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The Hatching of Insects from the Egg, and the Appearance of Air in the Tracheal System

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With 8 Text-figures.

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INTRODUCTION.

IT has recently been shown (Wigglesworth, 1930) that the extension of air into the tracheal capillaries of insects is determined largely by the osmotic pressure of the tissue-fluids in which these capillaries are bathed; and it was suggested that osmotic pressure might also be the force involved in the initial filling of the tracheal system with air at the time of hatching from the egg. It was argued, and the argument need not be repeated here, that, unless the nature of the tracheal wall changes later, the fluid present in the tracheae up to the time of hatching is

probably not 'serum', as it is usually said to be, i.e. a fluid containing both crystalloids and colloids like the tissue-fluids, but either pure water, or a solution in water of substances which could pass through the walls of the tracheal capillaries. The hypothesis was put forward that this fluid was an ultra-filtrate from the tissue-fluids into the tracheal system, the filtration being brought about by the hydrostatic pressure within the developing egg; and that after hatching, the hydrostatic pressure being reduced, this fluid was absorbed into the tissues by osmosis. This idea was suggested by the statement of Frankenberg (1915) that the developing larva of Corethra appeared to be subjected to great pressure.

The present work was undertaken in the first instance with a view to proving this hypothesis; and it may be said at once that it has proved to be unfounded. For it will be shown that in many insects the tracheal system fills with air while the larva is still inside the egg. On the other hand, we have made a number of observations upon the first appearance of air in the tracheae of a variety of insects, a subject about which comparatively little is known, and these observations provide a basis for discussion of the problem.

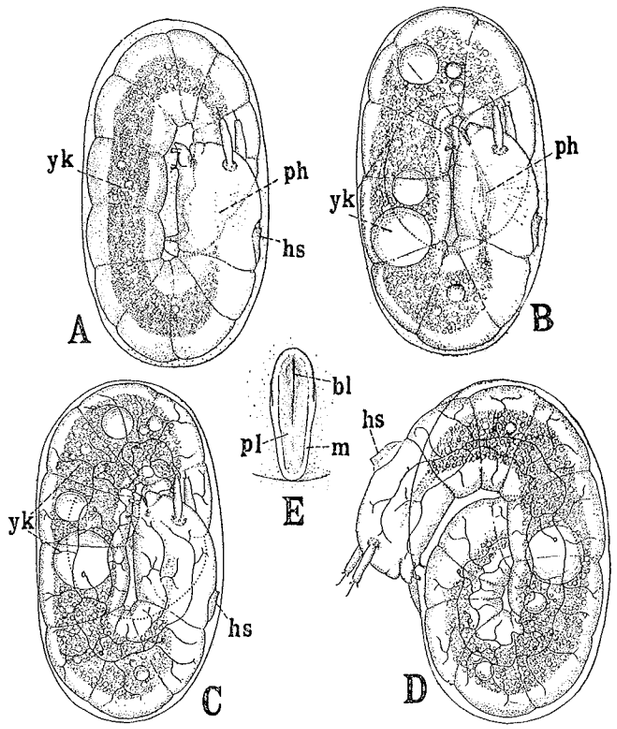
In the course of this work we have studied the mechanism of hatching in the insects dealt with. In this connexion we have added to the already long list of insects which possess 'hatching spines', and we have attempted some generalizations on the mechanism of emergence from the egg.

Hatching of Eggs of the Flea (Ceratophyllus wickhami) of the Grey Squirrel.

During its first instar the larva of the flea has on its head a powerful spine or egg-burster, figured, in the case of Ceratophyllus wickhami, by Sikes (1930). Although it has long been recognized (Künkel, 1873) that this structure is used by the larva to break through the chorion, its precise mode of action is not understood. Mitzmain (1910) observed the hatching of a single Ceratophyllus acutus larva, and gives a highly spirited account of the process; but our larvae followed the spectacular procedure there described only when the eggs

had been allowed to become too dry and therefore, judging by the many failures in hatching, were probably abnormal.

Text-fig. 1.



Hatching of egg of flea. A, egg about 12 hours before hatching. B, larva swallowing the amniotic fluid: it is longer and stouter, the gut contains large droplets of fat, the hatching spine is beginning to project. C, larva dry on surface, tracheae filled with air. D, larva has moved round in shell and cut a longitudinal slit through which it is emerging. Note projection of hatching spine. *hs*, hatching spine; *ph* pharynx; *yk*, gut contents. E, hatching spine viewed from above, consisting of a median tooth (*bl*) and an elongated footplate (*pl*), surrounded by a thin membrane (*m*).

Text-fig. 1, A, shows the fully developed larva curled up in the egg, about twelve hours before hatching. It is floating freely

in the amniotic fluid and does not fill the shell completely. The spiracles and tracheae are visible but contain fluid. The gut is filled with the remains of the yolk, in the form of rather small globules of fat and much granular material.

At about this stage, pulsating movements in the head of the larva commence, and it is seen to be swallowing the amniotic fluid (Text-fig. 1, B). When this watery fluid is added to the fatty contents of the gut, the fat globules run together to form large droplets. At the same time the larva increases both in length and girth, until all the free fluid in the egg has been consumed, and the distended larva fills the shell completely. Packard (1894) observed that the flea embryo grew much longer just before hatching, but he did not detect the cause.

Next, the remaining amniotic fluid begins to evaporate and the surface of the larva becomes dry.[1] The spiracles are now exposed to the air, which soon enters them and extends into the tracheal system, moving rapidly along the main trunks, but very slowly along the finest branches, so that it may be half an hour or more before the smallest capillaries contain gas (Text-fig. 1, C.)

The larva now becomes more active and begins to crawl round inside the shell. It has been noted by Packard (1894) that the hatching spine of the young flea larva arises from the floor of a depression, so that it does not normally project above the surface of the head. But the spine springs from an elongated plate of chitin (Text-fig. 1, E) more rigid than the remainder of the head-capsule, and surrounded by a thinner membrane; so that, at the time of hatching, and for some hours afterwards (i.e. while the body is distended with fluid), this plate lies almost flush with the general level of the head. Consequently, the spine projects well above the surface (Text-fig. 1, D),[2] and, as the larva moves forward inside the egg, is pressed against the shell. Sooner or

[1] The drying of the larva may be recognized by the fact that the spiracles and the shagreened surface of the segments become more apparent, while the tracheae and other internal structures are less distinct.

[2] This state of affairs is figured without comment by Harms (1912). A similar arrangement is present also in the larvae of Diptera Nematocera (Edwards, 1919).

later the shell is pierced. But the larva continues to move forward, and the sharp front edge of the spine, acting in the manner of a tin-opener, cuts a longitudinal slit in the chorion. If all goes well, the slit extends about two-thirds the length of the egg, and the larva escapes, head foremost (Text-fig. 1, D). But sometimes, particularly if the egg has been kept too dry, the spine may slip out of the cut; and the larva then moves forward with the spine inside the shell again. Eventually, it may re-enter the old cut, or fresh cuts may be made, and sometimes the egg-shell may be slit in many places before the larva finally makes its escape. So far as we could judge, the inner membrane of the egg is cut through at the same time as the chorion.

It is clear that, as a general rule, the tracheal system of the flea larva fills with gas directly from the outside air, and not with gas dissolved in the tissue-fluids. We may suppose that, whatever the nature of the force which is tending to absorb the tracheal liquid into the tissues (see 'Discussion'), it is not sufficiently great to break the column of liquid while the larva is still bathed in the amniotic fluid. When the spiracles are open to the air, the liquid is absorbed without difficulty.

The absorbing force is active, evidently, for a considerable time before hatching. For if a larva is extracted from the egg some twelve or twenty-four hours before it would normally have hatched, as soon as the surface of the larva is dry, air enters the tracheal system in the same way as in the normal larva.

On the other hand, if the egg, containing a fully developed larva, is immersed in water before the air has entered the tracheal system, the larva may emerge as usual, though with fluid in the tracheae. As soon as the spiracles are exposed to the surface of the water, however, the tracheal system fills with air. Indeed, under these circumstances, the air enters with extreme rapidity, and may fill the system in less than thirty seconds; and this increased rate of filling suggests that, as a result of this delay, the absorbing force also has increased in strength. The significance of this will be discussed later.

We have never observed the tracheal system fill with gas while the egg or larva was under water, as happens normally in

insect larvae with closed tracheal systems.[1] But this may well be because the conditions were not entirely right for the experiment (cf. Tenebrio, below).

Hatching of Eggs of the Mealworm (Tenebrio molitor) (Coleoptera).

In most particulars the hatching of the mealworm resembles that of the flea larva, and it may be described very briefly.

Text-fig. 2, A, represents the egg of the mealworm about forty-eight hours before hatching. The larva is enclosed in a membrane, the first or 'embryonic' cuticle (Wheeler, 1889), which envelops each limb in a separate, unsegmented sac; a sac which is too small, so that the limb is greatly crumpled. The claws are pale. The larva is not coiled in the egg like the flea larva, but the segments are very short and thickened.

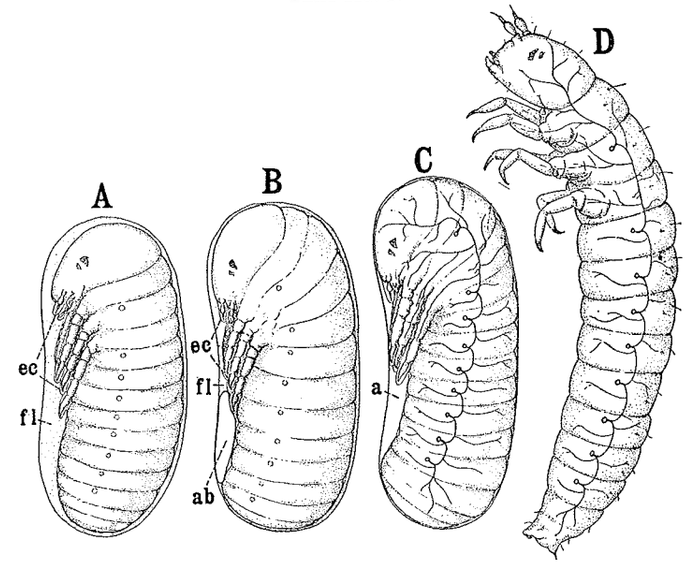
From time to time the larva swallows the amniotic fluid and, like the flea larva, comes to fill the shell more completely. The claws and mandibles slowly darken, and the body-segments become more elongated (Text-fig. 2, B), so that the head is flexed and the body somewhat curved. The outward form of the egg also is changed, becoming longer and thinner. Air enters the egg-shell, and occasionally some air may be swallowed by the larva; but this is not constant, and seems to play no essential part in the process of hatching. As the surface of the larva dries, air enters the spiracles and extends rapidly along the tracheae, though it often does not enter the finest branches in the legs until after hatching (Text-fig. 2, C).

The larva now becomes more active; perhaps it is invigorated by the improved supply of oxygen which follows the entry of air into the tracheal system. It forcibly extends the body, breaking off the head-end or the tail-end of the egg-shell, and so making its escape. The embryonic cuticle is shed shortly before, or at the time of rupturing the chorion. The larva does not swallow air after hatching, but it assumes its normal elongated form almost at once (Text-fig. 2, D), presumably by the

[1] Corethra (Krogh, 1911), Chironomidae (Pause, 1918; Keilin, 1924), Odonata (Calvert, 1898; Tillyard, 1916).

contraction of the transverse muscles of the body-wall. The mandibles play no part in opening the egg, and no hatching spines are present. Indeed, the chorion is so fragile that no such mechanism is needed.[1]

TEXT-FIG. 2.



Hatching of egg of Mealworm. A, egg about 48 hours before hatching: larva, enclosed in embryonic cuticle (*ec*) does not fill the shell. B, after swallowing amniotic fluid (*fl*): larva fills shell, body elongated, head flexed; a bubble of air (*ab*) has appeared in shell. C, larva and egg much elongated; surface of larva dry, shell containing air (*a*); tracheae filled with air. D, larva immediately after hatching.

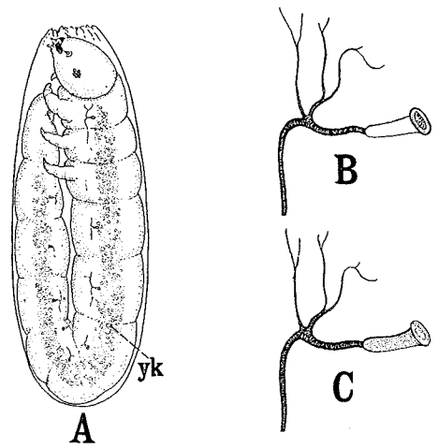
Thus, as in the flea larva, the tracheal system fills normally from the outside air. Like the flea larva, again, if the mealworm is removed from the egg before the amniotic fluid has disap-

[1] There is a row of short stout spines on either side of the clypeus, but these are present in subsequent instars, and so cannot be regarded as hatching spines in the ordinary sense. The same applies to the strong curved spines on the anal segment.

peared, the system fills with air as soon as the surface of the larva is dry.

On the other hand, if the egg is kept in water up to the time of hatching, provided it receives an adequate supply of air in

TEXT-FIG. 3.



Hatching of egg of Grain Moth. A, larva, with gut full of yolk (*yk*), biting its way out of the egg. Tracheae full of air. B, spiracle of larva still bathed in fluid; no air in the vestibule. C, spiracle when surface of larva dry; air extends to the outside.

solution (for example, if it is kept under a supported coverslip in a minute drop of water), the tracheae will fill with gas just as they do in aquatic larvae with the tracheal system closed.

Hatching of Eggs of the Grain Moth (Sitotroga cerealella).

The method of hatching in the Lepidoptera is well known, and the grain moth (Sitotroga cerealella) conforms to the general plan. When the larva is fully developed, it lies in an amniotic cavity entirely surrounded by a layer of yolk-cells. Shortly before hatching, it devours these cells, turning about in the shell with great activity.

Soon after these active movements have begun, the tracheae

are seen to have filled with air, although the larva is still bathed in fluid. The spiracles are closed and there is a clear space between the spiracles and the air-containing tracheae (Text-fig. 3, A and B.)

When the larva has consumed the yolk and the fluid contents of the egg, it proceeds to gnaw its way out (Text-fig. 3, A); the surface of the skin dries, the spiracles open, and the dark thread of air in the tracheae becomes continuous to the exterior. (Text-fig. 3, C.)

Hatching of Eggs of the Blow-fly (Lucilia sericata).

The embryology and hatching of Musca (= Calliphora) vomitoria have been described in detail by Weismann (1863), whose observations on the appearance of air in the tracheal system we can confirm; but since his observations on hatching were made on eggs from which the chorion had been removed, we can add some information on the method of hatching in the normal muscid egg.

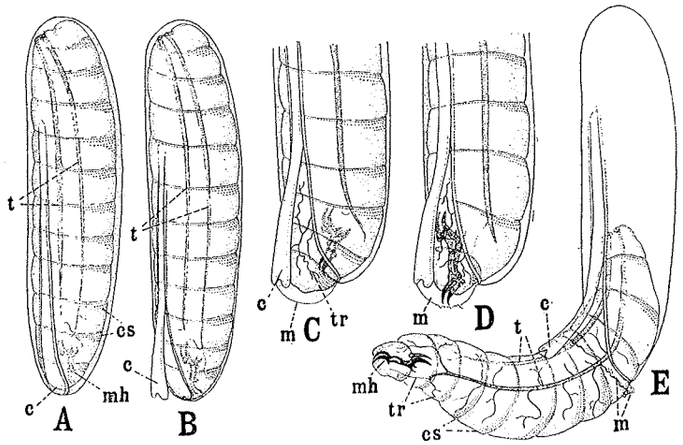
Along the dorsal surface of the egg, from the anterior pole backwards for about two-thirds of its length, the borders of the chorion are separated by a fissure, which is occupied by an elongated band tapering behind but broadening out in front (Text-fig. 4, A). We have found that this structure serves as a 'cap' precisely analogous to the cap on the eggs of Hemiptera or Anopleura.[1]

A few hours before hatching, the rows of spines on the integument of the larva darken, so that it can be seen through the sculptured shell of the egg, moving actively about. Shortly afterwards the larger tracheal trunks fill with air (Text-fig. 4, A). They fill at a time when the egg still contains fluid, and, as noted by Weismann, they will fill even if the egg is immersed in water. We have found, however, that this occurs only if the egg is near the surface. If the eggs are kept under a coverslip and flooded

[1] Hewitt (1914) describes the larva of Musca domestica as escaping through a longitudinal slit in the chorion to one side of this dorsal band. Prell (1915) observed a more or less circular cap on the egg of the tachinid (Parasetigena), quite different from the elongated band of Lucilia.

with water, the larvae in those eggs which are more centrally placed, cease to move; presumably they are asphyxiated, and their tracheal systems do not fill with air. When exposed near the surface of the water, however, they revive, and gas soon appears in the tracheae.

TEXT-FIG. 4.



Hatching of egg of Blow-fly. A, egg a few hours before hatching; cap (*c*) closed; egg contains fluid, but tracheal trunks (*t*) filled with gas; cuticular spines (*cs*) and mouth-hooks (*mh*) visible. B, the Y-shaped cap (*c*) lifted. C, the vitelline membrane (*m*) bulging through the opening; tracheal branches (*tr*) contain gas. D, larva has perforated the membrane (*m*) with its mouth-hooks. E, larva escaping from egg; air is entering the finest tracheal branches; vitelline membrane (*m*) is seen as a cuff round the mouth of the egg.

As the time of hatching approaches, the larva becomes increasingly active. It works its mouth-parts in and out with a piston-like movement,[1] directing its blows mainly at the broad end of the 'cap'. Eventually this cap springs open and then appears (Text-fig. 4, B) as a Y-shaped flap attached by an elongated hinge. The larva continues its movements, and the vitelline membrane, distended with fluid, begins to protrude through the

[1] Mitzmain (1913) describes similar movements in Tabanus during hatching.

opening (Text-fig. 4, C). The larva now directs its energies against this membrane, scraping and striking with its mouth-hooks until the membrane is pierced (Text-fig. 4, D). It then works its way out, enlarging the split and probably swallowing much of the fluid with which it was surrounded in the egg. As the larva emerges (Text-fig. 4, E) air can be seen extending slowly along the fine tracheal capillaries. Sometimes the larva swallows air after emergence, but this is by no means invariable.

Hatching of Eggs of the Bed-bug (Cimex lectularius).

The mechanism of hatching in the bed-bug does not seem to have been described, though the process is essentially the same as that known in other Hemiptera, in the Neuroptera (Smith, 1920, 1922; Withycombe, 1924), and in the Psocidae (Peyerimhoff, 1901; Huie, 1916). In none of these insects, however, has the appearance of air in the tracheal system been considered.

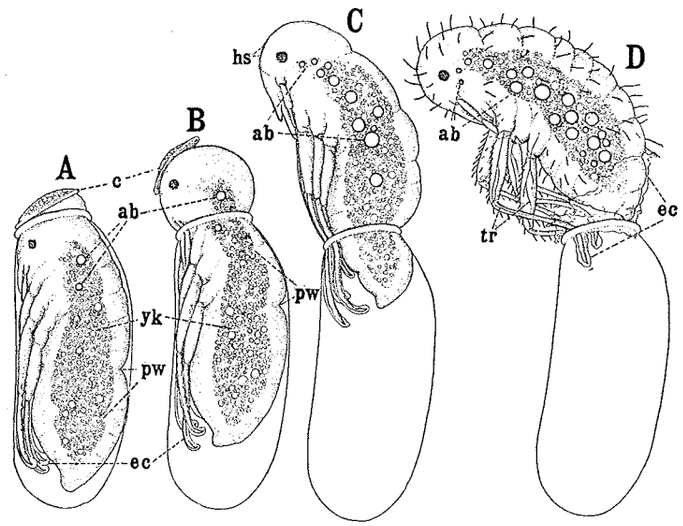
The larva of the bed-bug shows very little activity before hatching, and the sculpturing of the shell precludes any detailed observations upon it. But during the twenty-four hours before emergence the body of the insect comes to fill the egg more completely, the eyes moving forwards to the cap; the yolky contents of the gut become more apparent, the globules of fat becoming larger; and occasional pumping movements are visible in the head. It is probable, therefore, that the larva is swallowing the amniotic fluid during this period, like the other insects studied.

At the time of hatching, peristaltic, or rather anti-peristaltic movements of the gut, and similar movements of the abdominal wall, become very active, and drive the fluid contents of the insect into the region of the head. It is almost certainly this force which serves to dislodge the cap of the egg (Text-fig. 5, A); and the process is aided by the larva swallowing a certain amount of air, which increases the distension of the gut. As the cap is raised a small vesicle bulges through the orifice. This is composed of the escaping head and thorax, which are blown up like a bladder with the body-fluids. This bladder slowly enlarges as the larva squeezes its way through the constricted neck of the

egg (Text-fig. 5, B), and the process continues until only the tip of the abdomen remains inside (Text-fig. 5, C).

The insect, however, is still enclosed within its pre-larval skin or embryonic cuticle. It now begins once more to swallow large quantities of air (Text-fig. 5, C), distending the body more and more

TEXT-FIG. 5.



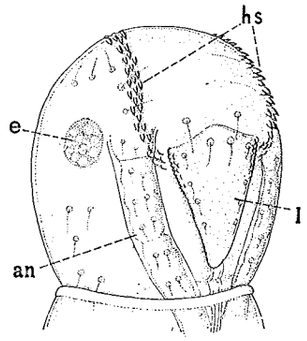
Hatching of egg of Bed-bug. A, cap of egg (*c*), being forced off; larva enclosed in embryonic cuticle (*ec*); abdomen and gut showing strong waves of peristalsis (*pw*) towards the head; gut contains globules of fat (*yk*) and a few small air-bubbles (*ab*). B, active peristalsis (*pw*) continues; head distended and bulging from the egg. C, larva, almost free from egg, has started to swallow air (*ab*) vigorously; hatching spines (*hs*) visible on front of head. D, embryonic cuticle (*ec*) has split and slipped backwards, allowing spines to stand erect; tracheae (*tr*) have filled with air; larva swallowing air (*ab*).

until the cuticle splits. The split appears over the top of the head and as the cuticle slips backwards, the bristles on the larva stand erect and the limbs and antennae become free (Text-fig. 5, D). During this process a layer of fluid can be seen between the larva and the cast skin; but on exposure, the surface of the

larva dries at once, and air enters the tracheal system for the first time. The air can be seen passing rapidly down the larger trunks, but more slowly along the finer branches, for example, in the distal portions of the legs.

When it has extricated itself from this first moult, the larva begins, for the third time, to swallow air most vigorously, until

TEXT-FIG. 6.



Head of Bed-bug, enclosed in embryonic cuticle, extruded from egg. *l*, labrum with serrated margins; *hs*, hatching spines on embryonic cuticle; *e*, eye of larva; *an*, antenna in separate cuticular sheath.

the entire gut is enormously distended. Meanwhile it alternately flexes and extends its body, assuming its characteristic flattened form and increasing in size until it far exceeds that of the egg from which it came. Within an hour or so, all this air has disappeared.

There is another feature of the hatching mechanism which has not been mentioned so far. Between the embryonic cuticle or 'inner egg-membrane' and the chorion or 'outer egg-membrane' is a third layer, presumably the vitelline membrane, conveniently called by Speyer (1929) the 'middle egg-membrane'. Before it can escape from the egg, the larva must rupture this membrane, and to this end it has a series of hatching spines. These arise from the embryonic cuticle, and take the form of a file-like margin to the prominent labrum and, more particularly, a series of small teeth which are arranged in two divergent

tracts running downwards and backwards from the vertex of the head (Text-fig. 5, c, and Text-fig. 6). This hatching mechanism could not be observed in action; but judging by its structure it is probably actuated by slight flexion of the head. It is possible that it may play a part in the separation of the cap, but we have no evidence on this point. On the other hand, it is possible that these small, backwardly directed spines play no part in rupturing the membranes, but serve merely to prevent the head from slipping back into the egg as the cap is lifted.

A very similar hatching mechanism is present in the blood-sucking Reduviid bug, Rhodnius prolixus; but in this insect the file-like structure on the labrum is not present and the divergent tracts contain many more teeth.

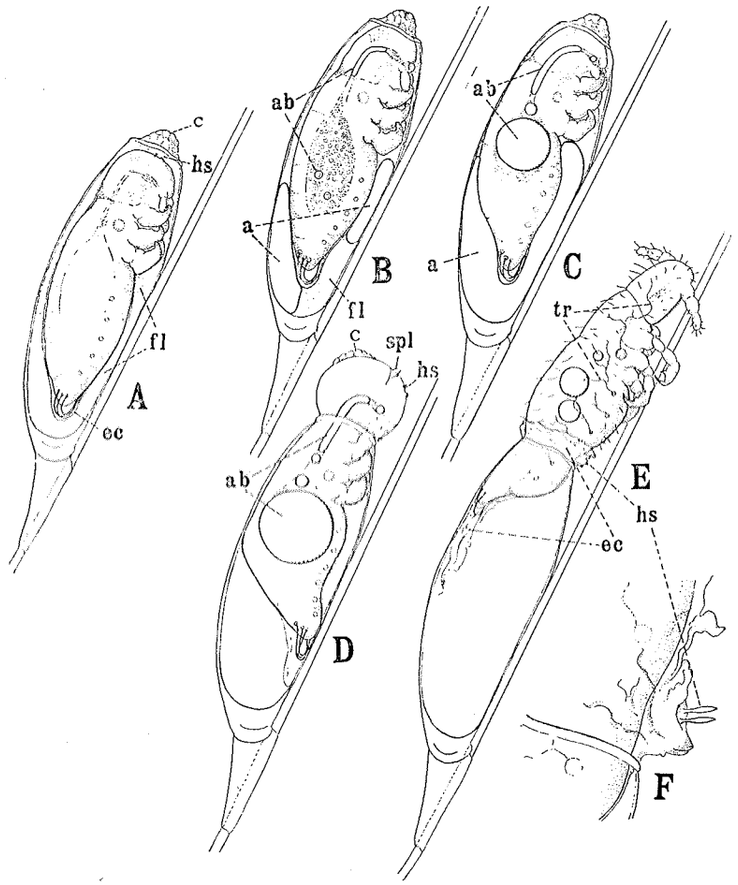
Hatching of Eggs of the Sucking-louse (Polyplax serrata) of the Mouse.

The hatching of the eggs of Pediculus has been described by Sikora (1915) and by Nuttall (1917), but these authors were not specially interested in the filling of the tracheal system. Further, the mechanism of hatching in Polyplax does not entirely agree with that given for Pediculus. Finally, we have found that all the genera of lice we have examined possess somewhat elaborate hatching spines, hitherto undescribed.

Text-fig. 7, A, shows the egg of Polyplax about twenty-four hours before hatching. The larva, enclosed within an embryonic cuticle which envelops each limb separately, is situated rather far back in the shell. The claws are pale. From time to time there are pulsating movements in the head, due to the insect swallowing the amniotic fluid. The hatching spines, which are shown in detail in Text-fig. 8, A, arise from a rigid area of the embryonic cuticle where it covers the front of the head. They consist of a pair of teeth, curved slightly upward; and, arising from the floor of a depression immediately above these teeth, a pair of lancet-shaped blades, side by side, directed straight forward.

During the last twenty-four hours of development the larva gradually moves forward, so that the blades of the hatching spines enter the cap of the egg. In so doing they must cut

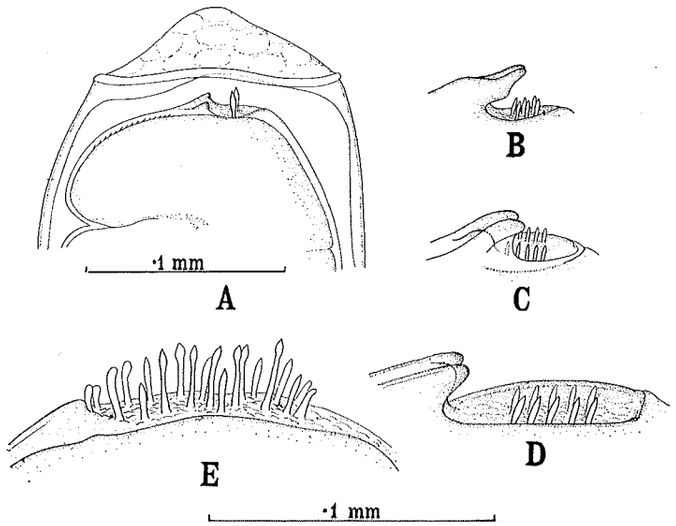
TEXT-FIG. 7.



Hatching of egg of Louse. A, egg about 24 hours before hatching, contains fluid (*fl*); larva, in embryonic cuticle (*ec*), well behind cap (*c*) of egg; hatching spines (*hs*) on front of head. B, larva has moved forwards; air (*a*) has appeared in shell; larva swallowing amniotic fluid and occasional bubbles of air (*ab*), body becoming distended. C, almost all fluid disappeared from shell; larva swallowing air (*ab*) very actively; gut distended with air. D, larva swallowing air and drawing up abdomen; cap (*c*) forced off and head bulging from the egg; embryonic cuticle has just split (*spl*). E, larva almost escaped from egg; tracheae (*tr*) filled with air; embryonic cuticle (*ec*) seen as a cuff bearing the hatching spines (*hs*). F, shows details of shed cuticle and hatching spines.

through the 'middle egg-membrane', and this is probably their main function. They now lie in the air-space within the cap. Meanwhile, one or more bubbles of air appear within the egg-shell, and as the larva continues to swallow the amniotic fluid

TEXT-FIG. 8.



Hatching spines on embryonic cuticle of various sucking-lice. A, Polyplax serrata; B, Pedicinus rhesi; C, Phthirus inguinalis; D, Pediculus humanus; E, Haematopinus sp.

and to press forwards to the front of the egg, these bubbles increase in size, until the space outside the embryonic cuticle is almost entirely filled with air. The larva continues to swallow, and, along with the fluid, it takes in bubbles of air (Text-fig. 7, B). Sikora and Nuttall state that in Pediculus this air is passed out through the rectum, accumulating behind the larva and so driving it from the egg. We have never seen this happen in Polyplax; indeed, seeing that the gut by this time is distended with watery fluid and fat-droplets, it would obviously require a very nice judgement to enable the insect to pass the

air while retaining the fluid. The bubbles which reach the stomach dissolve very rapidly and disappear. The oxygen they contain is doubtless used for respiration by the tissues of the larva. This will leave nitrogen with a high partial pressure, and, when the bubbles are small, under great tension, so that it will be quickly driven into solution.

Just before hatching, the larva begins to swallow air with great vigour, so that a large bubble accumulates in the gut (Text-fig. 7, C). At the same time it contracts the abdomen, and the increased pressure which results forces off the cap of the egg, and a little vesicle bulges out (Text-fig. 7, D). The swallowing of air and the contractions of the body continue, until the embryonic cuticle splits a little above the position of the hatching spines. The skin slips back, so that the hatching spines can be found attached to the cast skin at the mouth of the empty shell (Text-fig. 7, E).

The larva now works its way out of the cuticle and the egg, and as soon as the spiracles are exposed to the air, the tracheal system fills. (Text-fig. 7, E).

Text-fig. 8 shows the structure of the hatching spines in some other genera of lice. It is not necessary to describe each of these separately, but it will be noted that the general plan is alike in all, and that the number of blades is more or less in proportion to the size of the egg; ranging from a single pair in Polyplax to five pairs in Phthirus and Pediculus and about nine or ten pairs, irregularly arranged, in a large species of Haematopinus. The chief function of the lancet-shaped blades is probably, in all cases, to cut through the 'vitelline membrane', but the pair of teeth below them may perhaps assist in forcing off the cap of the egg.

DISCUSSION.

The Mechanism of Emergence from the Egg.

Heymons (1926) has reviewed the mechanism of hatching of insects from the egg, and van Emden (1925) has brought together all the examples of hatching spines which are known among insects, and has discussed their mode of action. The general conclusion reached by Heymons is that the chief force

which ruptures the shell is the internal pressure due to the growth of the embryo; and that the function of the various hatching mechanisms, whether they be 'egg-teeth' (Pentatomidae, &c.), hard plates (Sphodromantis, Mantidae), or soft distensible structures (Acridiidae), is merely to concentrate this increased pressure in one spot, and so cut through the chorion (e.g. Carabidae, Verhoeff, 1917) or to force off the cap of the egg (e.g. Pentatomidae). He compares the process with ecdysis, but claims that it differs therefrom in that the insect in the egg is never known to take up the outside air, either through the mouth or into the tracheae. Nor are any cases known in which the pressure is increased by the separation of gas from the tissues of the insect into the tracheal system. Thus, he says, important mechanisms for increasing the internal pressure are not available.

The observations recorded in the present paper allow some of these conclusions to be modified. In the first place, we have obtained no evidence that the growth of the embryo results in an increased pressure within the egg. In fact the reverse is probably the case. In Cimex, for example, a spoon-shaped depression often appears on either side of the egg a few days before hatching, and Nuttall (1917) records the same in Pediculus. The contracted form of the mealworm embryo gives the impression of its being under great pressure (cf. Frankenberg (1915) on Corethra), but even up to the time of hatching, the egg can be deformed by the slightest force; and the same applies to the egg of the flea.

The embryo certainly increases in size very rapidly before hatching. But it does so (Ceratophyllus, Tenebrio, Cimex, Polyplax) by swallowing the amniotic fluid. This, of course, will not increase the pressure in the egg, but it will give the insect a better purchase for its operations against the chorion. Although it has seldom been recognized before, the swallowing of the amniotic fluid before hatching is probably very common among insects. Thus Balfour-Browne records it in Hydrobius (1911) and Dytiscus (1913), Nuttall (1917) observed pumping movements in the head of Pediculus the day before hatching, and Huie (1916) saw this in Stenopsocus. Tillyard (1915) describes what he calls the 'cephalic heart' in the embryo of the dragon-fly, but concludes in a later paper (Tillyard, 1916) that this is a 'temporary modification of the oesophagus'; and the movements described by Verhoeff (1921) in the embryo of Carabus, and associated with disappearance of the fluid in the egg, are almost certainly swallowing movements.

In every case the force employed to break open the egg appears to be muscular. Even where the insect is apparently motionless (Cimex) and seems to be impelled from the egg by some invisible force, there are peristaltic movements in the gut or abdominal wall which drive the body-fluids into the head (cf. Tillyard, 1916; Smith, 1920, 1922; Withycombe, 1924; Speyer, 1929), causing this to act in the same manner as the cervical ampulla of the acridiid larva (Künkel d'Herculais, 1890 *a*) or the ptilinum of the muscid imago. Voss (1911) has described a special musculature in the embryo of Gryllus domesticus which performs this function alone, and then degenerates.

The swallowing of air may also play an important part in the emergence of the larva. It may subserve three purposes. It may distend the larva while it is still inside the egg, the air for the purpose diffusing in through the shell, and so assist those movements of fluid by which the cap of the egg is dislodged (e.g. in Cimex and Polyplax, vide supra; in Corydalis, Smith, 1920). Then, in those cases in which the larva escapes from the egg while still enclosed in the embryonic cuticle, it may swallow air a second time, blowing itself up until this cuticle is forced to split (e.g. in Cimex, vide supra; in Acridiidae, Künkel d'Herculais, 1890 *b*; in Psocidae, Huie, 1916; in Neuroptera, Smith, 1922). Finally, the young larva may swallow air a third time, until its skin has stretched and it has attained a size much greater than the egg from which it came (e.g. Cimex, vide supra; Benacus, Needham (1907); Psocidae, Huie (1916); Hydrobius, Balfour-Browne (1911); and Carabus, Verhoeff (1921).

We have shown that in many cases the tracheal system fills with air while the larva is still inside the egg; but it is highly

improbable that this has any material effect upon the pressure within the shell.

As regards the mode of action of the hatching spines, we can add little to the suggestions of van Emden (1925) and Heymons (1926). The spine in the flea larva certainly acts as a blade which cuts a longitudinal slit in the chorion, like the spines in Carabus (Verhoeff, 1917) and the dorsal spine in Psylla mali (Speyer, 1929), and not merely as a wedge ('Drückkante') to split the shell. The newly described spines in Cimex, Rhodnius, and the Anopleura, would appear to be of use chiefly for breaking through the middle egg-membrane, unless, as already suggested, those in Cimex and Rhodnius serve to prevent the extruded head of the embryo from slipping back into the egg.

The First Appearance of Gas in the Tracheal System.

Most of the observations, in the past, on the first appearance of air in the tracheae of insects relate to the closed tracheal system and are reviewed by Keilin (1924). The only detailed observations on the filling of the open system with which we are familiar are those of Weismann (1863) on the larva of Musca, and Davies (1927) on Sminthurus.[1]

The insects in which the process has now been observed fall into three groups: (i) those in which the surface of the larva dries while still in the egg, and the tracheal system fills, before hatching, from the outside air (Ceratophyllus, Tenebrio); (ii) those in which the larva at the time of emergence is enclosed in a cuticle which retains a layer of fluid beneath it, so that the air has access to the tracheae only when this 'embryonic cuticle' is shed, i.e. after hatching (Cimex, Polyplax); (iii) those in which the tracheal system fills with gas while the larva is still bathed in the fluid contents of the egg,

[1] Stadtmann-Averfeld (1923) describes a very improbable mechanism by which the tracheae of the mosquito larva fill with air. He supposes that muscular contractions squeeze all the fluid out of the tracheal system; and air is then taken in through the spiracle to replace it.

i.e. it fills like the closed tracheal system with the gases in solution (Sitotroga, Lucilia).

It is clear from the work of Weismann (1863) and Keilin (1924) that the fluid in the tracheal system is absorbed into the tissues; and it is probable that the mechanism of this absorption is the same in all cases.[1] Now the appearance of gas in the tracheae, especially in the larger trunks, is so rapid that it seems more likely that the absorption of fluid is due to some simple physical force than to secretory activity. It is probable, also, that the force which brings about this absorption is the same as that which normally keeps the tracheae more or less full of air. Now it has been shown (Wigglesworth, 1930) that the latter force is almost certainly the osmotic pressure of the tissue-fluids. The problem resolves itself, therefore, into two questions. How do the tracheae come to contain a fluid which can be absorbed by osmosis, i.e. a fluid whose constituents can pass through the walls of the tracheal capillaries; and is osmotic pressure a sufficient force to explain the filling of the system?

As regards the origin of the tracheal fluid, the notion which formed the basis of the present work, that the increasing hydrostatic pressure within the developing egg caused an ultrafiltration of the tissue-fluids through the walls of the tracheal tubes, has been shown to be fallacious. For an increased hydrostatic pressure does not exist (page 182), and in many insects the system fills with air before they leave the egg.

It was next thought that the fluid in the tracheae might be amniotic fluid. But it can readily be shown that the amniotic fluid of the mealworm egg contains proteins and salts like that of other animals.[2]

[1] That there is, indeed, no essential difference between the filling of the closed and the open systems is shown by the fact that, as described above, if the egg of the mealworm be kept in water, the tracheae may fill with the gases in solution.

[2] With the idea of changing the composition of the amniotic fluid or of the fluid in the tracheae, eggs of Ceratophyllus have been allowed to develop in aqueous solutions ranging from distilled water to one per cent. of sodium chloride; and the larvae have been allowed to hatch into these solutions with the tracheae still full of fluid. Prolonged exposure of the larva, after hatching, in any of these fluids may sometimes delay or entirely

If the tracheal liquid is not amniotic fluid, it must be produced within the tracheal system after closure of the spiracles. It has been shown by Weismann that the ingrowth of the ectoderm which becomes the trachea, has at first only a potential lumen; and that during development a cuticle is laid down and a lumen gradually forms. He assumes that as the lumen enlarges, fluid diffuses into it from the tissues. At a certain stage the cuticle of the larger trunks becomes impermeable to fluid. But the lumen goes on enlarging. Hence the space must be filled with gas, diffusing from the tissue-fluids; and in this way he explains the first appearance of gas in the tracheae. When development is complete, he supposes that the residual fluid is absorbed into the tissues through the finest tracheal tubes.

Before reading Weismann's work we had arrived at a somewhat similar hypothesis and obtained some evidence in its support. Mr. L. E. S. Eastham of Cambridge very kindly made some measurements of the tracheae of Pieris for us, at different stages of devlopment. That they might be comparable, these measurements were made as near the spiracle as possible in each case. The results, which confirm those of Weismann on Musca, are shown in Table 1.

TABLE 1.

Stage of Development.	*Diameter of lumen in μ.*	*Diameter of trachea in μ.*
1. Tracheal ingrowth newly formed . .	Capillary	12·0
2. A few days later	2·5	7·5
3. Shortly before hatching; cuticle formed	3·75	7·5
4. Newly-hatched larva	6·25	7·5

It will be seen that there is a progressive increase in the size of the lumen, with comparatively little change in the total diameter of the trachea. Assuming that the spiracles are closed, the fluid which occupies the lumen must have come from the tissues through the walls of the trachea. Now although these observations do not show whether this fluid is actively secreted

prevent the subsequent filling of the tracheae, an effect which is unexplained; but apart from this the experiments gave no positive results.

by the epithelial cells of the tracheae, or whether it is a simple filtrate or dialysate, as assumed by Weismann; yet in either case its constituents might be expected to pass back again through the delicate walls of the tracheal capillaries. In the final stages of development the formative cells of the tracheae and tracheoles degenerate and practically disappear; so that the fluid in the lumen is directly subject to the osmotic pressure of the tissue-fluids; and if, as seems probable, the osmotic pressure increases about the time of hatching, conditions will be appropriate for the absorption of the fluid by osmosis.

Turning now to the question whether osmotic pressure is a sufficient force to explain the filling of the tracheal system, it is clear, from experiments on the larva of the mosquito (Wigglesworth, 1930), that the osmotic pressure of the tissue-fluids can absorb the liquid in the tracheae until air extends into the fine capillaries. There is no reason to doubt, therefore, that it is great enough to bring about the initial filling of the tracheal system when this is open to the outside air. But in order to fill the system when the insect is under water, or, what comes to the same thing, the closed tracheal system, it will be necessary first to overcome the cohesion of water before the column of fluid in the tracheae can be ruptured and the dissolved gas set free.

Now the experiments of Dixon (1914) have shown that, even with air in solution, the cohesion of water amounts to about 200 atmospheres pressure; whereas the available osmotic pressure in the tissue-fluids can only be of the order of 10 atmospheres (Wigglesworth, 1930). But in order to obtain this high figure for adhesion and cohesion of water, very special precautions had to be taken to remove all grease from the apparatus and to ensure that every part was thoroughly wetted. If this was not done, free gas appeared when the pressure was only slightly reduced; and Dixon admits that air-bubbles are common enough in the conducting tissues of the stems of plants, although the negative tension (due to the transpiration force) to which the sap is exposed is only between 5 and 20 atmospheres. In practice, therefore, even these relatively small reductions in pressure may bring about the liberation of gas; and this in spite of the fact that, since the conducting tubes of plants

imbibe water, they must exert a very strong adhesion to the sap. The tracheal intima of insects, on the other hand, has a notoriously low adhesion to water, so that it is not unreasonable to suppose that the osmotic pressure of the tissue-fluids will be sufficient to overcome this adhesion and effect the liberation of the gases in solution.[1]

Keilin (1924), basing his argument on the high figures for the cohesion of water quoted above, considered that osmotic pressure could not account for the phenomenon; and he therefore invoked intramolecular changes in the protoplasm of the tissues, leading to an imbibition of water. But, as pointed out by Shull (1924) in the case of plants, it is difficult to see how imbibition could effect the removal of fluid from the closed tracheal tubes except through the secondary changes in osmotic pressure which it would induce.

If the insect is kept in water freed from gases, as was shown by Frankenberg (1915) on Corethra, the tracheal system does not fill. This was to be expected from the foregoing argument. But we are still faced with the problem why the tracheal system in some insects (e.g. Sitotroga, Lucilia) fills, like the closed system, with gases from solution; whereas in others (Cimex, Polyplax) it does not fill until after hatching.

It is notable that it is those larvae which show the greatest degree of activity within the egg whose tracheae normally fill with the gases in solution; and the most probable explanation is that it is the metabolites produced by this muscular activity which raise the osmotic pressure to the degree necessary to effect the liberation of gas. (Cf. Wigglesworth, 1930.)

Tillyard (1916) also noted that gas appeared in the tracheae of insects (dragon-fly larvae) at the time when muscular activity increased. He regarded the gas which first appeared as carbon dioxide, and explained its appearance at this time by its excessive accumulation in the active tissues. But in view of the great

[1] This point is well illustrated by a simple experiment devised by Dr. N. K. Adam (personal communication). Ordinary tap water is allowed to stand in two beakers, one thoroughly freed from grease with chromic acid, the other lined with paraffin wax. Bubbles of gas are soon liberated in the waxed beaker (at room temperature and atmospheric pressure) but not in the clean beaker.

solubility of carbon dioxide and its rapid rate of diffusion in water,[1] the explanation given above is more probable.

Again, the closed tracheal system fills, as a rule, with great rapidity, whereas Davies (1927) noted that in Sminthurus the process of filling might take several days. He attributed this slow rate to the fact that the air enters through a single pair of spiracles, but a more likely explanation is that the osmotic pressure in the tissue-fluids of this insect is exceptionally low; whereas in the closed system the osmotic pressure must reach a relatively high value before filling can begin at all.

As to the nature of the gas which first appears, a subject which has provoked much discussion, there is no reason to suppose that this is constant. Its composition will depend solely upon the partial pressures and the invasion coefficients of the various gases in solution in the tissue-fluids at the moment when filling begins.

Summary.

Hatching spines are described in the bugs, Cimex and Rhodnius; and in the lice, Polyplax, Pedicinus, Pediculus, Phthirus, and Haematopinus. In all these insects the spines occur on the embryonic cuticle which is shed at the time of hatching.

The mechanism of hatching is described in the following insects: the flea (Ceratophyllus), the mealworm (Tenebrio), the grain moth (Sitotroga), the blow-fly (Lucilia), the bed-bug (Cimex), and the sucking-louse (Polyplax).

In the light of these, and other observations in the literature, the general mechanism of the hatching of insect eggs is discussed.

The first appearance of air in the tracheae of these insects is described and the mechanism of the process considered. It is suggested that the fluid in the tracheae is absorbed by the osmotic pressure of the tissue-fluids, and that since osmotic pressure is increased by muscular activity, air appears earliest

[1] As soon as a bubble of gas has been liberated, the high coefficient of invasion of carbon dioxide between water and air will favour the accumulation of this gas more than the accumulation of oxygen or nitrogen.

in those insects which show the greatest activity while in the egg. It is argued that osmotic pressure will account also for the appearance of gas in the closed tracheal system.

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[1] More complete bibliographies on the hatching mechanisms of insects are given by van Emden (1925) and Heymons (1926).

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