consequently, only animals acquiring this weight relationship or more were included in the experimental groups. The day of feeding was considered to be Day 0. To follow the weight loss with time of drug treated and control groups the insects were weighed.
in groups at daily intervals up until nine days following Day 0. Individual weights were not recorded because this excessive handling of the organisms caused interference with the normal onset of moulting. The animals were also weighed on the fifteenth day after feeding. The criteria employed to assess effects on growth were onset and duration of moulting, as well as, successful moults and mortality.

(3) Electron Microscopy

For ultrastructural studies of the fat body animals from the four groups described above were sacrificed at twenty-four hour intervals following the nutrient meal, up until six days. Some insects were also sacrificed twelve hours after feeding. With fine scissors the body cavity was cut open along the dorsal-ventral midline and immediately some Dalton's fixative injected. The upper cuticule was detached, taking care not to loosen the net-like array of the fat body remaining attached to the cuticule and placed in a few drops of the fixative. With a scalpel this dorsal abdominal shell was divided into four quarters by cutting along the midline and between the third and fourth posterior segments. After one hour of fixation in Dalton's fixative, the tissue was dehydrated in ethanol and infiltrated in a mixture of epon-araldite. A special tissue orientation method was employed as a standard to ensure examination of large areas of the fat body cells. Embedding began by filling the lid of a Beem capsule with some embedding medium, into which the cuticule and adhering fat body were oriented in
such a way, that the fat body projected towards the plastic lid. The remainder of the capsule, without the pointed end, was now attached to the lid and filled with the embedding medium. After exposure of the capsule for one hour to a mild vacuum, trapped air from the tissue and liquid plastic was removed and the plastic polymerized at 70°C.

The blocks were sectioned with a Dupont diamond knife on a Reichert Om U2 ultramicrotome. After staining in uranyl acetate (saturated solution in 70% alcohol) and lead citrate solutions (Kay, 1965), the sections were examined in a Philips 300 electron microscope. To correlate the findings on the electron microscopic level 1µ sections from the plastic blocks were stained with toludine blue O (Kay, 1965) and hemotoxylin/eosin (Warner-Lambert Research Institute, Sheridan Park Research Community, Clarkson, Ontario, personal communication, 1971), or subjected to the periodic acid-Schiff technique (Kay, 1965) for light microscopic examination.
RESULTS

A. The Effects of BMVA on the Morphology of Rhodnius

(1) Body Weight

Fourth instar larvae of Rhodnius prolixus in all four experimental groups ingested about three times their body weight in blood (Table I, part 1). The series of average weight losses for the nine days after feeding and the fifteenth day are recorded in the second part of Table I. The values are expressed as percent loss of the original average weight recorded at Day 0, following the nutrient meal. Up until six days after feeding animals in the four experimental groups lose weight at a similar rate. In subsequent days the controls lose weight up to a maximum of thirty-eight percent of the original average weight noted at Day 0. The rates of decrease in Groups A and B behave in a similar manner, although at Day 6 animals in Group B lose more weight than the insects in Group A. An accelerated weight loss is apparent in Group C from the fifth day on, reaching almost half of the original average weight after the blood-drug meal.

(2) Onset and Duration of Moulting

Table II indicates that no significant change is observed regarding the normal onset of moulting, whether fed a blood-drug mixture or a blood meal alone; it takes place between thirteen and fifteen days after feeding. Also, the duration in which the animals moulted is normal. Only two
TABLE I. Body Weight and Weight Loss of *Rhodnius prolixus*
Following Administration of Drug Concentrations.

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<td>- Unfed</td>
<td>14.13±0.48</td>
<td>14.36±0.41</td>
<td>13.80±0.42</td>
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<td>- After Meal (Day 0)</td>
<td>54.90±1.97</td>
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<td>26</td>
<td>28</td>
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<tr>
<td>15</td>
<td>38</td>
<td>35</td>
<td>35</td>
<td>48</td>
</tr>
</tbody>
</table>

* Animals were treated as described. Controls received a human blood meal. Groups A, B and C received the same meal mixed with 10.5, 21.0 and 42.0 mg. of drug per 100 ml., respectively.

a. In milligrams, average ± S.E.M.

b. Percent difference from Day 0 values, recorded after feeding.

** Not determined.
larvae moulted beyond the twentieth day after feeding, on the twenty-first and twenty-fifth days.

(3) Moulting Ratios

The most significant morphological effect of BMVA is the inhibition of moulting to the fifth instar. One hundred percent of the insects in Group A and all but one in the control group moult normally. However, in Group B, eighty percent moult which is not mathematically significant, and only twenty-three percent in Group C (probability<0.005). Animals which moult after drug administration did not exhibit morphological abnormalities; no evidence of the formation of a cuticle can be noted in larvae which fail to moult. A conspicuous finding constantly observed is that any animal acquiring BMVA appears more static than the control group in the initial several hours following feeding.

(4) Mortality Ratios

It is apparent (Table II) that the mortality ratios are moderate in the controls and in Group A, and are more pronounced in the two groups receiving the highest drug concentrations. However, even though the percentage dying in Group C is twice that observed in the control group, no significance can be attributed to this figure (probability 0.995). Repetition of this series of experiments (Table III) reveal the same findings except for the death rate which is totally eliminated. It may be that excessive handling accounts for this mortality rather than the drug effects.
TABLE II. Onset and Duration of Moult, Moulting Ratio and Mortality of *Rhodnius prolixus* in Control and Drug Treated Groups.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>A</th>
<th>B:</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moult-Onset Day</td>
<td>13</td>
<td>13</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>-Last Day</td>
<td>20</td>
<td>23</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Moulting Ratio</td>
<td>16/17</td>
<td>18/18</td>
<td>9/11</td>
<td>3/13*</td>
</tr>
<tr>
<td>Mortality</td>
<td>3/20</td>
<td>2/20</td>
<td>5/16</td>
<td>5/18**</td>
</tr>
</tbody>
</table>

* P<0.005

** P<0.995 (not significant)
TABLE III Onset and Duration of Moult, Moulting Ratio and Mortality of *Rhodnius prolixus* in Control and Drug Treated Groups.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moult- Onset Day</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>--</td>
</tr>
<tr>
<td>- Last Day</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>--</td>
</tr>
<tr>
<td>Moulting Ratio</td>
<td>15/15</td>
<td>15/15</td>
<td>11/15</td>
<td>0/14</td>
</tr>
<tr>
<td>Mortality</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>
B. **Ultrastructure of Fat Body Cells Following Blood Nutrient Meals**

(1) **Nucleus**

Morphological investigations were undertaken of the liver cells following feeding of the larvae after starvation for eight weeks. The most obvious component of the cells, at all time intervals, is the nucleus. Except for the prominent nucleolus the nucleoplasm of the insect liver cells fixed in Dalton's fixative is homogeneous, which is similar when lead citrate alone is used as a stain. However, if uranyl acetate and lead citrate are employed in combination the chromatin material is well contrasted against the homogeneous nuclear matrix (Fig. 6). In starved animals and those fed nutrient meals, the chromatin is clearly recognizable as dense granular areas around the periphery of the nucleus and surrounding the nucleolus. The nucleolus is visible as a refractile structure, usually eccentrically placed in the nucleus and sharply demarcated from the remainder of the nucleoplasm. It forms part of an irregular shaped mass which stains intensely with basic dyes. Another component of this irregular mass is the nucleolus-associated heterochromatin (nucleolar satellites). The nucleolus proper seems to be made up of threadlike elements usually disposed as a network. Therefore, this nucleolus consists of coarse dense strands branching and anastomosing to form an irregular tridimensional structure. This nucleolonema is organized around one or more
Fig. 6. Electron micrograph of fat body cell 24 hours after receiving the human blood nutrient meal. The nucleus (N) demonstrates a large nucleolus (NU), as well as dense granular chromatin material (CM). (X 18,350)
spherical bodies, composed of closely compacted fine
granules of lower electron density than that of the other
elements of the nucleolus. This structure was referred to as
a nucleolar vacuole, and contains chromatin material which
seems to originate as a projection of the nucleolar associated
chromatin (Millen, 1967).

After a blood meal the first changes between twelve and
twenty-four hours are enlargement of the nucleolus and
nucleus, and accumulation of chromatin within the nucleus
and surrounding it in the cytoplasm. The increases in size
of the nucleolus and nucleus parallel enlargement of the
cell which about doubles in the six days following feeding.

In some nuclei distinct morphological structures are
observed. These structures seem invariably associated with
chromatin material, some being circular in shape and
surrounded by a double limiting membrane (Fig. 7). Within
such configurations a fine granular substance is evident
in a non-electron dense background. Many of such structures
are further divided into two to six compartments
(Figs. 8 and 9). The significance of these occurrences is not
understood. In these studies ten such configurations are
noted in over 900 electron micrographs, representing
thirty-two animals, examined over six days after feeding.
Based on these observations such inclusions in the nucleus
are rare and remain unexplained.
Fig. 7. Note an inclusion (arrow), bounded by a seemingly double limiting membrane within the nucleus of a fat body cell 120 hours after feeding. (X 29,520)

Fig. 8. Portion of fat body cell nucleus 12 hours following feeding. Nuclear inclusions (arrows) are associated with chromatin material. (X 29,520)

Fig. 9. Inclusion bodies of varying morphology and internal structure (arrows), 96 hours after blood meal. (X 29,520)
(2) **Endoplasmic Reticulum**

Changes in the endoplasmic reticulum are initiated shortly after feeding and reach various levels of expression in the following days. The rough endoplasmic reticulum (RER) occurs in two distinct morphological forms, as vesicular and cisternae types. The vesicular form does not constitute the continuous system of cytoplasmic channels, but is still considered to be part of the RER.

As time passes following feeding the cisternae type (Fig. 10), becomes distended and irregular in shape until at four days almost the entire cell is occupied by granular endoplasmic reticulum (Fig. 11). In starved insects few cisternae occur, mostly of single strands, but two to three layered lamellar structures are noted (Fig. 10). These organelles are concentrated for the most part around the periphery of the cell or close to the nuclear membrane. The vesicular distended variety occurs rarely in starved insects or twenty-four hours after the blood meal.

An increased quantity of cisternae appears as early as twenty-four hours after feeding (Fig. 12). Also here it is concentrated near the nuclear and cellular membranes, but now the cisternae display multi-layered structure. From the twenty-fourth hour on this variety of RER increases in amount and degree of distention to a maximum about four days post-feeding. The vesicular variety of RER becomes highly prominent by two days and continues to increase up to four days after the blood meal (Fig. 13).
Fig. 10. The cisternae variety of RER found in starved insects is located mainly around the nucleus or the periphery of the cell. The quantity of RER is highly reduced due to starvation. (X 18,350)
Fig. 11. Photomicrograph of fat body cell present at 96 hours post-feeding. Note the interdigitating processes (IP) of the cellular membrane, the position of many mitochondria (M), the large quantity of RER and the extracellular coat (EC), both on the cell surface and in the lumen (LU). (X 18,350)
Fig. 12. At twenty-four hours after a blood meal the cisternae type of RER is increased. Small quantities of the distended vesicular variety (arrows) are also evident. (X 18,350)
Fig. 13. Fat body cell cytoplasm 96 hours after feeding shows that much of the RER is of the distended vesicular type after 48 hours. (X 18,350)
In cells with large quantities of the vesicular and cisternae varieties, also the smooth endoplasmic reticulum occurs in the form of Golgi complexes (Figs. 14 and 17). Associated with the Golgi are vesicles containing an electron dense material. Similar small vesicles are present in close association with the plasm membrane. Between three and six days after feeding, in some areas of the fat body large smooth vesicles are located in abundance near the periphery of the cell (Figs. 15 and 16). These cells also demonstrate large vesicular RER of comparable size and density.

(3) Extracellular Coat

An electron dense band or coat is noted between most cells at two days after feeding (Fig. 17). As time progresses, by three days, such a band is also regularly observed around the periphery of the fat body organ (Fig. 18), which is most pronounced by four days after a nutrient meal (Fig. 11). Adjacent to the extracellular coat surrounding the organ in the lumen or hemocoel of the larvae, granules of varying configurations frequently occur (Figs. 11 and 18). Associated with this coat, composed of a granular material, interdigitating processes of the plasma membrane penetrate deep into the cytoplasm of the cell. The mitochondria of these cells, for the most part, concentrate near the interdigitating processes. Further the cisternae variety of granular endoplasmic reticulum constitutes the major component of the cytoplasm.
Fig. 14. Fat body cells, 144 hours after a blood meal, demonstrate a large number of Golgi complexes (G) relative to those up to 48 hours after feeding. (X 18,350)
Fig. 15. At 144 hours, in some areas of the fat body cells, large smooth vesicules (arrows) as well as distended vesicular RER occur. (X 18,350)
Fig. 16. The smooth vesicles (arrows) noted 144 hours after feeding accumulate at the cell periphery. 
(X 18,350)
Fig. 17. An extracellular coat (EC) appears between the fat body cells at 48 hours after a nutrient meal. (X 18,350)
Fig. 18. At 72 hours after feeding most cells demonstrate an extracellular coat (EC), both between the cells and on the periphery of the fat body. Note a similar material in the interdigitating processes (IP) and in the lumen (LU). (X 18,350)
A similar extracellular coat as described is occasionally noted in unfed larvae. However, in these animals starved for eight weeks, such a coat is highly reduced as is the quantity of interdigitating processes.

(4) Storage Materials

The fat body is the site of intermediary metabolic activity involving the synthesis and secretion of glycogen and proteins, as well as the uptake of proteins and the accumulation of lipids (Wigglesworth, 1953). Prolonged larval starvation causes a depletion of the fat body reserves (Millen, 1967). The increase in nucleolar, nuclear and cellular size as well as the quantity of storage materials after feeding depends upon the length of time of starvation. Millen (1967) reported that starving fourth instar larvae for six months lead to an increase of nucleolar, nuclear and cellular size by about 3.0 times at five days after receiving a blood meal. This applies also to the quantity of fat reserves. In these investigations the larvae were starved for only eight weeks and a one-fold increase of these conditions is observed by five days following feeding.

These investigations also demonstrate a metabolic cycle in the fat body of fourth instar larvae. Within twenty-four hours after feeding glycogen deposits increase considerably (Fig. 19), but by five days are reduced to a level found in starved animals (Fig. 20). In contrast, the lipoidal inclusions steadily increase over the same period.
Fig. 19. Large quantities of glycogen (GL) are found in the cytoplasm of the fat body cells 24 hours after the blood meal, which are not present in insects starved for eight weeks. (X 18,350).
Fig. 20. The glycogen deposits are seriously depleted in the fat body by the fifth day after feeding. (X 18,350)
Mitochondria

The changes in mitochondrial morphology following the blood meal are reported in Table III. It includes a mitochondrial total count and a distinction between a round and elongated variety of this organelle. The total number of mitochondria per cross-sectional area decreases between the twelveth and twenty-four hour after the blood meal. Only slight reductions are noted beyond twenty-four hours up until the sixth day post-feeding. It is also apparent from this table that between zero and twenty-four hours many mitochondria convert from the rounded type (Fig. 21) to the elongated variety (Fig. 22). Approximately twenty-five percent are of the elongated type at twelve hours, but subsequent periods demonstrate about double this percentage. In one animal four days after feeding, sixty percent is of the elongated variety. This increase in numbers of elongated mitochondria occurs when the insect liver cells become activated; therefore, this variation in shape could reflect an increase in mitochondrial activity.

The ultrastructural investigations of the mitochondria reveal slight increases in size at twenty-four hours after feeding, which remain fairly constant for the duration of the period of observation, as well as morphological features relating to the mechanism of mitochondrial genesis. The elongation of mitochondria as described seems to occur just before or during the formation of an extremely long cristae, initially extending from one side of the organelle.
### TABLE IV  Number and Types of Mitochondria at Various Time Intervals After Feeding

<table>
<thead>
<tr>
<th>HOURS</th>
<th>TOTAL MITO.</th>
<th>ROUND MITO.</th>
<th>ELONGATED MITO.</th>
<th>PERCENT MITO. ELONGATED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I  II</td>
<td>I  II</td>
<td>I  II</td>
<td>I  II</td>
</tr>
<tr>
<td>0</td>
<td>54.10 52.70</td>
<td>42.30 37.30</td>
<td>11.80 15.40</td>
<td>21.81 29.22</td>
</tr>
<tr>
<td>12</td>
<td>76.60 56.10</td>
<td>58.00 38.70</td>
<td>18.60 17.40</td>
<td>24.28 31.02</td>
</tr>
<tr>
<td>24</td>
<td>44.00 35.80</td>
<td>28.90 22.80</td>
<td>15.10 13.00</td>
<td>34.32 36.31</td>
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<td>48</td>
<td>39.90 34.70</td>
<td>22.30 19.70</td>
<td>17.60 15.00</td>
<td>44.11 43.23</td>
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<tr>
<td>72</td>
<td>40.20 38.60</td>
<td>19.00 17.10</td>
<td>21.20 21.50</td>
<td>52.74 55.70</td>
</tr>
<tr>
<td>96</td>
<td>25.30 33.00</td>
<td>10.50 18.40</td>
<td>14.80 14.60</td>
<td>58.50 44.24</td>
</tr>
<tr>
<td>120</td>
<td>29.20 33.70</td>
<td>15.40 20.80</td>
<td>13.80 12.90</td>
<td>47.26 38.28</td>
</tr>
<tr>
<td>144</td>
<td>31.30 36.20</td>
<td>18.00 18.00</td>
<td>13.30 18.20</td>
<td>42.29 50.28</td>
</tr>
</tbody>
</table>

Every value represents the mean number of normal mitochondria per 70 mm exposure (10 electron micrographs were counted in each case).

Two animals (designated I and II) were observed at each time.
Fig. 21. Insect liver cells 12 hours after feeding displaying that the majority of mitochondria are of the round variety. (X 18,350)
Fig. 22. As early as 24 hours after feeding the percentage of the mitochondria of the elongated type has increased. (X 18,350)
only (Fig. 23). Some mitochondria display such a crista extending across the entire structure to divide the matrix into two distinct compartments (Fig. 24). In still others are noted such partitions as well as constrictions (Figs. 25 and 26), suggesting that the continuation of a constriction results in a division leading to two organelles (Fig. 27). This line of argument is further supported by the observation that clustering of mitochondria is often noted, when such developments occurred as described (Fig. 28).

(6) **Lysosomes**

Another conspicuous cytological element noted in the fat body cells of fourth instar larvae are lysosomes. Structures classified as lysosomes are electron dense bodies surrounded by a single limiting membrane (Ham, 1969). In *Rhodnius* the great majority of these organelles show little or no internal structure (Fig. 29). The single membrane often seems separated from the contents of the organelle by a crescent gap which gives the appearance of a thin halo configuration (Fig. 30).

A great variety of other organelles occur, all classified as lysosomes (Ham, 1969). The reason these structures vary so greatly in appearance is that often other cytoplasmic inclusions are enclosed. In *Rhodnius* larvae some lysosomes display myelin figures within them (Fig. 31). Most of these "autophagic vacuoles" contain membranes which are being digested; further others show vacuoles and membranes and at times glycogen deposits (Fig. 32). Some
Fig. 23. Mitochondrion showing central cristae (arrow) as an outgrowth of the membrane. (X 29,520)

Fig. 24. Central cristae (arrow) seems to fuse with opposite double limiting membrane. (X 29,520)

Fig. 25. Note the constriction in the same area where a partition occurs. (X 29,520)
Fig. 26. The constriction becomes more pronounced. (X 29,520)

Fig. 27. Continued constriction leads to division of the mitochondrion (arrows). (X 29,520)

Fig. 28. Partitioned organelle (arrow) with a number of separated mitochondria in vicinity. (X 29,520)
lysosomes seem to arise from digestion of phagocytotic and pinocytotic vacuoles, resulting in multi-vesicular bodies with many vesicles contained in them (Fig. 34). Such "autophagic vacuoles" are most frequently noted within twenty-four hours after feeding and in starved animals; at later times most lysosomes are of the dense body variety.
Fig. 29. Most of the lysosomes found in the fat body are of the electron dense type showing little or no internal structure. (X 29,520)

Fig. 30. Many lysosomes of the dense variety display a crescent gap (arrow), which suggests a halo. (X 29,520)

Fig. 31. Electron micrograph of a lysosome with myelin figures in it. (X 29,520)
Fig. 32. This autophagic vacuole shows a myelin figure, a vacuole with glycogen deposits and bundles of membranes (arrow). \((X \ 29,520)\)

Fig. 33. Autophagic vacuole containing various bundles of membranes \((X \ 29,520)\)

Fig. 34. Multi-vesicular body with seemingly intact phagocytotic and pinocytotic vacuoles. \((X \ 29,520)\)
C. Ultrastructure of Fat Body Cells Following Blood-Drug Nutrient Meals

Drug-implicated changes on the ultrastructure of fat body cells could not be associated with cell size, the nucleus, nucleolus and Golgi complex. Also the events in cellular metabolism of glycogen and fat did not seem to alter quantitatively or qualitatively from animals when fed on blood alone.

Mitochondrial alterations are noted as early as twelve hours after drug application and for all concentration levels of the drug. While the ratio of elongated and round mitochondrial types is not affected, other morphological differences are occurring quite regularly and throughout all periods of observation. At twelve hours following feeding, some mitochondria of the elongated variety display long and wavey cristae, often in an orientation parallel to the long axis of the organelle (Fig. 35). Such mitochondria are often found in groups and the double limiting membranes are distorted, leaving an irregular electron dense residual body of little internal structure, such as cristae (Figs. 35 and 36). This pattern of a suggestive breakdown seems to lead to different mitochondrial forms. In some portions of the mitochondrial matrix a reduced electron density is displayed; in others the double limiting membrane reveals swellings, suggesting that the content of the organelle is lost into the cytoplasm (Fig. 37). By twenty-four hours after the blood-drug meals an increased number of
Fig. 35. Portions of a fat body cell 12 hours after a blood-drug feeding (42.0 mg drug per 100ml of blood). Many mitochondria are elongated with their cristae long and wavey, and orientated parallel to the long axis of the organelle (arrows). (X 16,350)
Fig. 36. Drug-fed animals (42.0 mg drug per 100 ml of blood), demonstrating degenerating mitochondria (DM) as a large electron dense mass with little internal structure. (X 18,350)
Fig. 37. As in animals fed a normal blood meal, 24 hour drug fed insects (21.0 mg drug per 100 ml human blood), show large glycogen deposits (GL). Mitochondria with irregular electron densities of the matrix (arrows) are however, unique to animals which received the various drug concentrations. (X 18,350)
mitochondria displayed either wavey and very long cristae or, tightly grouped cristae within the center of the organelle (Figs. 38 and 39). In other mitochondria no cristae of extended length are noted but rather very short entities of a round and swollen configuration (Fig. 40). Such mitochondrial changes in the drug treated larvae are by no means consistent for all cells of the fat body; mitochondria similar to those of the control group are found in most cells or even in the same cell together with the various forms of deformation. This finding suggests that a differential drug effect exists on different cellular and subcellular areas within the fat body of the growing larvae.

Some structural alterations of the RER are noted at all concentrations of drug, occurring between three to six days after feeding. The cristae are quite large and enclose a fine granular material (Fig. 41). However, such configurations of the RER occur irregularly and a pattern of its distribution could not be established.

Drug-fed animals reveal an increase in lysosomes at all times as compared to the larvae fed on blood alone. Most of these organelles are of the dense body variety with little internal structure (Fig. 42). Also, autophagic vacuoles are evident in greater abundance than found in the control group.

The events in activation of the insect liver cells described for animals receiving blood, are also observed in larvae fed the various drug concentrations. At
Fig. 38. Many mitochondria (M) in 24 hour drug-fed animals (21.0 mg drug per 100 ml blood), demonstrate concentrations of long cristae in the center of the organelle. (X 18,350)
Fig. 39. At 24 hours after feeding (21.0 mg drug per 100 ml human blood), large amounts of HER occur. Note also the unique mitochondrial configurations (arrows). (X 18,350)
Fig. 40. Electron micrograph of drug fed animal (42.0 mg drug per 100 ml blood) 24 hours following feeding. Many mitochondria display cristae of a swollen appearance and areas of little electron density (M). (X 18,350)
Fig. 41. At 120 hours (42.0 mg drug per 100 ml blood) some cells demonstrate swelling of the cisternae of the RER (arrow), and many mitochondria with broken membranes (arrow). Other cells contain RER and mitochondria similar to control insects. (X 18,350)
Fig. 42. All animals receiving any of the drug concentrations show an increased number of lysosomes (arrows) of the dense variety and little internal structure. This electron micrograph was taken 12 hours after feeding (42.0 mg drug per 100 ml blood). (X 18,350)
twenty-four hours post-feeding large concentrations of glycogen are noted (Fig. 36), which are reduced to the quantity found in controls and starved insects by five days following feeding. The quantities of chromatin material in the nucleus and cytoplasm parallels the events described for larvae when fed on human blood alone. Although the RER does illustrate morphological variations in some instances, the quantitative increase is similar to that observed in the control group. An extracellular coat is observed both between the cells and on the periphery of the fat body of drug fed animals (Figs. 43 and 44), but at no time is it as consistent and its quantity as great as in animals fed on blood alone.
Fig. 43. Drug fed animals 48 hours after feeding (42.0 mg drug per 100 ml blood) illustrating an extracellular coat (EC) both at the periphery of the fat body and between the cells. (X 18,350)
Fig. 44. Ninety-six hours following drug administrations (21.0 mg drug per 100 ml human blood), animals show a normal pattern of large quantities of RER, an extracellular coat (EC) and Golgi (G). (X 18,350)
DISCUSSION AND CONCLUSION

Development of a mass feeding technique was a prerequisite in the study of the drug BKVA in Rhodnius prolixus. The existing methods (Harington, 1960; Friend, 1963) were not only impractical when working with a large number of insects, but also failed to yield consistent results when human blood is used as the nutrient medium. In the feeding technique developed in this work some of the ecological requirements conducive to a successful response in conjunction with a stirring mechanism to keep the blood components in suspension, enables mass feeding of larvae and adults. Insects fed by this method did not differ in moulting time or mortality from those fed on the laboratory rabbit host, provided that the animals engorged a certain minimum amount of blood by either method; the minimum requirement for normal growth of the fourth instar larvae is at least two and a half times the body weight in human blood. In this rather simple and inexpensive method forty to sixty insects can be fed in thirty minutes; thus the basis for an artificial mass feeding program of Rhodnius is established as well as a bio-assay system to investigate the effects of drugs or other chemicals on insect development.

The effects of BKVA on fourth instar larvae were studied on organismic and subcellular levels. Considering first the organismic effects, animals which received 42 mg
of the drug show at least a ten percent greater weight decrease up to fifteen days after feeding than larvae acquiring less BMVA and controls. Another observation is that twice as many animals die in the two experimental groups receiving the highest drug concentrations as compared to the insects administered only 10.5 mg of drug per 100 ml human blood and controls. Most critical for insect development is the highest drug concentration of BMVA since only twenty-three percent of this group moult to the next instar. The medium concentration of the drug permits moulting of eighty percent of the animals; all insects in the group administered the lowest drug concentration and all controls undergo ec dysis.

These variations of the normal life cycle of Rhodnius prolixus may be due either to drug-related effects on the fat body or the physiology of the insect. Since BMVA is known to affect the mammalian liver in rats (Eades et al., 1966b), similar alterations on the insect fat body could account for the effects on organismic development. The ultrastructural study of the sequence of events occurring in the liver of Rhodnius after a human blood meal provides a basis for comparison with BMVA related subcellular effects.

In the six days following feeding of nutrient meals without drug additives, subcellular changes in the insect hepatocyte are quite evident. Prior to feeding the cells of the fat body, like many other parts of the insect, are in a state of diapause but are activated immediately by a blood meal...
meal. The starved insects demonstrate reduced quantities of fat and glycogen as compared to those animals twenty-four hours after feeding. The nutrient meal stimulates the accumulation of these substances within the first day after feeding. However, glycogen reserves are reduced by five days following feeding to the quantity noted in the animals starved for eight weeks. Therefore, glycogen is formed and mobilized within five days of receiving nutrients. This period corresponds to the time at which chitin is deposited in the new cuticle (Wigglesworth, 1953). The large vacuoles accumulating during the moulting cycle remain within the cells as fat reserves and may act as general energy source.

Activation is also characterized by increases in the size of the cell, nucleus and nucleolus. Eight weeks of starvation prior to receiving a blood meal leads to a doubling of cellular, nuclear and nucleolar size at twenty-four hours. Chromatin material increases at twenty-four hours, around the nucleolus and near the periphery of the nucleoplasm. At five and six days after feeding mitotic figures are observed.

Starvation reduces the quantity of endoplasmic reticulum present in Rhodnius fat body cells. Golgi material is rarely evident and the RER consists of single and double layers of cisternae disposed for the most part near the nuclear and plasma membranes. However, at twenty-four hours cells demonstrate large lamellar cisternae configurations of RER. A day later all cells display an even greater amount.
but now of the vesicular variety of granular reticulum and distended cisternae. The degree of distention with an electron dense substance enclosed may indicate an accumulated product within the RER. The Golgi complex, with small electron dense smooth vesicles, become prominent forty-eight hours after feeding and suggest a transport mechanism of a cell product leading to deposition in the forming cuticle.

In these investigations it is shown that after feeding, the number of mitochondria decrease relative to the cross-sectional area examined. However, fat body cells following feeding increase in size about one hundred percent, provided the animals were starved for eight weeks. Even though the mitochondrial number decreases within a portion of the cytoplasm, the number per cell is increasing due to the increase in cell size over the first twenty-four hours after feeding. These findings agree with the results of a mitochondrial increase after feeding reported by Wigglesworth (1953).

The fact that mitochondria tend to convert to the elongated variety after a blood meal, from the rounded type found in animals deprived of food for eight weeks, may reflect an increase in mitochondrial activity paralleling such an increase in the activity of the insect liver cells. Gansler and Rouiller (1956) used the electron microscope to study mitochondrial changes in the liver and kidney of rats, which were deprived of food for three to five days and of fasted animals after feeding a protein rich diet. Starvation leads to a rounding of the mitochondria; however,
after feeding many mitochondria become elongated and the process is essentially completed by twenty-four hours. These observations are similar to data accumulated of mitochondria in *Rhodnius prolixus*, when animals were starved for eight weeks and then fed a human blood meal. In the starved insects the majority of mitochondria (greater than seventy percent) are of the rounded type. Animals sacrificed at twelve hours after feeding also show such a proportion of rounded and elongated mitochondria, but as in the rat liver, at twenty-four hours many organelles have become the elongated variety. The number of elongated mitochondria is consistently much higher in animals fed a blood meal, from twenty-four hours up to the completion of the observations at six days. Therefore, as in rats the mitochondrial shape seems to be implicated in the state of activity of the organelle and the cells in which they are located.

The electron microscopic investigations also demonstrate several alterations of the morphology of the mitochondria, which can be related to the mechanism of mitochondrial genesis. Partitioned mitochondria of the type described in this study have been reported in the literature. In many of these investigations, the partition figures appeared at a time when mitochondria were increasing in number, and it has been suggested that they represent dividing organelles; a review of these studies was discussed by Tandler *et al.*, (1969). In some cases the replication of
mitochondria was experimentally induced. LaFontaine and Allard (1964) stimulated an increase in mitochondria in the liver of rats by feeding an azo 2-Me-DAB dye. Tandler et al. (1969) induced the replication of mitochondria in liver cells of riboflavin-deficient mice by injecting riboflavin. Onishi (1967) bled rats and observed degeneration and subsequent replication of cardiac mitochondria. Wigglesworth (1967) stimulated mitochondria to replicate in the fat body of *Rhodnius prolixus* by feeding severely starved animals. In the present study it is demonstrated that mitochondria divide in the fat body of fourth instar *Rhodnius* larvae during normal development from the fourth to fifth life stage. The two most significant observations which support this view are (a) partition figures appear only at the time when mitochondria are rapidly increasing in number (twelve to forty-eight hours after feeding), and (b) the size of the organelles remains quite constant during this period. These two observations rule out the possibility that partition figures represent stages in mitochondrial fusion. In addition, the presence of mitochondria with two or three partitions and the clustering of separated organelles nearby, suggest that these grouped mitochondria represent a clone.

Figure 45 illustrates a possible mechanism of division in the adult butterfly, *Calpodes ethlius*. The electron micrographs obtained in this investigation seem to support such a mechanism. Elongation of mitochondria after feeding of *Rhodnius prolixus* larvae may occur shortly before or
Fig. 45. Diagram of possible sequence of events leading to mitochondrial division (reproduced from W. J. Larsen, 1970): (a) The mitochondrion elongates slightly. (b) One centrally placed crista begins to grow across the mitochondrion. (c) This centrally placed crista fuses with the inner membrane around its entire periphery, thus separating the matrix into two distinct departments. (d) A constriction forms at the cite of the straight transecting crista or partition. (e) The constriction tightens uniformly around the mitochondrion. (f) Continuing constriction results in fission of the mitochondrion.
during the formation of the partition as noted in Figure 45a. As indicated in Figure 45b, the partition could form by the outward growth of one crista. This hypothesis is based on the observations of profiles with a straight transverse crista connected to only one side of the mitochondrion, which according to Tandler et al. (1967) fuses with the inner membrane on the opposite side as in Figure 45c. Since some mitochondria in the fat body possess a partition but demonstrate no constrictions, it is suggested that constriction follows the formation of the partition. Continuing constriction finally results in division of the mitochondrion (Fig. 45d, e and f).

After ninety-six hours the number of elongated mitochondria show slight decreases. Mitochondrial genesis of a single organelle in effect converts elongated mitochondria into two or three of the rounded type. Therefore, the figures reported in Table III seem to support the concept of genesis in *Rhodnius prolixus* illustrated by the sequence of electron micrographs. The greater number of rounded organelles after ninety-six hours may also reflect a decrease in the activity of the mitochondria since it has been established that structure and function are related.

Mitochondria in certain instances, mostly between seventy-two and one hundred and twenty hours after receiving a blood meal, take up a unique position within the fat body cells. Such findings have been noted previously with several epithelia engaged in active transport of ions (Ham, 1969).
Numerous mitochondria are lodged in narrow compartments or interdigitating processes of the cellular membrane in such a way, that they are brought into close association with the plasma membrane of the cell. The details of this process are not understood, but it is assumed that such an arrangement provides a favorable position of the energy source to the elaborately convoluted cell surface, in which the active transport mechanism resides. Such a close association of mitochondria with interdigitating processes of the plasma membrane is noted at times when large concentrations of extracellular material occurs, both between the fat body cells and on the periphery of the organ. Consequently, intensive active transport between the third and fifth days after feeding can be postulated. In this work it is confirmed that during this time interval glycogen deposits in the fat body cells decrease drastically. Wigglesworth (1953) further found that also the decrease in proteins parallel those of glycogen and he postulates that these events are related to the deposition of protein for the new insect cuticle. If this is correct then the constituents of the extracellular coat found in this work are composed of glycogen, protein as well as, precursors for their synthesis in the fat body cells.

These subcellular events in the fat body cells of fourth instar larvae up to six days post-feeding represent sequential stages of activation of this organ from diapause; by thirteen to fifteen days moulting to the fifth instar
occurs. Using these findings as the norm, minor deviations on the subcellular level are noted in animals fed human blood plus various concentrations of the drug BMVA. In its overall pattern in all drug treated animals, the degree of activation does not seem as pronounced, based on occurrences of deformed or degenerating mitochondria, RER and an increase in lysosomes of the autophagic variety. Such alterations occur at all time intervals following feeding and in most cells of the fat body. Golgi vesicles with electron dense inclusions seem to occur as frequently as in animals when fed on blood alone; however, the extracellular coat is highly reduced with respect to density and size suggesting less cellular synthesis and mobilization of cell product in animals receiving BMVA at various concentrations.

The effects of BMVA in the liver of fourth instar larvae of Rhodnius prolixus did not resemble those reported of the liver of rodents (de la Iglesia et al., 1969a). In insects, the drug did not induce microbodies comparable to such formations in rats. Also an enlargement of the liver in rodents could not be related to the increase in size found in Rhodnius fat body; such an effect of the drug is difficult to determine since an increase in size is normally occurring due to the activation of the organ after feeding.

Degenerating mitochondria in large quantities were noted previously (Millen, 1967), after feeding saline solution to Rhodnius prolixus. Accompanying these mitochondrial changes was an increased wastage of stored cell products.
Similarly, in this investigation degenerating mitochondria are abundant and increased wastage is apparent since the animals receiving 42 mg BMVA per 100 ml blood demonstrate a greater decrease in weight during the fifteen days following feeding. Much of this weight loss is due to the fluid and solid waste excreted by the insects. However, for such an increase in wastage a deranged metabolic capacity of the cells is a possibility. Increased wastage may therefore, relate to the subcellular changes described, particularly with respect to mitochondrial and RER degeneration.

The effects of BMVA on the ultrastructure of the fat body cells are not pronounced, neither on the basis of concentration nor within the period of time examined; while toxic effects on the ultrastructural level are apparent, they do not seem sufficient to affect moulting to the next larval stage. Yet some larvae feeding on a 0.02 percent concentration of BMVA in human blood fail to moult to fifth instars; this is even more pronounced when animals gorged on a 0.04 percent drug-blood mixture and nearly eighty percent of the insects are prevented from ecdysis. Such a drug related effect to moulting is further emphasized by the findings that larvae receiving the highest drug concentration also loss weight faster than the other experimental groups, between six and fifteen days after feeding. While a more pronounced drug effect on the insect fat body could still occur after six days of feeding, not investigated in this thesis, another mode of action
of the drug seems more plausible.

Moulting in *Rhodnius* larvae is initiated by the hormone ecdysone. If the insect is decapitated immediately after feeding ecdysone is not released and no moult occurs (Wigglesworth, 1953). However, removal of this cephalic pole exerts no effects on the fat body cells; the events of activation of this organ ensue without the presence of ecdysone (Wigglesworth, 1963). These experiments seem to be directly related to the investigations discussed here. Since the drug BMVA inhibits the biosynthesis of cholesterol (Eades et al., 1966), and since ecdysone is a cholesterol related compound it seems possible that the drug inhibits the synthesis of ecdysone, and as a consequence moulting. This hypothesis of a drug related effect on growth of the insect larvae of *Rhodnius prolixus* can be easily substantiated. The epithelium and intersegmental muscles of the larvae are target cites of the hormone; upon hormone release, these tissues become activated. Therefore, a study of these sites of hormone action would be conducive to the understanding of drug action or in further exploration of this drug as a potential advantage in insect control.
SUMMARY

The significance of the present investigations could be summarized briefly as follows:

1. A simple, inexpensive and reliable method was developed for mass feeding the haemophagus insect *Rhodnius prolixus*, on human blood.

2. This technique also allows the testing of drugs and other chemicals, having potential applications in human therapeutics, using *Rhodnius prolixus* as a bio-assay system.

3. Although no attempt was made to correlate the findings in this work with the pathogenesis of human disease, the field has been expanded towards the understanding of more elementary mechanisms, at the cellular or probably molecular levels in the relationship of cholesterol biosynthesis inhibition and hormone production.

4. Electron microscopic investigations revealed further information regarding the sequence of events in the fat body cells of fourth instar larvae of *Rhodnius prolixus*, following feeding of human blood as well as after meals of blood with biphenylyl methyl valeric acid as an additive.

5. This application of an experimental compound with well established metabolic properties revealed important interactions in insect development which suggests that
additional factors have an important role in insect growth, as well as potential advantage for insect control.
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