THE ORIGIN AND MOVEMENT OF GAS DURING ADULT EMERGENCE IN *AEDES AEGYPTI*: AN HYPOTHESIS

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ABSTRACT. Several events which precede adult emergence in *Aedes aegypti* were studied, including changes in buoyancy, changes visible through the pupal cuticle and changes at the foregut-midgut junction. Our data suggest that the gas which is present in the posterior midgut at the time of emergence originates in the tracheal system. During the process of emergence this gas moves into the exuvial space through the adult spiracles and then follows the exuvial fluid into the alimentary canal.

INTRODUCTION

Aspects of mosquito adult emergence (pupal to adult ecdysis) have been described (Nuttall and Shipley 1901, Marshall and Staley 1932, Brumpt 1941, Christophers 1960, and Clements 1963). Venard and Guptavanij (1966) and Gillett (1983) studied the fate of the gas which appears in the midgut during adult emergence. However, the source of gas in the midgut of newly emerged adults, and in addition, the source of gas which appears in the exuvial space prior to emergence from the exuvia remains unclear. We examined several events which precede emergence and present a new concept regarding the origin and movement of gas in the exuvial space and alimentary canal.

MATERIALS AND METHODS

Larval and pupal *Aedes aegypti* (Linn.) (Rockefeller strain) were reared from eggs on a diet of ground Purina gerbil chow, live brewers yeast and lactalbumin. All pupae examined were of known "ages" (±30 min) postpupation (larval-pupal-ecdysis) and all were females.

The rate of rise over a constant vertical distance in a column of water was taken as a measure of buoyancy. A pupa was killed by immersion for approximately 1 second in hot water (60°C), released at the bottom center of a 500 ml graduated cylinder. The time it took to rise along the same 121 cm interval was measured in 45 pupae ranging in age from 32 to 48 hours postpupation.

To observe changes in the exuvial space, from 38 hours to adult emergence, pupae were placed singly in vials of water and held at 27 ± 1°C in a Forma Scientific Incubator. They were examined on moist filter paper under a dissecting microscope at least once, but usually twice per hour.

Preliminary observation revealed the following: (1) as the time of emergence approaches, movements of the cibarial and pharyngeal pumps are visible through the cuticle, (2) pupal cuticle collapses against adult cuticle in places, and (3) the pattern of exuvial space gas spread can be observed. The following were then determined: (1) age at onset of pumping, (2) age at onset of cuticle collapse around the mouthparts (collapse is first evident here), (3) the pattern of cuticle collapse over the body, (4) the location of the first exuvial space gas appearance; (5) the pattern of exuvial space gas spread, (6) the age at complete proboscis sheath cuticle collapse, and (7) age at emergence. Usually, most or all parameters were observed in the same pupa.

Pupae of known ages (±30 min) were dissected in high viscosity Cargille Type B immersion oil to check for midgut gas. Serial paraffin sections of pupae of known ages (±1 hr) were prepared to study internal changes. Pupae were killed and fixed in hot (60°C) Bouin's solution, infiltrated with Paraplast®, mounted on slides and stained using the modified Azan trichrome method of Hubschman (1962).

RESULTS

Fig. 1 shows changes in rate of rise (buoyancy) for several hours prior to adult emergence. Mean rate of rise (±SD) in seconds is plotted against age in hours. The shorter the time, the greater the buoyancy. Note that buoyancy increase is particularly pronounced after 43.5 hr, all values being under 10 seconds. In contrast, values for pupae 32 to 43 hours old ranged from 9 to 18 sec. with wider variability. Mean (±SD) rate of rise after 43.5 hr, 7.8 ± 1.8 sec, was significantly lower than the same parameter, 13.6 ± 6.3 sec, prior to 43.5 hr ($t = 8.931$; 39 degrees of freedom; $P = 0.01$).

In Fig. 2 the mean (±SD) age at pumping onset was 42.8 ± 1.4 hr (range: 40.8-45.5, n = 15). Pumping movement was irregular at onset. In 5 individuals pumping was not evident until cuticular collapse. It is possible that pumping began earlier than observed in some individuals. During pumping, fluid movement was seen in the exuvial space.

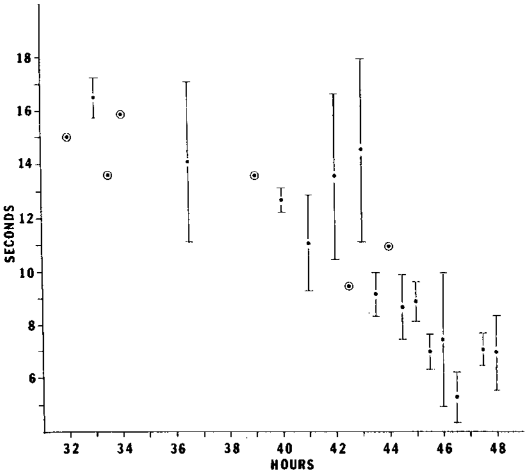

Fig. 1. Rate of rise (seconds) as a function of hours post-pupation. Solid circles, means; solid circles within larger circles, only one measurement taken; vertical lines, standard deviation; horizontal bars, ranges.

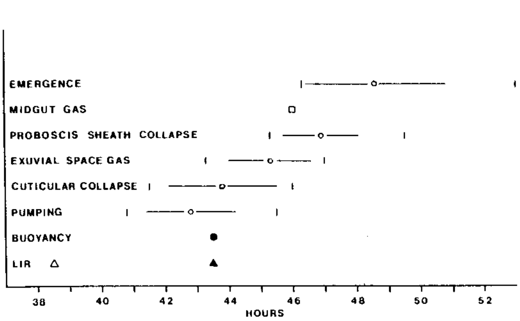

Fig. 2. Timing of events several hours prior to adult emergence. Open circles, means; horizontal lines, standard deviations; Vertical bars, extremes of ranges. LIR, open triangle represents earliest observation of reversal of larval intimal remnant and the solid triangle the time at which LIR reversal had occurred in all specimens. BUOYANCY, circle indicates the time at which buoyancy began to increase (see Fig. 1). PUMPING, onset of pumping. CUTICLE COLLAPSE, onset of cuticle collapse. EXUVIAL SPACE GAS, earliest observation of gas in exuvial space. PROBOSCIS SHEATH COLLAPSE, time of complete collapse. MIDGUT GAS, square indicates earliest appearance of gas in the posterior midgut. EMERGENCE, emergence of the adult from the exuvia.

Mean age at the beginning of cuticular collapse (Fig. 2) was 43.8 ± 1.7 hr (range: 41.5-46.0, n = 20). Cuticular collapse was first seen around the mouthparts, then around the wings and the legs. Although the first evidence of collapse is around the mouthparts, this region is the last to collapse completely. The abdominal cuticle collapses only slightly at about the time the mouthparts collapse completely.

Gas first appeared around the spiracles (Fig. 2, exuvial space gas) in the abdominal exuvial space (at a mean age of 45.3 ± 1.3 hr, range: 43.3-47.0, n = 21), then in the thoracic exuvial space around the wings, and finally around the legs and mouthparts.

By the time gas appeared around the mouthparts, the proboscis sheath had completely collapsed. Mean age of complete collapse of proboscis sheath cuticle (Fig. 2) was 46.9 ± 1.2 hr (range: 45.3-49.5, n = 26). There appeared to be little exuvial space fluid once the proboscis sheath collapsed.

Mean age at emergence (Fig. 2) in the sample of pupae observed for onset of pumping was 48.6 ± 2.1 hr (range: 46.3-53, n = 27). Sixteen pupae ranging in age from 44 to 49 hours were examined for gas in the posterior midgut. No gas was found in the posterior midgut before 46 hours. Gas was present in the following proportions of individuals: 46.0 hr (1/2), 46.5 (0/2), 47.0 (1/2), 47.5 (1/1), 48.0 (2/3), 49.0 (1/1).

Histological sections were examined with regard to the larval intimal remnant (Fig. 3) which is attached at the junction between the foregut and midgut (Romoser and Venard 1967). This structure remains from the incomplete shedding of the larval foregut intima at pupation. In young pupae it resembles a wind sock and "points" anteriorly into the foregut lumen (Fig. 3A). Later it reverses direction and "points" posteriorly into the midgut lumen (Fig. 3B). Examination of 58 pupae ranging in age from 32 to 46 hours revealed that larval intimal remnant (LIR) reversal began at 38.5 hours and had occurred in all individuals by 43 hours (Fig. 2).

Our data suggest the following sequence of events (Fig. 2): (1) larval intimal remnant (LIR) reversal, pumping onset, buoyancy increase, (2) beginning of cuticle collapse, (3) appearance of gas in the abdominal exuvial space and subsequently elsewhere, (4) complete collapse of proboscis sheath and appearance of gas in the posterior midgut, and (5) emergence from the exuvia.

DISCUSSION

Buoyancy increase appears to begin well before adult emergence and before the appearance of gas in the posterior midgut. Thus, while "late" buoyancy increase coincides with the presence of gas in the midgut, "early" buoyancy increase does not. However, the apparent onset of buoyancy increase (43.5 hr, Figures 1 and 2) coincides closely with the earliest observation of exuvial space gas (Fig. 2).

There are two regions in the mosquito pupa where an "early" increase in gas volume (and

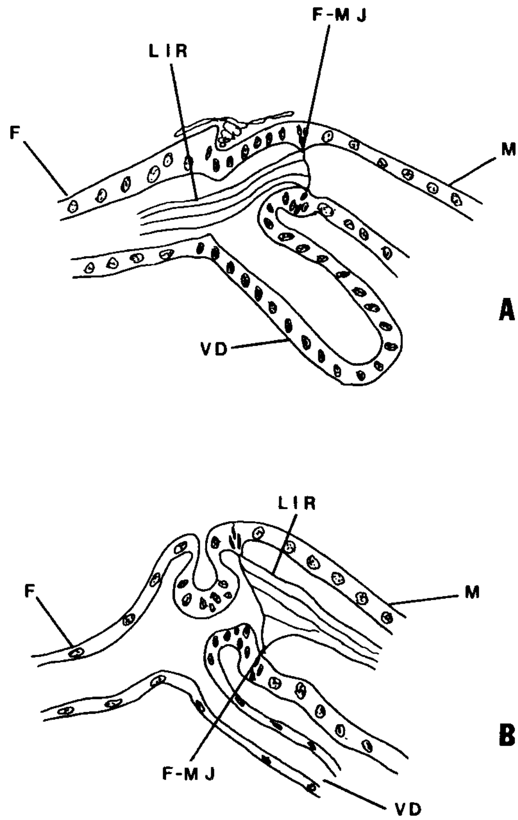

Fig. 3. Sagittal section in region of foregut-midgut junction. A, young pupa; B, pharate adult, F, foregut; F-M J, foregut-midgut junction; LIR, larval intimal remnant; M, midgut; VD, ventral diverticulum. Note the change in direction of the LIR in the pharate adult.

hence buoyancy) could take place, the ventral air space and the tracheae. The ventral air space could have gas added to it via the first abdominal spiracles, but Leung and Romoser (1979) showed that plugging these spiracles with paraffin does not inhibit ecdysis. Further, pupae are apparently unable to add gas to the ventral air space once it is filled during the process of pupation (Romoser and Nasci 1978). By elimination, the early buoyancy increase must indicate an increase in tracheal gas volume.

Brumpt (1941) first noticed gas around the respiratory trumpet bases. We found gas first in the abdomen and later around the trumpet bases. This discrepancy is probably due to the fact that we observed pupae laterally on moist filter paper. The appearance of gas in the abdomen before its appearance in the thorax precludes the thorax as the source of gas in the exuvial space in the abdomen. It thus appears that the only possible source of gas in the abdomen is from the tracheal system via the adult abdominal spiracles. The gas in the thoracic exuvial space probably also enters via spiracles instead of through breaks in the tracheae which connect the trumpets and thoracic spiracles as suggested by Brumpt (1941).

Reversal of the larval intimal remnant must be caused by something moving from the foregut into the midgut before the onset of emergence and implies the action of peristaltic contraction of foregut muscles. Such movement may also indicate ingestion of material. The temporal proximity of reversal of the larval intimal remnant and onset of pumping, the physical continuity between the exuvial space and foregut as described by Christophers (1960), and the visible movement of fluid in the exuvial space associated with pumping suggest that a pupa ingests exuvial fluid (and later gas) from the exuvial space. This would account for the observed collapse of the pupal cuticle against the new adult cuticle due to negative pressure generated by the removal of fluid from the exuvial space. The existence of negative pressure would in turn facilitate the movement of gas from the tracheal system via the adult abdominal spiracles into the exuvial space. Contraction of skeletal muscles may also be involved in "pumping" gas through the spiracles. It is also possible that some exuvial fluid is absorbed through the adult cuticle, but it seems more likely from our data that ingestion is involved. Both means of removal of exuvial fluid could be involved concurrently. It is, however, reasonable to assume that the forced movement of fluid by the action of the cibarial and pharyngeal pumps facilitates the subsequent movement of gas throughout the exuvial space.

Given evidence for the origin of gas in the exuvial space from the tracheal system and the ingestion of exuvial fluid and gas, we conclude that a major source of gas in the midgut is the tracheal system via the exuvial space. The ultimate source of gas in the tracheal system must be the external environment, gas entering through the respiratory trumpets.

In summary, we provide evidence that gas from the external environment moves into the tracheal system, inflates newly developed adult tracheae, passes through the adult spiracles into the exuvial space, and following the exuvial fluid enters the alimentary canal causing the expansion of the posterior midgut. The consequent expansion of the pharate adult within the pupal cuticle accounts, in part, for the way the emerging adult passively slides up and out of the pupal cuticle. A good analogy would be a hand swelling within a glove to the point where the glove splits along seams and the hand "emerges".

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