THE ABSORPTION OF FLUID FROM THE TRACHEAL SYSTEM OF MOSQUITO LARVAE AT HATCHING AND MOULTING

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(With Three Text-figures)

DURING development the tracheal system of insects is at first filled with fluid. About the time of hatching from the egg and at each subsequent moult this fluid is absorbed into the tissues. The mechanism of absorption was studied in a number of insects, and earlier work on the subject reviewed, by Sikes & Wigglesworth (1931), who concluded that although secretory activity might be responsible for the presence in the tracheal system of a fluid which could be absorbed by osmosis, the final absorption was probably brought about by the osmotic pressure of the blood. At the suggestion of Prof. A. Krogh, who has taken an active interest throughout this work, the problem has been reinvestigated with a view to differentiating the part played by secretion.

NORMAL HATCHING OF *AEDES* LARVAE

The mosquito, *Aedes aegypti*, has been used for all the experiments. The eggs of this species will hatch when submerged, and the tracheal system, unlike that of many insects, does not fill with air until the spiracles are opened at the water surface. It is therefore a very suitable object on which to investigate the mechanism of filling.

As is well known, the eggs of *Aedes*, which can be kept dry for weeks or months, hatch in a few minutes or a few hours after immersion in water. A circular cap is split from the anterior pole of the egg by means of the hatching spine on the head (Breslau, 1920; Stadtmann-Averfeld, 1923). The newly hatched larva at once makes its way to the surface of the water and exposes its spiracles to the air.

Fig. 1 D shows the apex of the respiratory siphon. The two spiracles, each of which appears to be guarded by a sphincter, open into a small vestibule which later always contains a minute bubble of air. The vestibule is formed by the apposition of a pair of valves, each of which bears on the outer side a long hair curved inwards at the tip. When the larva reaches the surface these hairs are held in the surface film and drawn apart so that the valves separate and the vestibule is exposed to the air (Fig. 1 E). Almost at once air enters the spiracles. Usually it extends first along one tracheal trunk to the front of the thorax and then along the other. The system is completely filled with air in about 15–30 min.

At this stage the larva has the outline shown in Fig. 1 B, but it soon swallows water and contracts its abdomen and thorax so as to drive fluid into the head until, within 1 or 2 hr., it assumes the form shown in Fig. 1 C.

THE EFFECT OF SUBMERGENCE ON FILLING OF THE TRACHEAL SYSTEM

The first question studied was the effect on the subsequent filling when the larvae were kept submerged for a varying length of time. Dried eggs were placed in distilled water, containing a very dilute infusion of Protozoa, in small tubes (0·7 × 2·0 cm.) closed with boulting silk held by a rubber ring; six eggs in each.

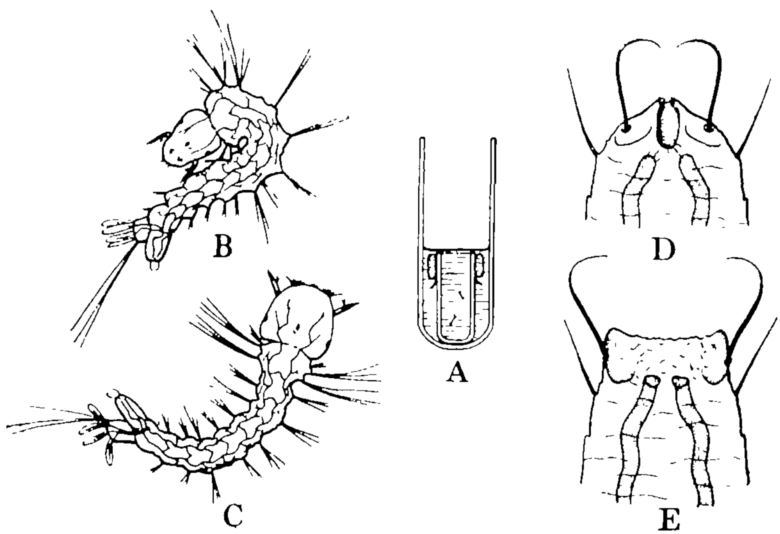

Fig. 1. A, method of keeping larvae submerged; explanation in text. B, newly hatched larva before expansion of head. C, the same with head expanded. D, tip of respiratory siphon in first-stage larva with valves opposed and spiracles closed. E, the same with valves separated and spiracles open.

Each tube was placed in an outer tube (1·5 × 6 cm.) and water added until it was just above the gauze top (Fig. 1 A). All air bubbles present in the small tube were sucked through the gauze with a pipette. The larvae were kept at 22° C., and on succeeding days they were taken out and exposed to the air in drops of water on a microscope slide and the entry of air into the tracheal system observed by transmitted light.

In larvae submerged in this way for 16–24 hr. the system began to fill with air immediately the siphon reached the surface. The main tracheal trunks were filled within 7 min., and the entire system was filled to the finest branches in 12 min. The rate of filling was almost identical in all the larvae (Fig. 2 A) and was rather more rapid than in larvae which had access to air immediately after hatching.

After submergence for 2 days filling began immediately; the main trunks were filled in 8–20 min. in different larvae; the entire system was filled in 20 min. to 1 hr.

After submergence for 3 days five larvae out of six began to fill almost at once; the main trunks were filled in 23 min. in three larvae, in 2 hr. in one larva, within 4 hr. in another, whereas in the sixth larva, in which air first entered the system 1 hr. after exposure to the surface, only a short segment of each trachea within the respiratory siphon filled with air. In only four of the larvae did the finest branches fill with air, and this took place between 5 and 16 hr. after access to the surface.

After submergence for 4 days the filling of the tracheal system in five larvae took place as shown in Fig. 2 B–F. In one larva the main trunks filled as far as

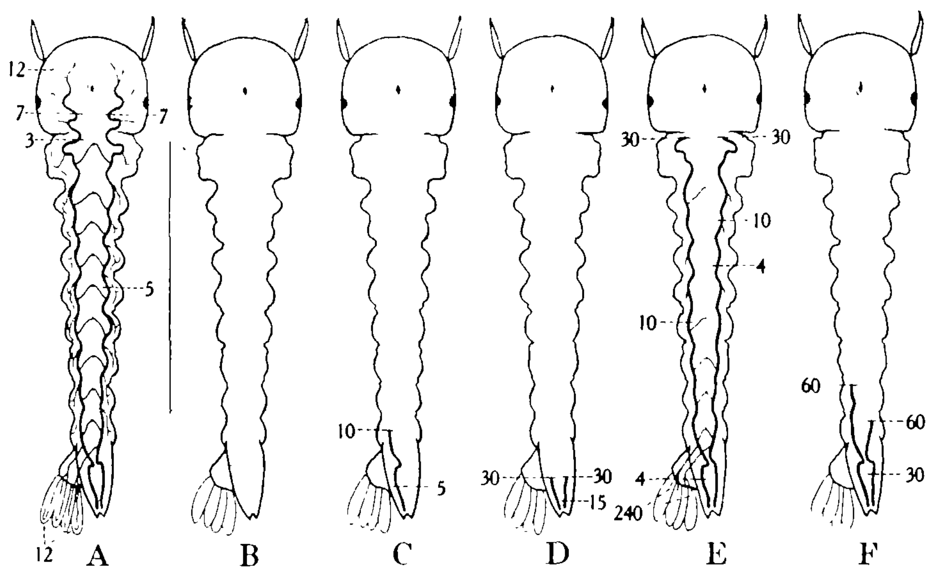

Fig. 2. A, first-stage larva exposed to the air after submergence for 16 hr. after hatching. B–F, after submergence for 4 days. The numbers indicate time in minutes after exposure to the air; they mark the level to which the columns of air in the tracheal system extended at these times.

the thorax; in three larvae only short columns of air entered the system; in one larva there was no filling at all. In none did air enter the finest branches.

It is clear from these experiments that if filling is deferred for several days the larvae gradually lose the power to absorb fluid from the tracheal system. This change is more evident and takes place more rapidly if the larvae are abundantly fed so that the tracheal system undergoes changes preparatory to moulting. But in the experiments described above the larvae were almost without food, and at the time they were exposed to the air there was no sign of moulting beginning; the failure of absorption was not due to that cause. These results confirm those of Sikes & Wigglesworth (1931), in which it was found that if the larvae of fleas were allowed to hatch into water and were kept submerged for several days the tracheal system would no longer fill with air when the larvae were dried.

THE SITE AND MECHANISM OF ABSORPTION

The main tracheal trunks of *Aedes* larvae are connected in each segment by a transverse trachea from which no tracheoles are given off. During filling it is quite common to see columns of air enter these transverse branches from both ends simultaneously. This proves that absorption of fluid probably takes place throughout the tracheal system and not only in the tracheoles. It is generally believed that the tracheae of insects, lined as they are by a hydrophobe cuticle, are later impermeable to water. This suggests that the development of impermeability in the cuticle of the tracheal system may be a factor in the disappearance of the capacity to absorb fluid.

An analogous change is seen in the cuticle of the body surface in *Rhodnius* during moulting (Wigglesworth, 1933; Wigglesworth & Gillett, 1936). Until shortly before the old cuticle is shed the new cuticle is freely permeable to water, and the moulting fluid is absorbed through it. At the time of moulting the cuticle is found to be almost impermeable to water, as judged by evaporation, and this change comes about without any visible alteration.

On the other hand, the tracheal endings remain permeable to water throughout life (Wigglesworth, 1930). If therefore the fluid in the tracheal system were absorbed simply by the osmotic pressure of the blood (Sikes & Wigglesworth, 1931), the system should fill with air in spite of the prolonged submergence of the larvae. This suggests that the essential factor in absorption is secretory activity by the cells bounding the tracheal system.

This has been tested by narcotizing submerged larvae with chloroform some 12 hr. after hatching and then exposing them to the air. Many of the larvae will rest with their spiracles open at the water surface, but no air enters the tracheal system while the larva is narcotized. The larvae recovered their activity in 20–30 min. but even then there was a further delay of 9 or 10 min. before the filling of the tracheal system began. As soon as filling started it was completed at the same rate as in the normal larva (Fig. 2 A, B).

The inhibition of absorption by narcotization suggests not merely that absorption is a secretory phenomenon but that the nervous system is concerned in starting the process. Further evidence of this is afforded by the behaviour of larvae which have been submerged so long that the tracheal system will no longer fill with air. Such larvae, which present a most agitated appearance, repeatedly brush the respiratory siphon with the mouth-parts as though to remove some imaginary obstruction.

If larvae 12 hr. after hatching are decapitated in a dilute Ringer's solution before they are allowed to bring the siphon to the water surface, they start to fill with air as rapidly as normal larvae. Clearly the cerebral ganglia are not necessary.

In order to test the absorptive capacity of the tracheal system, attempts were made to introduce different solutions into it. The larvae were submerged in the solution and exposed to 10% carbon dioxide to make them open the spiracles below the surface. In only one instance out of at least 100 larvae did a solution of indigo carmine enter the tracheal system. In this case there was a heavy precipitate of solid dye throughout the system, in large trunks and fine branches—again suggesting that absorption of water takes place in all parts of the system. But these experiments as a whole support the conclusion that the absorption of fluid is a complex reaction which is normally initiated only by appropriate sensory stimuli; i.e. when the curved spines of the siphon valves are caught in the surface film and drawn apart so that the vestibule is exposed to the air.

FILLING OF THE TRACHEAL SYSTEM AFTER MOULTING

The changes in the tracheae at moulting can be readily observed in the living larva. When the new tracheal system is fully developed it is seen to be separated from the old tracheal system, notably at the front of the thorax, by a wide sleeve of fluid (Fig. 3 C). If the system is followed to its terminations shortly before moulting, the delicate lining of the old trachea can be seen partially collapsed, with

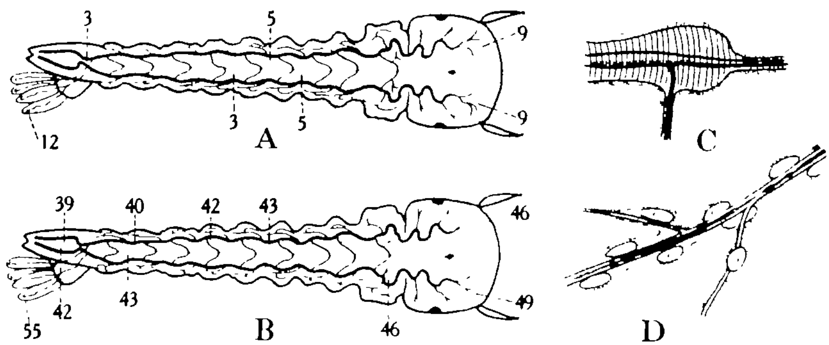

Fig. 3. A, normal first-stage larva submerged for 12 hr. after hatching and then exposed to the air. B, larva from the same batch narcotized with chloroform before exposure. The numbers indicate time in minutes as in Fig. 2. C, main tracheal trunk in the thorax of first-stage larva shortly before moulting; the new trachea, filled with fluid, forms a wide sleeve round the old trachea containing air. D, part of the central trachea in anal papilla of a third-stage larva just before moulting; the new trachea and its branches contain fluid; the old trachea is partially collapsed so that the column of air is interrupted.

the column of air contained in it often interrupted. The fluid between the cuticle of the old trachea and the new is here continuous with the fluid in the tracheoles (Fig. 3 D). At this stage the walls of the new main tracheae, although they can undoubtedly be deformed by the slightest pressure, show no sign of crumpling or collapse. There is clearly no absorptive force being exerted upon the tracheal fluid.

This state persists right up to the time of moulting. The larva then comes to rest at the water surface and proceeds to swallow water, at first slowly, but with increasing rapidity until its body becomes exceedingly tense. After swallowing a few times it will flex its head sharply until finally the capsule splits behind the clypeus. The larva will now be swallowing water every 2 sec. Peristaltic movements begin which advance the body, through the split in the head, away from the old skin. The main tracheal trunks rupture between each body segment and the fragments are drawn out through the two pairs of thoracic and eight pairs of abdominal spiracles. Usually when the old tracheae rupture air will escape from them into the new tracheal system; but sometimes the air seems to be drawn out with the old linings, and when the larva is examined immediately after moulting the new system contains only fluid, the main trunks being crumpled and collapsed.[1] In any case the larva at once comes to rest at the water surface, air enters the respiratory siphon, and the main tracheae resume their distended form as the air spreads along them. The fluid in the tracheal branches is then absorbed. The rate of absorption varies; it is usually complete and the fine branches are filled with air in from 3 to 5 min. During this absorption of fluid the muscles all over the body twitch continuously so that the whole body seems to be quivering.

The general features of the filling of the tracheal system after moulting are thus the same as after hatching from the egg. The absorptive force is first brought into play at a definite moment in development. Larvae which have been submerged so long after hatching from the egg that the tracheal system is incompletely filled with air, or contains none at all, will almost always become filled with air when they moult to the second stage. But if the first-stage larvae are kept submerged until they moult, the second-stage larvae will contain only fluid; and if they are then given access to the surface, the system fills with air for the first time when they moult to the third stage.

It was of interest to see how long the second-stage larvae, derived from air-free first-stage larvae, could defer filling of the tracheal system when they were held submerged. The exact time is uncertain, but it is undoubtedly very brief, for larvae allowed to come to the surface less than half an hour after moulting either took in no air at all or only into the tip of the respiratory siphon. Those larvae which are unable to fill their tracheal system, like the first-stage larvae in the same case, make repeated efforts to clean the spiracular openings.

There is therefore at the beginning of each stage a period during which the larva can absorb fluid from the tracheal system; but it is only in the first stage that absorption can be deferred. It is possible that this ability is associated with the habit of these larvae of remaining for weeks or months, fully developed, in the dried egg. An attempt to see whether the first-stage larvae of *Culex*, the eggs of which have not this property, are able to defer absorption was unsuccessful because the eggs failed to hatch when submerged.

A striking parallel with these changes in the tracheal system after hatching and moulting is seen in the adult fly *Calliphora*, as described by Fraenkel (1935). After emergence from the pupa these flies inflate their bodies and expand their wings, and then darkening and hardening of the cuticle takes place. But if the fly is kept beneath the surface of the earth so that it must continue to dig its way upwards, these processes are temporarily inhibited and can be deferred for many hours, though not indefinitely. As Fraenkel showed, the nervous system is concerned here also in controlling the chemical changes that take place in the cuticle after emergence.

[1] It is probable that much of the fluid occupying the main tracheal trunks is discharged with the old cuticle. Stadtmann-Averfeld (1923) describes this as happening in the first-stage larva after hatching, but that I am unable to confirm.

In a previous discussion on the mechanism of filling of the tracheal system (Sikes & Wigglesworth, 1931) the absorption of fluid was attributed to the osmotic pressure of the blood. But since the observations described in this paper show that in *Aedes* larvae, whatever may be the physical mechanism of absorption, it is subject to the control of the organism, that it is in fact a secretory process, much of the earlier argument is invalidated. The facts available do not yet justify a fresh discussion of the problem.

SUMMARY

At the hatching and moulting of *Aedes aegypti* the fluid present in the tracheal system is removed, apparently throughout the whole system, by an active absorption which takes place only when the respiratory siphon is open at the surface so that air can enter. The nervous system is apparently concerned in the initiation of this absorption.

First-stage larvae kept under water can retain fluid in the tracheal system for several days and absorb it when they get access to the air. This ability is gradually lost if filling is deferred for more than 3 days. The tracheal system then becomes filled with air for the first time after moulting to the next stage. Second-stage larvae must absorb the fluid within a few minutes of moulting or they are unable to do so.

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