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A Histological Study of the Malpighian Tubules of Gryllus Domesticus

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A HISTOLOGICAL STUDY OF THE MALPIGHIAN TUBULES OF GRYLLUS DOMESTICUS

By

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accepted!

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BIOGRAPHY

The writer was Born in Elgin, Texas. He received his elementary education in the public schools at Elgin. He received his High School education at Booker T. Washington High School, Elgin, Texas. After graduation from high school he attended Prairie View College from which he received the Bachelor of Science degree in May, 1942.

ACKNOWLEDGMENT

The author wishes to express his grateful appreciation for the valuable assistance rendered by many persons co-operating in this study. He is particularly indebted to Dr. Thomas P. Dooley whose constant guidance and generous help conveyed the entire work to its completion.

W. M. M.

DEDICATION

To my mother,
Mrs. B. J. Madison.

W. M. M.

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INTRODUCTION

Histological research has been carried on since early in the eighteenth century. Flemming is considered the pioneer in this field of research as his first works were completed as early as 1893. Tissue studied through fixed preparations as early as the eighteenth century make possible comparisons for work done by investigators of today.

Histology comprises microscopic investigation into the structure, the composition of tissue and its relationship to the healthy as well as the diseased tissue. The value of the results obtained from any microscopical study is directly dependent upon the quality of the technique employed in preparing the material.

Several investigators have made reports upon the structure of the excretory system of the Gryllus domesticus. Bordas, 1899, presented work on the Gryllus domesticus, while Stuart (7), presented work on the Melanoplus differentialis. The present work is based primarily on the work of the two above named workers. There are no discrepancies to be reported here on any previous works, but rather, this investigation tends to prove that the structure found in the malpighian tubules of the Melanoplus differentialis is similar to those found in the Gryllus domesticus.

Many of the methods in microscopical techniques have been handed down tradition-wise from one investigator to another. Some methods used today are the results of

accumulated experiences of several generations of work.

The work embodied in this investigation was done in the biological laboratories of Prairie View A.and M. College, Prairie View, Texas, under the supervision of Professor Thomas P. Dooley. I am pleased to express my appreciation to Dr. Dooley for the facilities afforded to me along with the kind criticisms of encouragement leading me to adopt the title chosen for this investigation.

To Walter L. Smith, M. S., Fellow-Student of Research, Prairie View A. and M. College, whose timely suggestions and kindly interests aroused my curiosity in histological research, I express my warmest thanks.

MATERIALS AND METHODS

Adult specimen of both sexes of the Gryllus domesticus were collected and grown in the laboratory. Living individual were anesthetized with ether and placed in Ringer's solution for dissection under the microscope. This was done in order that the living tubule might be observed in its normal state of activity. The malpighian tubules were seen along the region of the small intestine. Here the delicate tubules were amputated adjacent to the posterior intestine and immediately placed in the fixative. Some of the tubules were observed immediately, while others were processed through such fixatives as Bouin's, Zenker's, Regaud's fluid, and Mann's mercuro-osmic fluid. Zenker's fluid proved to be the best fixative for the preparation of malpighian tubules to show the general histological structure. Tissue fixed in Bouin's and Zenker's fluids were stained in Delafield's hematoxylin to show general histological structure. Tissue fixed in Regaud's fluid were stained in iron-hematoxylin demonstrating the presence of mitochondria. For the presence of Golgi apparatus tissue was fixed in Luford's modification of Mann's mercuro-osmic fluid. Impregnation was accomplished by subjecting the tissue to a 2% osmic acid solution for four days. Counterstains were used only following Delafield's hematoxylin stain. As a counter-stain, 5% alcoholic-eosin was used very effectively.

The paraffin method was employed. Using the microtome it was easy to obtain long sections of ribbons averaging eight microns in thickness. Oblique, longitudinal, and transverse section were made. After staining and subsequent dehydration the slides were immediately placed in xylene before being mounted in Canada Balsam.

OBSERVATIONS

The malpighian tubules of the Gryllus domesticus are very numerous and located on the so-called posterior intestine. Their function is primarily of an excretory nature. Some of the malpighian tubules are short, bent back at the edge, while others extended anteriorly forming hemispheric curves which project lateralward. The malpighian tubules of the Gryllus domesticus were seen lying free in the body cavity and bathed by the body fluid. Examination revealed them to be divided into a cephalic group extending as far forward as the gastric caeca, and a caudal group extending as far back as the rectum. Grossly they were observed to all enter the digestive tube just beyond the ilio-ventricular sphineter.

Transverse and longitudinal sections were made of the malpighian tubules showing them to be composed internally of an epithelium formed by large irregular cells, making of causing a rupture sometimes in the lumen of the tube (plate 2). The nuclie are voluminous and were seen to be surrounded by numerous inclusions comprising the general make-up of the cytoplasm. According to Stuart, (7), the lumen seems to be covered by a characteristic edging. The so-called cilia were not seen in this investigation, but according to Bordas (1) they are individually long, immotile, rectilinear, and are placed in clumps. I observed the lumen to be an irregular border probably of

the same description, (plate 2). Along the intermediary portion of the tubule the so-called cilia were shorter, pressed together, regular, and form a brush-like border, (plate 2).

In the Gryllus domesticus each tubule was seen to be yellowish or brown. There was found associated with iach a tracheole (plate 1) winding about the tubules in a spiral from the insetion to the tip and serving as a support ofr the delicate tubule and limiting the movements of the tubules by anastomosis at the tip with a large tracheae. This tracheole seems to be made up of many collapsible segments allowing for contraction and expansion, (7).

As results of tissue being fixed in Regaud's fluid, there were seen numerous cytoplasmic components which were present in the form of granules, rods, and fiaments. They seem to have been distributed throughout the cytoplasm. They were present in all the cells maintaining a striking regularity of form and position throughout the cells of the numerous malpighian tubules examined. Mitochondria stained dark as the results of iron-hematoxylin stain.

The Golgi apparatus were demonstrated by osmic acid impregnation following fixation with corrosive sublimate. The Golgi apparatus can be demonstrated by silver or osmic acid impregnation, but only after fixatives which lack fat solvents. Golgi apparatus usually consist of a meshwork of fibrils or surface membranes which occupy a definite position in the cells.

In the preparations observed in this investigation the Golgi bodies were seen to be somewhate granular and appeared black. A characteristic osmophilic as well as osmophobic area was revealed which is typical of the Golgi apparatus found in the Gryllus domesticus, (plate 3). In contrast to the Golge apparatus the mitochondria, nuclear structures appeared yellowish of brownish in the Golgi prepared tissue. The Golgi apparatus were found to occupy almost invariably a position between the nucleus and the free border of the cell. In form the Golgi bodies seemed to vary from a compact to a loosely arranged type of granular body, however they appeared characteristically circular displaying both an osophilic and an osmophobic area, (plate 3). As compact as they seemed to have been, the Golgi material at times appeared separate bodies.

DISCUSSION

When making histological or anatomical investigations of an animal supplying a tissue or an organ, I find that one should observe briefly the general arrangement of the internal viscera as to its anatomical gross and histological structure. In dealing with tissue we should study in the first instance the structure of the cell in general, as well as the general structure of the tissue constituting the organ.

The addition of a fixative to the tissue we endeavor to preserve as nearly as possible the appearance seen during the living stage. For the demonstration of the general histological structure of the malpighian tubules, Zenker's fluid proved most effective in this investigation. However, in order to observe numerous cell inclusions it was necessary to employ such means of preparation as Regaud's method for mitochondria, and Luford's method for Golgi apparatus. The compostion of the cell inclusions themselves will determine the type of fixative used. Numerous fallacies have been reported occuring in cell that have been "fixed" with reagents. Flemming, 1893, whom we regard as the pioneer in the field of histological research, has constantly laid stress on the point that we are not justified in regarding as normal all the structures as seen in cells which have been treated with reagents. Such fallacies may be due to the nature of the fixative used, and subsequently to the nature of the stain applied.

I have found that there are numerous disadvantages in the study of fixed material--namely, that the cell substance has been altered by coagulation through the process of preparation, and that it no longer remains in a functioning state. The observations recorded herein are in accord with the findings of previous investigators who have worked with fixed materials. In observing the malpighian tubules, Bordas, (1), states that the collecting reservoir has a structure that is similar to that found in the malpighian tubules but is very different upon histological examination. The two are not to be confused. In differentiating between the structure of the collecting reservoir and the malpighian tubules, Bordas states that the collecting reservoir possess clean lateral walls, (innersides), their nuclei are large, oval, and occupy the median of the cell. I found the nuclei of the cell of the malpighian tubules to be large, oval, and occupying a position in the median of the cell, (plate 4). The internal border of the malpighian tubule is nearly circular and has brush-like border. Bordas, (1) states that the hairs are short, regular, immotile, and form a casing characterized by its clear coloration.

The general histology of the malpighian tubules was plainly seen as shown on the accompanying plates. The plates were made by use of a camera lucida which gives the true picture of the topography of the cells. In most instances, structures like the nucleus appeared typically as spherical bodies situated near the center of the cell

of the malpighian tubules. No fixative will preserve all structures of the cell. I found the reagents used to be selective, that is, preserving certain and destroying other components of the cell. Staining reagents are likewise selective, and can be correctly interpreted only by a knowledge of the fixative with which they are used. Therefore it was necessary, in attempting to approach a complete description of structure from fixed material, to employ a variety of fixing and staining methods. I also found that the outstanding advantage of any fixed material is that they constitute a permanent preparation to which the investigator may return again and again for study. It is only through fixed material and records of same that a comparison of previous works may be made. Workers as early as the nineteenth century began to study preparations by similar methods of fixation.

In discussing mitochondria, I find that the functional claims for mitochondria have been so numerous in recent years that the whole subject has fallen into some disrepute. For example, mitochondria has been said to transform directly into structures such as myofibrillae, and fibrils of connective tissue; into pigment and yolk granules, and into secretory granules. They are considered by some to be the respiratory centers of the cell. A large body of the evidence, however, indicates that the mitochondria are in some way related to the metabolic, especially the excretory activities of the cell. Our knowledge is yet too limited to speak with assurance either

as to the origin of mitochondria, or their complete function. This investigation revealed only one thing certain, namely, that they are actually constituents of the cytoplasm of the cells found in the malpighian tubules of the Gryllus domesticus. Bordas, (1), does not give a description of the mitochondria and Golgi's apparatus to be found in the malpighian tubules of the Gryllus domesticus. In addition to his findings, we may add such cell inclusions as the mitochondria and Golgi's apparatus as seen in this investigation, (plate 5). With the exception of the Golgi bodies and the mitochondria, the findings are in keeping with those of Bordas. Cytologists of today deny the transformation of mitochondria into secretory products, but rather, regard them as dynamic centers which influence secretory activity. They are probably very susceptible to osmotic interchanges of the cell with its environment, frequently swelling and increasing with such activity, and being reduced during cell quiescence, (6). Among the literature today concerning mitochondria are the observations which ascribe to them independent growth, division, and in some instances a distribution to the daughter cell certain nuclear material. To my way of thinking, if some such generalization as this can be established, the mitochondria will become a valuable indicator of normal and pathological functioning of the cell.

Perhaps no structure of the cytoplasm has aroused more controversy than the Apparato reticlare interno which Golgi first described in 1898. It is now commonly known as

the Golgi apparatus. One can gain some insight into the status of Golgi's apparatus by knowing first the sequence of scientific opinion which followed its discovery. There was naturally a rush of investigators to discover it in all types of cells. Some of these were unsuccessful. Bordas, (1), after the announcement of the discovery of Golgi's apparatus in 1898, made his first report of investigation without including a report of this new discovery. Too much could not have been known at that time about this recent discovery, because the discovery of the Golgi apparatus was relatively new and extensive research had not been carried out. Again I find that the Golgi apparatus constantly appear throughout the cytoplasm of the cells as seen in the malpighian tubules of the Gryllus domesticus. The Golgi apparatus of the present investigation are not to be campared with the findings of Bordas for he found none, but in form and appearance the Golgi apparatus found in the present investigation agrees with fixed preparations of other workers, (4), (plate 3).

In most of the preparations observed in this investigation, generally, the cytoplasm appeared to be granular. Different methods of preparation only demonstrated the presence of different types of cell inclusions as was previously stated. Such inclusions as mitochondria, the Golgi apparatus, plus the general histological structure of the cell were all seen and recorded by aid of the camera licida. The general outline of the cells found in the malpighian tubules of the Gryllus domesticus was in

keeping with those found by Bordas, (1) and Stuart, (7). Plate number 1 shows the tracheole winding as described by Stuart in his description of the malpighian tubules of the melanoplus differentialis. The same structure exists in regard to the tracheole windings on the malpighian tubules of the Gryllus domesticus. The epithelium found bordering the lumen of the tubules of the Gryllus domesticus was found to be polygonal cells with the nuclei occupying a position in the median of the cell. This again was in keeping with the findings of Stuart as shown on plate number 4. Bordas, (1), spoke of distinct cell outlines revealing clear wall and membranes, but in longitudinal sections as seen in plate number 2, a distinct cell wall was not detected. No comparison is made of the Golgi bodies found in this investigation as they are the original findings of the author and are presented herein as an addition in comparison to the findings of Bordas and Stuart.

SUMMARY

The malpighian tubules of the Gryllus domesticus are very numerous. Each tubule is yellowish or brownish. There is associated with each tubule a tracheole which winds about the tubule in a wide spiral form. The tracheole seems to limit the movements of the malpighian tubules. In cross section typical malpighian tubules are made up of, first, a single layer of polygonal epithelial cells which surround the lumen. They have large deeply staining nuclei and somewhat indistinct cell outlines; secondly, an outer covering consisting of flattened cells; and thirdly, sections of the tracheole windings.

The mitochondria appeared to be heavily stained granular structures distributed evenly throughout the cytoplasm of the cells. Excellent results were obtained from Regaud's method for the demonstration of mitochondria.

The Golgi apparatus were easily impregnated with osmic acid after fixation with acqueous corrosive sublimate. Close examination revealed the characteristic osmophilic and osmophobic areas of the granules. They were also seen to have an even distribution throughout the cell.

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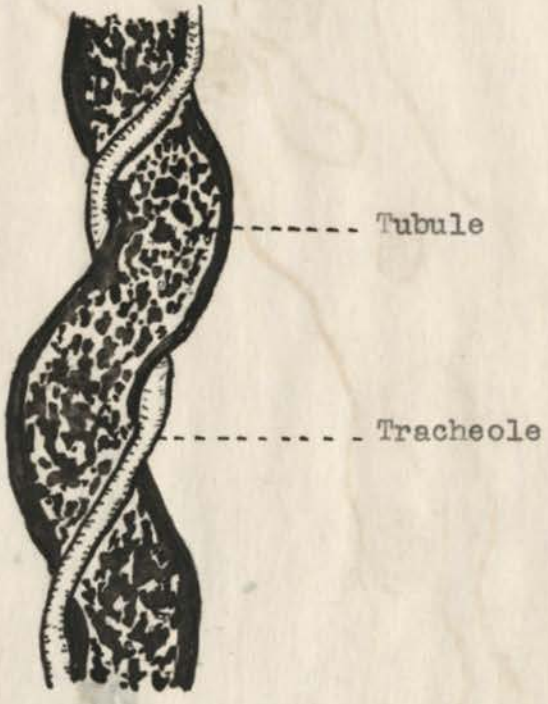
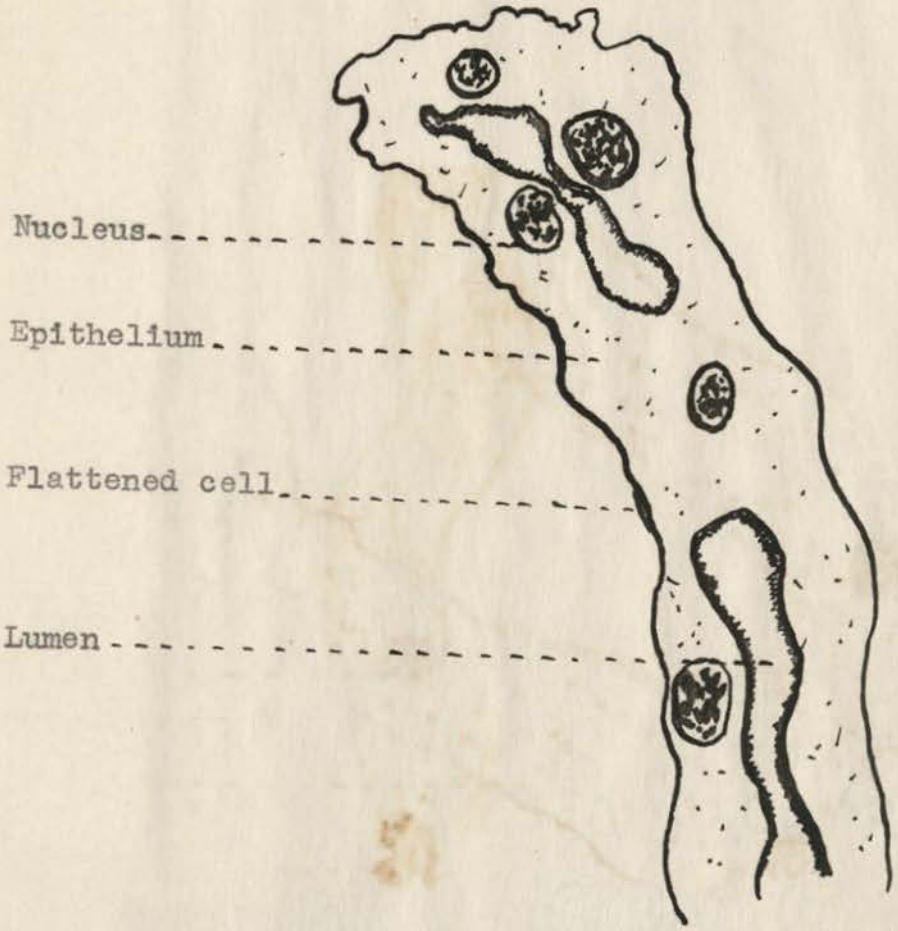
PLATE 1



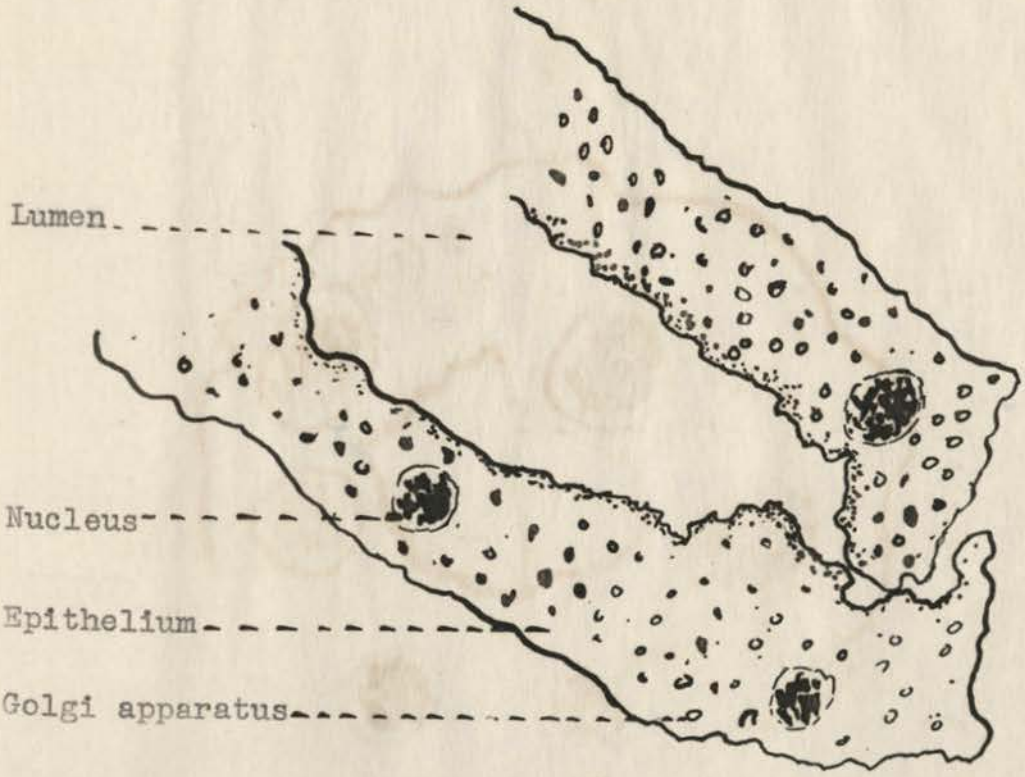
Above is a surface view of a Malipghian tubule as seen in the Gryllus domesticus. (50X)

PLATE 2



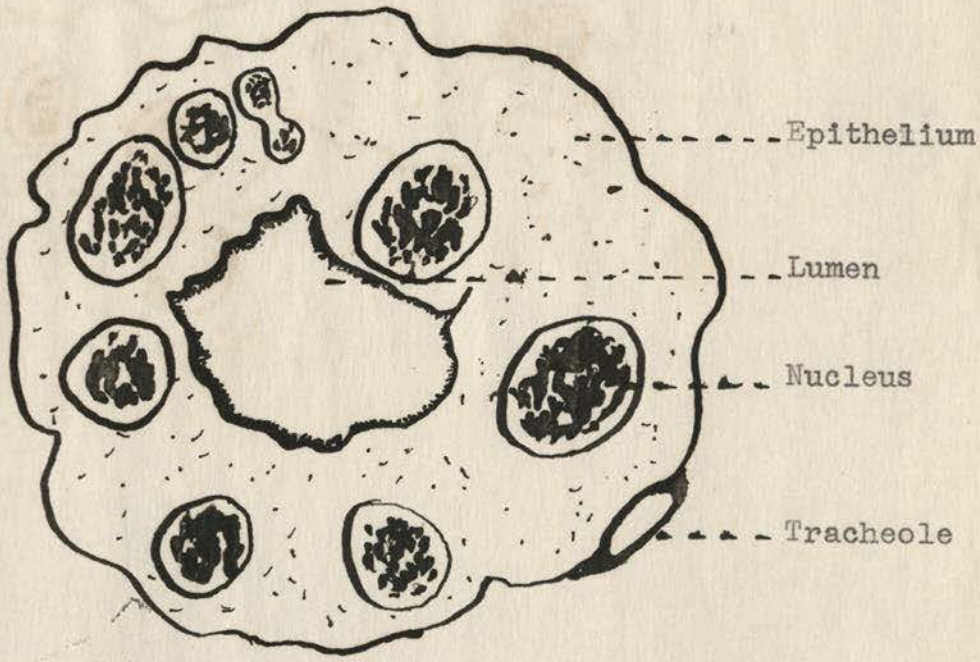
Longitudinal section through the distal end of the Malpighian Tubule. Note large irregular cells and brush-like border of Lumen.

PLATE 3



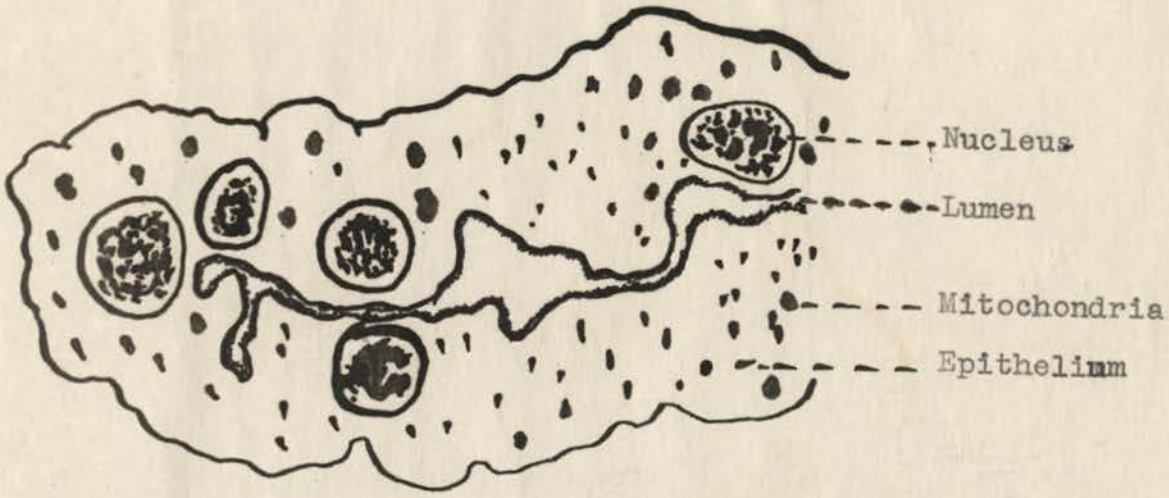
Golgi Apparatus as seen through camera lucida. Note Characteristic Osmophilic and Osmophobic areas throughout their even distribution.

PLATE 4



Cross section of the Malpighian Tubule of the Gryllus domesticus. Note large, oval Nuclei occupying a position in the median of the cell.

PLATE 5



Section through Malpighian Tubule showing Mitochondria. Note even distribution of granules throughout cytoplasm.