On the Ptilinum of the Blow-fly (Calliphora erythrocephala).

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With 14 Text-figures.

CONTENTS.

1. INTRODUCTION.

FLIES of the group Cyclorrhapha Schizophora possess, in the anterior region of the head, an extensive membranous organ known as the ptilinum or frontal sac. It serves, by its alternate distension and contraction, to rupture the puparium and to assist in the emergence of the imago. After emergence, this sac remains retracted as an invagination in the head-cavity. First described by Réaumur in 1738, brief accounts of the nature and use of the ptilinum have been numerous. None of these accounts describes the complete musculature, very little information is available as regards the development of the ptilinum, while the changes that follow the ultimate withdrawal of the organ within the head have not been investigated. An attempt has been made to study these aspects of the subject as fully as the means at my disposal would allow and the results are embodied in this paper. The investigation was carried out

at the suggestion of Dr. A. D. Imms, F.R.S., who has assisted me throughout the work. I wish to express my sincere thanks to Dr. Imms for his encouragement and help, and to Newnham College for the opportunity of conducting this research.

2. Review of the Literature.

The first reference to the ptilinum is that of Réaumur (1738) in his illustrated account of the emergence of the blow-fly. He remarked on the systole and diastole of the 'museau', which we now know as the ptilinum, and the associated dilation of the adjacent areas of the head. He pointed out the presence at the end of the 'museau' of 'un petit enfoncement qui marque apparemment l'endroit par lequel il est tiré quand il rentre sous le crâne'. It is evident in this observation that he refers to the base of the ptilino-oesophageal muscle. The distension he thought to be caused by air-pressure. The obvious purpose of the 'museau' was, he said, to break open the puparium and to assist in the emergence of the imago. He further suggested that it aided in the circulation of the body-fluids. He showed also the importance of the 'museau' in facilitating the escape of flies of another species, living in galls on thistles. The fact that the 'museau' did not again appear during the remainder of the insect's life did not escape his notice.

In 1911 Knab gave a short review of the literature concerning the emergence of Cyclorrhaphous flies from their puparia. From this account it appears that little had been added, between 1738 and 1911, to our knowledge of the ptilinum. Three new points, however, emerged. First, Joly in 1846 pointed out that the inflation took place by blood-pressure and not by air-pressure. Secondly, Künckel d'Herculais (1875) stated that an apparent ptilinal mechanism was found in the Syrphids (Syrphus, Volucella, and Eristalis), but all traces of the ptilinum disappeared before the insects were ready for flight. Thirdly, this same author described the structure of the ptilinum as being, apart from its coloration and transparency, identical with that of the surrounding integument. Observations on the rate of pulsation of the ptilinum have since been made by

Graham-Smith (1916) in his description of the emergence of the blow-fly.

Little attention has been given to the muscle-supply of the ptilinum except by Lowne (1890–5), who attributed the movements of the organ to three sets of muscles. Lowne mentioned: (1) two large fan-shaped muscles arising from the lower edge of the occipital foramen and inserted partly on the genae and partly on the ptilinum; (2) compressor fibres covering the inner surface of the ptilinum; and (3) two bundles of muscles arising from the ptilinum and inserted on the oesophagus. These muscles, (3), he termed 'retractors of the fulcrum'. The same paired muscles were described and figured by Graham-Smith (1930) 'retractors of the oesophagus', and he attributed to them the function of withdrawing the loop of the oesophagus during retraction of the proboscis. Previous to Graham-Smith, but apparently unknown to him, Mercier and Villeneuve (1925) had announced that muscle-fibres extending from the pharynx to the ptilinum, their 'muscle ptilino-pharyngien', were responsible for retraction of the ptilinum. They held that the fibres belonged to the same group of muscles as the fronto-pharyngeal, which were dilators of the pharynx, and that after invagination of the ptilinum, the ptilino-pharyngeal muscle played only the rôle of a pharyngeal dilator. Lowne further stated that most of the muscles were subsequently absorbed, though the fan-shaped muscles remained for one or more weeks after emergence.

Although both Lowne and Hewitt (1910) termed the opening of the ptilinal invagination the 'lunula', or 'lunule', the name generally adopted is frontal or ptilinal suture. The term lunule is applied to the small crescentic sclerite between the ptilinal suture and the bases of the antennae. It is, according to Becher (1882), the well-cuticularized lower border of the ptilinum. The division of the Cyclorrhapha, into Aschiza and Schizophora, was founded by Becher upon the differences in the frontal region of the head. In the Aschiza there is no ptilinum or associated suture in the mature fly, while in the Schizophora both ptilinum and its suture are present.

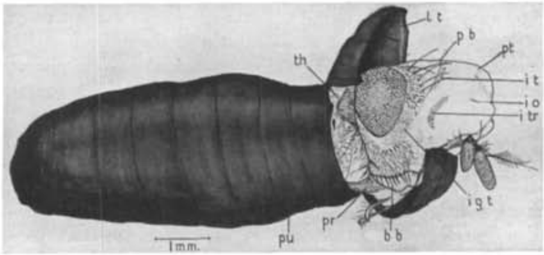
As regards the development of the ptilinum, the only direct reference appears to be that by Wahl (1915) who states that 'the median hindmost part of the frontal sac comes to the anterior end of the pupa and later becomes again intucked as the ptilinum'. In this case, 'frontal sac' refers to the vesicle containing the cephalic imaginal disks, which, when evaginated, forms the head of the imago. In the account by Pérez (1910, p. 176) of the metamorphosis of Calliphora is a description of the development of the ptilino-oesophageal muscle. Pérez termed it a dilator muscle of the pharynx, and showed that it developed, chiefly, from the remains of the corresponding larval pharyngeal dilator.

3. Material and Technique.

(*a*) Material.—Larvae of Calliphora erythrocephala Meigen were reared, usually on liver, in shallow vessels containing a layer of dry sand and kept in an incubator at 25° C. After four days' development at this temperature, the larvae left the meat which was then removed from the vessel. When pupae of known age were required, the fully fed larvae were placed in similar shallow flat-bottomed containers on a layer of sand, insufficient in depth for them to bury themselves. By examining the larvae hourly on their sixth day—when the majority pupated—it was possible to remove those which had contracted into the pupal barrel-shape, in order to separate them, and to label them with their time of pupation. When, 9 or 10 days afterwards, the adult flies began to issue they were removed from the incubator so that their emergence might be observed and specimens fixed with the ptilinum expanded.

The adult flies were fed with yeast and sugar; water soaked into cotton-wool was also provided, together with liver for oviposition.

(*b*) Methods.—Carnoy's fluid, used cold, gave the most satisfactory fixation. Pupae were pierced in the thoracic region of the puparium, immediately upon immersion in the fixative, and were removed from this shell an hour later. Fixation in vacuo was found useful in removing air from the heads of one day or older imagines. Of stains, the most satisfactory was Delafield's haematoxylin counterstained with eosin: iron haematoxylin, Mann's methyl-blue-eosin, and Mayer's haemalum were also used. Paraffin sections were cut 6μ or 10μ in thickness. In order to avoid tearing of the sections of 1, 2, or 3 day-old flies, double-imbedding in celloidin and paraffin was tried but this method gave results no better than single imbedding in



TEXT-FIG. 1.

Blow-fly in act of emergence, lateral view. ×9·7.

REFERENCE LETTERING to Text-figs. 1–14.

a, antenna; *b.b.*, buccal bladder; *b.t.*, body of the tentorium; *c.*, cuticle of the ptilinum; *d.f.c.*, disintegrating fat cells; *f.*, fulcrum; *f.c.*, fat cells; *fr.c.*, fronto-clypeus; *f.t.*, fronto-tentorial muscle; *g.t.*, geno-tentorial muscle; *h.*, epidermis of ptilinum; *i.f.t.*, insertion of fronto-tentorial muscle; *i.g.t.*, insertion of geno-tentorial muscle; *i.l.*, inner layer of cuticula of vertex; *i.l.m.*, insertion of lunular muscle; *i.o.*, insertion of ptilino-oesophageal muscle; *i.t.*, insertion of ptilino-tentorial muscle; *i.tr.*, insertion of transverse ptilinal muscle; *l.*, lunule; *l.e.*, left compound eye; *l.m.*, lunular muscle; *l.t.*, larval trachea; *oc.*, occipital foramen; *oe.*, oesophagus; *o.l.*, outer layer of cuticula of vertex; *p.b.*, boundary between ptilinum and vertex; *pc.*, phagocyte; *p.c.f.*, posterior cornu of fulcrum; *ph.*, pharynx; *p.l.*, pigment layer of vertex; *p.o.*, ptilino-oesophageal muscle; *pr.*, proboscis; *p.ru.*, ptilinal rudiment; *pt.*, ptilinum; *pt.i.*, ptilinal invagination; *p.t.*, ptilino-tentorial muscle; *pu.*, puparium; *r.a.*, retractor of antenna; *r.e.*, right compound eye; *s.g.*, spheres of granules; *s.o.g.*, supra-oesophageal ganglion; *sp.*, spinules of ptilinum; *t.*, tendon; *th.*, thorax; *t.m.*, transverse ptilinal muscle; *t.v.*, trans-vertex muscle; *v.*, vertex; *v.r.*, vertex retractor; *w.a.s.*, wall of air sac.

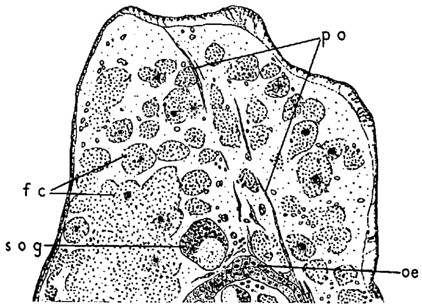
paraffin. Dissections of the heads of the imagines in alcohol and clearing in xylol or cedar-wood oil, with the ptilinum inflated, gave preparations very valuable for the study of the musculature.

4. Development of the Ptilinum (Text-figs. 2–8).

During the first day of the pupal period the paired cephalic buds, bearing antennal and eye rudiments on their walls, fuse into a single median dorsal cephalic vesicle. Between 24 and 36 hours after pupation at 25° C., the cephalic rudiment becomes evaginated through the opening of the larval pharynx and appears externally as the imaginal head. The most anterior part of the head, in front of the eyes and antennae, which before evagination was the posterior blind end of the cephalic vesicle, gives rise to the ptilinum. The head membrane is at first thin and distended with blood-plasma, spheres of granules ('Körnchenkugeln'), and fat cells. The antennal rudiments are lateral, their insertions being separated by more than one-third of the width of the head. The epidermis (hypodermis) is not obviously demarcated into ptilinum-forming and other areas, although to the most anterior part are attached fibres destined to form part of the ptilino-oesophageal muscle (Text-fig. 2).

Between 1½ and 2 days after pupation, the shape of the head changes in that the antennal bases move to a more median position and the epidermis immediately above them becomes a many-celled layer, which represents the imaginal bud of the future ptilinum. Between the cells of this thickening numerous spheres of granules, and fragments of these, are present (Text-figs. 3 and 8).

On the third day after pupation invagination of the ptilinal bud begins (Text-fig. 4). The process of invagination is gradual, being connected with the growth of the sac, and is not performed by the still-rudimentary muscles. Insinking occurs first around the upper and outer edge of the thickening, the membrane extending underneath and parallel to the vertex. As the surface increases the thickness gradually decreases (Text-figs. 5 and 6), the increased area of the membrane accommodating itself by becoming crumpled as it extends into the head. Before the fifth day the ptilinum already bears cuticular spinules, while the developing muscles, attached to its walls, are repre-



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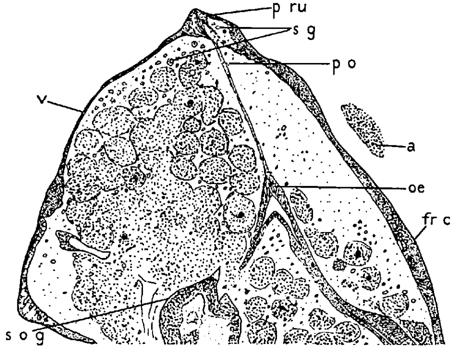

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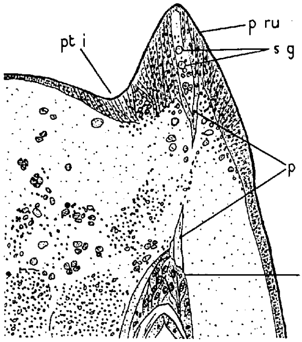
TEXT-FIGS. 2—3.

FIG. 2. Longitudinal section through head of pupa 1½ days after pupation at 25° C. ×32·9. Pupal integument not shown in this or other figures of pupae.

FIG. 3. Longitudinal section through head of pupa, 2½ days old. ×32·9.

sented by linear chains of nuclei surrounded by a small amount of unstriated cytoplasm (Text-fig. 7). In the completely developed organ the cuticle becomes greatly thickened and the



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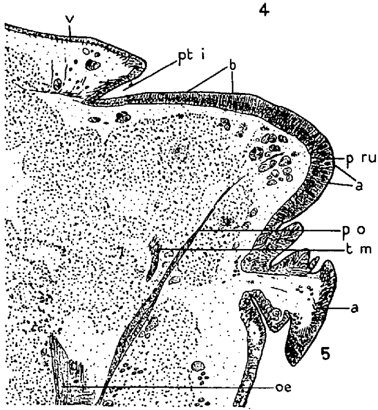
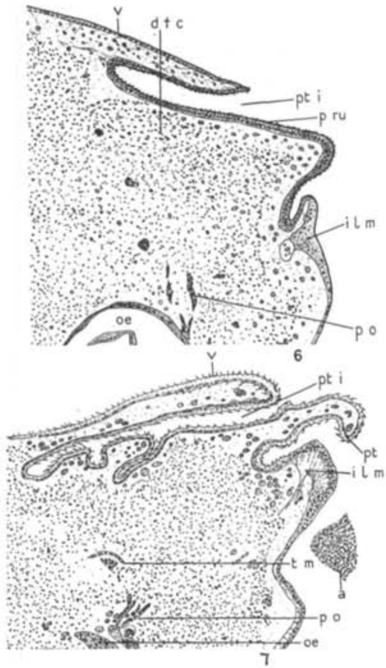


TEXT-FIGS. 4—5.

FIG. 4. Anterior part of head of pupa, longitudinal section, $3\frac{1}{2}$ days old. $\times 78$.

FIG. 5. Anterior part of head of pupa, longitudinal section, 4 days old (early). $\times 78$.



TEXT-FIGS. 6—7.

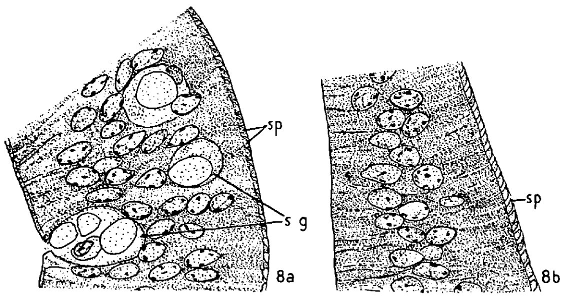
FIG. 6. Anterior part of head of pupa, longitudinal section, 4 days old (late). × 78.

FIG. 7. Anterior part of head of pupa, longitudinal section, 5½ days old. × 78.

epidermis reduced to an attenuated layer (Text-fig. 9). The blood-plasma, on the fourth and fifth days, is laden with a thick suspension of the contents of the disintegrated larval fat cells and spheres of granules, which in the earlier part of the pupal period retained their integrity.

5. The Ptilinum and Associated Muscles.

The chief surface of the ptilinum lies between the antennae and vertex. A narrow extension also passes ventrally on either side, between the gena and the fronto-clypeus, to about the level of the tip of the pendulous third antennal joint. After emergence from the puparium, this whole area of spiny membrane is invaginated into the head cavity, the external evidence



Text-fig. 8.

(*a*) Enlargement of part of ptilinum of fourth-day pupa in position indicated at (*a*) Text-fig. 5. ×775. (*b*) Enlargement of part of ptilinum of fourth-day pupa in position indicated at (*b*) Text-fig. 5. ×775.

of its presence being the narrow mouth of the invagination. This forms the ptilinal or frontal suture which curves as a ∩-shaped loop around the fronto-clypeus. Immediately above the sockets of insertion of the antennae is the lunule. It is a small crescent-shaped area of thickened cuticle forming the lower margin of the median part of the suture. It appears on the inflated ptilinum as a slightly pigmented area immediately above the antennae in the median line.

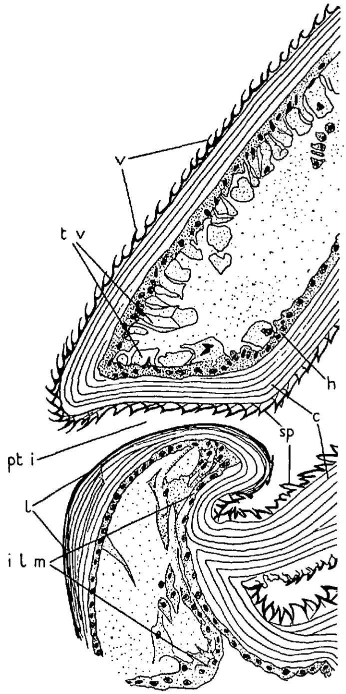
The structure of the ptilinum in the newly emerged adult is very similar to the integument of the genae, vertex, and frons with which it is continuous. The chief difference is that the spinules which cover the surface of the sac are shorter and less slender than those of the vertex. There are no differences in the shape of the spines in different parts of the organ such as those described by Jobling (1932) in the ptilinum of Glossina. The wall of the ptilinum consists of (*a*) spinules, regularly arranged, unstained with haematoxylin, with an average width at their bases of 20μ and a height of 10μ; (*b*) a layer of cuticle $25–30\mu$ in thickness, without pigment and staining purple with haematoxylin, and secreted by (*c*) a syncytial epidermis (Text-fig. 9).

On the inner surface of the wall of the ptilinum are the insertions of certain muscles, all of which function as retractors of the organ. These muscles are as follows:

1. Transverse Ptilinal Muscle (*t.m.*) (Lowne's 'compressors').—The fibres extend horizontally across the cavity of the ptilinum and have a long dorso-ventral attachment to each side. When the organ is invaginated the muscle forms a compact mass over its posterior surface.

2. Ptilino-oesophageal Muscle (*p.o.*).—This arises on the oesophagus between the fulcrum and the brain. It has a wide base of insertion on the most anterior part of the inflated ptilinum in a narrow band of wide lateral extent. It is these fibres which Lowne termed 'retractors of the fulcrum', Graham-Smith 'retractors of the oesophagus', and Mercier and Villeneuve 'muscle ptilino-pharyngien' or 'm. retractor ptilini'. My own observations corroborate the opinion of Mercier and Villeneuve in indicating that the fibres play an important part in the retraction of the ptilinum. The French authors further supposed that, after invagination of the ptilinum, the ptilino-pharyngeal muscle played the rôle of pharyngeal dilator. Since, however, from dissections and sections of heads of flies, several days after emergence, all traces of ptilinal muscles are gone, it seems probable that the only function of the ptilino-pharyngeal muscle is the retraction of the ptilinum.

3. Ptilino-tentorial Muscle (*p.t.*).—This is a paired muscle arising from a tendon which is attached to the strong cuticular body of the tentorium, at its junction with the border of the occipital foramen. It is inserted on the ptilinum at each side, dorso-laterally, in a narrow band immediately in front of

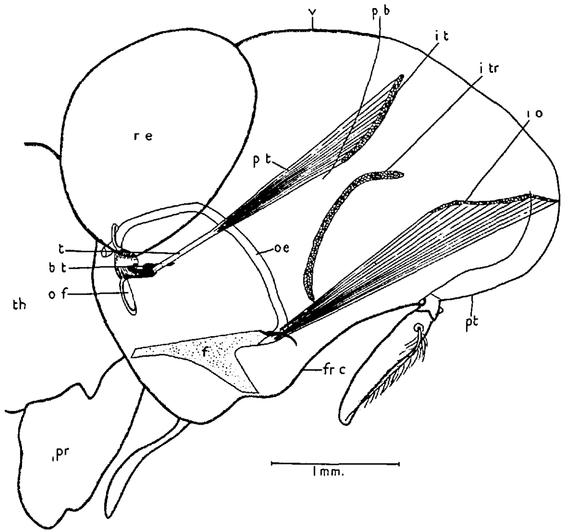


TEXT-FIG. 9.
Vertical section passing through mouth of invaginated ptilinum of newly emerged imago. × 163·7.

the line of demarcation between the vertex and the ptilinum. This pair of muscles is evidently homologous with Lowne's large fan-shaped retractors.

Although not inserted on the ptilinal membrane there are, in the head, six other groups of muscle-fibres which appear to be connected with the distension and contraction of the ptilinum, and other parts of the head, during emergence (Text-figs. 12 and 13). These are as follows:

(1) Geno-tentorial Muscles (*q.t.*).—The fibres arise from the same tendon as the ptilino-tentorial muscle and from the occipital sclerites. They are inserted over a wide area on

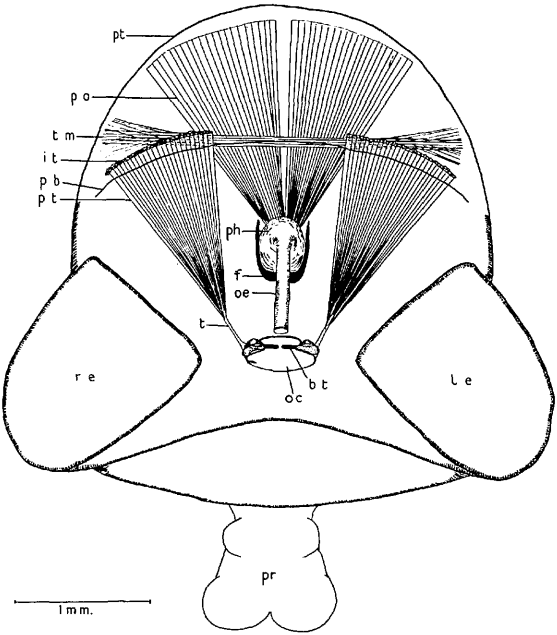


TEXT-FIG. 10.

Diagram of right half of head of adult, with ptilinum inflated, indicating position of intrinsic ptilinal muscles. ×21.

each gena between the eye and the frontal suture. Contraction of the muscles results in the approximation of the gena and occiput and, when blood is being withdrawn into the abdomen, in a return to normal in the shape of the head. When the head is distended with blood, the contraction of these muscles appears to result merely in the flattening of the genae. Fluid is thus pressed forward into the ptilinum which is thereby rendered more turgid.

(2) Fronto-tentorial Muscles (*f.t.*).—The fibres arise

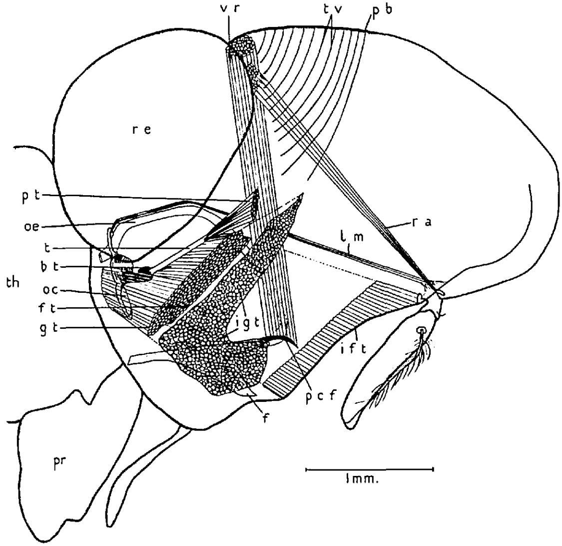


TEXT-FIG. 11.

Diagram of head of adult from above, with ptilinum inflated, indicating position of intrinsic ptilinal muscles. ×22.

from the same tendon and occipital sclerites, though more centrally than (1), and are inserted in a large group just below the base of each antenna on the fronto-clypeus. Contraction of these muscles also results in diminution of the volume of the head, as in (1).

(3) Trans-vertex Muscle (*t.v.*).—This is a muscular



TEXT-FIG. 12.

Diagram of right half of head of adult, with ptilinum inflated, showing position of accessory muscles connected with head pulsation. Base of cut ptilino-tentorial muscle indicated. Fibres to gena and fronto-clypeus are cut across near their origin. ×21.

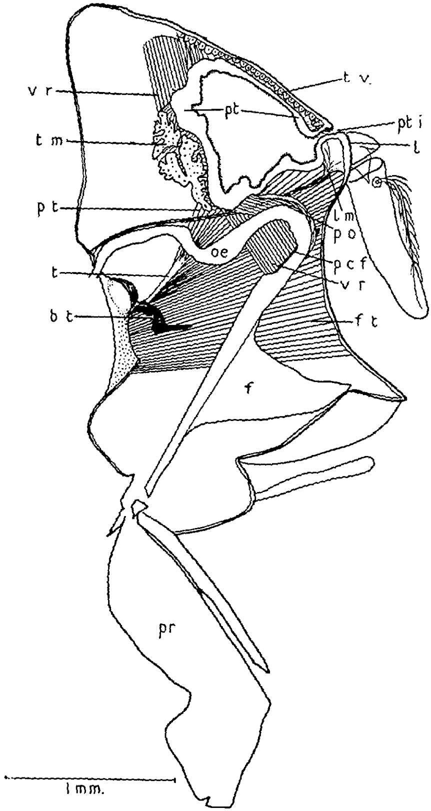
layer underneath the epidermis of the vertex, immediately posterior to the median part of the frontal suture, and extending transversely between the frontal bristles. By contraction of these fibres the volume of the head is diminished.

(4) Lunular Muscle (*l.m.*).—This muscle is described by Jobling in Glossina under the name 'retractors of the fronto-

clypeus'. His account is as follows: 'an unpaired, long, thin muscle arises from the dorsal edge of the occipital foramen, passes through the canal of the cephalic ganglion towards the fronto-clypeus where it is attached above the antennae close to the ventral edge of the ptilinal suture. It retracts the fronto-clypeus and ptilinum inside the head after hatching. Non-functional in adult insect.' This description applies equally well to the homologous muscle in Calliphora. The fibres are inserted by means of two minute roots on a cone-shaped mesh of strands of cytoplasm and nuclei lying beneath the cuticle of the lunule. This muscle was described by Mercier and Villeneuve (1926) in Calliphora as 'muscles gubernateurs de la lunule'. They held that the origin is on the body of the tentorium. According to these authors the tendonous insertions of the fibres, just referred to, serve as the framework for a sensory organ of the same nature as Johnston's organ in the second antennal joint.

(5) Retractor of the Antenna (*r.a.*).—According to Jobling this muscle 'arises from the latero-vertex and passes through the scape, being inserted into a short process of the dorsal part of the posterior border of the pedicle'. At the time of emergence this muscle may assist in the deflation of the head, by approximating the vertex and fronto-clypeus. Subsequently it functions only as the antennal retractor.

(6) Retractors of the Vertex (*v.r.*).—The fibres arise from the hind surface of the posterior cornua of the fulcrum and are inserted on the lateral regions of the vertex, behind the origin of the retractor of the antenna. The diameter of the fibres is very small. Contraction results in inpulling of the vertex during deflation of the head. These muscles appear to be the 'muscle fronto-pharyngien' of Mercier and Villeneuve (1925), although they state that the origin of the fibres is on the pharyngeal wall. In their diagrams (p. 883, figs. i and ii) fibres appearing in transverse section underneath the vertex are labelled as part of the fronto-pharyngeal muscle. The sections are actually, however, of the fibres of the trans-vertex muscle.



TEXT-FIG. 13.

Diagram drawn from thick vertical section of newly emerged adult; ptilinum invaginated. ×29.

6. Emergence of Fly from Puparium and use of the Ptilinum (Text-fig. 1).

Emergence of the adult fly occurs after a pupal period averaging 10 days at 25° C. The puparium is ruptured in two fissures—a longitudinal and a circular. The former extends around the anterior end and along the sides, external to but in the same line as the main tracheal trunk of the larva, until it meets the circular fissure, which runs completely around the anterior margin of the fourth visible segment. The ruptured anterior end may hinge back, or break off, in two pieces, a dorsal and a ventral, or more rarely it may separate as a single bowl-shaped portion. The ruptures are effected by inflation and retraction of the ptilinum, the pulsation of which continues regularly until the legs are free, whereupon the fly scrambles from the puparium. The inflation is brought about by pressure of blood forced into the head by contraction of the abdomen; the whole head, including the proboscis, is very much distended and a small buccal bladder appears at each side above the maxillary palps. The head is first distended principally along the antero-posterior axis, then, apparently by contraction of the muscles inserted on the genae, it becomes flatter and the ptilinum seems to be more turgid and possibly of greater strength as a buffer. Expansion of the abdomen results in withdrawal of blood from the head, and the ptilinum is invaginated by contraction of the muscles inserted on it. The decrease in bulk of the posterior part of the body by its contraction may facilitate its passage through the opening of the puparium, which is not at the point where its diameter is greatest.

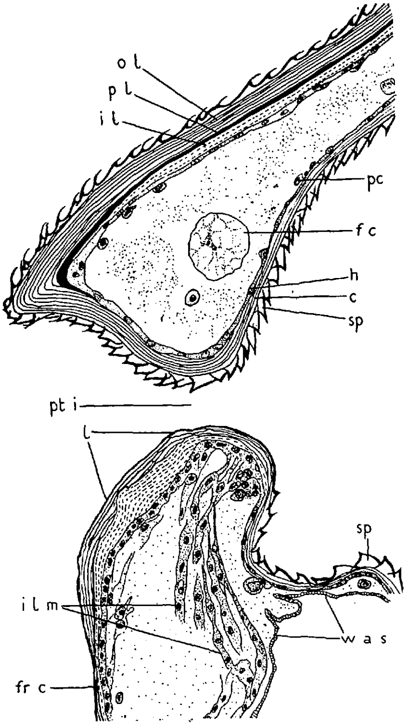
The observed times of rhythmic pulsation were approximately 3 seconds in the inflated and ½ second in the deflated condition. The shortest time taken by a fly to emerge under observation was 2 minutes; the usual time is about 5 minutes. Occasionally, when difficulty was experienced, the process occupied more than an hour.

Pulsation continues after the insect has emerged from the puparium only if there is some obstacle in front of its head, or if buried in sand. Under such circumstances the ptilinum assists the insect in making its way to the surface, and irregular pulsations continue so long as the remains of the pupal skin cling to the legs. Generally, however, once the fly is free of the pupa case, the ptilinum is drawn into the head cavity where it remains thereafter in a functionless condition.

7. Subsequent changes in the Ptilinum and Associated Muscles.

It has previously been mentioned that the cuticula of the ptilinum, in newly emerged flies, is very similar in structure to that of the neighbouring integument. In fully hardened and pigmented flies the cuticle covering the vertex is seen to consist of: (1) an outer yellow refractive layer bearing minute spinules externally: this layer is not stainable with Delafield's haematoxylin and it contains an inner stratum of pigment; (2) a thinner, less refractive, inner layer which stains readily with haematoxylin. If the head cuticula be followed to where it becomes continuous with that of the invaginated ptilinum its structure is seen to change. On the ptilinum it is composed only of the outer layer which becomes less refractive and is stainable with haematoxylin but contains no stratum of pigment. After the third day the cuticula of the ptilinum undergoes marked dissolution, its thickness changing from 25μ to 5μ (Text-fig. 14). Fourteen days after emergence the greater part of this cuticula has disappeared and the only covering of the ptilinal wall is the very thin layer which serves to connect the bases of the spinules.

Before two days have elapsed after emergence, all the muscles connected with the ptilinal mechanism, with two exceptions, have completely disappeared. The lunular muscle alone remains in a condition the same as at eclosion. The functional muscles of the proboscis and the intrinsic muscles of the antennae are unchanged. The retractors of the antennae, however, are still present although non-functional, since the cytoplasm is reduced to a very thin covering around the degenerated nuclei. They can be detected partly enclosed among the creasings of the ptilinum. The head-space, formerly occupied by muscles, is now filled by air-sacs. These are applied closely against the



TEXT-FIG. 14.

Vertical section passing through mouth of invaginated ptilinum of imago three days after emergence. × 163·7.

contours of the ptilinum; they surround the brain and extend from the vertex ventrally to the muscles of the fulcrum. In the few narrow blood-spaces that still remain are small phagocytes and fat cells. There are no phagocytes distended with ingested muscle débris such as are numerous during histolysis of the larval muscles.

The degeneration of the muscles seems to proceed most rapidly during the fly's second day of adult life, since sections of the heads of flies 24 hours old show the fibres with but little change in their structure. The actual process of histolysis has not been followed in any detail, and for this purpose a very complete series of sections made at intervals of every few hours during the second day after emergence is necessary. The onset of histolysis appears to manifest itself in loss of staining capacity in the muscles concerned: their striae soon become lost, the cytoplasm becomes spongy, and finally complete breaking-down results. Although phagocytes are present, both free in the blood and associated with fat cells, none were found in intimate relation with degenerating muscles. The actual beginning of degeneration is probably associated with disuse of the muscles concerned after the final retraction of the ptilinum has been accomplished. In this connexion the distension of the cephalic air-sacs, which results in cutting off much of the blood-supply, may be significant. If the foregoing observations be correct it would appear that histolysis of the muscles in question takes place by some process other than phagocytosis, which is so prominent in the pupa (Pérez, 1910). It may also be mentioned that it is only in relatively few cases that phagocytosis has been proved to occur as a histolytic process among insects. In other cases it has been much disputed whether phagocytes play any part at all.

8. Discussion.

From a comparative study of the head-capsules of Diptera, Peterson (1916) was not able to determine the origin of the ptilinum, although he made the following suggestions: 'It seems evident that the frontal suture was once a membranous area which became invaginated to form a membranous pouch or ptilinum. If this is the case the mesal membranous area of the fronto-clypeus of Sepsis, Oecothea, Calobata, and Desmometopa would be very significant. The ptilinum might have originated from some form similar to Scenopinus in which the ventral margin of the chitinized vertex is located dorsad and laterad of the antennae. It seems quite possible that the membrane along this margin became invaginated in the early stages of the development of the ptilinum.' The 'mesal membranous area' here mentioned is that between and below the antennae and continuous with the ptilinum. Both from Peterson's views and from the development of the ptilinum from the head integument, it seems evident that the origin of this sac was by the enlargement of a membranous area in the region of the antennae. Quite possibly it arose as an extension of the membrane surrounding the antennal sclerites, similar to but greater than that found in various members of the Nematocera (e.g. Rhabdophaga, Mycetophila, Chironomus, Mycetobia). The presence of such a membranous area may have been of use originally during emergence in allowing distension of the head with blood from the thorax and abdomen. This area, having become highly flexible, would no longer serve as a rigid base for those of the pharyngeal dilator muscles that originated from it. The contraction of the fibres of such muscles would result not in altering the contour of the pharyngeal cavity so much as in pulling inwards the membranous area of the head. It is, therefore, possible that, with the formation of the ptilinum from the membranous area mentioned, certain of the bundles of the original dilator muscles of the pharynx have changed their function so as to constitute the ptilino-oesophageal muscle. In this connexion it is of interest to note that in some of the Nematocera (Culicidae, Knab, 1911) the pharyngeal dilators are used in the process of emergence. By the contraction of these muscles air is sucked into the alimentary canal and serves to distend and to harden the body. Contrary to what might be expected, a study of the head in a typical Syrphid fly does not afford any clue to the origin of the ptilinum.

In Eristalis tenax there is a delicate strand of muscle passing from the vertex to the oesophagus in the median line. On account of its relations it may be termed the vertico-oesophageal muscle. It is apparently homologous with the ptilino-oesophageal muscle of Calliphora, but it is uncertain whether it is functional. The vertex, to which this muscle is attached, is well-cuticularized and non-distensible, continuing so to the insertions of the antennae. In the median line, immediately above the antennae, is the crescent-shaped lunule to which, as in Calliphora, are attached the two minute roots of the lunular muscle. In Eristalis, however, the fibres do not unite into one median muscle, but diverge and are inserted on the inner marginal area bordering each eye. The vertico-oesophageal and lunular muscles are to be seen in sections and dissections of the heads of pupal and imaginal Eristalis. Mercier and Villeneuve (1927) drew attention to the lunular muscle and considered it significant in proving the homology of the lunule of Calliphora and Eristalis. In the Schizophora the lunule forms the lower median border of the ptilinum while in Eristalis the sclerite is directly continuous with the vertex, no ptilinal membrane intervening. Even in the pupa of Eristalis the integument above the lunule appears indistensible, while within the upper part of the head-cavity there are no functional muscles. It seems, therefore, highly improbable that eclosion is assisted by a ptilinal mechanism, as Künckel d'Herculais recorded. Becher denied this, arguing that the lower part and sides of the face of Eristalis were distended with blood during eclosion, and that it was their activity which Künckel d'Herculais had mistaken for that of a ptilinum. Support is lent to Becher's contention by the fact that the lower part of the frons and genae, and the integument between these sclerites and the proboscis, form a crumpled thin membrane covered externally with numerous minute scale-like structures. In Calliphora a similar membranous area swells during emergence as a buccal bladder at each side of the mouth frame.

The Aschiza and Schizophora, therefore, as exemplified by Eristalis and Calliphora respectively, possess in common the faculty of assisting their eclosion by distending the head, but the process takes place by different methods. By the development in the Schizophora of a ptilinal membrane, which is without its counterpart in the Aschiza, the two groups have become separated. From the foregoing evidence it seems more reasonable to conclude that the Aschiza never possessed a ptilinum, rather than to assume that, in common with the

Schizophora, they developed such an organ which subsequently atrophied.

9. Summary.

1. An account is given of the morphology, development, and changes following emergence of the ptilinum of the blow-fly.

2. The ptilinum in *Calliphora erythrocephala* develops from the integument of the anterior part of the head. Differentiation from the surrounding integument begins first as a local thickening of the epidermis. The structure of the thickened area is similar to that of an epidermal imaginal bud. At the end of the third day after pupation at 25° C. this epidermal thickening grows inwards and, as its surface increases, its walls become gradually thinner. Rudiments of the ptilino-oesophageal muscle are obvious even immediately after eversion of the head; other muscles do not appear until the fourth day after pupation.

3. In the newly emerged fly the ptilinum is continuous with the integument of the frons, genae, and vertex. Two unpaired and one pair of retractor muscles are inserted on the ptilinum; six other muscles appear to be accessory in connexion with pulsation of the head during emergence.

4. By rhythmic expansion and contraction of the ptilinum the puparium is ruptured and emergence of the fly assisted. After final retraction of the ptilinum into the head-cavity, and hardening of the surrounding head integument, no more use is made of the organ.

5. During the adult life of the fly the ptilinal integument is reduced to a layer of spinules. Before two days have elapsed after eclosion practically all the ptilinal and accessory muscles have disappeared. Their disappearance does not appear to be due to phagocytic activity. From histological evidence the cause and mechanism of muscle degeneration cannot be ascertained.

6. A possible method of origin of the ptilinum from a membranous area above the antennae is suggested. *Eristalis*, without ptilinum, is compared with *Calliphora*. From the evidence brought forward it is suggested that the Aschiza and Schizophora represent two independent lines of development.

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