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POST-EMERGENCE CHANGES OF THE OESOPHAGEAL DIVERTICULA IN Aedes TRISERIATUS (SAY) (DIPTERA: CULICIDAE).

The Ohio State University, Ph.D., 1966
Entomology

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POST-EMERGENCE CHANGES OF THE OESOPHAGEAL DIVERTICULA
IN Aedes triseriatus (Say)
(Diptera: Culicidae)

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Pensri Guptavanij, B.Sc.(Hons), M.D., M.Sc.

* * * * * *

The Ohio State University
1966

Approved by

[Signature]
Adviser
Department of Zoology and Entomology
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VITA

May 12, 1934  Born - Bangkok, Thailand

1956 ....  B.Sc. (Hons.) in Biology, Department of Sciences
            Chulalongkorn University, Bangkok, Thailand

1960 ....  M.D. Faculty of Medicine and Siriraj Hospital,
            University of Medical Sciences, Bangkok, Thailand

1960 ....  A staff member of Faculty of Tropical Medicine,
            University of Medical Sciences, Bangkok, Thailand

1962 ....  Scholarship to study Medical Entomology in the
            United States of America

1964 ....  M.Sc. Department of Zoology and Entomology,
            The Ohio State University, Columbus, Ohio

1965-1966  Research Assistant, Mosquito Project, Department
            of Zoology and Entomology, The Ohio State
            University, Columbus, Ohio.

FIELDS OF STUDY

Major Field:  Medical Entomology.
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INTRODUCTION

Mosquitoes, from the standpoint of human health, are of outstanding importance as vectors of organisms pathogenic to man. The mosquitoes belong to the family Culicidae in the order Diptera, and have been successful in their distribution throughout the world from the Arctic to the temperate zones and in the tropics. They have been known as intolerable pests; more important than this, they play an important role in the transmission of human diseases. They have been found to act as vectors of malaria, filariasis, dengue, yellow fever, and several kinds of viral encephalitis.

Since the mosquitoes are important to human welfare, various subjects have been studied by many investigators; the internal anatomy of the mosquito is one of these. The alimentary canal of various species of mosquitoes has been intensively studied. The recent work on the development, anatomy, histology, and physiology of the oesophageal diverticula, carried out at The Ohio State University, has stimulated this study which involves the changes and the histological detail of the oesophageal diverticula of the imago after emergence.

The oesophageal diverticula are three evaginations of the oesophagus immediately before the junction of the stomach and the oesophagus. The two dorsal sacs, situated dorso-laterally in the thorax between the flight muscles, are small and diverging forward and
laterally from the oesophagus. Each has its own duct and orifice which opens into the oesophagus. The ventral diverticulum, the largest of the three, has a long slender neck which expands into a long sac which, when fully expanded, reaches about the sixth abdominal segment. The wall of a diverticulum consists of an inner delicate intima, a middle layer of epithelial cells, and a thin outer network of muscle fibers.

The function of the oesophageal diverticula in relation to food intake has been observed by a number of investigators. In general it is found that ingested blood goes directly to the stomach, while all kinds of sugar solutions go first into the diverticula to be later delivered to the stomach.

Lane (1963) and Romoser (1964) studied the development of the oesophageal diverticula in *Aedes aegypti* and *Aedes triseriatus*, respectively. They described the origin, growth, and development of these organs in the various ages and in the newly emerged adult up to about four hours of age. Romoser (1964) found that the adult emerges with the walls of the oesophageal diverticula greatly folded. He suggested that the inner surface of the epithelial layer of this ventral evagination becomes irregular due to the cytoplasmic extensions into the lumen. Later the extensions increase, and the epithelial layer becomes quite folded, and the nuclei become very flattened. Subsequently, the diverticula are inflated by aspirating air. He did not explain how these highly irregularly shaped cells changed into squamous cells. However, he did find a long spindle-shaped muscle cell in an adult female three to four hours after emergence.
The work, reported here on *Aedes triseriatus* (Say), was carried out in order to determine how these cytoplasmic extensions and muscle cells change their shapes, how long it takes for this to occur, and additional details concerning the oesophageal diverticula.
LITERATURE REVIEW

The internal anatomy of the mosquito has been studied by several investigators. The functions of the oesophageal diverticula and the distribution of ingested foods have been discussed by de Grandpre and de Charmoy (1900), Nuttall and Shipley (1903), Wright (1924), Grassi (1924), Falleroni (1926), Roy (1927), de Boisseyzon (1930), MacGregor (1930, 1931), Marshall and Staley (1932), Pawan (1937), Roy and Ghosh (1940), Denisova (1943), Bishop and Gilchrist (1946), Fisk (1950), Trembley (1952), Day (1954), and Hosoi (1959).

Information on development, morphology, and histology of the alimentary canal of the mosquito is given by Hurst (1890), Christophers (1901), Nuttall and Shipley (1903), Thompson (1905), Roy (1927), de Boissezon (1930), Salem (1931), Trager (1937), Pawan (1937), Richins (1938, 1945), Snodgrass (1959), Christophers (1960), Jones (1960), Lane (1963), Clements (1963), and Romoser (1964). Few of these papers are reviewed in this report.

Nuttall and Shipley (1903) were the first who gave detailed accounts of the emergence of Anopheles maculipennis. They also gave an extensive description of the alimentary canal of Anopheles. The position, form, and structure of the oesophageal diverticula were described. They found that the organs are muscular and lined with an exceedingly delicate layer of chitinous intima, and that the organs performed
peristalsis. The organs were called food reservoirs, and they were described as being impervious to the passage of water through their walls.

Thompson (1905) studied the alimentary canal in the larva, pupa and adult. He described histological changes that occurred in the pupa.

Marshall and Staley (1932) observed the condition of the oesophageal diverticula and midgut both before and during emergence; they found that shortly before the imago emerges, air accumulates under the pupal skin. The air is swallowed by the imago and passed directly into the stomach which distends the abdomen and pushes the thorax forward so as to rupture the pupal skin. After rupture of the pupal skin, the imago continues to swallow air, which causes the stomach (and therefore the abdomen) to expand further and thereby to facilitate the emergence of the imago. Within an hour after complete emergence of the imago, they reported, the air in the midgut commences to pass forward into the oesophageal diverticula, and after 12 to 22 hours the midgut no longer contained air. They agreed with Nuttall and Shipley that these organs were food reservoirs.

Brumpt (1941) did not accept the statement of Marshall and Staley about the origin of the air in the oesophageal diverticula. He pointed out that the oesophageal sphincter would seem to prevent the passage of air from the stomach to the oesophageal diverticula; that there is more air in the stomach than in the diverticula and it seemed probable that the air was released from the stomach through the anus; and that the air which inflates the diverticula is newly aspirated like that which inflated the stomach.
Venard and Guptavanij (1966) studied the inflation of the oesophageal diverticula and reported that in newly emerged *Aedes aegypti*, the oesophageal diverticula were inflated by air which the mosquito aspirated. Their experiments showed definitely that the air in the stomach left the mosquito through the intestine and anus.

There is no information in regard to the morphological and histological changes of the oesophageal diverticula after emergence of the imago. Most of the papers on these organs are concerned with their functions and the destination of ingested food by adult mosquitoes.
MATERIALS AND METHODS

The tree-hole mosquito, *Aedes triseriatus* (Say), Alabama Strain, was used in this study. The colony, originally obtained from the U. S. Public Health Service, Montgomery, Alabama, has been maintained in the Department of Zoology and Entomology, The Ohio State University, for over five years.

Larvae and pupae were reared in a plastic waterbath designed by Lane (1963). The waterbath, 12 1/2 X 10 X 4 in. in size, contained an aluminum platform (1/32 in. thick) with several perforations for circulation of water. Twenty plastic cups were placed on this platform. These cups were placed in 5 columns with 4 cups in each column. The volume of each cup is approximately 50 ml. Thirty milliliters of tap water were poured into each cup. The columns were separated by glass rods nine inches in length. The columns were numbered one through five. A thermometer, held by two pieces of plasticine, was placed in the water in the waterbath. Tap water was added to the waterbath to the level of the water in the cups. In order to maintain a constant temperature and volume of water, a plastic cover was placed over the waterbath. The waterbath was placed in the rearing room or in a B. O. D. incubator overnight to stabilize the temperature of the entire assembly at 27°C before larvae were placed in it.
The technique used for the rearing of larvae and handling of pupae is similar to that used by Lane (1963) and Romoser (1964); however, the larval diet was changed somewhat. In this study blood powder was tested as larval food. To compare the blood powder with the diet of guinea pig chow used by Lane (1963) and Romoser (1964), an experiment was carried out. Each scoop, as designed by Lane (1963), was made from a short piece of plastic tubing connected to the tip of a wooden handle. Scoops, made in this manner, were used to transfer food to each cup. The blood powder and guinea pig chow were first sifted through a 40-per-inch wire mesh; a scoop of food was taken and leveled off. The contents were then weighed to determine the capacity of the scoop. The number-one scoop held 9-10 mg; the number-two scoop 23-25 mg; and the number-three scoop 38-40 mg.

Eggs of *Aedes triseriatus* on a strip of paper toweling were placed in a plastic cup 3 11/16 in. in diameter and 2 5/8 in. in depth and the cup was half-filled with water. About 160 mg of hatching medium, a mixture of baker's yeast and guinea pig chow (1:1), was added to the cup. On the following day, when many eggs had hatched, 20 first-instar larvae were transferred to each rearing cup in the waterbath. The larval food was added using the scoops described above. In column one of the waterbath, each cup received a scoop number-one of blood powder; each in column two received a scoop number-two of blood powder; each in column three received two scoops of number-three of blood powder; each in column four received a scoop number-two of guinea pig chow; and those in column five received a scoop number-three of guinea pig chow. On the third day after hatching when the larvae were about the second instar, this feeding procedure was repeated each day.
for five days except for column two. The larvae in column two were fed only once. The larvae pupated on the eighth day after hatching, and the adults emerged two days later. The results of this experiment are presented in Table 1. Scoop number-two, furnishing 23-25 mg of blood powder daily, gave the best results with an adult emergence of 96.25% and, therefore, this schedule was employed in this study. It should be noted that the adults reared from blood powder were healthier in appearance and larger in size than those receiving the guinea pig chow. A scum formed during the first two days of rearing, but no scum was present on the rearing media during the remainder of the larval period.

Variation between rearing larvae in a B. O. D. incubator at 27 ± 0.5°C and in the rearing room at 27 ± 2°C was carried out in two waterbaths, each with 20 larvae per cup. One waterbath was placed in the B. O. D. incubator, and the other was placed in the rearing room. The larvae in each cup were fed 23-25 mg of dry blood meal daily. The results presented in Table 2 indicate little difference between the number of larvae pupating and their time of pupation in the two waterbaths. In the following studies all larvae were reared in the rearing room.

After determining the rearing and feeding techniques, methods were considered for holding, killing, and fixing adults of known ages. Newly formed pupae were transferred from the rearing cup to a holding cup (a plastic cup 3 11/16 in. in diameter and 2 5/8 in. in depth) half-filled with water; the pupae were held until the adults emerged; then the adults were transferred to a second holding cup with a lid of
Table 1. Testing the effect of larval diets as indicated by the percent of adult emergence.

<table>
<thead>
<tr>
<th>Rearing Media</th>
<th>Number of Larvae</th>
<th>Adults Emerged</th>
<th>Percent Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-10 mg of blood powder daily</td>
<td>80</td>
<td>74</td>
<td>92.50%</td>
</tr>
<tr>
<td>23-25 mg of blood powder daily</td>
<td>80</td>
<td>77</td>
<td>96.25%</td>
</tr>
<tr>
<td>80 mg of blood powder (Initial feeding only)</td>
<td>80</td>
<td>72</td>
<td>90.00%</td>
</tr>
<tr>
<td>25 mg of guinea pig chow daily</td>
<td>80</td>
<td>54</td>
<td>77.50%</td>
</tr>
<tr>
<td>40 mg of guinea pig chow daily</td>
<td>80</td>
<td>63</td>
<td>78.75%</td>
</tr>
</tbody>
</table>
Table 2. Testing the difference of rearing in a B.O.D. incubator and in the rearing room.

<table>
<thead>
<tr>
<th></th>
<th>In B.O.D. incubator</th>
<th>In rearing room</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of larvae started</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>July 30, 1965. Number of pupae appeared</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>July 31, 1965. Number of pupae appeared</td>
<td>198</td>
<td>173</td>
</tr>
<tr>
<td>August 1, 1965. Number of pupae appeared</td>
<td>134</td>
<td>161</td>
</tr>
<tr>
<td>Total pupae appeared</td>
<td>374</td>
<td>368</td>
</tr>
</tbody>
</table>
plastic screen. Adults were kept in the rearing room until sacrificed. The emergence time and the number and sex of the adults were recorded. Adults of desired age were taken from the holding cup with an aspirator and anesthetized with nitrogen. The mosquitoes were killed in vials 2 1/4 X 3/4 in., which were half-filled with Bouin's picric-formal solution. This solution was heated to 60°C for rapid killing and fixation. The specimens were held in the solution for 3 to 6 hours. The wings and legs were removed and the specimens were transferred to 70% ethyl alcohol for storage until the process of paraffin infiltration was begun. Five to ten adults were stored in each vial which was labeled according to age and sex.

Four batches of *Aedes triseriatus* were reared in the rearing room at 27°C. The first rearing was on March 19, 1965. Adults of different hourly ages were collected, fixed, sectioned, and stained. After studying the sections it was decided to make the intervals shorter during the first hours after emergence, because the changes occurred rapidly at that time. For the purpose of this study it was decided to select the newly emerged mosquitoes at fifteen-minute intervals for the first two hours and at one-hour intervals for the next 24 hours. The pupae were inspected at fifteen-minute intervals for adults. Newly emerged adults were removed immediately and killed or held for a desired age. Table 3 illustrates this procedure. Three additional rearings were carried out: June 10, 1965 through June 22, 1965; July 24, 1965 through August 4, 1965; and November 5, through November 17, 1965. Males and females were collected.
<table>
<thead>
<tr>
<th>Time of emergence</th>
<th>Number of adults emerged</th>
<th>Time killed</th>
<th>Age when killed (hrs.)</th>
<th>Code of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 15, 1965</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07:30</td>
<td>3♂</td>
<td>13:30</td>
<td>6</td>
<td>♂ 406</td>
</tr>
<tr>
<td>09:45</td>
<td>3♂</td>
<td>13:45</td>
<td>4</td>
<td>♂ 404</td>
</tr>
<tr>
<td>10:15</td>
<td>3♂</td>
<td>13:15</td>
<td>3</td>
<td>♂ 403</td>
</tr>
<tr>
<td>10:30</td>
<td>1♂</td>
<td>12:30</td>
<td>2</td>
<td>♂ 402</td>
</tr>
<tr>
<td>10:45</td>
<td>1♂</td>
<td>12:45</td>
<td>2</td>
<td>♂ 402</td>
</tr>
</tbody>
</table>

| November 16, 1965 |                        |             |                        |                   |
| 07:15             | 3♀                      | 16:15       | 9                      | ♀ 409             |
| 08:00             | 1♀                      | 14:00       | 6                      | ♀ 406             |
| 08:30             | 2♀                      | 14:30       | 6                      | ♀ 406             |
| 09:00             | 2♀                      | 14:00       | 5                      | ♀ 405             |
| 09:15             | 1♀                      | 14:15       | 5                      | ♀ 405             |
| 10:00             | 3♀                      | 14:00       | 4                      | ♀ 404             |
| 10:15             | 4♀                      | 13:15       | 3                      | ♀ 403             |
| 11:00             | 3♀                      | 13:00       | 2                      | ♀ 402             |
| 11:00             | 2♀                      | 12:30       | 90 min.                | ♀ 490             |
| 13:30             | 1♀                      | 14:45       | 75 min.                | ♀ 475             |
When the rearing was complete, the paraffin impregnation was accomplished in the same vial using the dioxane dehydrating and impregnating technique with OSU embedding mass, a special mixture of paraffin and latex. All specimens were arranged for longitudinal sectioning and embedded in high melting point paraffin by using plastic embedding rings and metal base molds. The base molds were treated with mold release compound. Infiltrated specimens were kept in vials of melted paraffin at 60 ± 3°C in a waterbath. A specimen was transferred from the vial to the base mold with a medicine dropper and the desired position of the specimen was arranged by using a hot dissecting needle. The embedding ring, tagged with the label of the number of batch, age, and sex, was placed on the mold; melted paraffin was added to the level of the embedding ring. When a scum had formed on the surface of the paraffin block a few minutes later, it was transferred to ice water for rapid cooling. The solidified paraffin block was released from the mold and stored in the refrigerator until it was sectioned. The block was trimmed as small as the specimen permitted and it was sectioned at seven-micron thickness. Seven microns is approximately the thickness of the majority of the cells making up the tissue and so the sections, about one cell thick, were considered appropriate for this study. The ribbons were stored in a cardboard box and kept in the refrigerator. Subsequently, the ribbons were cut about 33 mm long or about two-thirds of the length of a 22 X 50 mm cover-glass. Ribbon mounting was performed by placing slides on a warming table heated to 40 ± 3°C. Diluted albumin, as an adhesive for the sections, was spread uniformly on the slide by a dropper pipette. A moistened camel hair brush was used to
place the ribbons on the slide which was temporarily removed from the warming table. About two to three rows of ribbons can be floated on the slide; this number of ribbons was limited by the 22 X 50 mm cover-glass area. The slide was then returned to the warming table to allow the ribbons to expand. When a ribbon was curved, straight rows of sections were acquired by cutting the curved ribbon and realigning the segments. When expansion of the ribbons was complete, the excess albumin dilution was drained from the slide by a gauze sponge. The slide was labeled and left on the warming table for about 24 to 48 hours. Finally they were stored in a micro-slide box.

The staining procedure was performed by using a modification of the Azan technique (Hubschman, 1962), and picrolyte was used as a mounting medium. These permanent prepared slides were dried under two 75-watt light bulbs for about 48 hours. At the end of this period, the preparation of the slides was finished and the slides were ready for studying. Two hundred and twenty eight females and ninety four males were prepared and examined. Only female specimens were thoroughly studied.
THE ALIMENTARY CANAL OF THE LARVA

The development of the oesophageal diverticula in the larva and the pupa in Aedes triseriatus has been described in detail by Romoser (1964). Therefore, in this study there is no attempt to repeat his work; only the general characteristics of the alimentary canal of the larva and pupa will be reviewed.

The alimentary canal of the mosquito larva is a relatively simple tube (Fig. 1) and consists of three parts: ectodermal stomodaeum, an endodermal mesenteron, and an ectodermal proctodaeum. Passing backwards, the stomodaeum begins with a preoral cavity which Christophers (1960) refers to as a functional mouth of the larva, a slit-like true mouth, a funnel-shaped and muscular pharynx followed by a narrow oesophagus that goes through the neck into the thorax, where it forms an invagination with the cardia. This invagination is often referred to as the proventriculus. Following the proventriculus is a long straight tube, the stomach or ventriculus, which extends back into the seventh abdominal segment. The anterior end of the ventriculus bears radially arranged gastric caeca, eight in number. Wigglesworth (1930) has shown that the cell walls of the cardia secrete the peritrophic membrane which surrounds the food materials. The proctodaeum or the hind gut consists of an ileum with its anterior end dilated in a funnel-shaped structure forming, as in the adult, a pyloric ampulla,
into which the five Malpighian tubules open at its upper end. These Malpighian tubules are commonly used as the landmark of the end of the midgut and the beginning of the hind gut. Following the ileum is a saclike anterior enlargement of the rectum, which finally proceeds as a narrow tube to the anus.
Figure 1. Diagrammatic drawing of a mosquito larva, showing the alimentary canal.
THE ALIMENTARY CANAL OF THE PUPA

Hurst (1890) stated that the alimentary canal of a young pupa more resembled the digestive tract of the larva than that of the adult. It undergoes a complete reconstruction during the pupal life to convert into the imaginal canal. The changes begin in the late (prepupal) stage of the larva by the withdrawal of the invaginated portion of the oesophagus from the proventriculus (Christophers, 1960). Thompson (1905) and Romoser (1964) found that the oesophagus undergoes no changes in its epithelial layers during the pupal period. The larval pharynx is lost, and an enlargement in the back of the head forms the post-cerebral sucking pump of the adult. The oesophageal diverticula of the adult grow out from the oesophageal wall. Romoser (1964) states that the development of the diverticula begins in the third and fourth larval stages where the cells of the anterior imaginal ring and that of the reflected wall of the oesophagus are found to be actively dividing. These cells later develop into the oesophageal diverticula. The larval gastric caeca are absorbed and not replaced in the adult. According to Richins (1945), the larval epithelium of the stomach degenerates and is cast off into the stomach lumen; a new epithelium is formed by permanent regenerative cells. The five Malpighian tubules of the larva go over into the adult without change. In the wall of the rectal sac of the pupa, there are six invaginations which become the rectal papillae of the adult.
THE IMAGINAL ALIMENTARY CANAL

The alimentary canal of the adult mosquito (Fig. 2) is quite different from that of the larva, but it still consists of three parts: stomodaeum, mesenteron, and proctodaeum. The anterior region of the alimentary canal contains two powerful pumps: the cibarial pump lies under the clypeus and the pharyngeal pump which is narrow anteriorly but expands considerably behind the brain. These two pumps are the sucking apparatus of the mosquito. Following the pharyngeal pump is the oesophagus which is a narrow tube and it terminates by a valve-like oesophageal sphincter which marks the beginning of the midgut. Just anterior to this oesophageal sphincter arise two small dorsal diverticula which lie between the flight muscles, and a large ventral diverticulum which lies partly in the thorax and partly in the abdomen. The midgut consists of two parts: the anterior portion of the midgut is a narrow tube and under all conditions it remains a narrow tubular structure. The posterior region of the stomach is wider and capable of great distension. When it is distended, it may be ballooned to occupy most of the swollen abdomen. Immediately following the stomach is the funnel-shaped pyloric ampulla which is the beginning of the hind gut. Opening into the pyloric ampulla are the Malpighian tubules, five in number. These tubules are also used as the landmark of the hind gut.
The remainder of the hind gut consists of the muscular ileum, the rectum, and the anal canal. The rectum has a thin epithelium with rectal papillae which project from its dorsal wall, four in the male, and six in the female.
Figure 2. Diagrammatic drawing of the alimentary canal of mosquito adult (From Snodgrass, 1959).
THE STATE OF THE OESOPHAGEAL DIVERTICULA
AT THE TIME OF EMERGENCE

A. The ventral diverticulum

The adult *Aedes triseriatus* begin to emerge at some time between two to three days after pupating. The males emerge a few hours before the females. At the time of emergence, the ventral diverticulum has a long neck and an enlarged body at its posterior end but it is confined to the thorax (Figs. 3, 5, 9, 10). The enlarged body portion is in a folded and compacted state. The wall consists of an inner cellular layer which will become an epithelium and an outer muscular layer of longitudinal and circular fibers. A non-cellular intima lines the lumenal surface of the cells.

The inner layer is composed of cells with indistinct boundaries; thus it is not possible to recognize individual cells with clarity. In places two or three nuclei are located one above the other so that this layer appears to be two or three cells deep (Figs. 7, 8). The lumenal surface of the cells are very irregular in shape due to what Romoser and Venard (1966) call cytoplasmic extensions. These cytoplasmic extensions (Figs. 12, 13) are finger-like protrusions of the cytoplasm with a variety of shapes. They are quite transparent because they take much less strain than the remainder of the cytoplasm. Most of the cells appear to have a single cytoplasmic extension but two or
three may be present. It is the cells which will be in the sac-like portion of the diverticulum that have the cytoplasmic extensions. They are not present in the cells which will form the neck of the diverticulum. These cytoplasmic extensions make it possible for a cell of large volume to have a small diameter and to occupy a small area at its base. At this time the diverticulum has a distinct lumen (Fig. 7) but it is very small and in places it is obliterated (Fig. 11) due to the fact that the cells on opposite surfaces come into contact.

In the neck region of the diverticulum the epithelial cells are in a single layer. These cells are somewhat cuboidal in shape and they bear slender spines that are 10 to 30 μ long (Fig. 4). The spines arise from the entire surface of the neck and a single cell may give rise to several spines. The spines are arranged with their free ends directed anteriorly. Both sexes have these spines. A group of similar spines was found arising from the epithelium of the oesophagus immediately anterior to the oesophageal sphincter. These spines are of the same shape and size as those in the neck of the diverticulum but their free ends are directed posteriorly.

Day (1954) observed a group of spines in the neck of the ventral diverticulum of a whole mount of Aedes aegypti, and he stated that their presence would assist in keeping erythrocytes out of the diverticulum when the circular muscles at the neck were contracted. The observations in this study seem to agree with Day's statement. As pointed out, the spines along the neck region of the diverticulum are pointed anteriorly and those of the oesophagus are pointed posteriorly and this could have something to do with the flow of blood cells. Because of this
arrangement of spines, the blood cells would have a tendency to flow into the stomach rather than into the ventral diverticulum where the blood cells would be blocked by the spines in the neck region.

Along its entire length the diverticulum is bounded by longitudinal muscle fibers. Circular muscle fibers also exist running transversely round the sac; they are situated at fairly definite intervals along the length of the sac (Figs. 7, 21). These muscle fibers in cross section are polygonal in shape with round nuclei. The striations of these muscle fibers are clearly evident.

At the time of emergence no evidence of inflation by air can be seen.
B. The dorsal diverticula

The dorsal diverticula at the time of emergence are, like the ventral diverticulum, also in a folded and compacted state (Fig. 27). They lie in the thorax between the flight muscles. Like the ventral diverticulum, the dorsal diverticula have walls that consist of a non-cellular intima resting on a layer of epithelium and external to the epithelium is a layer of muscles.

The epithelial layer of the dorsal diverticula is quite different from that of the ventral diverticulum. It is only one cell layer in depth (Fig. 28). The cells are somewhat cuboidal in shape with large nuclei. The cytoplasmic extensions are present and they almost fill the cavity of the diverticula (Figs. 27-30). Spines are found at the orifice of each dorsal diverticulum but they are stouter, not so long, and less numerous than those of the ventral diverticulum.

The outermost layer of the dorsal diverticula consists of muscle fibers (Figs. 28, 30). They form a strongly developed layer of circular fibers which are markedly striated. The muscle fibers are interconnected with branches and anatomoses and in this way the muscular coat forms a complete investment to the dorsal diverticula.
THE INFLATION OF THE DIVERTICULA

A. The mechanism of inflation and resulting histological changes

Both male and female mosquitoes emerge with the oesophageal diverticula confined to the thorax and in the folded and compacted state as described above. There is no air in them either prior to, or during the emergence of the imago. In *Aedes aegypti* following emergence, the imago begins to aspirate air through the oral aperture and inflates the oesophageal diverticula (Venard and Guptavanij, 1966).

An experiment was performed to discover if the oesophageal diverticula of *Aedes triseriatus* are inflated by air aspirated through the oral aperture is the same manner as in *Aedes aegypti*. The mouth of the newly emerged adult was sealed with paraffin in a few seconds after emergence. Then, after a certain period of time the mosquito was dissected. The results are presented in Table 4. In the control group (Table 4, A) the air bubbles were observed in the diverticula 15 minutes after emergence and all oesophageal diverticula contain air one hour after emergence. The air left the stomach 18 hours after emergence. In the group with the mouth sealed at emergence (Table 4, B) no air bubbles were found in the oesophageal diverticula at any time. The air bubbles also left the stomach in 18 hours after emergence. The ventral diverticulum of one specimen was filled with meconium. There was meconium in the stomach in all mosquitoes examined.

27
Table 4. Distribution of air in the stomach and the oesophageal diverticula of adult *Aedes triseriatus* at specific intervals of time following their emergence.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after emergence when dissected</th>
<th>Number of mosquitoes dissected</th>
<th>Air present in stomach</th>
<th>Air present in diverticula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vd. Dd. Dd.</td>
<td></td>
</tr>
<tr>
<td>A. Controls; no</td>
<td>0-1 min</td>
<td>5</td>
<td>5</td>
<td>0 0 0</td>
</tr>
<tr>
<td>treatment</td>
<td>15 min</td>
<td>28</td>
<td>28</td>
<td>20 9 3</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>11</td>
<td>11</td>
<td>11 10 9</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>6</td>
<td>6</td>
<td>6 6 6</td>
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<tr>
<td></td>
<td>3 hr</td>
<td>11</td>
<td>11</td>
<td>11 11 11</td>
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<tr>
<td></td>
<td>6 hr</td>
<td>17</td>
<td>15</td>
<td>17 17 17</td>
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<td>18 hr</td>
<td>8</td>
<td>0</td>
<td>8 8 8</td>
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<tr>
<td></td>
<td>24 hr</td>
<td>24</td>
<td>0</td>
<td>24 24 24</td>
</tr>
<tr>
<td>B. Experiment;</td>
<td>15 min</td>
<td>10</td>
<td>10</td>
<td>0 0 0</td>
</tr>
<tr>
<td>mouth sealed at</td>
<td>30 min</td>
<td>7</td>
<td>7</td>
<td>0 0 0</td>
</tr>
<tr>
<td>emergence</td>
<td>1 hr</td>
<td>6</td>
<td>6</td>
<td>0 0 0</td>
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<tr>
<td></td>
<td>3 hr</td>
<td>12</td>
<td>12</td>
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<td>6 hr</td>
<td>23</td>
<td>17</td>
<td>0 0 0</td>
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<td>18 hr</td>
<td>9</td>
<td>0</td>
<td>0 0 0</td>
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<td></td>
<td>24 hr</td>
<td>23</td>
<td>0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

*aOne mosquito had meconium in the ventral diverticulum.*
The inflation of the diverticula begins to show in sectioned material 15-45 minutes after emergence (Figs. 14-18). The ventral diverticulum begins to unfold and stretch out into the first abdominal segment. The cells along the neck region and the anterior half of the diverticulum begin to change their appearance, while the cells in the posterior part remain the same. When a bubble of air enters the diverticulum (Figs. 19, 20), it changes the shape of the epithelial cells with which it comes into contact. The individual cell is stretched by forces acting on its sides so that its sides become farther apart and the inner and outer surfaces of the cell come to lie close together.

In other words the cell is changed from a shape that is somewhat columnar to a shape that is squamous. As this change in shape occurs the cytoplasmic extensions disappear. As these cells change in shape the diverticulum increases in length. Additional air is aspirated and, as more cells change shape, the diverticulum showly expands into the abdominal cavity (Fig. 19). Finally, the contained air is in contact with all of the epithelial cells and inflation of the diverticulum is complete. It now extends to the fifth abdominal segment. No more cytoplasmic extensions can be observed. In longitudinal sections of the diverticulum the epithelial cells form a very thin layer with flattened nuclei; no cell membranes can be seen.

In the unexpanded ventral diverticulum the muscle cells are seen at fairly regular intervals (Fig. 7). Their nuclei are surrounded by a layer of cytoplasm as thick as or thicker than the nucleus. When the diverticulum is expanded the muscle cells are stretched so that they become much elongated and the diameter of the cell is approximately
the same as the diameter of the contained nucleus. The muscle cells are branched and will be described later. The striations in these cells show distinctly.

The dorsal diverticula have the same mechanism of inflation as that of the ventral one; but there is a difference in the way of expanding. All the cells undergo their change in shape at the same time rather than in a progression from anterior to posterior as in the ventral diverticulum. The cytoplasmic extensions disappear from all cells simultaneously (Fig. 32). The dorsal diverticula originate from the lateral wall of the oesophagus and as they are inflated they extend posterio-dorsally for the first 30 minutes and then they expand upward between the flight muscles and into the anterior and dorso-lateral areas of the thorax. Figures 33, 34, 35, and 37 show the state of expansion at 1, 2, 4, and 12 hours respectively.
B. The sequence of inflation of the oesophageal diverticula

The dorsal and ventral diverticula are free of air when the imago emerges from the pupal skin. The commencement of inflation of the ventral diverticulum precedes the dorsal diverticula (Table 4, A 15 min). At about 15 minutes after emergence 20 mosquitoes out of 28 were found with small air bubbles in the ventral diverticulum, while only 9 mosquitoes had air bubbles in the dorsal diverticula. However, the dorsal diverticula are fully inflated before the ventral diverticulum. About 30 minutes after emergence, the dorsal diverticula are considerably expanded (Fig. 32). The evidence of air bubbles can be observed; the muscle cells and the epithelial cells are expanded. At the same interval of time only traces of air bubbles can be seen in the ventral diverticulum (Fig. 14); the muscle cells in the cross section still retain their polygonal form and the epithelial cells are also in irregular forms with their cytoplasmic extensions. The inflation of the dorsal diverticula continues and the completion of inflation is reached at about 6 hours after emergence (Fig. 36). The ventral diverticulum expands gradually and reaches the completion of inflation about 12 hours after emergence. Because of the size of the dorsal diverticula which is many times smaller than that of the ventral diverticulum, they need only small amount of air for their inflation and they are completely inflated six hours before the ventral diverticulum.
A DESCRIPTION OF THE ANATOMY OF THE FUNCTIONAL DIVERTICULA

In adult mosquitoes 12 hours or more of age the oesophageal diverticula appear as transparent sacs containing air bubbles of various sizes or if collapsed, as the ventral diverticulum may be in older females filled with eggs, as solid wrinkled organs.

Christophers (1960) stated, "In sections the walls are seen to consist of an inner layer of epithelium with small nuclei and an outer elasto-muscular layer." The muscle fibers "... consist of rather broad flat bands passing circularly around the sac and ending in branches consisting of small isolated fibres. The bands are spaced at regular and rather considerable intervals and arranged so that the branched thicker parts alternate. ... Besides the terminal branches there are numerous fine cross fibres linking the muscular bands."

Clements (1963) stated, "The wall of the diverticula are highly elastic and consist of a thin epithelium lined with cuticle. Circular muscle fibres circumscribe the diverticula and cause peristalsis of these organs, and in the neck regions they act as sphincters although they are not concentrated into bands."

A. The muscular layer

Before the diverticula are inflated cross sections of muscle cells can be observed quite distinctly at fairly regular intervals (Figs. 7, 8). These muscle cells encircle the diverticulum. Also
there are longitudinal muscle cells that consist primarily of branches from the circular muscles. The muscle cells form a network which encloses the remainder of the diverticulum. As the diverticulum is inflated the meshes in the network become increasingly larger. Such a network is difficult to understand from a study of sectioned materials.

Whole mounts were made of diverticula which were distended with sugar solution. The organs were stained with Delafield's hematoxylin and basic fuchsin. In such preparations, the musculature could be studied. There are approximately twelve bands of muscle tissue that encircle the ventral diverticulum (Figs. 38, 40). Between the circular bands, in a longitudinal orientation, are numerous muscle fibers (Fig. 41). These longitudinal muscle fibers may extend from one circular band to the next or they may extend across a circular band without joining it. The longitudinal fibers frequently branch and anastomose. The muscle fibers as illustrated in Fig. 41 form a network which surrounds the diverticulum. The dorsal diverticula have a similar network although it does not show nearly as clearly in whole mounts.

The nuclei of the muscular fibers are superficial or partially buried within the body of the fibers (Figs. 42-44). There are two sizes of nuclei, one is small and round in shape and the other is oval and larger. The size of the round nuclei is approximately 10 μ in diameter. The nucleoplasm contains fine granules scattered around the nucleus; these fine granules take about the same degree of stain. The oval nuclei are found less frequently than the round ones. They are
approximately 19μ in diameter. These nuclei are probably polyploid. The nucleoplasm contains coarse granules and some stain dark and others light. The nuclei of both sizes are situated either peripherally or centrally in the fiber.

There is no evidence of a sphincter muscle in the neck of the diverticulum, but the circular bands in this area serve a sphincter function.
B. The epithelial layer

In the functional diverticulum the epithelial cells are in a layer one cell deep (Fig. 25). The nuclei stain less deeply than the round nuclei of the muscle cells and they are larger (Figs. 42, 43). The size is about 12-14 μ in diameter and they are usually oval in shape. Also elongate epithelial nuclei can be seen. This shape (Fig. 42) appears in areas of the diverticulum that are stretched. Apparently the stretching of the cells affects their nuclei. The nucleoplasm contains granules of different sizes, and these granules take the stain in different degrees so that some granules are stained deeper than the others. No nucleolus can be seen. The cytoplasm is finely granular and it stains light blue. Cell membranes have not been seen so that the boundaries of the cells could not be distinguished. Attempts to study cell boundaries in temporary preparations of fresh tissue and in permanent whole mounts (which were very useful in studying muscles) have not been successful to date. Both the standard light microscope and the phase contrast microscope have been used.
C. The intima

The innermost layer of the diverticulum is a very thin, delicate, and non-cellular layer which is probably a secretion from the luminal surface of the epithelial cells. In the standard histological preparations the intima appears as a thin and wavy line which does not stain; the reagents apparently affect it adversely because in places it will be absent.

The intima is best demonstrated by feeding a mosquito a colored sugar solution and removing the ventral diverticulum. Sometimes it is possible to dissect away the cellular layer of muscle and epithelium and leave behind the intima enclosing the sugar solution (Fig. 39).

The intima can stretch but it is quite inelastic. In a partially deflated diverticulum containing colored sugar solution, the intima can be seen due to its numerous folds.
D. The innervation

The innervation of the ventral diverticulum can be observed in the whole mounts. There are two nerves, one on each lateral wall (Figs. 45, 46). On each side a nerve can be traced from the neck to the posterior part of the diverticulum. There are about 9-12 small nuclei along the nerve. These nuclei are elongate in shape (Figs. 47, 48), about 4 X 12 μ in size, and the nucleoplasm contains fine granules which take a uniform deep stain. These nuclei appear to be the nuclei of sheath cells. A large spherical nucleus also can be found; this nucleus is about 13 μ in diameter and it stains poorly (Figs. 49, 50). Only one large nucleus can be found in each nerve. The cell containing the large nucleus is terminal and it has branches, so that it is a multipolar nerve cell (Fig. 50).
E. The fine structure from electron micrographs

Preliminary observations have been made on the fine structure of the ventral diverticulum of *Aedes aegypti* (L.) by using the electron microscope. The ventral diverticulum was fixed in osmic acid, dehydrated in alcohol, embedded in maraglas, and sectioned on a microtome with a glass knife.

The muscle fiber appears to be composed of one myofibril (Figs. 51, 52). The myofibril is in the contracted state and so only A and Z bands can be seen. Longitudinal filaments stretch between the Z bands. The mitochondria tend to be concentrated under the sarcolemma and at the periphery of the fiber; they are almost absent from the interior of the fiber. Occasionally, there are finger-like projections (Fig. 52, P) from the periphery of the muscle fibers. These projections occupy the space between the Z bands. In these projections there are several mitochondria of various sizes. The Z band is usually seen as a dense region limiting the sarcomeres and bisecting the I band when identifiable. Huxley and Hanson (1960) described a Z band as a dense band composed of an amorphous material. The I band could be examined only rarely and then it was not clear. The myofibrils seem to terminate at the I band. The nuclei are located next to the sarcolemma in a peripheral position (Fig. 51, N).

The fine structure of insect muscle has been studied by several authors. Generally, two types of filaments appear to be present; they differ in their location, diameter, and protein composition. The thicker filaments extend from one end to the other of the A band and they account for the high density and birefringence of that band; they
consist largely of myosin. The thinner filaments extend from the Z-line, through the I-band, into the A-band, up to the edge of the H-Zone; these filaments contain actin. When the muscle shortens, these thin filaments are apparently drawn further into the array of the thick filaments which form the A-band. Huxley and Hanson (1956) studied the flight muscle of Calliphora. They found that only Z lines were visible, and between them stretch longitudinal filaments. There were two types of filaments which are thick and thin filaments. Huxley and Hanson show that the structure of the muscle corresponds to their model for vertebrate striated muscle with the difference that the thin filaments are more numerous and are so arranged that each thin filament is shared by two of the thick type. However, Hodge (1955) and Philpott and Szent-Gyorgyi (1955) described only one type of filament. Shafiq (1963) studied Drosophila flight muscle and showed that there are two types of filaments arranged in the same pattern as described for Calliphora by Huxley and Hanson. In this study of the muscle of the ventral diverticulum of Aedes aegypti, there are two types of filaments. The Z band and the I band can be observed. The details of these muscle fibers remain to be studied.

The epithelial layer, from the electron micrographs, appears to be attached closely to the intima (Fig. 53). The basement membrane lying on one side of the epithelium, and the intima lying on the other side. The intima appears as a heavy line; it is probably composed of osmophilic materials which in general are unsaturated fatty acids. However, it might be electron dense materials which will be seen as a heavy line too.
The above description is based, as indicated, on *Aedes aegypti* and the structure of this species is probably very similar to *Aedes triseriatus*.
THE FUNCTIONS OF THE DIVERTICULA

Most people know that mosquitoes bite and suck the blood of many vertebrates, including man, but many do not realize that only females do so. All males and both sexes of a few species live on nectar or other sugary fluids alone. The sugary solutions are an important source of energy for all mosquitoes; blood is the source of protein required for egg production. In general, when the mosquito feeds, blood passes directly to the midgut, while sugary solutions pass to the diverticula and from there they gradually pass to the midgut.

Nuttall and Shipley (1903) gave the name "food reservoirs" to the three oesophageal diverticula and this indicates their function of storing food which Nuttall and Shipley demonstrated by a series of experiments. They also reported the walls of the diverticula were extremely impervious to the passage of water. They stated that the diverticula of Culex pipiens, dissected out and allowed to dry at room temperature, still contained fluid after some months. They observed peristalsis of the ventral diverticulum. They also commented on the air bubbles and speculated about their origin. They proposed to study the air bubbles at a later time but apparently they did not publish anything about them.

MacGregor (1930), discussing certain functions of the diverticula, found that in normal feeding blood passed directly to the stomach but water, fruit-sap or other sugar-containing fluids passed to the
diverticula. He decided that the diverticula also function as air separators. In discontinuous or intermittent feeding, air-locks occur between the break in the column of fluid in the food canal. This air, he stated, is separated in the diverticula to prevent its entry into the stomach, and may be seen in the form of numerous air bubbles. MacGregor apparently did not realize, as we observed, that the mosquito takes considerable air into the diverticula in addition to that obtained during discontinuous feeding and that at intervals air passes from the diverticula into the stomach and out of the anus. The fact that ingested air passes into the diverticula does not prevent this air from eventually reaching the stomach. During emergence, as previous stated, ingested air passes directly into the stomach.

The importance of the function of the oesophageal diverticula in the blood-sucking Diptera as a reservoir for water and sugar solutions, has been pointed out by Denisova (1943). He has shown that the injection of water into the body cavity of Anopheles maculipennis and of Tabanus, of amounts greater than 50% of the body weight produces an osmotic shock. Tabanids are more susceptible than Anopheles. He concludes that the crop, having a water-impermeable wall, represents a valuable adaptation which controls the expenditure of the consumed water and its concentration in the haemolymph. The function of the crop, therefore, enables the blood-sucking Diptera to consume hypotonic solutions, such as water and plant-juices, without the rapid absorption of these fluids through the midgut and into the haemolymph.

Day (1954) also pointed out the function of the diverticula as containers of ingested sugar solutions and as air separators as
discussed by MacGregor. Also he wrote, "In the majority of species female mosquitoes require a blood meal in order to mature their eggs. The opportunity to bite a suitable vertebrate to provide the blood meal will come at irregular intervals and the insect must be capable of utilizing the opportunity when it is presented. Nectar is more generally available and is sufficient to keep the insect alive. But the ability to take a blood feed in spite of a recent nectar meal would seem to be of survival value, and ... a crop containing some nectar does not preclude the taking of a blood meal should this become available."

Clements (1963) referred to Hocking's (1953) calculations that the capacity of the ventral diverticulum varies from 0.91 mm$^3$ in Aedes communis to 3.39 mm$^3$ in Aedes punctor, and this represents 45% and 95% of the insect's basic weights, respectively. The mosquito, like certain other Diptera, stores sugary fluids in the oesophageal diverticula and blood in the stomach. Clements stated, "This separation of nectar and blood permits females to store a meal of nectar in the diverticula, passing it slowly to the midgut for absorption, while leaving the stomach empty to receive a blood meal at any time."

Christophers (1901, 1960) suggested that the ventral diverticulum, in addition to the food reservoir function, probably serves as a protective device in the form of an adjustable air cushion. He stated (1960), "The newly gorged insect with an amount of blood that may be equal to or greater than its own weight is in a position where trauma may readily occur. When, too, as digestion proceeds and the gut shrinks and eventually the ovaries are empty, the ventral diverticulum comes to occupy a large part of the abdomen. Thus it appears to play the part of an
adjustable air cushion in the abdomen that might well be purposive in
effect." He also suggested that, because of the position of the dorsal
diverticula which occupy space between the flight muscles, it is pos-
sible for them to serve as a cushion to this area. Also, Christophers
(1901) suggested that the ventral diverticulum serves as an air chamber
which lightens the body of the mosquito.

As far as the literature is concerned and from our own observa-
tions the functions of the oesophageal diverticula in relation to food
intake are that they serve as a reservoir for all kinds of sugary fluids.
This function seems important to the mosquito for maintenance of the
concentration of the haemolymph, and it provides for the stomach having
room for a blood meal even though the mosquito has recently fed on
sugary material. All three diverticula usually contain some air, often
they contain much air, and this air reduces the specific gravity of the
insect. The significance of the diverticula in forming adjustable air
cushions appears to have considerable importance in maintaining the vol-
ume of the body cavity. When the stomach is filled with blood, the air
in the ventral diverticulum is compressed and gives room in the body
cavity to the stomach. As the blood is digested and the stomach is
empty again, the air in the ventral diverticulum increases and it
occupies more of the body cavity. When the ovaries are filled with
eggs and occupy the body cavity, the air in the ventral diverticulum
decreases until it may be very small in amount. After oviposition the
air increases in amount. Thus, it seems that if there were no adjust-
able air cushion, that when the stomach or the ovaries are empty, the
body cavity would tend to collapse. Also, great changes in the volume of the body cavity would effect the circulation of haemolymph. Therefore, the function of the diverticula as adjustable air cushions is probably of much value to the mosquito.
Figure 3. Longitudinal section of a female at emergence. The ventral diverticulum, indicated by the arrow, is compact with folded walls and confined to the thorax. 75X.

Figure 4. Enlargement from Fig. 3 showing spines at the neck of the ventral diverticulum indicated by the arrow. 727X.
Figure 5. Longitudinal section of a female at emergence. Similar to Fig. 3. 75X.

Figure 6. Enlargement from Fig. 5 showing spines at the neck of the ventral diverticulum indicated by the arrow. Similar to Fig. 4. 727X.
Figure 7. Enlargement from Fig. 5 showing the circular muscle cells indicated by the arrows. 322X.

Figure 8. Enlargement from Fig. 7. Posterior part of the ventral diverticulum showing muscle cells indicated by the arrows. 727X.
Figure 9. Longitudinal section of a female at emergence. Similar to Figs. 3 and 5. 75X.

Figure 10. Longitudinal section of a male at emergence. The ventral diverticulum is indicated by the arrow and, as in the female, it is confined to the thorax. 75X.
Figure 11. Longitudinal section of a ventral diverticulum of a female at emergence. The lumen is obliterated. 727X.

Figure 12. Longitudinal section of a female at emergence showing cytoplasmic extensions of the ventral diverticulum indicated by the arrows. 727X.
Figure 13. Diagrammatic drawing made from Fig. 12 showing cytoplasmic extensions indicated by arrows.
Figure 14. Longitudinal section of a female 30 minutes following emergence. The ventral diverticulum contains some air indicated by the arrows. 75X.

Figure 15. Enlargement from Fig. 14. 322X.
Figure 16. Longitudinal section of a female 45 minutes following emergence. The ventral diverticulum contains some air indicated by the arrows. 75X.

Figure 17. Longitudinal section of a female 45 minutes following emergence. Similar to Fig. 16. 75X.
Figure 18. Enlargement from Fig. 17. 322X.

Figure 19. Longitudinal section of a female 1 hour following emergence. Approximately half, the anterior half, of the ventral diverticulum is inflated. 75X.
Figure 20. Enlargement from Fig. 19. 322X.

Figure 21. Enlargement from Fig. 20. Showing posterior part of the ventral diverticulum; a circular muscle band is indicated by the arrow. 727X.
Figure 22. Longitudinal section of a female 2 hours following emergence. Except for a small portion indicated by the arrow, the ventral diverticulum is inflated. 75X.

Figure 23. Longitudinal section of a female 4 hours following emergence. The degree of inflation is slightly more than in Fig. 22. 75X.
Figure 24. Enlargement from Fig. 23. Showing the epithelial cells indicated by the upper arrow, and the muscle cells indicated by the lower arrow. 727X.

Figure 25. Longitudinal section of a female 12 hours following emergence. Showing the muscular layer indicated by the upper arrow, and the epithelial layer indicated by the lower arrow. 727X.
Figure 26. Longitudinal section of a female 12 hours following emergence. Showing the nucleus of a muscle cell indicated by the arrow. 727X.

Figure 27. Longitudinal section of a female at emergence. A dorsal diverticulum is indicated by the arrow. 322X.
Figure 28. Enlargement of Fig. 27. Showing a dorsal diverticulum. 727X.

Figure 29. Longitudinal section of a female at emergence. Similar to Fig. 27. 322X.
Figure 30. Enlargement from Fig. 29. 727X.
ecn, epithelial cell nucleus;
mcn, muscle cell nucleus.

Figure 31. Longitudinal section of a female 15 minutes following emergence. The slightly inflated dorsal diverticulum is now pear-shaped. 322X.
Figure 32. Longitudinal section of a female 30 minutes following emergence. The dorsal diverticulum is more inflated than in Fig. 31, the wall is thinner, and its apex is directed posteriorly. 322X.

Figure 33. Longitudinal section of a female 60 minutes following emergence. A dorsal diverticulum, indicated by the arrow, is now directed anteriodorsally. Further inflation can be traced in the next four illustrations. 75X.
Figure 34. Longitudinal section of a female 2 hours following emergence. Inflations of a dorsal diverticulum, indicated by the arrow, is greater than in Fig. 33. 75X.

Figure 35. Longitudinal section of a female 4 hours following emergence. Inflation of a dorsal diverticulum, indicated by the arrow, is greater than in Fig. 34. 75X.
Figure 36. Longitudinal section of a female 6 hours following emergence. Inflation of a dorsal diverticulum, indicated by the arrow, is greater than in Fig. 35, and inflation is complete. 75X.

Figure 37. Longitudinal section of a female 12 hours following emergence. Inflation of a dorsal diverticulum, indicated by the arrow, is approximately the same as in Fig. 36. 75X.
Figure 38. A whole mount of the ventral diverticulum treated with Delafield's hematoxylin and basic fuchsin which stains the muscle and the nuclei of the epithelial cells. The longitudinal striae are creases in the epithelial layer and the intima. 75X.

Figure 39. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin, showing the intima after the muscle and the epithelial layers have been removed. 75X.
Figure 40. Diagrammatic drawing of the musculature of a whole mount of the ventral diverticulum.
Figure 41. A diagramatic drawing of the musculature of a whole mount of the ventral diverticulum. cm, circular muscle; ln, longitudinal muscle; n, nucleus of the circular muscle.
Figure 42. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin showing a circular muscle band; the nuclei of the epithelial cells indicated by the upper arrows, and the nuclei of the muscle cells indicated by the lower arrows. 322X.

Figure 43. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The nuclei of the muscle cells are indicated by the upper arrows, and the nuclei of the epithelial cells are indicated by the lower arrows. 727X.
Figure 44. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The nuclei of the muscle cells are indicated by the arrows. 727X.

Figure 45. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The nerve on the left side is indicated by the arrow. 75X.
Figure 46. The same diverticulum illustrated in Fig. 45. The nerve on the right side is indicated by the arrow. 75X.
Figure 47. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The nerve with the nucleus of a sheath cell is indicated by the arrow. 322X.

Figure 48. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The nerve with the nucleus of a sheath cell is indicated by the arrow. 727X.
Figure 49. A whole mount of the ventral diverticulum stained with Delafield's hematoxylin and basic fuchsin. The terminal nerve cell is branched and the nucleus is indicated by the arrow. 322X.

Figure 50. Enlargement from Fig. 49. The nucleus of the terminal nerve cell is indicated by the arrow. 727X.
Figure 51. An electron micrograph of the ventral diverticulum of *Aedes aegypti* showing a portion of a muscle cell. I, I band; M, mitochondria; N, nucleus of the muscle cell; S, Sarcolemma; Z, Z band. 23,328X.
Figure 52. An electron micrograph of the ventral diverticulum of *Aedes aegypti* showing a portion of a muscle cell. M, mitochondria; P, fingerlike projections. 23,328X.
Figure 53. An electron micrograph of the ventral diverticulum of *Aedes aegypti* showing the epithelial layer with intima. B, basement membrane of the epithelial layer; In, intima. 67,400X.
SUMMARY

Post emergence changes of the oesophageal diverticula in Aedes triseriatus (Say) were studied. Adult mosquitoes of known ages were reared and prepared for serial sections and for whole mounts of ventral diverticula and for other observations.

At the time of emergence the ventral diverticulum is in a folded and compacted state confined to the thorax. The wall consists of an inner epithelial layer two to three cells in depth, a lining of non-cellular intima, and an outer muscular layer of circular and longitudinal fibers. The lumenal surface is very irregular due to cytoplasmic extensions. The epithelial cells of the neck region are somewhat cuboidal without cytoplasmic extensions, and they bear slender spines 10 to 30 μ in length. These spines are arranged with their free ends directed anteriorly. The diverticulum is bounded by longitudinal and circular muscle fibers. The circular muscle fibers are located at fairly definite intervals along the whole length of the sac. The dorsal diverticula, like the ventral diverticulum, are in a folded and compacted state at emergence. The wall has an organization similar to that of the ventral diverticulum. However, the epithelial layer is only one cell layer in depth and the spines are stouter and less numerous than those of the ventral diverticulum. The circular muscle fibers are
strongly developed and form a complete investment to the dorsal diverticula. There is no evidence of air in any of the diverticula at the time of emergence.

The inflation of the diverticula begins 15 to 45 minutes after emergence of the imago. Air is aspirated and the cells of the anterior part of the ventral diverticulum which are in contact with the air bubbles change in appearance from columnar to squamous and the cytoplasmic extensions disappear. As inflation increases the ventral diverticulum expands slowly into the abdominal cavity and reaches the fifth abdominal segment when the inflation is complete. The dorsal diverticula are similar to the ventral diverticulum in the process of inflation, except that all the cells undergo their changes at the same time and the cytoplasmic extensions disappear from all cells simultaneously. The commencement of inflation of the ventral diverticulum precedes the inflation of the dorsal diverticula but the dorsal sacs reach the completion of inflation at about six hours after emergence while the ventral sac takes about twelve hours to complete its inflation.

A description of the anatomy of the functional diverticula is given. The walls of the diverticula consist of a thin epithelium lined with non-cellular intima and an outer muscular layer. The muscular layer consists of circular and longitudinal fibers which form a network enclosing the diverticula. There are approximately twelve bands of circular muscles and numerous longitudinal fibers between the circular bands. These longitudinal fibers frequently branch and anastomose and they may extend across a circular band without joining it. The nuclei of the muscle fibers are of two sizes and 10 and 19 in diameter.
These circular bands of muscle serve a sphincter function at the neck of the diverticulum. The epithelial layer is one cell deep; the nuclei are about 12 to 14 in diameter and they are usually oval in shape. The cell boundaries could not be distinguished. The intima, a non-cellular layer, is the innermost layer of the diverticula; it appears in sections as a thin and wavy line. The intima is inelastic and in a partially filled diverticulum it can be seen due to its numerous folds.

The ventral diverticulum has one nerve on each lateral wall. Nine to twelve small nuclei of sheath cells are observed along the length of the nerve. Each nerve has one multipolar nerve cell located at the posterior end of the diverticulum.

The fine structure was observed from electron micrographs of Aedes aegypti. The muscle fibers are composed of one myofibril, and only I and Z bands can be seen. The mitochondria are concentrated peripherally next to the sarcolemma. There are finger-like projections in the space between the Z bands; these projections contain many mitochondria of various sizes. The epithelial layer has a closely attached intima on one side and the basement membrane on the other side. The intima appears as a heavy line probably composed of osmophilic or other electron dense materials.

The functions of the oesophageal diverticula are discussed. They serve as food reservoirs for all kinds of sugary fluids and they serve as adjustable air cushions which maintain the volume of the body cavity.
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