

STUDIES IN RELATION TO MALARIA.

II (*concluded*).

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(Anopheles maculipennis Meigen.)

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THE DIGESTIVE ORGANS.

THE alimentary canal of *Anopheles* consists of the following parts:—
 1. Mouth, 2. Buccal cavity, 3. Pharynx or Pumping-Organ, 4. Oesophagus, with which are connected, 5. Three food reservoirs (two situated dorsally and one ventrally), 6. Oesophageal Valve, 7. Mid-gut, 8. Ileum, 9. Colon, 10. Rectum, 11. Anus. (See Plate VI, Fig. 1.)

Judging by analogy with other insects, the chitinous lining of the parts numbered 1—5 (mouth to oesophagus inclusive) indicates that they arise from the embryonal stomodaeum. The mid-gut has its origin from the mesenteron. The termination of the mid-gut and commencement of the colon correspond with the point where the Malpighian tubes are connected with the alimentary canal. The chitinous lining of these tubes indicates their origin from the proctodaeum or hind-gut. The alimentary canal lies above the ventral nerve-cord and ganglia and beneath the heart or dorsal vessel.

The salivary glands do not communicate with the alimentary canal.

The examination of a series of sections of the proboscis of a female *Anopheles maculipennis*, passing from the tip to the base, shows that the various parts—maxillae, mandibles, labrum-epipharynx, hypopharynx, labium, even the maxillary palps—have their origin at about the same level. That level is a little way behind the anterior limit of the clypeus, and a section through this region shows all these parts closely adpressed one against the other but still discrete. A section or two further back shows that these parts become fused with one another and that they surround the beginning of the alimentary canal.

The Mouth and Buccal Cavity.

(See Plate VII, Fig. 5.)

The region where the mouth-parts coalesce, as described above, we style the *mouth*¹. The *buccal cavity* extends from the mouth to the valvular arrangement situated at its juncture with the pharynx. The general direction of the buccal cavity is upward and backward; the portion which approaches the pharyngeal orifice is directed more suddenly upward. Close to the mouth the lumen of the buccal cavity resembles that of a railway tunnel, measuring (in the female) but 33μ across its floor and 25μ in height, it then becomes complicated by the origin of two wing-like chitinous processes which project upward and serve as apodemes for the insertion of muscles. The lumen next narrows to a horizontal slit in the middle, but pointing upward and outward at the sides. This change of form is due to the stout chitinous floor projecting upward at the sides. Passing beneath the floor is the well-developed paired muscle running forward to the salivary "pump" and having its origin from a chitinous flange which is continuous with the chitinous floor of the buccal cavity. Towards its posterior end the lumen of the

¹ It will be noticed that we divide the fore-gut into more parts than is usual, this being merely a matter of convenience. In insects the alimentary canal may be divided into three main divisions, in accordance with their embryonic origin, viz. fore-gut, mid-gut, and hind-gut; derived respectively from the stomodaeum, mesenteron, and proctodaeum. The fore-gut, in a typical insect, is usually divided into regions known as the mouth, pharynx (which includes our buccal cavity), oesophagus, crop (ingluvies), and proventriculus (gizzard). The hind-gut, where more highly developed than in *Anopheles*, has been divided into ileum (short intestine), long intestine, colon, and rectum.

Dimmock styles our buccal cavity "pharynx," and includes our pharynx with the oesophagus. Annett and Dutton describe our buccal cavity as the "ascending portion of the pharynx." The structure of the parts we have described separately as buccal cavity, pharynx, and oesophagus, with their sharp limitations, lend themselves best we think to the divisions we have given, our terminology having the advantage of terseness.

buccal cavity again becomes circular in section, and it is at this point that the flange just referred to projects from the floor, the flange bending forward and the paired muscle being attached to its concave surface. Whereas the floor of the buccal cavity is lined throughout with stout chitin, its roof is less chitinous. Beginning again with the mouth, Annett and Dutton (1901, p. 84) describe and figure low papillae, which they term "taste-papillae" projecting from the chitinous roof, or palate as we shall term it, and apparently consisting of chitin, judging from their figure. They appear to represent blunted chitinous protuberances, and we do not know on what evidence the authors named attribute a sensory function to these minute structures, which they found in *Anopheles costalis*.

In *Anopheles maculipennis* we have noted the existence of an interesting structure in this region. The anterior portion of the palate is lined with thick chitin, this being followed by a "soft palate," (Fig. 5, Plate VII) lined with a distinct membrane, on the wrinkled surface of which there is a delicate layer of chitin, such as is demonstrable in the oesophagus by maceration in caustic potash. Posterior to the soft palate, thick chitin again makes an abrupt appearance, and this is continuous back to the pharyngeal valve. Anteriorly, the thickened chitin of the palate projects backward somewhat in the form of a trowel, the convexity being directed ventrally. The end of the trowel is continuous with the lining of the soft palate. In longitudinal section, the trowel is seen to end in a series of blunt projections apparently due to transversely folded chitin, the structure being very minute, and only clearly visible by the aid of a $\frac{1}{12}$ th immersion. These correspond to the so-called "taste-papillae" of Annett and Dutton. Here the soft palate begins. Anterior to the protuberances just mentioned are situated short and minute spines of chitin, apparently about six in number and directed slightly forwards. The spines are hollow and are moveable, being articulated upon or within a ring of thickened chitin which is continuous with that of the palate. These spines appear to be in direct communication with strands of the anterior pair of the ten palatal muscles. In the chitinous floor of the mouth, directly opposite, a depression (seen in cross section) is noticeable which has a corresponding form to that of the structure just described, so that it appears that the lumen of the buccal cavity in this region may at times be almost occluded. Transverse sections midway through the soft palate moreover show two similar spines protruding, some distance apart, from

discrete annular thickenings of chitin, the spines being directed downward into the lumen of the buccal cavity. The significance of these structures is not clear, possibly they act as teeth or as strainers of particles of food ingested.

The wrinkled surface of the membrane lining the soft palate indicates that the lumen of the cavity may be considerably increased at times, this being clearly effected by the palatal muscles attached to the dorsal surface of the palate and running upward to their origin from the inner surface of the roof of the clypeus. What we have termed the palatal muscles are those which Annett and Dutton call the "oblique central fibres of the pharyngeal muscles." These authors do not figure the very marked membrane of the soft palate, stating that it is lined by flattened epithelium only and no chitin.

The thin layer of chitin lining the palate renders it easy, we consider, for the palatal muscles to draw the palate upward, and away from the floor. These muscles are five in number and paired. The structure we describe indicates that the buccal portion of the alimentary canal may well exert a suction action.

The valvular arrangement at the entrance to the pharynx was first described by Dimmock (1881, p. 13), who thought it "a valve to prevent the return of fluids to the mouth during the pumping process." This valve lies just behind the posterior end of the clypeus, the buccal and pharyngeal cavities uniting at an angle with regard to their axis at this point. There is evidence that the clypeus may be slightly moveable dorso-ventrally upon the head, and consequently the angle at which these cavities join may vary somewhat during life.

In this region the latero-dorsal chitinous wings are conspicuous, the chitinous structure being kept in position by two paired muscles. Two of these pass close to one another in the middle line of the vertex, outwards to each chitinous wing. The other pair of muscles arise each from the under surface of the chitinous wing, and, after a short course, are inserted into the tubular struts which pierce the head (see *Journal of Hygiene*, Vol. I. p. 482, Plate IX, Fig. 13, *tu*). Immediately in front of the two fine muscles running to an apodeme in the dorsal middle line, and the anterior line formed by their insertion into the dorsal surface of the buccal tube, lies the buccal ganglion. Close behind these two muscles are a second pair which run down parallel to one another from the middle of the vertex to be inserted into the anterior part of the pharynx.

In *Anopheles costalis*, according to Annett and Dutton (p. 85 and *Journ. of Hyg.* III

Plate XVIII), at the junction of buccal cavity and pharynx, and attached at the angle of junction, ventrally, there is "a peculiar ridge of chitinous stout hair-like processes, which curve forwards so that their tips lie in the angle formed between the upper parts of the first (our buccal cavity) and second parts (our pharynx) of the pharynx. The hairs are of two kinds, an anterior large set—probably a single row—and a posterior, small, fine set situated in a clump immediately behind the former. The larger hairs consist of a short stout shaft firmly embedded in the chitinous pharyngeal wall; this shaft supports a cup with a free rim curved outwards; within the cup lies the oval-shaped bulbous extremity of the base of the hair; this bulbous extremity contains a single large cell. The remaining free portion of the hair curves forwards and tapers to a fine point, and appears to have a central shaft enclosed within a chitinous cuticle from which barb-like processes project. The hairs of the posterior set are much finer and shorter, and are much more numerous; they appear to be simple in character. In transverse section this structure presents to some extent the appearance of 'rods and cones.' The suboesophageal ganglion lies in close proximity to this structure, but no nerve-fibres have been traced to communicate with these specialised hairs, although such probably exist." These hairs appear to be sensory, and moreover they should aid in rendering the valve more effective, for the reason that they project into a space formed by the chitinous portions opposite. We have not as yet observed this interesting structure in *A. maculipennis*, and propose, when we have more material, to study the structure of the valve more closely.

*The Pharynx or Pumping Organ*¹.

(See Plates VI, VII, and VIII.)

The pharynx extends from the valve just described, to near the back of the head, where it joins the oesophagus. The anterior portion is

¹ Packard (p. 303) states that Meinert ("Tropi Dipterorum") described the pharynx as the principal, and in most Diptera as the only part of the pumping apparatus (antlia). Meinert appears to have recognised the nature of the mechanism. His figure of the pump, given by Packard (p. 78, figure 81) and the muscles attached thereto, the "musculis antliae," taken from *Culex pipiens*, is fairly accurate. A similar apparatus is also present in other flies, in Hemiptera and Lepidoptera. Dimmock (1881, p. 19) rightly states: "This bulb is the chief sucking organ in the female *Culex*"; he is in error however when he states (p. 13) that it is absent in the male insect.

tubular, and is strengthened by longitudinal chitinous ridges (Plate VII, Fig. 1). This part shows a dorsal flexure, which is better marked in the male insect (Plate IX, Fig. 2). The chitin with which it is lined throughout is continuous with that of the buccal cavity, and is seen to consist of a dorsal and two latero-ventral plates, which widen and again narrow posteriorly. Separated from one another, by dissection, or crushing beneath the coverglass, the three chitinous plates have somewhat the form of a narrow ping-pong racket, the distal or broadened end adjoining the oesophagus, showing rodlike sinuous chitinous ridges, the chitin ending in very fine spines, giving it a somewhat comb-like appearance (Plate IX, Figs. 1 and 2); cross sections in this region show that the chitinous rods project backward and slightly into the lumen of the commencement of the oesophagus, where they doubtless act like combs in straining out particular matter. This region is surrounded by a sphincter muscle. This structure has not been as yet observed (see Plate VII, Fig. 4). The study of the structure of the pharynx is greatly facilitated by the use of caustic potash, the soft parts being removed. Instructive specimens may be prepared, showing its relation to the other chitinous portions of the head, by macerating the heads of the insects, and afterwards cutting away the eye or other parts so as to expose the pharynx *in situ*. In this way also the pharynx may be dissected away from the rest of the head together with the appendages (Plate IX, Figs. 1 and 2). Viewed in cross section through the enlarged portion the three plates are seen to be connected together at their edges, which show delicate serrations running to the margin, and are curled outward. The junction of the plates is effected by means of a thin grooved gutter of chitin (V-shaped in section) in such a way as to permit of the expansion of the lumen by the muscles which are attached to and draw the expanded plates asunder. As will be seen by reference to Figs. 2 and 3 (Plate VII) the chitinous plates are curved, so that when the divaricating muscles are at rest the plates by virtue of their elasticity narrow the triradiate lumen of the pharyngeal bulb.

The pharynx is considerably larger in the female than in the male insect. In the male the tubular portion is relatively longer, the whole length of the pharynx is but $\frac{4}{5}$ that of the female's. The width of the plates which compose the bulbous portion is about twice as great in the female as in the male, and the plates are composed of much thicker chitin, as will be seen by reference to Figs. 1 and 2, Plate IX.

We add some actual (averaged) measurements in round numbers, made on specimens treated with caustic potash :

Length of pharynx in two males = ·32 mm.

Width of widest portion of plates = ·09 mm.

Length of pharynx in three females = ·40 mm.

Width of widest portion of plates = ·20 mm.

The pharynx is obviously the chief organ by means of which the mosquito sucks up fluid food or blood. The most conspicuous and powerful muscles in the head are those whose function it is to draw apart the three walls of the bulbous portion of the sucking pharynx (Plate VII, Figs. 2, 3 and 5). Two, the posterior dorsal dilators, run side by side, straight downward from the occiput behind the brain and eyes to the median dorsal plate. Two others, the anterior dilators (or in the female two pairs), run in front of the brain from the vertex to the pharynx. The latero-ventral dilators consist of five distinct muscles on each side which run from the lateral posterior angle of the head inwards, upwards, and a little forwards to be inserted in the latero-ventral plates. When these muscles contract simultaneously, the almost slit-like triradiate lumen of the pharynx must become nearly circular in cross section, and suction will take place through the epipharynx, so that if the insect has its proboscis fixed in any animal, plant, or fluid—blood, sap, or the fluid will be drawn in. When the muscles relax, the food will be propelled backward into the oesophagus by the elasticity of the released plates.

The lumen of the end of the pharynx is triangular, that of the oesophagus which follows is rounded. The anterior tubular portion of the pharynx passes through the ring formed by supra- and infra-oesophageal ganglia and their commissures.

The Oesophagus.

The oesophagus is short, and extends from the posterior end of the chitinous pharynx to the oesophageal valve. Anteriorly, where it traverses the insect's neck, it is narrow, it then gradually widens, hardly to the extent that it can be called a crop. The oesophagus is plentifully supplied with bands of muscles, the posterior part being thrown into transverse folds when the circular fibres are contracted (as is indicated in Plate VI, Fig. 7). The oesophagus may be seen to undergo vigorous rhythmical contractions for hours when dissected out from the body and

placed in salt solution. In hardened sections it at times offers a spacious lumen, at others it is all but occluded. The shape of the epithelial cells lining the tube varies according to the degree of contraction or expansion. Their average outline is roughly cubical, their nuclei being sharply defined and slightly oval. The general structure corresponds in detail with that of the mid-gut to be considered presently.

The Food Reservoirs.

(Plate VI, Figs. 1, 4, and 7.)

The posterior end of the oesophagus lies on about a level with the origin of the first pair of legs, as pointed out by Grassi, and here are given off the three food reservoirs. The large ventral reservoir¹ opens into the oesophagus by means of a simple pore in the middle line, the two small latero-dorsally situated reservoirs open laterally into the oesophagus. Beginning at its junction with the oesophagus, the large reservoir (Plate VI, Fig. 1) is seen to extend backward under the alimentary canal as far as the 6th or 7th abdominal segment when completely filled. The anterior portion is narrow, posteriorly it widens into a fusiform sac. The dorsal reservoirs extend dorsally from the oesophagus, outside the large mass of dorso-ventral muscles, between them and the body-wall. The relative size of the reservoirs will be seen by reference to the plate.

Before proceeding to describe these organs more fully it appears necessary to refer to what has been stated with regard to their function by other authors. Grassi (*Studi*, etc. 1901, 2nd edition, p. 108, plate 4, figures 9 and 10) describes and figures them. In his schematic figure the dorsal reservoirs are very small, and the ventral reservoir extends but a little into the abdomen, viz. to near the posterior margin of the second abdominal segment. He refers to the first as lateral "succhiatoio accessori," to the latter as "succhiatoio principale o stomacho succhiatore o ingluvie." He notes that the sacs have very thin walls, they are lined with a delicate chitinous cuticle, followed by a layer of

¹ According to Packard (p. 305) this sac is always on the left side in Diptera. In our sections it appears median. It appears that such sacs are present in most Diptera and Lepidoptera, where they are falsely called a sucking stomach. In the Lepidoptera they generally contain only air. Newport found the sac filled with food in the flesh-fly and in *Eristalis*, the latter having fed on pollen. Graber also saw food enter the reservoir in flies, the food being coloured. In Hymenoptera the reservoir occurs as a pouch communicating with the oesophagus, and in the honey-bee it is to be distinguished from the "honey-sac," which is the crop or proventriculus. Nevertheless, in bees the reservoir has been seen to be filled with honey.

much flattened epithelium covered externally by muscular fibres. He found them to contain air, mixed with a little colourless fluid, or blood if the insect was examined immediately after sucking blood.

De Grandpré and de Charmoy (1900, p. 21) state that the sacs open into the under side of the oesophageal swelling, that two are situated on the right side (*sic*) and are always less dilated than the third. The oesophageal diverticula, as they style them, contain air-bubbles, at times blood when the stomach is replete therewith. The large diverticulum is on the left side (*sic*), it extends into the abdomen, contains air-bubbles, and it is not infrequently full of blood when the stomach is also full.

Giles (1902, 2nd edition, p. 101) speaking of the oesophagus, says: "It is not uncommon, in dissecting, to bring away attached to it a pair of delicate bags of air-bubbles, the true nature of which will be referred to in the description of the respiratory organs." On pages 103-105, he refers to the "aspiratory vesicles" or, preferably, "the pneumatic sacs," and goes on to say: "This structure is not as has been stated in any way peculiar to gnats, but is, I find, often even better developed in the midges, and other allied insects; moreover, it is not a median, but a paired structure, and I believe that its size, which has hitherto been absurdly underestimated, is inversely proportional to the size and power of the wings in the different species in which it is found. The reason that it has hitherto been mistaken for a single median sac is that, owing to the pressure of the contained air, the two sacs come to lie one behind the other. They have no true organic connection with the oesophagus and the only reason why they are often brought away attached to it, owing to the fact that the fibrous base of the sacs, which connects them together across the middle line, is divided into two bands, leaving between them an opening through which passes the oesophagus, a little behind the valve in which the latter commences. To the naked eye they look like clusters of minute air-bubbles, and when intact their walls rival in tenuity those of a soap-bubble."... "Instead of being as they have usually been figured, barely larger than the salivary glands, they occupy during life as much or more space than the digestive canal when at its utmost distension. Moreover the more gorged the insect, or the heavier it be with eggs, the larger will these sacs be found, as together they occupy a large space and fill out the entire ventral portion of the body-cavity from the front part of the thorax to the end of the fourth or fifth abdominal segment." Giles does not understand why some should claim the function of these organs to be suctorial, he thinks they act like air-spaces (!) in birds. Continuing (p. 105), he writes, "Into the base of the sacs may be traced large tracheae, and these split up and become continuous with a brush of dichotomously dividing fibres of which the base of each sac is composed." These fibres are chitinous. In a collapsed sac the bubbles of air remain, and under a high power are seen to be entangled in "dilatations of their continuity...in other words these fibres...are extremely elastic and distensible tracheae, which swell out into bubble-containing dilatations wherever their mutual pressure permits of their doing so. Apart from a few loose connective tissue elements, the sacs consist of nothing else but these curiously modified tracheae."

It will be seen from what follows that we do not agree with Grassi in considering these organs suctorial. Their structure points entirely to their being organs which can *expel* fluid, being comparable in this sense to the mammalian urinary bladder. De Grandpré and de Charmoy are in error as to the disposition of the organs in the body, when they place the dorsal reservoirs on one side, the ventral one on the other. Giles claims that there are two sacs, not one, whereas there are three. He denies that they are connected with the alimentary canal, which is very astonishing, for the reason that the simplest feeding experiment or dissection, if skilfully carried out, not to mention serial sections, show absolutely that the sacs are connected with the alimentary canal. It is still more surprising that he attributes to them a function analogous to the air-sacs in birds, and that he has claimed to trace direct connections between the "aspiratory vesicles" as he terms them, and the tracheal system, as it has been impossible for us to find any such connections. In fact the reservoirs compared for instance to the alimentary canal appear to be entirely without tracheae! We can trace no relation whatever between the amount of air in the sacs and the number of eggs it contains.

The name we have given to the three oesophageal diverticula, namely "food reservoirs," indicates what we consider to be their function. There is no evidence that they are suctorial organs, and indeed such a function would be superfluous when we consider the mechanism of the buccal apparatus and the powerful pumping pharynx and highly contractile oesophagus. If an unfed imago is observed under a low power by transmitted light the ventral reservoir can easily be made out, owing to its usually containing numerous small air-bubbles, which move backward and forward owing to irregular contractions of the sac whilst the insect is at rest. In some cases the contractions are very slight, and, as stated, they occur irregularly. Thus in a single insect, observed continuously, 18 slight contractions occurred in two minutes. When fed the coloured fluid food was seen to enter the sac. An hour later, the insect having been left undisturbed, the sac was seen to be contracting powerfully, continuously and more rapidly, 30 contractions being counted in the space of one minute, there being but three pauses. The peristaltic wave travelled backward. In unfed insects 48 hours after their having emerged from the pupal covering the sac was seen to contain much air. On dissection, the stomach was contracted and empty, the three reservoirs frequently being fully distended with air-bubbles.

To facilitate the study of these organs, which for brevity's sake we shall call sacs, imagoes were fed with blood serum and sugar, either alone or together with carmine, or neutral-red. Sometimes the feedings took place alternately on coloured and uncoloured food. Most of these experiments were made upon *Culex pipiens* because we could spare few *Anopheles* for the purpose, both genera however possess the same organs, and they behave similarly.

Experiment 1. Insect fed on sugar-carmine-serum. Killed at once after it had fed very fully, on dissection all three sacs contained food, as did also the intestine. There were 32 very small bubbles in the ventral sac.

Experiment 2. Repetition of the above. No bubbles in the sacs, they contained all the carmine, intestine empty.

Experiment 3. Repetition of the above, insect fed on serum-sugar. Ventral sac half filled with bubbles, 3 bubbles in one small sac, none in the other. Little food in the intestine, nearly all of it in the sacs. This insect had fed but moderately.

Experiment 4. Insect fed on sugar-carmine-serum. Fed again after 2 days with sugar-serum, and killed immediately. No bubbles in the much distended ventral sac, which contained carmine. Small sacs distended with bubbles. Much aggregated carmine in the stomach. No carmine in other portions of alimentary canal.

Experiment 5. Repetition of the preceding: 54 large bubbles, and much carmine in ventral sac. Bubbles distending small sacs. Little carmine in intestine, contained some clear serum, and numerous bubbles along its whole length.

Experiment 6. Insect fed with sugar-carmine-serum. Killed *after one hour*. Some carmine in small intestine, close to stomach. Most of the carmine in the ventral sac, very little in intestine. The carmine in this and other similarly-fed insects could be seen to become deposited at the bottom of the ventral sac, the living insects, resting on the sides of a tube, being viewed under a low power by transmitted light.

Experiments 7 and 8. Insects fed on sugar-serum. Killed *after 24 hours*. Moderate amount in the stomach, mostly in ventral sac, bubbles in small sacs.

Experiment 9. Several insects fed with sugar-carmine-serum, killed after 24 hours, showed carmine in intestine down to rectum, besides in ventral sac; at times a few grains of carmine in small sacs.

Experiment 10. Several insects, treated as in 9, were killed *after 48 hours*, there being more carmine in the intestine and rectum.

Experiment 11. Four insects fed on sugar-serum, killed *after 48 hours*. All contained serum in ventral sac, the small sacs also distended, and containing but a bubble or two, or no bubbles.

Experiment 12. Insects fed on sugar-carmine-serum. Killed *after 48 hours*. Large sac contained 24 bubbles, small sacs filled with bubbles. Carmine in large sac chiefly, also considerable amount in intestine and rectum.

Experiment 13. Three insects fed with sugar tinged with neutral-red. Killed *after 24 hours*, contents of ventral sac red, many bubbles almost filling it. Stomach contracted.

Experiment 14. Six insects fed as in the preceding case were fed again after 24 hours on clear sugar-serum. The result was very striking. The contents of the ventral sac were coloured red, that of the stomach yellow, so that there could be no doubt but that the second meal had been almost entirely taken up by the stomach. This could be easily seen in living insects with the naked eye as they rested on the walls of a glass vessel in which they were contained. On killing the insects within a few minutes after feeding, and dissecting them, the stomach was found to contain clear serum, the ventral sac coloured serum and bubbles.

The foregoing evidence suffices to utterly disprove Giles's assertion with regard to the function of these organs. The continued contractions or peristaltic movements of the ventral sac observed in living resting insects indicate that the fluid they contain must for some reason be kept moving. The bubbles are not as yet explained. They must have their origin from the outside, that is they must come in through the mouth-parts, either in the process of feeding or afterwards. During feeding air might very well enter if the pumping action of the pharynx, etc. were continued even for a moment after the removal of the proboscis from the fluid which is being ingested. It appears reasonable that an insect will seek to clear its mouth-parts and buccal cavity by sucking up the remainder of the food they contain. It is not impossible moreover that the saliva may be expelled from the tip of the hypopharynx after a meal has been completed, the secretion being drawn back again through the labrum-epipharynx. The small size usually shown by the bubbles argues in favour of their having been carried in through the small oral aperture. We propose to pursue this question, which possesses considerable interest. In the figure shown in Plate VI, Fig. 7 the bubbles are larger than usual. This may depend upon the nature of the fluid food imbibed, a viscid fluid would

prevent the bubbles combining into large ones in a way which a less viscid fluid would not do. This also requires study.

When enclosed in the body of the insect the ventral sac is elongated, as shown in Plate VI, Fig. 1, when removed from the body it shortens and widens, for obvious reasons. When dissected out it continues its peristaltic movement for half-an-hour or more if kept in saline solution. The contractions are due to bands of muscles running transversely but not completely round the sac, and situated at definite intervals along the length of the sac (see Fig. 4). The contractions are very powerful at the end of the sac, which may contract down so as to have a very small lumen. The bands situated alongside each other usually contract alternately, the fluid contained in the sac being churned backward and forward. The system of transverse bands continues along the whole length of the sac until it joins the oesophagus. The bands terminate by spreading outward into isolated fibrils over the sac. Delicate diagonally-placed intercommunicating and interlacing fibrils secure a certain amount of shortening in the length of the sac. The muscle bands are readily shown, by simply allowing a dissected sac to dry outwardly on the coverglass and then staining with an aniline or other stain, the appearance presented being as in the figure. The small dorsal sacs are also traversed by small bands of muscles, the distribution not being so symmetrical, and the bands but few in number.

If a ventral sac is removed to a slide, and the salt-solution or water in which it suspended is allowed to dry, the sac does not collapse. The fluid it contains does not evaporate for a considerable time. Such sacs have been kept for several weeks in the laboratory, exposed to room-temperature and unprotected, and still were found to contain fluid after as long as two months, by which time they had shrunk somewhat. This is evidently due in part to their being lined with an apparently impervious, but exceedingly delicate layer of chitin throughout. Nevertheless it is not the chitin which prevents evaporation of the contents, for if such a sac is placed in caustic potash so as to remove the soft parts, it will soon dry up when exposed to the air, being completely dried in a few hours. The chitinous lining is demonstrated most clearly by the use of caustic potash, a sac treated therewith retaining its shape after being macerated for days in that fluid, provided evaporation of the contents is prevented. No structure could be made out in this delicate chitinous sac. Similarly a chitinous lining of great delicacy can be demonstrated in the two small dorsal sacs, and parts of the alimentary canal anterior to the oesophageal valve or sphincter.

What we have stated with regard to these sacs appears to justify the conclusion that they are food reservoirs. When feeding, the greater part, or all the material ingested, may find its way into the sacs, and is thence gradually supplied to the digestive canal proper. This is effected by the contractions of the muscle-bands already described, which force the fluid back into the highly muscular oesophagus which doubtless contracts at the pharyngeal end so as to impede the flow towards the pharynx, and contracting backward towards the orifice to the intestine, forces the food into it through the relaxed sphincter.

That blood is also taken up into the sacs has already been stated by Grassi, and de Grandpré and de Charmoy, cited above. This may be of some practical importance. We have seen that insects which were given two good meals collected the first in the sacs, the second directly in the stomach. Of course a certain amount of mixture of the food ingested may take place, and all insects will not behave alike in this respect. Assuming that the first meal is of blood containing malarial parasites, then the parasites might be retained chiefly within the ventral sac, and they would have difficulty in getting out of it. The chitinous lining would prevent the exit of the vermicules, and many parasites would die within the sac; a few might of course be pumped out of the sac later and thus reach the stomach, but by that time many might have died. Of course this is only an hypothesis, but it may possibly explain some of the negative results obtained by various investigators who have failed to successfully infect mosquitoes with parasites. Apart from this, the nature of the food previously ingested by the insect may very well exert a deleterious effect upon malarial parasites entering the food reservoirs subsequently; a matter which appears worthy of consideration by those engaged in infection experiments upon mosquitoes. A suitable opportunity offering, we propose to further investigate the function of these sacs, which does not appear to have been hitherto appreciated. Many insects appear to draw blood directly into the stomach, corpuscles being expelled with a small drop of fluid from the anus toward the end of feeding¹. Schüffner (1902, p. 93) describes an especially blood-thirsty species of *Anopheles* from Sumatra which ejects 4—5 times as much blood from its anus, whilst feeding, as it requires for a meal, so that if several of these insects are feeding on a hand it is spotted all

¹ It is of interest to note that Schoo (1902, Feb.?) considers that it is chiefly serum which is ejected. He weighed *A. maculipennis* before (weight 1.9–4.2 mg., average 3 mg.) and after feeding (weight 3.6 to 6.4 mg.), concluding that the amount of blood ingested weighed 1.4 to 2.9 mg.

over with blood. Schüffner adds that the ejected blood does not coagulate.

Grassi (p. 111) casually states that the air is expelled from the reservoirs just before the insect bores its proboscis into the skin, as a preliminary to feeding. He gives no evidence as to how this may be accomplished, and it is difficult to judge of whether or no he has made any observations to back the statement. We have seen in our feeding experiments that bubbles were at times absent immediately after feeding, but this is not always the case. The mechanism requires further study. We have not been able to decide how the air is expelled, that is, whether it passes out *per os*, or whether it passes out through the alimentary canal backward. When the insects rest, as they usually do, with their heads uppermost upon a vertical or inclined surface, or even when hanging by their legs from a surface like a ceiling, all the bubbles in the ventral sac, at any rate, collect towards its exit, and would be readily expelled at will. The fact that air-bubbles may at times be observed in the mid-gut indicates that some of the air at any rate may be expelled posteriorly¹. In some insects, evidently in consequence of fermentative processes taking place within the sacs and intestine, these cavities may be greatly inflated. This condition appeared at times to lead to the death of the insects.

The Oesophageal Valve.

This structure, which appears to be homologous with the proventriculus of many insects, as pointed out by Christophers (1901, p. 14), serves as a valve between the oesophagus and mid-gut². Viewed externally the structure is seen to produce a marked annular thickening of the intestinal wall. It does not appear to be lined with chitin as are the preceding structures. The thickening is partly due to powerful annular muscles which act as a sphincter, occluding the lumen by their contraction, and rendering it patulous when they are relaxed for the passage of food from the oesophagus. The thickening is also due to the intussusception of the gut at this point, the invagination protruding into the tubular mid-gut, somewhat after the manner of the cervix uteri into the vagina in man. Attached to this portion of the alimentary

¹ According to Packard (p. 324) Dragon-flies, Orthoptera and Lepidoptera swallow some air with their food.

² Weissmann (1864, cited by Packard, p. 311) already regarded the proventriculus of flies as an intussusception of the oesophagus.

canal are six small protuberances, which are more or less marked in different insects, these are reduced caecal appendages which are well-marked in the adult larva, where they are also distributed in a circle about the gut. They are indistinct in the imago, whereas in the larva they form elongated cavities communicating directly on one side and by a pore with the lumen of the gut. Grassi says there are many, and figures the caeca to the number of nine, whilst Christophers (p. 13) says they are absent. The lumen of the valve opens directly into that of the mid-gut. The sphincter, just referred to, may continue to undergo rhythmical contractions for two or more hours after the gut has been removed to salt solution, the parts not being subjected to pressure from a coverglass.

The Mid-gut (or "Chylific Ventricle"), including the "Stomach."

(Plate VI, Figs. 1 and 7, Plate VIII.)

The mid-gut runs as a simple straight tube from the oesophageal valve at about the level with the first pair of legs to about the level of the posterior limit of the sixth abdominal segment, as also found by Grassi. The relative position with regard to the parts of the exoskeleton just mentioned will vary somewhat according to the degree of distension of the gut with food. The mid-gut can be compared to a long-necked flask, the anterior portion of which is narrow and tubular, the posterior portion dilated. It is the posterior portion of the mid-gut which is usually styled the "stomach" by medical writers, and the term is convenient because of brevity, for it is in this portion of the mid-gut, chiefly in its posterior two-thirds, that malarial parasites develop in their insect host. We shall therefore refer to this portion of the mid-gut in future as the stomach. The anterior tubular portion of the mid-gut ("colo del stomaco" of Grassi) has a slight dorsal flexure, to give room to the ventral reservoir described above. The dilatation or stomach begins on about a level with the second abdominal segment. When there is food in the mid-gut it accumulates in the stomach. Grassi observed that after a meal of blood the corpuscles accumulated in the posterior $\frac{3}{4}$ of the stomach, the serum being contained in the anterior portion. Giles (1902, p. 102) states that in the recently emerged imago the stomach may contain remains of food ingested by the larva.

The mid-gut has a similar structure throughout. It is not lined by chitin, as can be seen when the gut is macerated in caustic potash. There is no trace of the chitinous tube, known as the peritrophic

membrane, which is found in the larva. The change of food from solids to fluids no doubt accounts for this.

According to Grassi the internal coat consists of a delicate *cuticula*, which he figures and describes (Plate VIII, Fig. 2). The cuticula is figured but not described by Christophers. Packard states that it is always present in insects. The second coat, which forms the greater part of the thickness of the wall, is made up of a single layer of large cylindrical or cubical *epithelial cells*, with large oval nuclei. The form of the epithelium may be altered by pressure exerted by the food within or parasites without, as has been shown by Christophers and Grassi. When the stomach is distended the cells are flattened. Pressure from parasites causes a distortion of the cells corresponding to the form of the parasites which press upon them. Christophers writes (p. 13) of the epithelial cells:—"They have a finely-reticulated protoplasm, which stains more deeply toward the free border. Stained with Heidenhein's haematoxylin alcohol-hardened specimens are seen to contain numerous stained granules collected especially in the outer portion of the cell. They are especially abundant in the anterior portion of the mid-gut. They have also very frequently a number of small clear vacuoles (droplets) which become more frequent and of larger size towards the free border of the cell. The most marked feature of the cell is the clear striated border which is present in all the cells of the mid-gut, but absent in all other portions of the alimentary canal. The striated border is best marked in the undistended organ, and becomes almost invisible in the fully distended state when the cells are much flattened. The nucleus of these cells is large and centrally situated. The chromatin is arranged in small stellate masses arranged circumferentially and centrally and connected with one another by fine threads of chromatin. There is a body which stains less deeply generally to be made out (karyosome) in the centre of the nucleus. Occasionally young cells are seen near the basement membrane." Our observations accord with these.

External to the epithelium lies an elastic basement membrane, which Grassi styles the *elastic-muscular tunic*, for the reason that the muscular fibres appear to lie embedded within its substance. That the basement membrane is elastic is indicated by the fact that it stretches when the intestine is distended and the epithelium upon it is flattened. It appears to be structureless. The membrane can be removed, as Grassi has shown, from the epithelial layer, thus facilitating the examination of the coat for malarial parasites, the view being impeded by the epithelial cells with their large, darkly-staining nuclei. The

bands of muscles cannot be separated from the membrane, for the reason that they are apparently ensheathed in its substance. Grassi (1901, pp. 175—176) is of the opinion now that the amorphous substance forming this outer tunic constitutes what has hitherto been considered the capsule of the oocysts of the malarial parasites, and this for the reason that the "capsule" stains like the basement-membrane and is continuous therewith. This appears reasonable because of the probability that the parasites nourish themselves upon the products of digestion which pass through this membrane from the mid-gut to the coelom.

Muscular fibres run around and longitudinally upon or within the amorphous layer, forming a loose network (see Plate VIII, Fig. 1) such as was already figured in Ross's earlier papers. Where the intestine is contracted (see Plate VI, Figs. 1 and 7) the circular fibres throw the surface into a large number of transverse folds, each muscle-band forming an annulation. When dilated with food the play of the separate muscle-bands may be observed in the excised stomach in salt solution. When the bands contract the stomach is indented by the separate bands. Ovoid when dilated, the stomach may contract about the centre, assuming the form of an hour-glass, etc. The muscular bands are very long and of remarkably uniform width. Christophers (p. 13) states that all the muscular bands of the alimentary canal are striated¹. The outer surface of the mid-gut shows numerous large branched cells in which the small tracheae end, and from which bundles of exceedingly minute structureless air-capillaries pass into the wall of the mid-gut. These cells are often well shown according to Christophers, in specimens stained with gold chloride. Such cells occur throughout the viscera in connection with the tracheal endings and have been described in other insects (see figures in Packard, p. 436). If we except the anal glands, the mid-gut is more plentifully supplied with tracheae than any other portion of the alimentary canal, large dividing spiracles occurring plentifully, especially over the stomach, the smallest spiracles measuring anywhere from 2 to 6 μ across. The fat-body which is more or less marked according to the stage of nutrition of the insect, is not organically connected with the

¹ This requires further study. Packard (p. 316) states of some insects that the inner (circular) layer of muscles is unstriated, the outer (longitudinal) striated. On p. 324 occurs the remarkable statement: "Suctorial insects draw in their liquid food by the contractions followed by the dilatations of the mid-intestine," a conception which is obviously false.

mid-gut about which it may in part lie. The gut seems to lie freely in the body-cavity, being obviously in a measure kept in place by the tracheae which arise from its surface and pass outward to the larger air passages and stigmata. It is not impossible that delicate muscles, such as are described by Lyonet (Packard, p. 297) as "retractores ventriculi," may also afford additional loose fixation, but we have been unable hitherto to detect them.

The Hind-Gut.

The hind-gut begins at the junction of the Malpighian tubes with the end of the mid-gut. The lumen, which is wide in the stomachic dilatation of the mid-gut, here suddenly narrows. The hind-gut is divided into the ileum, colon, and rectum, and ends with the anus. The ileum curves dorsally, the succeeding bend in the intestine representing the colon. The rectum is dilated into a sac, but narrows where it approaches the anus.

The *ileum* is very short. It may be somewhat dilated near the mid-gut. It is lined with flattened epithelium. It is very transparent, the contents being readily seen through its walls. As Grassi (p. 109) states, it is lined with a chitinous cuticula, which appears thickened in undulating lines or ridges. When dilated he notes that the ridges, which are very close to each other, are due to cuticular thickenings on a level with the lines which mark the anterior and posterior margins of the epithelial cells. The ridges to a certain extent invade the contiguous cells. We have seen this portion of the intestine undergo active contractions for hours after removal from the body, its activity in this respect being comparable to what has been noted in the oesophageal valve.

The *colon* succeeds the ileum, without there being any line of demarcation. The colon is lined by a single layer of epithelial cells of cubical form. Christophers (p. 14) notes that the nuclei of these epithelial cells are similar to those of the mid-gut, although they possess a more open arrangement of the chromatin. "The protoplasm is finely reticular, and stains less deeply than the cells of the mid-gut. Stained with Heidenhein's haematoxylin no granules are present as in the cells of the mid-gut. They have no striated border." The muscular coat is well developed in this region, showing a well-marked fenestration, or crossing of fibres.

The *rectum* (Plate VI, Fig. 2) forms a spacious oval chamber into

which the colon suddenly opens. Its lumen is diminished by the protrusion into it of six large ovoid papillae¹. The cavity is lined with flattened epithelium. Each of the papillae consists of a number of large cells, modified from the ordinary lining cells of the rectum. A bundle of minute tracheae passes up through the centre of the papilla, between the cells (see Plate I, Figs. 2 and 3), the tracheae being distributed from the apex backward, and apparently uniting again into a bundle, returning to the same large trachea whence they had their origin. The papillae are covered with chitin.

The rich supply of tracheae to the rectal papillae indicates that these organs must fulfil some active and important function. What this function is is not clear. Such papillae are widely distributed amongst insects. Miall and Hammond write (1900, p. 107), "Chironomus has two, most other Diptera four, Pulex, most Hymenoptera, Neuroptera, and Orthoptera six, Lepidoptera 60—200, Coleoptera and Hemiptera none. They are absent in larvae with few exceptions." The abundant supply of tracheae and the analogy of the anal respiration of such insects as the larvae of dragon-flies have been put forward as supporting the view that the rectal papillae are respiratory organs. Others believe they function as glands, but as Minot points out their structure lends little support to this view. Fernald (cited by Packard) regards them as valves, but although the papillae must offer some obstruction by their presence to the passage of the intestinal contents, they have nothing of the usual structure of valves, at any rate in *Anopheles*.

The rectum narrows just before the anus, the narrowed tube being well beset with muscles, both longitudinal and circular. The anus is just ventral to the orifice of the reproductive organs, and is guarded by two short lateral papillae. It is "situated in the last segment of the body, under the last tergite or suranal plate," a position which Packard (p. 297) says is invariable in insects.

The Malpighian Tubes.

The five Malpighian tubes, which are already fully developed in the larva, open into the hind-gut at the same level, at its junction with the termination of the mid-gut. The fact of their containing chitin points

¹ Giles (2nd ed. 1902, p. 103) is evidently in error when he states that there are four anal papillae. He states that they are connected by short ducts to the intestine, and that they probably secrete some "fluid accessory to digestion."

to their being derived from the embryonal proctodaeum. They lie bathed in the fluids of the haemocoel and are slightly coiled, making one or two loops, so that in cross section the same tube is not infrequently cut twice. As a rule the number of Malpighian tubes is even in insects; Packard (p. 350) citing *Culex* and *Psychodes* as "remarkable exceptions" in possessing five. *Anopheles* we see, also possesses five, and if Eysell (Oct. 1902, p. 341) is right *Aedes* possesses a similar number, for he distinctly states of *Aedes cinereus* Hoffmg., that the intestinal canal and its appendices are exactly as in *Anopheles* and *Culex*. The usual number of tubes in Diptera is four.

Commencing at the entrance of the tubes in the intestine, they are seen to be lined for a short distance by cells continuous and similar to those of the gut. Secretory cells however soon appear, which presumably excrete the waste nitrogenous matter from the body. Each of these cells is very large, the nucleus being conspicuous. The tubules consist of a double row of cells, arranged alternately and enveloping the excretory duct. The alternate arrangement of the cells gives the tube a wavy appearance. The lumen of the tube is usually somewhat flattened, being lined by chitin, which is supposed to be perforated by porosities. In some sections the lumen is dilated, apparently owing to excreted matter. The cells rest upon an apparently structureless basement-membrane.

No muscular fibres appear to be present in these organs. They usually have a pale yellow colour when viewed by transmitted or reflected light, the colour being due to the matter excreted. Packard (p. 352) states that the colour varies according to the nature of the food, turning red for instance in certain insects when fuchsin is mixed with their food. Uric acid and other renal products have been found in these organs, as also concretions, etc. The Malpighian tubes are richly supplied with tracheae.

The Salivary Organs.

The salivary apparatus in adult mosquitoes is not connected with the alimentary canal¹. The saliva issues from tip of the hypopharynx (*Journal of Hygiene*, Vol. I, p. 464, and Plate IX) when the insect feeds. Examined under a low power the hypopharynx appears to possess a duct, situated centrally, and running from near its tip to its

¹ Giles (2nd ed. 1902, p. 100), speaks of the salivary duct as "a chitinous tube prolonged from the lining of the buccal cavity," this being incorrect.

base, a duct being figured in the plate referred to above. When cross sections of the hypopharynx are examined under a high power (magnification 500 or over), the supposed duct is seen to be a groove, or "salivary gutter" as it has been styled by Annett and Dutton. We have figured a cross section of the hypopharynx highly magnified on Plate VII, (Fig. 6), the section having been made about midway along its length. It will be seen that the groove in the chitin serves the purposes of a duct, for, although open dorsally, delicate chitinous lamellae arch over the opening, and overlap, practically closing it. The groove is of remarkably uniform width throughout the length of the hypopharynx; the lumen, in several specimens, measured 5.8 to 6.6 μ .

At the base of the hypopharynx we find a very interesting structure, connecting the common salivary duct and the groove (Plate VII, Fig. 6a). It has been described as the "salivary receptacle" by various authors, including Macloskie (1888), and Annett and Dutton (1902); the latter appear to have been the first to rightly understand the mechanism. The structure is more than a receptacle, it constitutes a *pump*, the mechanism of which corresponds to that of the pharyngeal pump in a sense, that is, it depends upon the action of powerful voluntary muscles which overcome the elasticity of a chitinous membrane which, when released by the muscles becoming relaxed, rebounds or returns to its original form, as a bow does when the pull on the bow-string is released. The chitinous mechanism is best studied in dissected parts which have been treated by caustic potash. It is very difficult to cut the valve in sections for the reason of its being highly chitinized, the structure being frequently torn out of its situation by the knife. It will be seen then that the common salivary duct ends (lumen 5 μ) in the centre of a chitinous membrane, the junction being strengthened by a chitinous thickening of annular form. The membrane is continuous with a highly chitinized cup, which tapers anteriorly, and is continuous with the hypopharynx, an opening therein connecting it with the groove described above. We shall not here consider the other chitinous structures surrounding the salivary pump. As is shown in Fig. 6a spicules of chitin occur about the duct on the pump-membrane, these serving for the attachment of the powerful muscles presently to be described. The thickened chitin surrounding the membrane is flattened on its dorsal surface which is applied to the floor of the buccal cavity. The pump-membrane is covered in the centre by the insertion of two stout bundles of muscle-fibres which pass backwards, parallel

with one another, to their origin on the anterior surface of the chitinous flange which projects ventrally from the floor of the buccal cavity. (Plate VII, Fig. 5.) When the muscles contract a partial vacuum is produced within the cup, saliva flows in from the glands, and when they relax the membrane rebounds forward, driving the saliva out of the cup into the salivary channel along the hypopharynx. In an earlier part of this paper we referred to these muscles as retractors of the hypopharynx (Vol. I, p. 464), a statement which must now be withdrawn. The relation of the base of the hypopharynx to the bases of the other mouth-parts admits of no free movement of this organ. The hypopharynx is probably withdrawn with the other piercing stylets, this withdrawal being mainly effected by the bracing action of the insect's legs lifting the head and body away from the object bitten as we already stated elsewhere (Vol. I, p. 467). We believe this to be the case from observations on living insects which have been allowed to suck our blood, being closely watched during the process. Proceeding backward from the salivary pump the common duct (lumen $4\ \mu$) passes beneath the buccal cavity to a point beneath the valve which separates the buccal cavity from the pharyngeal pump. Here it divides into two ducts of similar structure (lumen $3\ \mu$) which run closely side by side, beneath the infra-oesophageal ganglion and ventral nerve-cord along the ventral wall of the neck into the thoracic cavity, where they diverge and branch into the salivary glands.

The glands appear in cross sections close to the neck, and reach back to a little beyond the oesophageal valve, being situated laterally with regard to the oesophagus. Each branch from a secondary duct divides into three smaller ducts (lumen about $2.5\ \mu$) immediately before entering a corresponding number of glands, see Plate VI, Fig. 6. Taking the one set of glands, we see, in cross sections, that they are arranged in a triangle at first, one gland being dorsal, the other two ventral and close to each other (see Plate VI, Fig. 8, where the dorsal or *central* [see below] gland is on the left). The glands then gradually shift their positions, so that the dorsal gland comes to lie between the two previously ventral glands, these being placed, the one ventrally, the other dorsally. This is due to the glands having to accommodate themselves to the position of the powerful thoracic muscles which move the wings. The glands are surrounded to a varying degree by the fat-body.

When the salivary glands are removed from the mosquito (methods to be described later), they present an appearance such as is figured in Plate VI, Figs. 5, 6, and 7. They are very large in proportion to the

size of the insect. If the dissection is skilfully performed the two sets of glands with their (secondary) individual ducts and common duct are brought away together. At times a certain asymmetry is observed in the glands, as figured (Fig. 7). In Fig. 5 it will be seen that the central gland is smaller than the lateral, and this is also shown in Fig. 7, where this gland (as can be seen by looking closely at the junction of the three ducts quite near the glands) is displaced to the left. This smaller, central gland ("tubulo intermedio" of Grassi), as we shall for convenience call it, is the one which appears to occupy a dorsal position in sections near the neck, an intermediate position further back, with regard to the two other glands. The central gland differs also from the others histologically, as we shall presently see.

The size of the glands as a whole varies with the dimensions of the individual insect. The glands are considerably larger in the female than in the male, the salivary pump being moreover small in the male. Measurements made of the fresh salivary glands of a female insect of average size showed a width of $85\ \mu$, the lateral glands being $880\ \mu$, the central gland $510\ \mu$ long. In a male, the largest gland measured 51 by $212\ \mu$. The measurements were made longitudinally through the axis of the gland. With the exception of Grassi (p. 110), who states that the lateral salivary glands of the female *A. maculipennis* measure 1 mm. in length, we are not aware that the size of these glands has as yet been determined. It will be seen that Grassi's measurement agrees fairly well with ours of the lateral gland, allowing for variations in the size of the insect and contraction of the gland, which may be observed to take place after its removal to salt solution, especially when the anterior portion is dilated as shown in Fig. 5 (Plate VI), the drawing having been made immediately after removal.

The common duct and secondary duct are chitinous and surrounded by a well-marked sheath¹, within which they pursue a slightly serpentine course which indicates that the substance of the sheath is not firm. Outwardly the sheath possesses a basement membrane, and within, cells with clearly staining nuclei. In unstained specimens the substance around the duct itself appears slightly granular and homogeneous. These ducts throughout their length, and for a little distance along the three branches of the secondary ducts into each set of glands, show a structure somewhat similar to what is seen in the tracheae. Some

¹ See Plate VI. This is not shown in the diagrammatic cross-sections of the head in Plate VII, Figs. 1-3, nor in Fig. 4.

authors have described the chitinous ducts as showing spiral thickenings, as in the tracheae, but this is not the case. On examination with a high power the thickenings are seen to be incomplete hoop-like bands. These thickenings project slightly outward like the tracheal cartilages of mammals. Like the tracheae the ducts possess a considerable degree of elasticity, as can be seen in the process of dissection.

The presence of the chitinous ducts leading to and into the glands is explained by the fact that the salivary glands are developed like the fore-gut from the embryonal proctodaeum.

The salivary glands of a mosquito (*Culex taeniorhynchus*) were first described by Macloskie (1887) who figured them, dissected out, and showed the arrangement of the ducts in longitudinal section through the head. He noted a difference in the appearance of the central gland, to which he attributed the toxic action of the salivary secretion of the mosquito, there being no obvious ground for such an assumption. His figure shows that a chitinous duct runs through the axis of the gland to near its base.

Considerable attention was drawn to these glands by the investigations of Ross, who first demonstrated the presence of the sporozoites or "blasts" of the avian malarial parasite (*Proteosoma*) within the cells of these glands. Subsequently Grassi, Bignami and Bastianelli found the sporozoites of human malarial parasites in a similar situation in *Anopheles* infected with malarial blood. The parasites of aestivo-autumnal malaria, as also of tertian and quartan ague, have been found as sporozoites within the cells of the salivary glands, by the Italian observers, as also by Ross and others since. See Fig. 3, Plate IX.

In their essential structure the three glands are similar, consisting of acini. Each acinus is surrounded by a basement membrane, which can be clearly demonstrated in fresh glands by placing them in non-isotonic fluid so that the epithelium which rests upon it contracts away from the enveloping membrane. It may also be seen at times in hardened sections. The membrane is extremely delicate and apparently structureless, although de Grandpré and de Charmoy (p. 29) claim to have found muscle-fibres, both longitudinal and circular, therein. A single layer of epithelial cells rests upon the basement membrane and surrounds a central lumen, which can be made out in fresh glands, through the transparent structures. The lumen is occupied by a chitinous intra-glandular, or intra-acinar duct, communicating anteriorly with the secondary duct, which is joined at once by the three

intra-glandular ducts at one point. As was first pointed out by de Grandpré and de Charmoy (1900, p. 21) there is a difference with regard to the intra-glandular duct in *Anopheles* and *Culex*. These authors, as also Christophers since (1901), have figured the difference which they describe. As Macloskie showed, the duct in the central gland of *Culex* possesses a uniform width down to the base of the acinus. In *Anopheles* it broadens soon after entering the acinus. In other words the gland is sacculated in *Anopheles* and not in *Culex*. Whereas Eysell (p. 341) states that there are also three glands in *Aedes cinereus* Hoffmg., he says nothing regarding the structure of the ducts in this insect. In fresh glands mounted in glycerine, which increases the transparency of the structures about the duct (Plate VI, Fig. 6), the intra-glandular ducts are seen to at times give off short lateral branches, which may be longer if the gland possesses a small lateral acinus, such as we have figured. These branches appear to terminate blindly, as do the main intra-glandular ducts in *Culex*. On the other hand the chitinous lining of the duct would appear to end in *Anopheles* before it has reached down half-way into the lateral glands, and even sooner in the central gland, as is shown in our figure. Either the chitin actually ends at the points figured, or it abruptly grows so thin that it can no longer be discerned in the posterior portions of the acini. We are not aware that this has as yet been noticed. Grassi (p. 111) states that he was once able to distinguish minute pores in the delicate cuticula which forms the intra-glandular duct, on examination of fresh specimens. Giles (p. 101) examining these ducts in an undetermined species of *Culex*, writes "it can be distinctly made out that, minute though it be, this chitinous wall of the tube is pierced by spirally arranged minute perforations, each corresponding to the point of origin of a cell." His figures of the gland and ducts do not agree in several respects with what we have observed in glands of *Culex pipiens* and *C. annulatus*, and we have been unable to determine any "spiral arrangement" of the pores in question. We have detected the minute pores both in *Anopheles* and *Culex*, especially in ducts treated with caustic potash, the soft parts of the glands which had previously been dissected out being thus removed. To see these minute pores requires careful illumination and a magnification of about 1000. Viewed under such conditions the intra-glandular duct is seen to be perforated by minute circular pores, and to be roughened on the outside by extremely delicate chitinous spicules, which possibly serve for the attachment of epithelial cells, it being apparently true as Giles

states that individual cells may remain adherent to the duct in glands which have been injured.

The individual epithelial cells can be made out in unstained glands, to which they give a beaded appearance. The lateral glands may broaden toward the front, this being apparently due to the glands being filled with secretion, the gland serving as a reservoir, in the absence of a true receptacle. It is a misnomer to refer to the pump already described as a salivary receptacle. The central gland narrows anteriorly to a neck (Fig. 5, Plate VI).

The *lateral glands* possess a similar structure histologically. The acinus is more or less filled with secretion (Plate VI, Fig. 8), and the cells surrounding the lumen broaden toward the base, near which the nucleus lies. These glands, when examined stained or unstained, are seen to differ from the central gland. Their secretion certainly does not stain with eosin as does that of the central gland, as has been shown by Grassi, a fact we can confirm. The bulk of the acinus and consequently of the epithelial cells is composed of the mass of secretion, the cell protoplasm and nucleus being forced backward by it against the basement membrane. When a fresh gland is crushed the secretion escapes in the form of clear refractile drops. In hardened sections the clear secretion, as has been pointed out by Christophers (p. 15), appears as a granular mass filling the greater portion of the cell. It stains poorly with haematein, and exhibits a coarse reticulum and isolated globules, probably, as he says, due to precipitation or coagulation of the secretion by alcohol. Grassi (p. 111) found the portion of the glands near the exit of the duct to contain a secretion which was more refractive than that contained towards the blind end of the acinus. Stained with haemalum the portion toward the exit of the duct became diffusely coloured, this not being the case in the posterior portion. In glands preserved directly in absolute alcohol as also in fresh preparations when examined late, the secretion appeared granular or showed delicate filaments, which must be considered artefacts. As both Grassi and Christophers point out considerable variations exist in the appearance of the granular secretion in different mosquitoes and in one and the same gland. We also find as stated by Christophers that the greater portion of the gland is filled with granular substance, and for this reason this author has referred to these glands as belonging to the "granular type" in contradistinction to the following, which he styles "clear or colloid type." In cross-sections through the thorax, stained in various ways, these differences between the central and lateral glands are

brought out strikingly, it being possible to compare the glands which lie side by side. In Plate IX, Fig. 3, a small part of a lateral gland is shown above the central gland which contains parasites.

The *central gland*, as we have seen, is shorter than the lateral ones, and narrows anteriorly where its duct emerges. Christophers notes of the fresh gland that the cell contours are not so distinct nor the secretion so refractive (Grassi says it is more refractive) as in the lateral glands. The secretion almost entirely fills the cells, which however contain more protoplasm than do those of the lateral glands. The hyaline secretion stains intensely with eosine, Heidenhein's haematoxylin, and also with haematein. The cells at the neck, as Grassi pointed out, do not stain with eosin, this being striking as the secretion in this portion stains therewith. Christophers notes that the nuclei of the secretive cells are less inclined to present degenerative appearances than in the lateral glands. He found that the secretion in the dilated central duct at times presents an appearance of faintly stained sporozoites, this being apparently something like the artefacts Grassi noted, as stated above. Christophers (p. 16) states that in freshly hatched insects the cells in both types of acini contain a large centrally situated nucleus, the protoplasm containing a large number of coarse granules staining with haematein. "This is the commencement of the large mass of secretion which, in the mature gland, occupies the entire cell."

Both Grassi and Christophers have studied the glands of insects which have fed and have remained unfed, but noted no especial difference in the appearances. It would therefore seem as if but a very small amount of secretion were given off. Viewed in fresh specimens Grassi thought to note that the central gland seemed somewhat more contracted (narrower) in insects which had fed than in unfed insects.

We have not as yet had an opportunity of studying an appearance of the glandular epithelium, casually referred to by de Grandpré and de Charmoy (p. 26), namely, "les prolongements ciliformes de l'épithélium que Bordas décrivait dès 1894 chez plusieurs familles d'Hyménoptères et en 1896 chez les Orthoptères et que Lecaillon vient de retrouver en 1899 chez le *Culex pipiens*." These "prolongements ciliformes," it appears, are not vibratile and are seen in thin sections stained with magenta-red and afterwards with indigo-carmin. Each process penetrates into the cell and connects with a deeply-staining body. No reference is given to the Journal where Lecaillon's paper appeared.

The Effects of the Salivary Secretion.

The effects of mosquito-bites are well known to most people. The method of feeding has been described elsewhere (this *Journal*, Vol. I, p. 467). Immediately after the insect has withdrawn its stylets, a small area about the puncture whitens, and after a minute or two becomes pink, swelling commencing. Soon afterwards the spot begins to burn and itch, the itching being relieved by pressure, only temporarily relieved and subsequently increased by scratching. In tropical countries especially the scratching of the spot may lead to the formation of wounds which heal badly, possibly owing to infection through the lesion. It is notorious that new-comers are more affected than aborigines in mosquito-infested countries. There is evidence that a certain degree of immunity may be acquired to the poisonous action of the mosquito saliva. Some persons show a high degree of susceptibility, others apparently a considerable degree of natural resistance to the effects of the toxin. Several cases of death due to being very severely bitten are recorded. Where the bites are numerous the swellings due to each bite become confluent, and there may be considerable oedema. For instance in a hunting expedition in Canada one of the writers was so severely bitten some years ago that one eye was closed, as also one ear through oedematous swelling, the face on one side being so swollen as to render him utterly unrecognizable. Experiments conducted here in England by one of us with *Anopheles maculipennis* have demonstrated what had been proved elsewhere for this insect, that its saliva is distinctly toxic. An hour or so after being bitten, provided the parts are not rubbed, the itching may cease, although the elevation around the puncture does not subside. At night-time, and in the morning, doubtless owing to the heat of the bed, the spots begin again to itch and grow red. Bites subjected to the friction and heat of clothing also tend to remain irritated. In some cases this irritability of the spots was renewed every night for a week or ten days, and all trace of the bites had only disappeared after 11 to 14 days. Of course the intensity of the effects and their duration vary with different individuals. It should be noted that in these experiments the insects were allowed to feed and afterwards withdraw their stylets without being disturbed, this being done for the reason that it is not infrequently asserted, as we see without reason, that if the insect is allowed to feed without being disturbed there may be no after-effects

whatever, the mosquito supposedly having time to withdraw the saliva it pumps into the wound.

Leeuwenhoek thought that the inflammation following the bite of *Culex* was due to the nature of the wound inflicted. Réaumur (1738), who cites him, considered that the effects were due to a toxic fluid injected by the mosquito with the object of increasing the blood-flow. Macloskie (1887) considers the salivary secretion "seems by inflaming the tissues to determine a flow of blood and also to prevent the coagulation of blood or other proteid" on which the insects usually feed. The blood, he notes, subsequently coagulated in the insect's stomach. It has also been suggested by others that the secretion might prevent the coagulation of the blood, which would clog the mouth-parts, to the detriment of the insect.

The problem was not approached experimentally, until last year when one of us (G. H. F. N.), seconded by Dr Graham-Smith, made some preliminary investigations which will be continued this year. Fragmentary though they be, the data recorded do not support the hypothesis that the salivary glands, at any rate of *Culex pipiens*, contain a substance which prevents coagulation.

*Some Experiments with Emulsions of the Salivary Glands
of Culex pipiens.*

1. Six sets (36 acini) of glands were dissected out of freshly killed insects, and placed in a drop of salt solution. The drop was allowed to dry, it being thought that the salt crystals would facilitate the grinding up of the glands with the end of a small glass rod, this being done under microscopic control. After grinding up, a small drop of water was added of the size of the original drop of saline, and an equal volume of human blood taken from the clean finger-tip was quickly mixed therewith, and the whole drawn up into a capillary tube. Clotting was not prevented and no haemolysis occurred. Salivary gland emulsion added to a dilute suspension of corpuscles did not lead to haemolysis.

2. Twenty-two sets (172 acini) of glands were emulsified as before, and added to guinea-pig blood, both pure and diluted. The result was the same as before.

3. Twenty-one sets (160 acini) were ground up with powdered glass-wool on a hollow-ground slide with a couple of drops of salt solution. The glass was removed by centrifugalization. Human blood, both pure

and diluted, was added to the gland-emulsion, and drawn up into capillaries. The blood clotted and was not haemolysed.

4. After 24 hours the contents of these tubes were injected subcutaneously in two places into a guinea-pig, but no swelling occurred at the seats of inoculation.

5. A capillary containing some of the above gland-emulsion alone was placed beneath the skin of a rabbit's ear and broken. One-half of the tube removed after 24 hours was found to contain unclotted blood, haemolysis had occurred, some corpuscles appearing as shadows, others being crenated. There were many leucocytes at the entrance to the tube. These effects may have been due to *Streptococci*, which were found microscopically in the tube-contents.

As stated above, these experiments are of a preliminary character. As soon as the season is sufficiently advanced they will be continued. It remains to be proved if the salivary secretion ferments starch or not; it appears to do so in some insects.

Concluding Note.

When the first part of this study of the "Structure and Biology of *Anopheles*" appeared in the first volume of the *Journal of Hygiene* the writers did not expect that it would take so long to reach a conclusion. The delay in publishing the different parts has been partly due to lack of material, especially during 1902, when *Anopheles* proved exceptionally scarce. The descriptions we have published apply to matters regarding the anatomical features and biology, which should interest medical readers. We have not as yet described the nervous, circulatory, respiratory, and reproductive systems, nor the structure of the eye, etc. A description of these appears somewhat too technical for the purposes of this *Journal*, and for this reason it will be given elsewhere. In the meantime we would add that we shall probably from time to time publish additional observations on the subject of *Anopheles*, confining ourselves however to matters of medical interest. Such communications will take the form of separate papers. This paper may therefore be regarded as concluding the "Studies in relation to Malaria" which have appeared serially in this *Journal*.

The "Studies in Relation to Malaria" comprise Part I: "The Geographical Distribution of *Anopheles* in relation to the former distribution of ague in England" (*Journ. of Hygiene*, Vol. 1), and Part II: "The Structure and Biology of *Anopheles*" (*Journ. of*

Hygiene, Vol. I—III). The investigation was originally planned to include a Part III: relating to malaria-infection experiments upon *Anopheles* indigenous to England. It is to be hoped that these will be ultimately carried out successfully. Several attempts have been made in the last two years to infect *A. maculipennis* with malarial parasites, but they have failed. These experiments were made by one of us (G. H. F. N.) in conjunction with Mr Strangeways of Cambridge, and members of the staff of the London School of Tropical Medicine, the late Mr Patrick Thurburn Manson, and Dr C. W. Daniels.

The announcement that the *Index Medicus* will be revived this year relieves us of the necessity of giving further bibliographies of recent literature, such as follow in the Appendix to this paper. It has appeared desirable to publish these bibliographies in view of the scattered nature of the literature. The labour spent in their compilation should facilitate the work of others who are engaged in the study of malaria and its prevention, as also in mosquitoes.

We gratefully acknowledge the able assistance of Mr Edwin Wilson, F.E.S., in the work of illustration. His technical skill and keen interest in the subject have materially furthered the morphological studies on *Anopheles*.

The expenditures entailed in the prosecution of the "Studies in Relation to Malaria" have been largely defrayed by grants from both the Government Grants Committee of the Royal Society, and from the John Lucas Walker Fund, Cambridge.

EXPLANATION OF PLATES.

Illustrating the paper of G. H. F. Nuttall and A. E. Shipley on
"The Structure and Biology of *Anopheles maculipennis*."

PLATE VI.

- Fig. 1. Schematic longitudinal section of a female insect showing the relations of the various parts of the alimentary tract to each other, and to the exoskeleton, as also the salivary glands of one side with their duct joining the common duct, which is prolonged into the hypopharynx. Ventral reservoir filled, the stomach contracted. One of the dorsal reservoirs is cut off near to where it joins the oesophagus.
- Fig. 2. Longitudinal section through the posterior portion of the abdomen, showing a portion of the colon, the rectum with the papillae of one side, as also voluntary muscles about the anal orifice.
- Fig. 3. Anal papilla as seen in fresh specimen, showing distribution of tracheae therein.
- Fig. 4. The ventral reservoir, filled with food, has been removed to a slide, and allowed to dry externally, being then stained with fuchsin. The sac had broadened through lack of its usual support, but nevertheless measures roundly 4 mm. in length, the neck being included. The muscle-bands do not completely encircle the sac.

- Fig. 5. Freshly dissected glands (from one side) of a female insect. Viewed in salt solution without a coverglass. The central gland is small, and narrows to a neck where it joins the secondary duct. The lateral glands broaden anteriorly. After about 15 minutes these glands grew narrower anteriorly (secretion expelled?). Note the undulating course of the chitinous duct within its sheath, both in this and the succeeding figure. The junction of the secondary with the common duct is also shown in both figures.
- Fig. 6. The same as the preceding, the specimen having been mounted in glycerine, which has rendered the soft parts hyaline and permits a study of the duct-structure. The central gland has been displaced to the left of the beholder, as can be seen by the torsion of its duct where it joins the secondary duct together with the intra-glandular ducts of the lateral glands. The chitinous duct within the central gland is shorter than in the lateral glands, but in all three there is a terminal broadening, and the ducts appear annulated for a short distance after entering the glands. Note the lateral branches of the intra-glandular ducts, and, on the right, an accessory lobe corresponding to a well-marked branch.
- Fig. 7. Sketch of the alimentary canal and salivary glands as they appeared when successfully dissected out. Viewed in salt solution without a coverglass, the only addition being the schematized head in outline. By reference to Fig. 1 the different parts will be readily distinguished. The reservoirs contain large air-bubbles. The posterior portion of the ventral reservoir is contracted down. Scarcely a trace of food is contained in the stomach.
- Fig. 8. Semi-schematic cross-section through the three glands of one side in a female insect. The central gland is situated on the left of the observer, as can be readily seen by the stellate arrangement of the secretion, which, resting on the secreting cell, runs in the form of a cone towards the central duct. In the lateral glands the lumen is filled by darkly-staining granular secretion.

PLATE VII.

Figures 1 to 3 inclusive represent cross sections of the head of *A. maculipennis*.

- Fig. 1. Section at the commencement of the pharyngeal pump close to the pharyngeal valve. Above are seen elevator muscles (dor. val. in Fig. 5) whose action appears to be to open the valve, by raising the dorsal plate of the chitinous tube. Below are seen muscles in transverse section which move the maxillae. The common salivary duct situated in the median line, its sheath not being shown. The eye-facets at this point cover almost the entire surface of the head.
- Fig. 2. Section through the enlarged portion of the pump, cutting through the anterior portion of the posterior dorsal dilator muscles and posterior upper portion of the dorso-cephalic or "supra-oesophageal" ganglion (refer to Fig. 5). The relaxed muscles permit the chitinous walls to form a triradiate lumen. Above is seen the dorso-cephalic ganglion. Attached to the upper chitinous plate are the posterior dorsal muscles which run upward and backward. To the lateral plates are attached the lateral dilator muscles. Below are the paired ventral nerve-cords, and outside of these the two secondary salivary ducts, and again outside of these the muscles which retract the maxillae. tr., tracheae.
- Fig. 3. Section posterior to the preceding, avoiding the dorso-cephalic ganglion and including the posterior dilator muscles from their origin at the roof of the head to their insertion on the surface of the dorsal plates of the pharyngeal pump.
- Fig. 4. Cross-section through the contracted posterior portion of the pharyngeal pump where it joins the oesophagus. [Small chitinous hairs protruding backward and

slightly into the lumen of the alimentary canal indicate the point at which the section is taken in the longitudinal section of the head (Fig. 5)]. The section runs somewhat obliquely, consequently there appears to be more (black) chitin on the upper side of the figure than below. The chitin here suddenly thins, a section or two further back shows a round lumen and the chitin seems to disappear, being of extreme tenuity. Viewed laterally (see Plate IX, Figs. 1 and 2), the chitin shows irregular external longitudinal ridges which correspond to the finger-like protrusions which point upward from the (black) chitinous plate in the upper part of the figure. Exceedingly fine chitinous hairs are attached to the thinning ends of the plates, and project *backward* beyond them, as seen in the lower wall of the lumen in the figure. The hairs lie in bunches in grooves formed by folds of the delicate chitinous cuticula which lines the commencement of the oesophagus. The structure is surrounded by what appears to be a ring-like sphincter muscle. The whole structure, including the muscle (measured from left to right as seen in the figure), measures but $60\ \mu$. The hairs are only visible with an immersion, by regulating the light, and focussing up and down. (Draw with the aid of Zeiss, $\frac{1}{2}$ apochromatic oil-immersion, Ocular 8.) Evidently when the sphincter contracts the chitinous hairs will serve as a sieve.

Fig. 5. Longitudinal schematic median section through the head of *A. maculipennis* (female), showing the mouth, buccal cavity, pharyngeal valve, pharynx, and commencement of the oesophagus. The dorso-cephalic and ventro-cephalic ganglia (s.g. and i.g.) are represented without detail. The labium-epipharynx (Ep. lab.) and hypopharynx (hyp.) are cut off near the base with the other mouth-parts. Note the thick chitinous lining to the pharynx and greater part of the buccal cavity, the "soft palate" with its chitinous tooth (there are two side by side), and anterior to this the chitinous spines on the thick chitinous upper "lip." The muscles figured are the elevator of the labium-epipharynx, the elevators of the palate, the muscle running to the upper part of the valve (see Fig. 1) and the anterior and posterior dorsal dilators of the pharynx. Beneath the buccal cavity runs one of the paired muscles (hy.) which works the salivary pump (s.p.), the structure of which is not represented (see Fig. 6 a). The muscular insertion covers too much of the membrane in the figure. Running backward from the pump-membrane is the common salivary duct, which bifurcates at the ventro-cephalic (infra-oesophageal) ganglion.

Fig. 6. Transverse section of the hypopharynx, showing the salivary groove. Highly magnified.

Fig. 6 a. Salivary pump and connected structures, as seen in a dissected specimen which has been treated with caustic potash. Viewed slightly from behind and from its ventral aspect. The common salivary duct enters (in the figure) from above, broadening where it joins the membrane, and being surrounded by chitinous spicules which serve for the attachment of muscles. In the absence of muscular action the membrane protrudes into the hollow cup, which narrows below and opens directly into the groove in the hypopharynx, which is bent to one side and cut off. The epipharynx, also cut off, is shown beneath the hypopharynx. Highly magnified. (Drawn as seen, except where the hypopharynx and the epipharynx are cut off.)

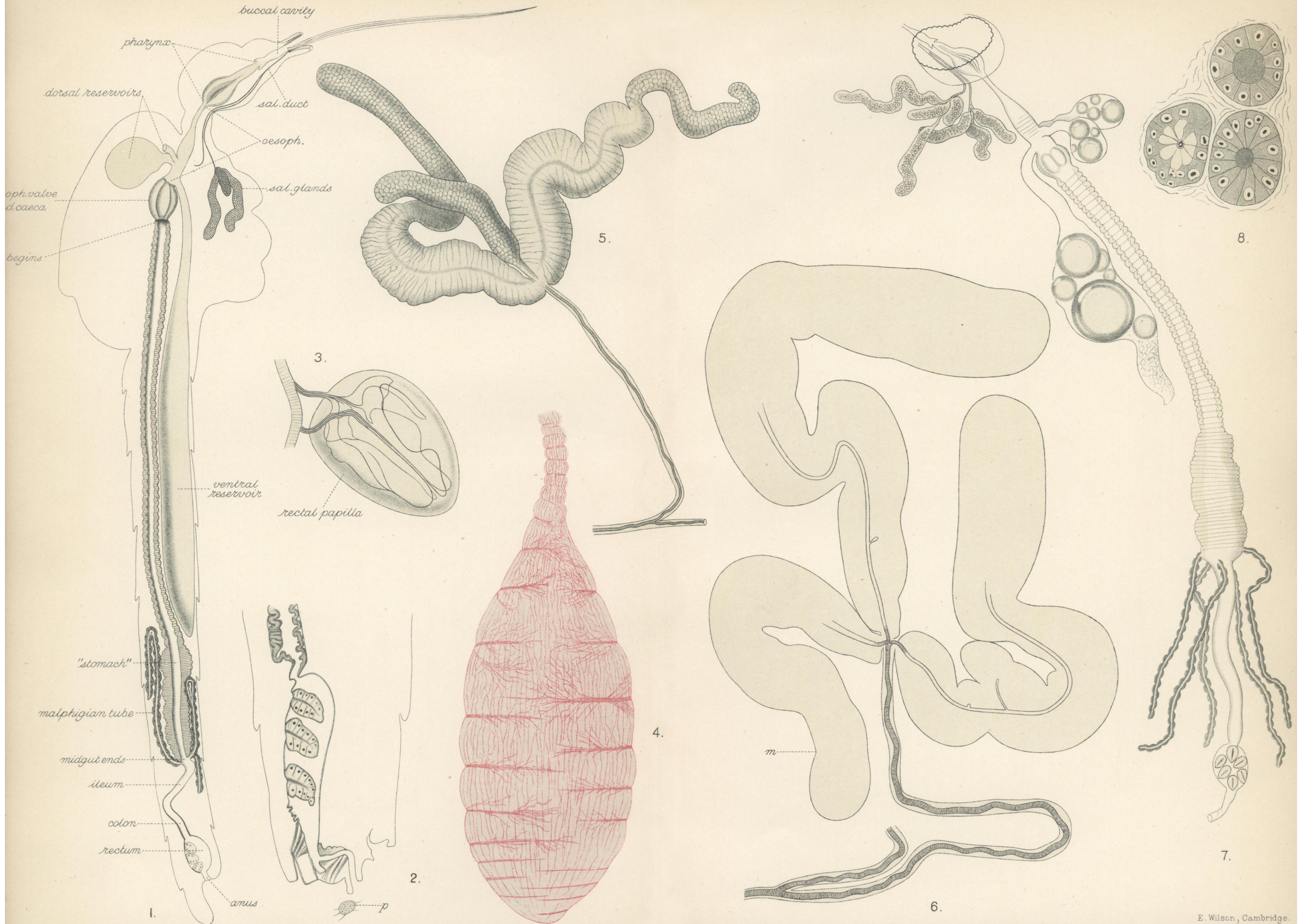
Fig. 7. Tip of the epipharynx, seen from the ventral surface. The widest portion measured externally $43\ \mu$. The structure resembles a quill-pen in general outline, the walls being of varying thickness as indicated by shading. Exceedingly minute chitinous teeth are symmetrically placed about the tip, which doubtless increase its boring power, they are borne on chitinous thickenings which offer some resemblance to an articulation. (Zeiss, apochromatic oil-immersion $\frac{1}{2}$, Ocular 8, reduced from a drawing to scale by G. H. F. N.)

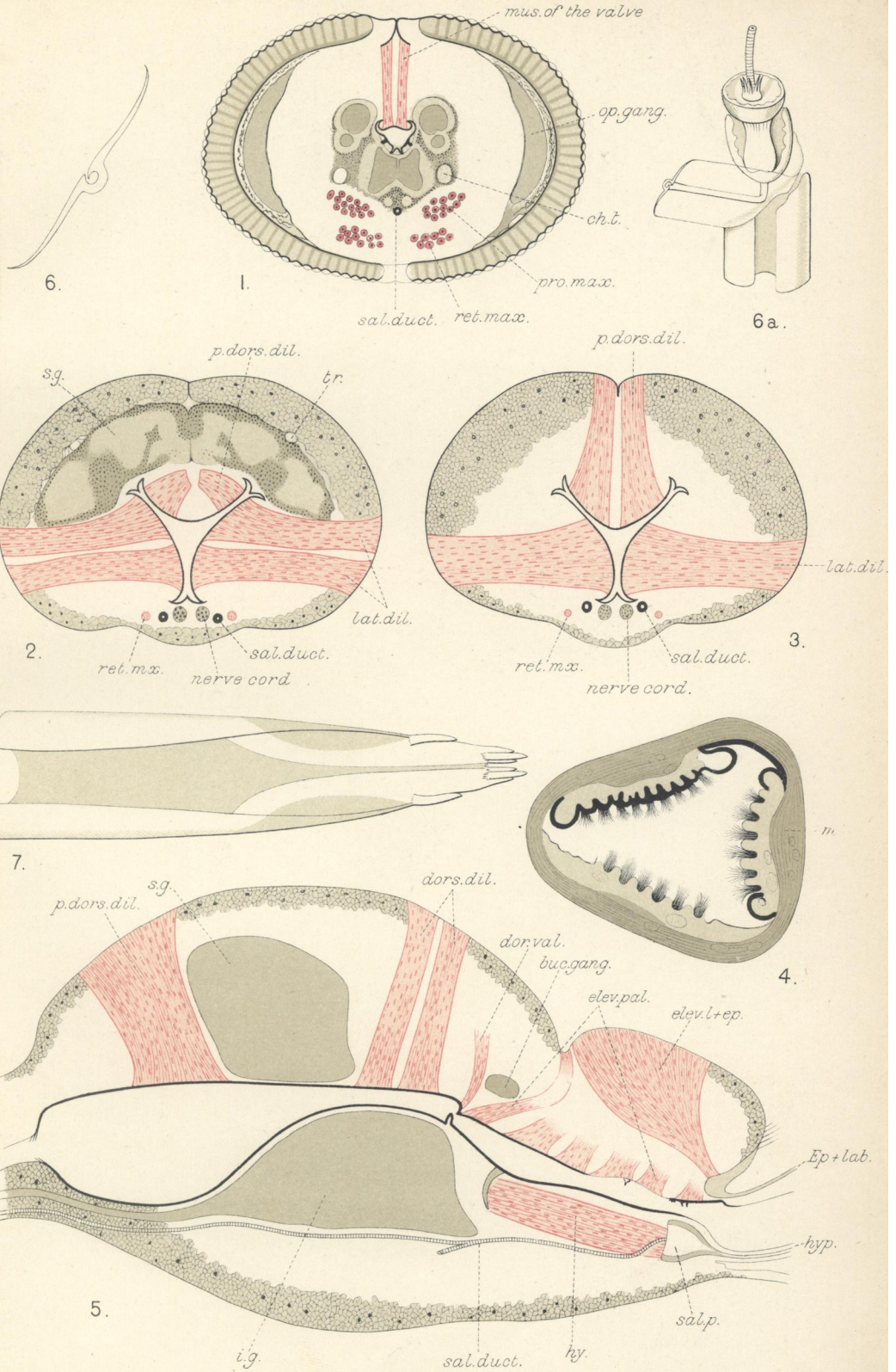
PLATE VIII.

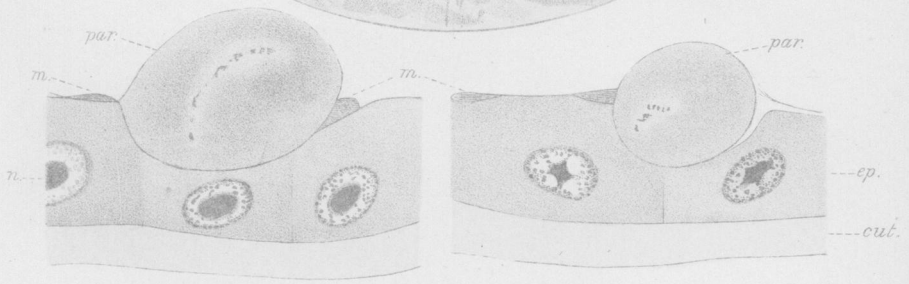
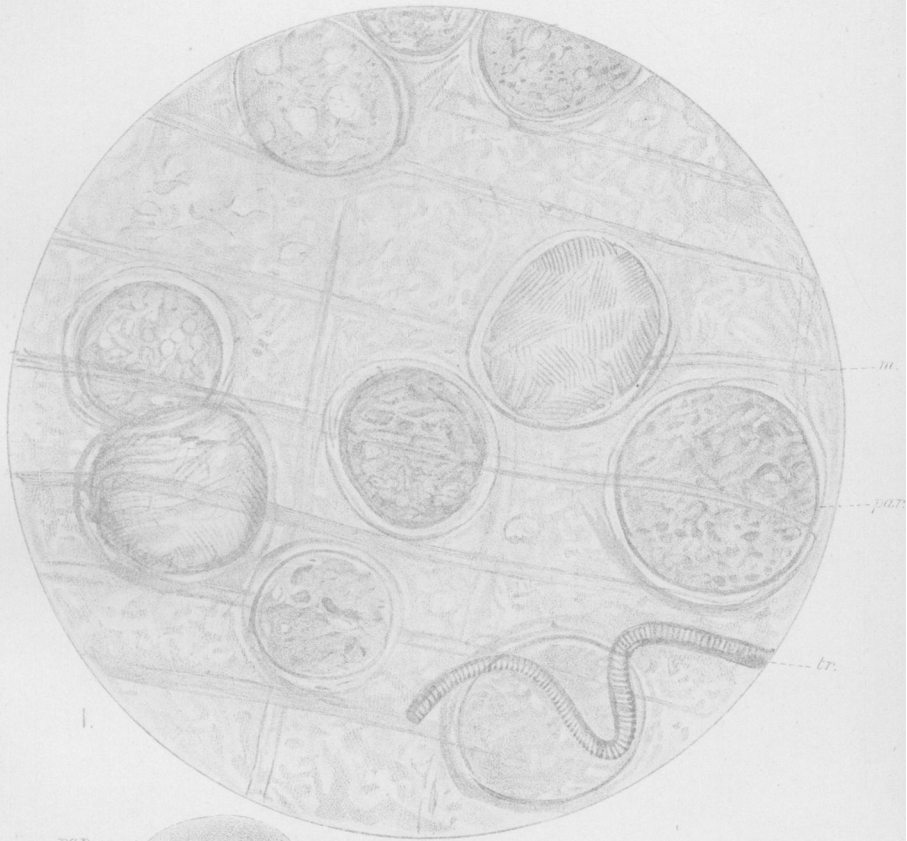
- Fig. 1. Unstained preparation of the stomach-wall of *Anopheles costalis*, showing ten oocysts of malarial parasites (par.), transverse (m.) and longitudinal muscle-bands, and a small trachea (tr.) below. The greatest measurement of the large oocyst on the right (par.) equalled 43μ ; the smallest oocyst measured 28μ . Two of the oocysts are seen to contain sporozoites. All the parasites were seen in a single field, the drawing representing exactly what was seen with the aid of focussing. This represents an intense infection of the insect. One of the authors is indebted to Professor Ronald Ross, F.R.S., for this specimen, which was prepared at Wilberforce, Sierra Leone. (Zeiss apochromatic immersion $\frac{1}{15}$, Ocular 8. Drawn to scale by G.H.F.N.)
- Fig. 2. Sections through the stomach wall of *Anopheles maculipennis*, infected with malarial parasites (par.) to show the position which these occupy. Below we have the cuticula (c.) which lines the gut, next the layer of epithelial cells with their large nuclei (n.) covered externally by the elastic amorphous basement-membrane within which the muscle-bands (m.) appear to lie embedded. (After Grassi, *Studi*, etc. 1900, Plate 2.) The pressure exerted by the parasites is seen to deform the epithelial cells. This semi-diagrammatic figure shows what we have seen if we except the parasites.

PLATE IX.

- Figs. 1 and 2 represent chitinous parts only, owing to treatment with caustic potash. They represent the pharyngeal pumps of a female (Fig. 1) and male (Fig. 2) *Anopheles maculipennis in situ*, parts of the exoskeleton of the head, which would impede the view, having been dissected away. The pump is seen to be much larger in the female, as well as more highly chitinized (darker in the photograph). The dorsal flexure of the tubular portion of the pharynx is well-marked in Fig. 2. Some of the delicate chitin lining the oesophagus is seen in Fig. 1 attached to the posterior portion of the pharynx, the very transparent tube being scarcely discernible and folded upon itself. In both figures the ends of the bulbs are seen to bear delicate ridges, regarding which see description of Fig. 4, Plate VII. In Fig. 1, the situation of the pharyngeal valve is indicated by a small black mass on the top of the chitinous tube leading to the right of the figure, the direction of the tube, corresponding to that of the buccal cavity, also changes at this point. In Fig. 2, above and on the left is seen the clypeus, and beneath it the mouth appendages, including the labium which is covered by scales and hairs. The photographs were both taken to the same scale. Our thanks are due to Mr Walter Mitchell, attendant in the Pathological Laboratory, for the pains he has taken in their reproduction.
- Fig. 3. Transverse section through the central salivary gland of *A. maculipennis*. On the right, running up and down is a mass of muscle. Above, a small part of the darkly staining (granular type) lateral (here dorsal) gland is seen. Below the central gland the presence of a large trachea is faintly indicated in cross section. Situated about the periphery of the gland, which is indented, one sees the darkly staining nuclei of the salivary cells. In the centre lies the intra-glandular duct, which together with the secretion of the cells contains innumerable small bodies, which in a few cases are seen to be elongated. These are sporozoites, the insect having been infected with the parasites of aestivo-autumnal fever. One of the authors is indebted to Professor Grassi for this specimen, which was given to him some three years ago, and it will be seen that it almost exactly reproduces a very beautiful drawing given by Grassi (Plate II, Fig. 19) in the first and second editions of his *Studi* (1900). The microphotograph ($\times 500$) is the excellent work of Professor Carl Günther, of the Hygienic Institute, Berlin.







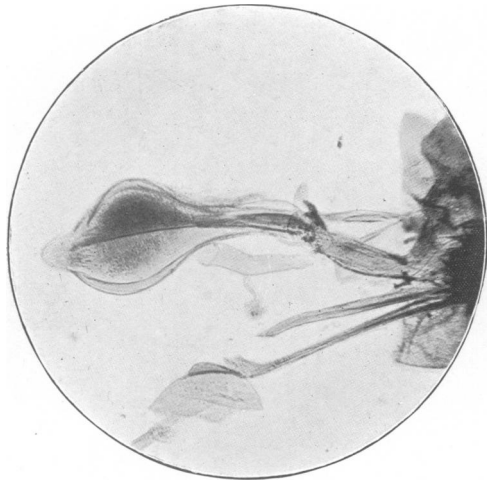


Fig. 1.

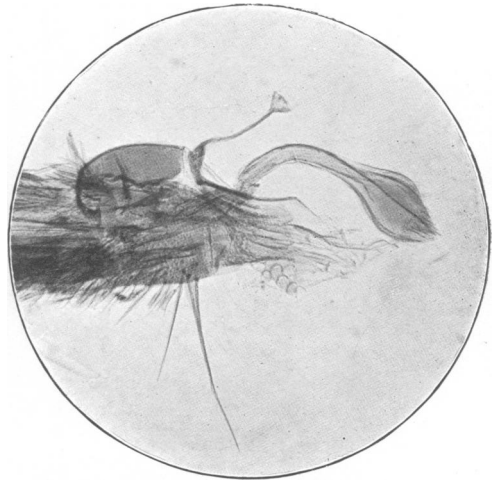


Fig. 2.

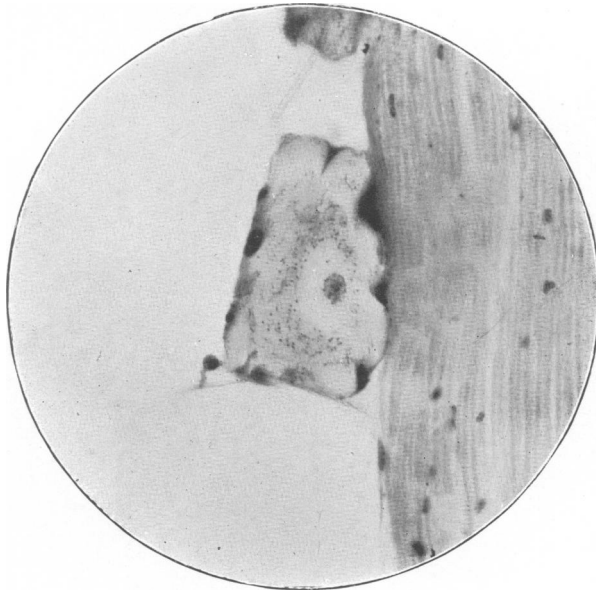


Fig. 3.

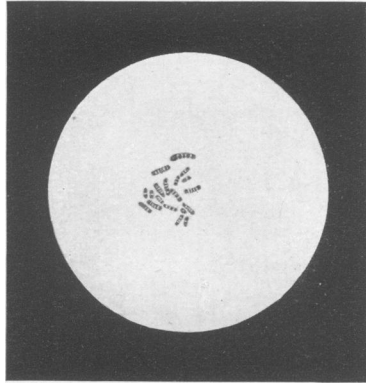


Fig. 1. Hofmann's Bacillus (Pseudo-diphtheria type)
24 hour culture on serum at 37° C.

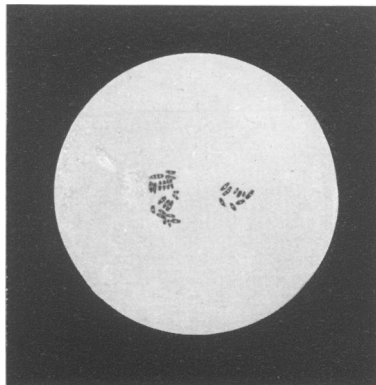


Fig. 2. The same as Fig. 1, subculture 24 hours old on serum. This represents the typical Hofmann's Bacillus. (Both figures drawn with aid of camera lucida, stained with Löffler's methylene-blue (diluted 1 : 5). Zeiss $\frac{1}{2}$ in., No. 4 oc.)

1 2 3 4 5 6 7 8 9

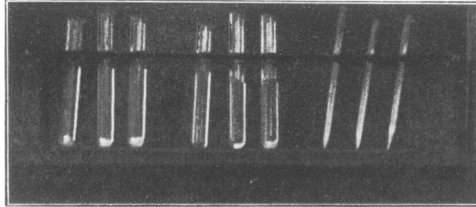


Fig. 1.

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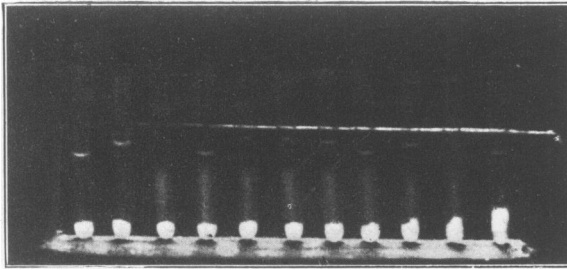
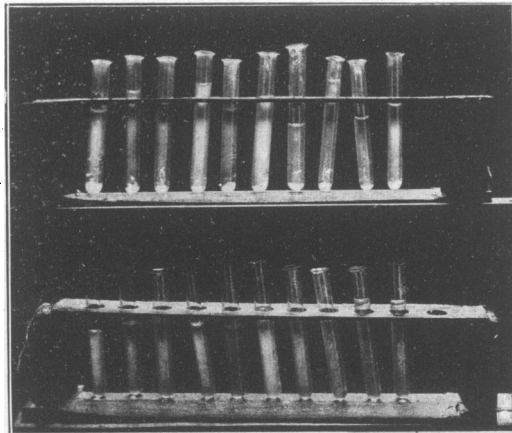


Fig. 2.

1 2 3 4 5 6 7 8 9 10



11 12 13 14 15 16 17 18 19 20

Fig. 3.

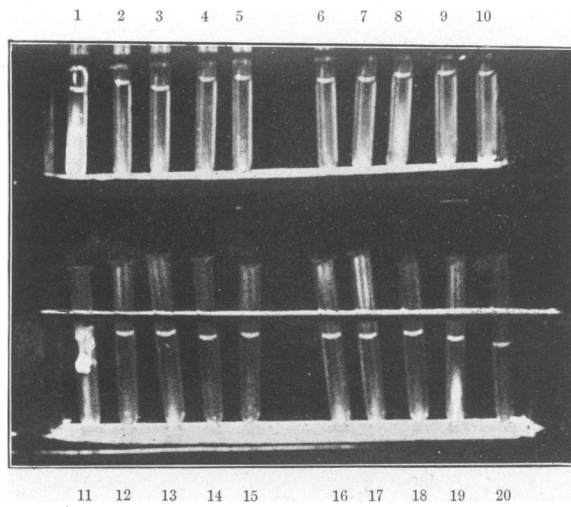


Fig. 4.

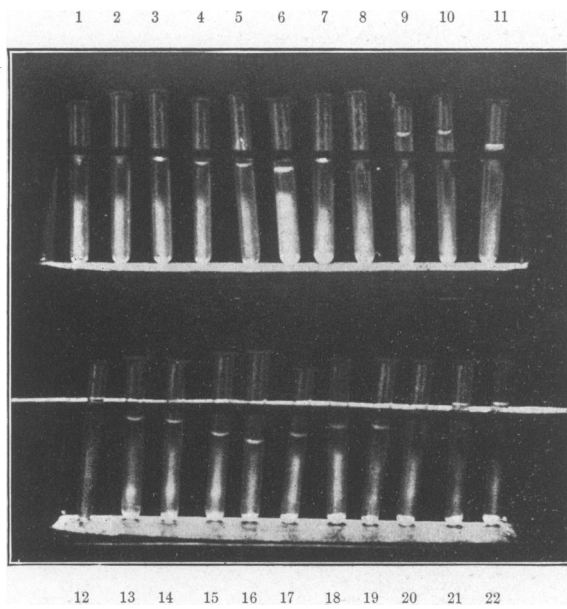


Fig. 5.

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- SCHOO, H. J. M. (rec'd 14. III. 1902), La malaria in Olanda. *Atti per la Società della Malaria*, vol. III. Reprint, 22 pp., 6 figures.
- SCHÜFFNER, W. (1902), Die Beziehungen der Malariaparasiten zu Mensch und Mücke an der Ostküste Sumatras. *Zeitschr. f. Hygiene*, vol. XLI. pp. 89—122. 4 plates of microphotographs. (Studied ague in Sumatra, where he found two species of *Anopheles* which he describes. He gives some data relating to their biology, successfully infected *Anopheles* with malarial parasites, describes his methods, and figures the parasites in the insects by means of microphotographs of sections.)

APPENDIX.

Bibliography of recent Literature on Malaria, and relating chiefly to Prophylaxis, Epidemiology, and Mosquitoes,

by G. H. F. N.

In the following bibliography, which practically ends with 1902, a number of papers are included which we reomitted in our earlier lists. Owing to lack of space, but a few of the papers are accompanied by comments regarding their contents; in most cases the titles are sufficiently suggestive.

- BENTLEY, C. A. (Jan. 1902), *Anopheles* mosquitos in Tezpur, Assam. *Indian Med. Gazette*, vol. xxxvii. pp. 15—16. (1 figure.) (Describes what may be a new species of *Anopheles*. Figure bad.)
- BERKELEY, W. N. (1902), Laboratory work with mosquitoes. (New York, Pediatrics Laboratory, 254 W. 54th St.) (A short guide, which I have been unable to consult.)
- BERNEGAU, L. (1900), Zur Bekämpfung der Moskitos. *Bericht der deutsch. pharmak. Gesellsch.* p. 210. *Rev. Arch. f. Schiffs- und Tropen-Hygiene*, vol. vi. p. 70. (Author protected himself against mosquito bites in West Africa by use of 5% kreolin-water applied to hands, neck and ankles. Clove-oil, camphor, and naphthalin with soap had no effect. Better results obtained with petroleum, but not found practical because of its smell and the danger of fire. (Original inaccessible.)
- BETTINETTI, G., and BORDONI-UFFREDUZZI, G. (1901), La malaria a Milano e nei dintorni. *Atti d. Soc. p. g. Studi d. Malaria*, vol. II. pp. 139—145.
- BEYER (26. VI. 1902), Zur Frage der Bekämpfung der Malaria in unseren westafrikanischen Kolonien. *Deutsche med. Wochenschr.*, Jahrg. xxviii. pp. 472—474.
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- BILLET, A. (1900), Sur un hématozoaire endoglobulaire de *Platydictylus*. *Compt. rend. Soc. Biol.* Reprint, 3 pp., 1 figure.
- BILLET, A. (1901), Paludisme. Contribution à l'étude de la fièvre intermittente de type quarte. *Bulletin méd. de l'Algérie*. Reprint, 25 pp., 1 plate.
- BILLET, A. (9. III. 1901), A propos de l'hématozoaire endoglobulaire pigmenté des *Trionyx*. *Haemamoeba metchnikovi* (Simond). *Compt. rend. Soc. de Biol.* Reprint, 3 pp., 1 figure.

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- BILLET, A. (IX. 1901), Sur l'apparition simultanée des moustiques du genre *Anopheles* et des premiers cas de paludisme dans la région de Constantine. *Compt. rend. Acad. des Sciences*. Reprint, 2 pp.
- BILLET, A. (7. XII. 1901), Sur la présence constante de l'hématozoaire de Laveran dans le paludisme en Algérie (Constantine). *Compt. rend. Soc. de Biol.* Reprint, 3 pp.
- BILLET, A. (1902), Contribution à l'étude du paludisme et de son hématozoaire en Algérie (Constantine). Note préliminaire. *Ann. de l'Inst. Pasteur*, vol. XVI. pp. 185—194.
- BIRDWOOD, G. T. (III. 1902), Some practical suggestions for the prevention of malarial fevers. *Indian Med. Gazette*, vol. XXXVII. pp. 81—85, also *Indian Med. Record*, 5. II. 02.
- BLANCHARD, R. (7. VI. 1902), Note sur les moustiques de la Réunion. *Compt. rend. Soc. de Biol.*, pp. 643—644.
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- BOCHICCHIO, A. (1902), La malaria nel Melfese (Basilicata) nel 1901. Note epidemiologica. *Atti d. Soc. p. g. Studi d. Malaria*, vol. III. pp. 448—456.
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