STUDIES IN RELATION TO MALARIA.

II.

THE STRUCTURE AND BIOLOGY OF ANOPHELES
(*Anopheles maculipennis*).

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(*From the Pathological and Morphological Laboratories of the University of Cambridge.*)

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III. The Pupa.

External Structure.

The larva about to turn into a pupa comes to rest, the thoracic region becomes swollen, and the pupa gradually issues through a dorsal slit in the larval cuticle, which is ultimately thrown off with all its exterior chitinous appendages as well as those parts of the spiracles etc. which are superfluous.

A favourable specimen threw some light on the change from larva to pupa, it was in fact a very young pupa which had half cast off its larval skin and was in the act of freeing its tail. The collar round the base of the head of the larva had split in the middle dorsal line and a gape had appeared, this gape was continued forward, shaped like a **Y**,

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along the lines of the posterior sides of the diamond-shaped area already described on the head, in this way the centre of the upper surface of the head projected as a kind of lid or flap. The skin of the body had also split along the back and the young pupa was just freeing its tail from the cast-off skin. This pupa measured 6·5 mm., of which the head and thorax measured 2 mm. As the pupa matures the head and thorax increase in size up to about 3 mm. in length, the abdomen looks relatively small, more like a tail, and is bent round along the ventral surface of the sac.

During the last larval stadium the various pupal organs are being formed. When the larva gives rise to the pupa the head and thorax are already in their "sac," the respiratory trumpets are there, the tail fins, mouth parts, and limbs are enclosed in their sheaths, the former showing no relation to the mouth parts of the larva.

Whereas the larva breathed through two spiracles which ran along the back, gradually increasing in size and terminating in two stigmata at the posterior extremity (see p. 64), the whole respiratory system of the pupa is reversed, together with the position of the insect, when it assumes the pupal form. The insect now breathes through two respiratory trumpets issuing laterally from the anterior dorsal surface (Plate II, fig. 10), these forming apparently the only external openings. The insect does not feed during the pupal stage, during which it only undergoes its metamorphosis into the imago.

The pupa is a tadpole-looking object, but the comparison would be more correct if we imagine the tadpole has its tail flattened in a horizontal plane and folded under the body. The whole of the head and thorax is enveloped in a thin and semitransparent membrane, within which the various appendages (and even the scales upon them) can be seen coiled up in a symmetrical manner. We shall call that part of the body included in this membrane the "sac."

The head is folded down upon the breast. The most conspicuous organs of the body are the eyes, which are already black and consist of an increasing number of ommatidia. Just anterior and above the eye the antennae emerge from the head and are folded backwards, crossing the upper part of the head, and passing backwards across the origin of the three pair of limbs. They then lie parallel and between the posterior pair of legs and the anterior edge of the wings.

As the pupa increases in age it perceptibly darkens, and the darker parts are usually first apparent at the ends of the appendages and on the wings. The pupa is occasionally green. We have seen quite an

old specimen in which all the internal parts were coloured green and shone through the transparent cuticle.

The mouth parts are very long and are coiled symmetrically. The maxillary palps do not reach so far forward as the base of the anterior pair of legs, but their great length is provided for by the palps being sharply bent back and their curving forward again in a somewhat *S*-shaped manner.

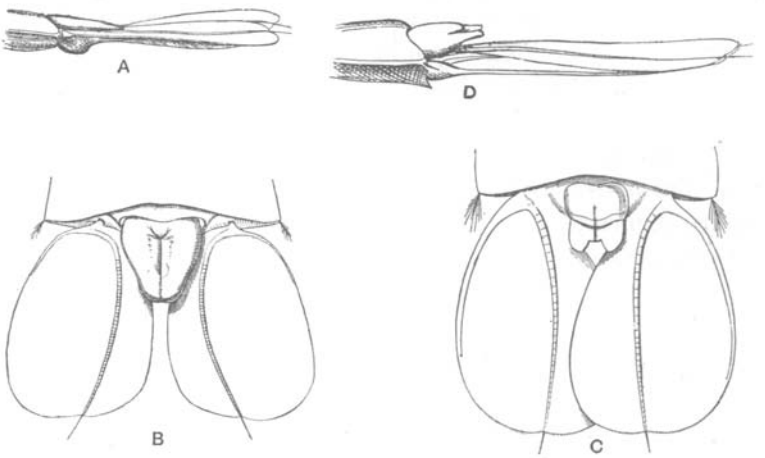
The labrum, mandibles, maxillae, labium and hypopharynx all pass backward like the trunk of an elephant curled in, in the median plane. On each side of this middle organ lie the legs, all passing backward and converging inwards, at the hinder end. As the legs are too long for the case they bend gracefully upwards and forward in a quite symmetrical manner. The sac extends ventrally as far as the division between the 2nd and 3rd post-thoracic segment and hides the sterna of the anterior abdominal segments, but dorsally the terga of the last thoracic and first two abdominal segments are quite distinct.

The breathing trumpets have their origin from two very stout and conspicuous tracheae which run backward and inwards parallel with the base of the wing; at its inner end this trachea is slightly constricted just where it passes the integument to open into the external organ or trumpet. This is not really trumpet-shaped, but more like a cornucopeia of paper, that is, on the face directed inwards there is a *V*-shaped slit of very graceful form. The edges of this slit are, when the pupa is breathing, supported by the surface film. The edges sometimes overlap one another for a little near the base, and the surface of the whole trumpet is marked out into very delicate spindle-shaped areas.

Wedged into the space between the wings is the tergum of the first abdominal segment. The ventral part of this segment is included in the sac and does not stand clear. The second abdominal segment is free, and this is followed by six other segments all more or less resembling one another. The eighth segment bears posteriorly two large flaps or fins measuring usually about ·8 mm. in length. Each fin has a chitinous ring at its base and a weakly chitinized transversely striated bar runs through about the centre of the fin as a strengthening skeleton, it thins out at the periphery and terminates in a single hair. The right fin overlaps dorsally the left. (Fig. *C*.)

The ninth segment bears a pair of blunt processes within which the gonapophyses develope. These processes are situated in front of and between the fins, below and in front of the anus. The processes are much larger and of a different form in the male pupa, so that it is possible

even on naked-eye examination to determine the sex of the living pupa by viewing it through the sides of the aquarium. The accompanying figures serve to show this difference. Figures *A* and *B* represent these structures in the male (lateral and ventral views), *D* and *C* the corresponding parts in the female pupa. Figure 10 on Plate II. represents a male pupa viewed from the side, *x* representing the processes which contain the gonapophyses.



From the second to the eighth the segments have much the same structure. The narrow chitinous tergum of the larva has increased and forms a large plate, stretching from side to side and covering from before backward three-quarters of the area. At each side the chitin of the tergum is thickened and darkened, and at the posterior angle it is produced into a stout, backwardly directed hair. The thickening gives the pupa sharp sides to its body, the cross section of which is no longer, as in the larva circular, but somewhat spindle-shaped. Posteriorly the terga bear four symmetrically arranged branched hairs which project back over the soft skin which forms the posterior fourth of the segment uncovered by the tergum. Anterior to these is a pair of similarly three or four times branched hairs, and some long single ones. The tergum does not cease laterally at the thickened sharp lateral edge but bends under and forms a small plate increasing in width from before backwards. The inner posterior angle of this plate bears a hair.

When viewed sideways the pupa of *Anopheles* presents a comparatively smooth dorsal outline, but in *Culex* the edge where each tergum

joins posteriorly the soft integument which unites it with the succeeding tergum stands out as a ridge, and the dorsal outline presents a series of salient angles. We might add that Howard[1] (1900, p. 40) draws attention to the fact that the pupa of *Culex* assumes a more vertical position in the water than that of *Anopheles*, and that its respiratory trumpets are not so broad terminally.

The sterna are of some size, not so broad as the terga but stretching over the middle three-quarters of the ventral surface, anteriorly rather rounded, they are posteriorly sharply cut where they unite with the soft intersegmental membrane. In the older pupae they bear numerous rows and patches of minute hairs, symmetrically arranged, these hairs point backwards and outwards, and the central ones on the eighth abdominal segment point inwards and are longer than the others.

Similar symmetrically arranged hairs also pointing backwards and outwards are found on the terga. Since each hair articulates by a deeply pigmented base, there is an increase in the coloration with the appearance of these hairs.

As the pupa increases in size more hairs appear and additional chitinizations arise in the skin, a median small plate occurs behind each tergum, and this is produced laterally into two side dorsal plates which look as though they were perforated.

Just in front of the anterior abdominal tergum, which as has been said is dovetailed into the dorsal part of the thorax, is another small tergum which is continued on each side into a flattened process which bends in and is concealed under the wings. This would seem to be the segment which bears the halteres. It and the first abdominal segment are hollowed out, bent in as it were like the small of the back. This hollow is the surface which lies uppermost when the pupa is in its usual position floating at the surface of the water, and the creature is maintained in this position by a pair of those conical shaped hairs which played the same part in the larva. The hairs are stalked and bear secondary hairs, like the ribs of an umbrella turned inside out, but in the pupa the secondary hairs are filiform, not flattened, as they are in the larva. They are black and do not form a complete cone, one quarter being absent.

The pupa, until about the time when it gives rise to the fly, floats quietly at the surface, breathing through its respiratory trumpets. The trumpets slightly indent the film, over which at times the dorsal surface

[1] See Bibliography, p. 75.

of the pupa may protrude. When disturbed the pupa shows great activity, and apparently purpose, in its movements, as is seen when the attempt is made to capture it by means of a pipette. It darts rapidly with a series of quick intermittent strokes of its muscular abdomen to the bottom of the vessel. On account of its great buoyancy it again quickly rises passively to the surface as soon as it ceases to move. It advances tail first, and owing to the motion of the abdomen being apparently limited, that is dorso-ventral, it only moves downward, counteracting the buoyant action of the air vesicle and the air contained in the respiratory trumpets and tracheae which keep the anterior portion of the body uppermost. The effectiveness of the tail as a swimming organ is materially increased by the broad flaps with which it terminates. When extended the abdomen assumes the position which the abdomen of the fly occupies in relation to the thorax. This is best seen in dead pupae, where owing to the relaxation of the parts the abdomen becomes extended in a straight line. Locomotion is effected by means of powerful muscles situated ventrally within the abdomen. Besides the air in the respiratory trumpets and tracheae there is a considerable reservoir of air at the posterior end of the sac. This air is ventral, but extends some distance up each side of the body. It acts as a very efficient float, keeping the animal right way upwards and enables it to float up to the surface the moment it ceases swimming. The position of the pupa in rising to the surface is determined by the air vesicle, the bubble of air being visible through the pupal covering, especially of young pupae. Owing to its great buoyancy and lack of weight so to speak in its "keel," combined with irregularities in locomotory movements, the pupa at times floats sideways beneath or near the surface, moves sideways and downwards etc., but soon rights itself when it ceases to struggle.

The movements of the pupa are so powerful that if placed in a thin glass vessel containing water it produces an audible sound by striking against the walls of the vessel. Pupae which have retreated from the surface frequently show two minute air-bubbles at the ends of their trumpets, these bubbles bursting when they come in contact with the surface again.

The surface of the sac becomes darker with age and develops numerous small hairs, and a fairly conspicuous bunch of longer hairs, which arise just inside the base of the respiratory trumpet and are closely adpressed to the surface of the pupa. All along the top of the head, stretching from the "forehead" back between the respiratory

trumpets almost to the end of the "sac" is a special area bounded by two slightly elevated lateral ridges and divided into two equal halves by a strongly marked ridge. The areas each side of this latter are faintly marked out into small squares.

Duration of pupal stage.

The pupal stage observed in 37 insects kept isolated and separately observed, was found to last from 3 to 4 days. The pupae were removed from tanks immediately after transformation and placed in bottles containing clear water. The temperature at which the pupae were kept was found to exert a considerable influence upon the rate at which metamorphosis took place. Three pupae kept at 23·7° C. developed on the fourth day. Twenty-three kept at an average temperature of 25·2° gave rise to flies on the third day. Two of the latter group actually gave rise to flies on the second day, the one fly a male (small), the other a female of medium size. Three flies which issued on the fourth day were large females. Generally speaking the development of small flies is more rapid than that of large ones independent of sex.

Adding the time required for pupal development to the time spent in the larval stage, the insect requires under the conditions stated about 20 to 25 days for its development from the time it issues from the egg to the issuing of the fly from the pupal covering. Naturally other conditions of temperature and natural surroundings will exert a material influence upon the rate of development.

Grassi[1] (1900, p. 84) found that the development of *A. maculipennis* from the time that the larva issued from the egg to the exit of the imago from the pupal case lasted about 30 days at 20—25° C. After about 20 days these flies in turn laid eggs. The development was more rapid in summer, how rapid is not stated. He says (p. 70) that the pupal stage lasts about 3 days. Howard[1] (1900, p. 40) found the pupal stage of the same species (no temperature given) to last not less than 5 days and in several cases as much as 10 days. Finally Ross, Annett and Austen (1900, p. 20) found a species of *Anopheles* in Sierra Leone to undergo pupal metamorphosis (no temperature given) in 48 hours.

The influence of temperature upon the rate of pupal metamorphosis is very evident from our last observation this season. Two full-grown

[1] See Bibliography, p. 75.

larvae, the only two met with, which were caught in the Granta on October 20th became converted into pupae on the 21st. The imago issued from one pupa on the 27th, from the other on the 28th, that is on the 7th and 8th days respectively. The temperature in the Granta about this time measured 8·3° near the surface. The temperature in the laboratory where the insects were subsequently kept varied between 13 and 17° C.

To be continued.

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