INFLATION OF THE OESOPHAGEAL DIVERTICULA AND ELIMINATION OF AIR FROM THE STOMACH IN NEWLY EMERGED MOSQUITOES [1]

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Observations by several investigators on a variety of species of mosquitoes permit one to generalize that the adult mosquito emerges from its pupal skin with its stomach inflated with air and its oesophageal diverticula devoid of air. Also it may be observed that an hour or less after emergence the diverticula acquire some air and within a few hours they are well inflated; while this is taking place, the stomach loses the air it contained.

Marshall and Staley (1932) studied the distribution of air in the oesophageal diverticula and intestine of mosquitoes and its relation to emergence, feeding, and hypopygial rotation. They observed, as had others before them, the presence of air underneath the pupal skin and air in the stomach shortly before the adult emerges. Within an hour after emergence of the adult, Marshall and Staley state that the air in the stomach commences to pass forward into the oesophageal diverticula and this continues until the diverticula are inflated. Their hypothesis that the diverticula in the new adult are inflated by air from the stomach, has been widely accepted and quoted in the literature on culicid biology (Snodgrass, 1959; Christophers, 1960; Clements, 1963).

Brumpt (1941) raised objections to the hypothesis of Marshall and Staley. He pointed out that the structure of the oesophageal valve at the junction of oesophagus and stomach would seem to prevent the passage of air from stomach, to

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oesophagus, to diverticula. He believed that the amounts of air in the three diverticula total less than the amount held by the stomach and that the difference would have to be released from the mouth of the insect. He suggested that the air in the stomach could pass from that organ into the hind-gut and out of the anus, and that the air which inflates the diverticula is newly aspirated. In opposition to his suggestion Brumpt stated that he had never observed any air in the hind-gut. He studied several species of mosquitoes, including *Aedes aegypti,* and he made a number of excellent observations.

The objective of this paper is to demonstrate that the oesophageal diverticula are inflated in the new adult *Aedes aegypti* by air which enters through the mouth and passes directly into the diverticula, and that the air in the stomach leaves the mosquito by way of the hind-gut.

OBSERVATIONS. The observations reported in this paper were made on the Rockefeller strain of *Aedes aegypti.* The adults were picked up as they emerged from their pupal skins and usually within seconds they were used in an experiment.

The following experiments were performed on newly emerged adults to test our hypothesis that the diverticula are inflated with air that enters through the mouth and that the air in the stomach leaves by the hind-gut.

Experiment 1. The mouth of the adult was sealed, usually within a few seconds and rarely more than a minute or two after emergence, by liberally covering the mouthparts with stopcock grease or melted paraffin. This treatment prevented the

TABLE 1.—Distribution of air in the stomach and the oesophageal diverticula of adult *Aedes aegypti* at intervals of time following their emergence after (A) no treatment, (B) mouth sealed at time of emergence, and (C) mouth and anus sealed at time of emergence.

Treatment	Time after emergence when dissected	Number of mosquitoes dissected	Air present in stomach	Air present in diverticula		
				Vd	Dd	Dd
A. Controls; no treatment	0–1 min	6	6	0	0	0
	15 min	11	11	6[a]	4[a]	1[a]
	30 min	12	12	12[a]	7[a]	5[a]
	1 hr	9	9	9	9	9
	3 hr	6	6	6	6	6
	6 hr	3	0	3	3	3
	18 hr	4	0	4[b]	4	4
	24 hr	3	0	3[b]	3	3
B. Experiment 1; mouth sealed at emergence	30 min	12	12	0[c]	0[c]	0[c]
	1 hr	5	5	0[d]	0[d]	0[d]
	2 hr	9	9	4[a]	1[a]	0
	3 hr	6	6	0	0	0
	6 hr	6	2[a]	0	0	0
	18 hr	9	0	0[e, f]	0[f]	0[f]
	24 hr	9	0	0[c]	0	0
C. Experiment 2; mouth and anus sealed at emergence.	6 hr	10	10	0	0	0
	12 hr	13	0	0[f]	0[f]	0[f]
	18 hr	5	0	1[a]	0	0
	24 hr	8	0	1[a]	0	0

[a] The air consisted of one to six tiny bubbles.

[b] The ventral diverticulum inflated and extended to the fifth abdominal segment.

[c] Two mosquitoes had stopcock grease in all three diverticula and a third mosquito had stopcock grease only in the ventral diverticulum.

[d] One mosquito had stopcock grease in all three diverticula.

[e] The ventral diverticulum extended into the first abdominal segment indicating it had increased in length without inflation. Previous to this time it remained in the thorax.

[f] Meconium found in all three diverticula of one specimen.

entrance of air by the oral route and the objective was to learn what effect, if any, this would have on inflation of the diverticula. The result for all periods of time, up to and including 24 hours after treatment, was that no mosquito had inflated diverticula. In fact there was total absence of air in the diverticula except in a few instances (Table 1, B, 2 hrs) where several very tiny bubbles were present in four of nine mosquitoes.

The presence of these tiny bubbles is explained as follows. Within a few minutes after emergence, because of action of the pharyngeal pump, some air is pumped into the oesophagus from which it passes into the diverticula. When mosquitoes are dissected within a few seconds after emergence (Table 1, A, 0-1 min) no air is found in the diverticula. Fifteen and thirty minutes following emergence (Table 1, A, 15 min and 30 min) tiny bubbles are present. Referring back to the mosquitoes in Table 1, B, 2 hrs, and also noting that there are two similar occurrences in Table 1, C, 18 hrs and 24 hrs, the air in their diverticula can be explained easily by the aspiration of a little air before their mouths were sealed. Sometimes, when the operation does not proceed smoothly, several minutes may pass between the time of emergence and the sealing of the mouthparts.

The stomachs of these mosquitoes were inflated with air, as is true for all mosquitoes that have normal emergence, and it was not passed forward into the diverticula. The valve between the oesophagus and stomach is apparently very effective in preventing the passage of air from stomach to diverticula.

If the mouth is not sealed (Table 1, A), the diverticula are inflated; if the mouth is sealed (Table 1, B and C), the diverticula are not inflated. Therefore it must follow that the air which inflates the diverticula enters the mosquito by way of the mouth and it passes directly from the oesophagus into the diverticula.

The air in the stomach at the time of emergence is present in considerable amount for 3 hours; thereafter it rapidly diminishes, and at 6 hours it is usually gone (Table 1, A and B). This observation suggested a second experiment.

Experiment 2. Both the mouth and the anus were sealed a few seconds after emergence to see if the air in the stomach would be retained by that organ.

Six hours after emergence 10 treated mosquitoes had some air remaining in the stomach; 12 to 24 hours after emergence 26 mosquitoes had no air in the stomach (Table 1, C). Thus, the air was not retained in the stomach. This raised the question of how the air left the stomach and the mosquito. As pointed out in Experiment 1, the air was not passed forward into the diverticula, and no trace of it was found elsewhere in the alimentary canal. In Experiment 2 the air left the stomach gradually; the large bubble of air became smaller until it disappeared. Our explanation for its disappearance is that the oxygen diffused into the tissues where it was utilized; and the nitrogen, also obeying the laws of diffusion, passed into the abundant tracheae in the stomach wall and from there it was forced out of the insect by respiratory movements.

Experiment 3. In untreated mosquitoes, under usual conditions, does the air in the stomach leave by way of the hind-gut and anus? It is common knowledge that if mosquitoes are observed while feeding, and shortly after feeding, droplets of liquid leave the anus. Occasionally bubbles form so there is no doubt that air can be passed from the anus. Does the original air in the stomach of newly emerged unfed mosquitoes leave in similar fashion? In this experiment newly emerged mosquitoes were kept under the microscope with the tip of the abdomen in water and they were constantly observed for bubbles at the anus. None was seen. Dr. Alan P. Brockway suggested that if air left the anus in small amounts it could be absorbed by the water and would not appear as bubbles. He recommended the use of mineral oil instead of water. With the tip of the abdomen of the newly emerged mosquito surrounded

by mineral oil, small bubbles of gas can be observed leaving the anus. The air in the stomach, therefore, normally leaves by this route.

Experiment 4. Mosquitoes were studied to see if the meconium was eliminated soon after emergence and, if this was the case, is its elimination related to the discharge of air from the stomach. Adults were collected one at a time as they emerged from their pupal skins. Each was attached by the mesonotum to the tip of a toothpick with a droplet of glue. By manipulating the toothpick in a lump of clay, the mosquito was positioned with a cover glass underneath it; a second cover glass, at right angles to the first one, barely touched the tip of the abdomen. Thus, any expelled excreta struck one or the other of the cover glasses. Examination of these cover glasses revealed droplets of meconium within 15 to 20 minutes and a large amount was passed in two hours. This meconium was expelled along with gas and the source of gas is the air in the stomach. In the newly emerged adult the air is in the anterior portion of the stomach. The meconium is in the posterior portion of the stomach and in the intestine. It is reasonable to believe that the elimination of the air from the stomach helps free that organ and the hind-gut of most of the meconium. Mosquitoes held without water or other food and dissected 24 hours after emergence, usually have a little meconium remaining in the stomach and somewhat more in the hind-gut.

DISCUSSION. The adult mosquito at emergence from the pupal skin has air in its stomach. Under normal conditions this air leaves the stomach within a period of 6 hours after emergence. As the air is leaving the stomach, the oesophageal diverticula are inflated, but the long-held assumption that the diverticula are inflated with air from the stomach is in error.

When the newly emerged mosquito is prevented from taking air by way of the mouth it does not inflate the diverticula, although the stomach is filled with air. The air which inflates the diverticula enters the moquito by way of the mouth and it passes directly from the oesophagus into the diverticula. The air in the stomach leaves this organ by passing out of the hind-gut and anus. However, if the anus and mouth are both sealed, the air still leaves the stomach and it does not pass into the diverticula because they remain uninflated. To account for the disappearance of air from the stomach when both mouth and anus are sealed, it is postulated that the oxygen is used by living tissue and the nitrogen diffuses into the tracheae of the stomach and is eliminated from the insect by way of the spiracles.

If the anus is not sealed and the tip of the abdomen is placed in a drop of mineral oil the appearance of bubbles of gas at the anus is evidence that the air from the stomach is discharged from the insect by the way of the hind-gut and anus. This can be observed in normal mosquitoes, in which case the diverticula will be inflated; or it can be observed in mosquitoes with the mouth sealed, in which case the diverticula remain uninflated. We have not actually observed air in the hind-gut but observation of this organ *in situ* in the living mosquito has not been successful and observation of this organ dissected from the mosquito is not very meaningful. In this matter our negative direct observations agree with Brumpt.

The use of stopcock grease, with a melting point of 200 C, was discontinued in favor of paraffin embedding compound, with a melting point of 56–57C, because some mosquitoes pumped the stopcock grease into their diverticula. It is not only of interest that the mosquito could pump this grease but it is of particular interest that it was always forced into the diverticula and none into the stomach. Sometimes the grease was found only in the ventral diverticulum and at other times it was found in all three.

Reverse peristalsis can take place in the stomach because the meconium of the stomach was found in the diverticula of 2 out of 92 mosquitoes with sealed mouths. Although this occurred only rarely, and

then not sooner than approximately 12 hours after emergence, it is clear evidence that this material can be forced from the stomach into the diverticula. This fact would suggest that the mosquito could also force air from the stomach into the diverticula, but they never did so; the air left the stomach approximately 6 hours before any meconium was forced into the diverticula.

The role of the air in the stomach in helping the imago out of the pupal skin and giving shape to the compact and limp new adult has been covered by various authors; Christophers (1960) gives a good review. To the above functions of this air we add that it makes a little oxygen available at a critical time when the tracheal connections with the respiratory trumpets are severed, and when the remaining air passes through the alimentary canal it helps to clean the stomach and intestine of the meconim.

SUMMARY. According to the hypothesis of Marshall and Staley (1932) the oesophageal diverticula of newly emerged adult mosquitoes are inflated by the air from the stomach. This hypothesis has been widely accepted in the literature on culicid biology. Experiments are described which demonstrate that this hypothesis is incorrect. The diverticula are inflated by air which is newly aspirated. The air which inflates the stomach, instead of passing forward into the diverticula, leaves the mosquito by way of the hind-gut.

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