STUDIES ON THE ALIMENTARY TRACT OF THE FEMALE OF THE BITING MIDGE *CULICOIDES NUBECULOSUS* MEIGEN (DIPTERA : HELEIDAE = CERATOPOGONIDAE).

By

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Introduction

The adult blood-sucking females of a number of species of Culicoides are pests in many parts of the world. The irritation and annoyance caused by their attacks on man and his animals are often so great as to constitute a serious problem. The chief significance, however, of Culicoides spp. is as vectors of filarial and virus diseases to man and his animals. The medical and veterinary importance of these insects has been comparatively recently recognised : in 1913, Patton expressed the view that "as far as is known at present, no species of Culicoides is even suspected of being the invertebrate host of any pathogenic parasite". Several species have been incriminated as vectors of human filariasis by Sharp (1928), Buckley (1933, 1934), Dampf (1936), Henrard & Peel (1949) and Hopkins & Nicholas (1952). Other species have been incriminated as the vectors of filarial diseases to horses and cattle by Steward (1933), Buckley (1938), and Moignoux (1952). Also, serious virus diseases of horses and sheep are transmitted by Culicoides spp. (Du Toit, 1944). A species of Culicoides is incriminated as vector of a virus causing fowlpox of domestic fowls and turkeys (Tokunaga; after Hill, 1947). C. nubeculosus was found by Steward

(ibid.), in England, to be the vector of Onchocerca cervicalis, which infests the cervical ligament in horses and is the principal cause of nuchal disease (fistulous withers and poll-evil). It is also incriminated by Moignoux (ibid.), in the south of France, as the vector of O. reticulata, which infests the suspensory ligament of the pastern joint of horses.

In view of the economic importance of these insects as pests and as vectors of disease, much attention is being paid to their study in many parts of the world. In the United Kingdom, the midge-work has taken several directions : a- surveys of the British species and descriptions of new ones (Edwards, 1926, 1939; A.E. Cameron et al., 1946; Downes & Kettle, 1952); b- the structure of the head-capsule and the mouth parts of the adults (Jobling, 1928; Gad, 1951); c- ecological studies on the adults (Parker, 1949; Kettle, 1950, 1951 a & b); d- the life-cycle and habits of several species (Hill, 1947; Downes, 1950); the anatomy, morphology and systematics of the early stages (Lawson, 1951; Kettle & Lawson, 1952); f- studies on the eggs of several species (Parker, 1950); g- midge repellents and the effect of insect- :icides (E. Cameron, 1946; Hill & Roberts, 1947; Kettle, 1948).

Works on C. nubeculosus, in particular, in this country include those of Downes (1950) on the habits and life-cycle; Roberts (1950) on artificial feeding in the laboratory; Lawson (1951) on the anatomy and morphology of the early stages, and of Weitz & Buxton (1953) on the rate of digestion of blood meals as indicated by the precipitin test. Pomerantsev (1932), in the USSR, studied the morphology and anatomy of the genitalia.

Hitherto, however, no attention has been paid to the study of the alimentary tract, especially that of the female, in any species of Culicoides. The references on this subject are a figure of the alimentary tract of C. kiefferi (Plate XXll) and an elementary account of the Malpighian tubes given by Patton & Cragg (1913), and some brief class-notes and figures of the alimentary tract and salivary glands (pp. 135, 140, 149) of C. varius (?) (probably C. obsoletus Mg., syn. Ceratopogon varius Winnertz, 1852, Linn, Ent., 6, 35; vide Edwards, 1939, p. 143) given by Patton & Evans (1929). It is clear that the alimentary tract constitutes the environment of the ingested parasites, and has therefore a special importance from this point of view. For this reason it has been studied in many blood-sucking flies. The present work

attempts to extend our knowledge to the alimentary tract of Culicoides and relate this to the previous studies on other biting flies. C. nubeculosus was chosen for this study be-:cause it is the only species which, hitherto, has been maintained as a laboratory culture, and adults can be obtain-:ed at intervals throughout the year. As males are of no importance as vectors of diseases, the study was confined to the females.

The present work is divided into four sections. The first includes a description of a simple culture method which provides adequate numbers of adults, and some accounts of the biology of the species in the laboratory. The second deals with the morphology and histology of the alimentary tract, the Malpighian tubes and the salivary apparatus. The third deals with the changes which occur in the mid-gut epithelium in flies which fed on blood as well as in flies which refused to feed. The fourth deals with the function of the oesophageal diverticulum.

SECTION 1.

CULTURE METHOD AND NOTES ON THE BIOLOGY OF *C. NUBECULOSUS* MG. IN THE LABORATORY.

CULTURE METHOD AND NOTES ON THE BIOLOGY OF C. NUBECULOSUS MG. IN THE LABORATORY.

Hill (1947) reviewed four previous attempts to breed Culicoides, including C. nubeculosus, in the laboratory; these met with varying, but never complete success; none of the workers quoted maintained the species studied through more than one generation. Hill herself reared C. impunctatus and C. obsoletus in the laboratory in pots containing medium in which each species was found to breed in nature. She was, however, dependent upon wild females, already gravid or fed in the laboratory, for obtaining the eggs. Females reared in the laboratory did not survive long enough for complete development of the ovaries; throughout, only two female C. impunctatus and one C. obsoletus survived to lay their eggs.

The only species which, hitherto, has been successfully maintained in the laboratory is C. nubeculosus. A strain of this species collected around Chideock in Dorset, has been established in the Department of Zoology, University of Glasgow, by Mr. J.A. Downes in 1947. It has been successfully maintained, except for one hiatus, since then.

It may be noted here that since the middle of 1953 this strain has shown symptoms of deterioration. The majority of

the gravid females refuse to lay their eggs, the batches of eggs laid and the percentage of hatching are becoming smaller. This deterioration may be an after-effect of the culture method used, but it is most probable that it is due to a long period of inbreeding without any introduction of a new blood.

The basic idea of the method used was to simulate the natural habitat of the species. Richly manured soil was collected from a farm near the field-station at Loch Lomond. This was either used at once, or spread out on trays to dry up for storage, and then made up with water when required. The mud was kept in any type of unglazed earthenware pots, filled to a depth of several inches. Each prepared pot was kept standing in a basin of water, and exposed to an electric lamp until a fair algal growth formed. When ready for use, eggs or first instar larvae were introduced into the culture pot and kept well lit at 20°C.

When I began my work, I found the job of inducing algal growth tedious and uncertain. Even when the pots were pre-:pared many weeks beforehand, there was no encouraging success. When such pots were used, the rate of larval mortality was great. Only a few adults, many of them undersized, emerged,

and they were just sufficient for re-stocking. This fact suggested that the medium was deficient in some way or the other. Acoording to Howland (1930), the part played by algae may be one of balance, turning an otherwise nearly suitable environment into an eminently suitable one; or it may be important only indirectly, supplying decomposition products which dissolve in the water and are absorbed by the larvae (mosquito) with other organic matter. Assuming that algae play both parts, the idea of adding powdered charcoal and dried autolyzed yeast to the mud suggested itself. When the idea was put into practice, fairly large numbers of adults were obtained. The yeast enriched the medium directly, as it is a well established fact that yeasts contain growth stimulat-:ing factors (vitamins of the B-complex), and indrectly by encouraging the growth of bacteria and other micro-organisms; in several cases rich algal growth also occurred. The yeast used was "Dried Autolyzed Yeast - D.C.L. - Distillers Edin-:burgh" - but probably any dried yeast would do. The mud used was obtained from the same farm near the field-station; any mud, preferably from near a byre, is probably as good. To prepare the mud, it is spread out on a tray in the labor-:atory and is left to dry. The dried mud is then powdered in

a mortar, sieved through a 10 meshes/inch sieve in order to exclude big objects as gravel and pupae ... etc., and is stored till required. The pots used are earthenware un-:glazed 6 x 1 inch pots without a drainage hole.

The laboratory temperature was maintained at 20°C., which was fairly constant except during the hot spells, when it sometimes reached 25°C., and during very cold spells, when it usually dropped below 20°C., but rarely below 15°C.

Preparation of the culture pots

After several preliminary trials a simple method has been adopted. For each pot the following mixture was used:

a - powdered mud to fill a depth of approximately 4 mms. (about 50 grams).

b - 2 moderate teaspoonfuls of dried autolyzed yeast (about 6 grams).

c - 1 moderate teaspoonful of powdered charcoal (about 4 grams).

The ingredients are mixed thoroughly in a mortar, the mixture transferred to the pot and levelled, and ample water is added to it; the water usually takes some time to penetrate the surface of the mixture, but there is no need for stirring. The prepared pots are then transferred to an insect-proof

(psychodids breed heavily in this medium), airy and naturally lit cage in the open; the decay of yeast gives an offensive stinking odour. The medium should be kept well watered until required. Within a week or so, the yeast rises to the surface of the medium and forms an amber-coloured or brownish skin. This skin frequently encourages fungal growth which, when great, is unfavourable to the larvae. It is preferable to stir the medium well once or more as necessary, so as to inhibit the formation of this skin. The culture pots, however, should be prepared about 3 weeks beforehand.

For use, the pots are brought into the laboratory where the recently hatched larvae are introduced into them. The practice of directly introducing batches of eggs into the culture pots was not followed because a- very frequently, the females lay batches of eggs which fail to hatch; b- it was desirable to know the number of larvae added to each pot and the percentage of emergence relative to the number of larvae; and c- it was also desirable to know the percentage of hatching in the laboratory. The number of larvae introduced into each pot varied from 70 to 220 larvae. The stocked pots should not be disturbed, should be well irrigated and desiccation of the medium should be avoided.

The layer of water, however, should not reach the edges of the pot, otherwise some of the larvae may escape and perish. From the beginning of the third week of the larval stage only a thin layer of water is needed. Much water at this time hampers pupation, and may damage some of the emerging adults. Any fungal growth on the walls of the pots should be wiped off as many newly-emerged adults become entagled in it.

Each stocked pot is put in a "rearing-cage", which is a glass tank 20 x 11½ x 11½ inch, with the mouth covered with muslin. It is preferable to put the pot in an empty basin so that the drainage water does not spoil the cage. It is not advisable to surround the pots with water; this may lessen evaporation from the pots, but many adults drown in this water. As the adults are phototropic, some source of light, e.g., a window should be provided at the other end of the cage to facilitate the collection and to avoid the escape of the adults when the cage is opened.

Maintaining the collected adults

The emerging adults are kept in a "collecting-cage", which is similar to the breeding-cages. It contains several Petri dishes provided with a layer of wet cotton-wool which is covered with filter paper, to serve as a supply of water,

and to maintain a relative humidity of 80 - 90 per cent. Some raisin is also provided to supply the adults with carbohydrates.

Feeding

The females of C. nubeculosus feed readily in the laboratory. The majority of the females feed during the second and the third days after emergence, but a proportion of the females feed during the first day after emergence. Occasionally, some females may refuse to feed at all.

I am myself not very sensitive to the bites and consequently found it easier to feed them on my own arms than to use a lop-eared rabbit or the artificial method described by Roberts (ibid.). The females (and also males to ensure mating) are sucked into a tube 3 x 1 inch, and the tube is inverted on the under-side of the fore-arm. One should not put many females in one tube; at the time of feeding and for some time afterwards, the females drain themselves and if there are many of them in one tube they become stuck to the condensation water on the walls and may be damaged. If males are present they become badly injured.

Mating

Mating occurs readily in 3 x 1 inch tubes. It rarely occurs in the cage. It does not depend upon feeding. The unfed females, as well as females which have already fed, appear to resent the approach of the males, which chase them persistently in the tube till they either succeed in copu-:lating or give up. However, as soon as the genitalia are engaged, the females become quiescent. On the other hand, females engaged in feeding usually help the males by separating the wings a little and raising the tip of the abdomen, so as to facilitate copulation.

The males of C. nubeculosus are lusty creatures. Very frequently, and many times successfully, they will attempt to mate soon after disengaging; it is not known whether they produce a spermatophore in such cases. Homosexuality is a rather common phenomenon in the males of this species, with the normal copulatory position being assumed.

Production of eggs

A full blood meal is necessary for the development of the ovaries. A small meal does not cause any perceptible development. In some rare instances, however, females which fed on blood to satiety had undeveloped ovaries. The cause

of this undevelopment of the ovaries is obscure, but it seems most probable that the size of the meal was inadequate. The females of C. nubeculosus feed only once in each gonotrophic cycle.

For egg-laying, tubes 3 x 1 inch with a layer of plaster of Paris at the bottom are used. The plaster is moistened and covered with a disc of filter paper, and a strip of filter paper is inserted in the tube leaning on the wall. Two fed females and two males are put in each tube, the tube is then plugged with a bored cork covered with muslin, with a small piece of raisin pinned to its inner surface. It was found that if only one female and one male were put in each tube, the females often refuse to lay their eggs. The prepared tubes are kept in the laboratory at 80 - 90 per cent. relative humidity. The eggs are laid on the disc of filter paper; condensation water should be avoided otherwise the eggs may be laid on the walls of the tube.

It should be noted that observations emphasize the fact that the gravid females will never lay their eggs on a dry surface, and it is essential, therefore, to maintain the plaster and the disc of filter paper moist all the time. On the other hand, it has been observed that the presence of

males, if the females have really mated, and of raisin are not essential. Fertilized females laid eggs as usual, in the absence of the males. However, it was found labour-saving to put the males with the females in the egg-laying tubes; pairs in copula are frequently seen in the egg-laying tubes and, undoubtedly, the majority of the females are mated more than once before they lay their eggs. Blood-fed females which had no access to raisin (carbohydrates) laid viable eggs; however, raisin was supplied as a source of energy, especially for the males.

The period between feeding and oviposition varies from 3 - 16 days, 3 - 4 days being the more common. In sections, the oocytes appear to be fully developed in flies fixed 3 days after a full blood meal. A good number of the gravid females, however, commonly refuse to lay their eggs. The number of eggs laid per female per batch varies from 40 - 206 eggs, with an average in the region of 135 eggs. Ovi-:position is usually complete, but occasionally, however, from 1 or 2 eggs up to 70 eggs may be retained. Also, occasionally, immature eggs (white or grey) are laid.

As stated by Downes (ibid.), the females are ready to bite after oviposition and in due course to lay. In the

present work, however, the females in each tube were dis-
:sected as soon as eggs were seen on the disc of filter paper, to see whether the laid eggs belonged to one or both females, and also to see if oviposition was complete or there were any eggs retained.

The disc of filter paper bearing the eggs is transferred to a solid watch-glass, and the eggs are kept just moist for the first two days then are covered with a thin film of water. The watch-glass cover should be smeared with petroleum jelly so as to avoid the evaporation of the water and the subsequent desiccation of the eggs; the petroleum jelly should be re-
:moved thoroughly from the edges of the watch-glass before turning the hatching larvae into the pots. The eggs hatch 3 - 6 days after oviposition, 3 - 4 days being more common. The number of the larvae varies from 26 - 96 per cent. of the eggs of each batch, the average being in the region of 55 per cent. It should be noted that batches of eggs frequently fail to hatch and, sometimes, only a few eggs may hatch. The viable eggs of any given batch usually hatch within a short time of each other, but, occasionally, a period of 1 or 2 days may be necessary for the eclosion of all the viable eggs in the batch.

The life-cycle as studied in the laboratory

The life-cycle of individual adults which belong to a given batch of eggs varies considerably. This variation is due to the difference in the rate of development of the larvae. It is usual that a proportion of the larvae lag in development behind their fellows and, therefore, while adults are emerging from a given culture pot third and fourth instar larvae may still be seen. The egg stage usually occupies 3 - 4 days, and the pupal stage occupies 4 days though in rare instances it appeared to occupy about a week. However, in the period January - October the first adult (or adults) to emerge from any given culture pot does so 18 - 36 days from the beginning of the first larval instar, and the last adult (or adults) emerges 30 - 57 days from the first larval instar; the period of emergence, however, extends from 7 - 35 days. Adding the period occupied by the egg stage, the earliest adults to emerge complete their life-cycle in a minimum period of 3 weeks, with 6 weeks as a maximum; the latest adults complete it in a minimum period of $4\frac{1}{2}$ weeks, with $8\frac{1}{2}$ weeks as a maximum.

On the other hand, the larvae obtained during October commonly take a long time for their transformation. A very

few adults may emerge in the scheduled time, but usually emergence begins in late December, January and February. As stated previously, the laboratory temperature drops in winter time but rarely below 15°C. It is not known if this drop in the temperature, or the short length of day light, is responsible for this delayed development; at any rate, the larvae do not become dormant and they may be seen feeding or swimming at the surface of the medium. The earliest adult (or adults) emerges 38 - 75 days from the beginning of the first larval instar; the latest adult (or adults) emerges 85 - 124 days from the beginning of the first larval instar. In other words, the first adults to emerge complete their life-cycle in a minimum period of 6 weeks, with 11 weeks as a maximum; the latest adults complete it in a minimum period of 13 weeks, with 18 weeks as a maximum. The period of emergence occupies 14 - 82 days.

In general, from 6 to 8 generations of C. nubeculosus can be obtained in the laboratory each year, the last gen-:eration, i.e., from the October larvae, usually occupies a period of several months. The number of the adults emerging from any given culture pot varies from 17 - 91 per cent. of the larvae reared, the average being in the region of 45 per

cent. However, from time to time, some pot (or pots) may yield only a few adults, or none at all. It is probable that such pots are in some way unfavourable for the develop-:ment of the larvae, but it is also possible that the larvae themselves are weak and unfit for completing the life-cycle. In some cases, several prepupae failed to transform into pupae; Lawson (1950) recorded an unsuccessful pupation in C. heliophilus, and this may be common in nature (see, how-:ever, Weerekoon, 1953). The first adult to emerge may be a male or a female; sometimes males and females are collect-:ed, but it has not been ascertained whether they emerged at the same time. It may be noted that the number of larvae reared in a given pot (within the range of 70 - 220 larvae) does not appear to affect the percentage or the period of emergence; in Anopheles, Bates (1941) states that in an unfavourable medium the results may be greatly influenced by the concentration of the larvae, but in favourable media larval density does not seem to be so important.

Behaviour of the larvae in the culture pots

The observations described here were made on third and fourth instar larvae; a free surface layer of water helps to show the described activities.

The larvae appear to spend the greater part of their life buried in burrows at the surface of the substratum with the head and a part of the thorax protruding; it is also usual for a part or most of the abdominal segments to be protruded, but the hindermost extremity, at least, remains within the burrow. However, the larvae sometimes leave their tunnels and swim freely in the surface layer of the water, but sooner or later each excavates a tunnel by penetrating through the loose medium either with its head or with the end of the abdomen, and then withdrawing the rest, or a part, of the body. In the less inundated spots of the substratum the larvae are usually buried except for the head, and the mouth parts are seen working through the surface as vibrating dots. Sometimes the larvae are seen creeping on the surface, or on the wall of the pot.

The larvae of C. nubeculosus are phototropic, and they are to be seen in large numbers showing conspicuous activity when the pot is exposed to an electric light. They are very easily alarmed, the slightest jarring or vibration makes them dash into their tunnels. Carter et al. (1920) described more or less similar activities in the larvae of C. accraensis.

These larvae are, commonly at least, top feeders con-

:centrating their feeding activities on the surface of the substratum and several millimeters downwards, and through the surface of the water. They seem to spend most of their time in feeding. Whenever they were observed in a given pot, they were helping themselves from the considerable variety of living and non-living organic matter available without any discrimination. In the flooded spots the larvae were seen to protrude the greater part of the length of the body from the burrows and work their mouth parts through the water surface where a film of iridescent micro-organisms, which consists mainly of bacteria with infusions of ciliates and flagellates, moving their bodies in every direction and sometimes leaning on either side or bending the head upwards or downwards. Some larvae leave their tunnels and swim for a while till they reach the wall of the pot and browse on what is found of algal and little fungal growths on it. Also, they may creep on the surface of the substratum and still go on feeding. Other larvae are seen burrowing in the heaps of organic materials just below the water layer and browse rapidly on bits of it; sometimes the larva takes a large piece which entangles the mouth parts so that the larva struggles to get rid of

it by shaking the head vigorously.

The behaviour of the larvae in the uninundated spots is exactly the same except that the larvae either withdraw the whole of the body to reappear in another spot, or leave the tunnel, creep on the surface and tunnel again in the new spot.

The larva while feeding is very restless. It starts feeding in a given spot and then after a while and very abruptly makes a jerk with the free portion of its body and starts to feed in the new site for a while and so on. Several such shifts can be observed within a circle of about 1 cm. in diameter. Because of this restlessness, it was found very difficult to observe the feeding mechanism. All that could be observed was that the surface of the medium was vibrating, indicating the movements of the head and mouth parts. It was possible to see through the head-capsule the backwards and the forwards piston movements of the epipharynx described by Lawson (ibid.) in Tetraphora (= Dasyhelea). The bolus is very easily seen through the rather transparent integument, and as in Tetraphora, it moves slowly and smoothly through the fore-gut until it reaches the stomodaeal valve (at the posterior end of the metathorax), where it

remains for a little while before being admitted into the mid-gut. The bolus varies in colour : whitish, yellowish, brownish or rather dark according, of course, to the colour of the eaten substance. More than one bolus, each with a different colour, could be seen passing down the oesophagus with a conspicuous space between each other. The culture pots studied contained amorphous organic matter, a little growth of algae, a very little fungal growth with white hyphae and occasional sporangia, a film of bacteria and infusions of ciliates and flagellates. The larvae were not observed to feed on the fungus spores or the Protozoa, but the dead bodies of the latter, at least, may contribute to the nourishment of the larvae. However, it is certain that the larvae avail themselves of the other matters mentioned, and they pay particular attention to the bacterial film.

A larva was once seen while defaecating. The region of the alimentary tract in the penultimate segment and a little before was seen containing a greenish amorphous matter bathed in a surplus of fluid. Then, due to peristaltic movements, this load was discharged. The greenish colour of the faeces may indicate the indigestibility of at least some of the ingested algae (or green flagellates; Phytomonadina). As

the larvae feed indiscriminately and excrete some undigested food, one can suggest that they feed for so long in order to obtain their nutritional requirements.

Behaviour of the pupae in the culture pots

Pupae, white or pigmented, are usually seen buried in tunnels in damp but not inundated spots of the substratum, with the cephalothorax and the breathing trumpets protruding, or with only the breathing trumpets protruded. They can also be seen lying on the surface of the medium, but the last abdominal segment (or segments) is almost always buried. Not uncommonly, pupae are to be seen in good numbers lying on the walls of the pots in different positions : pupae lying across the walls of the pots were observed to have the cephalothorax and the last abdominal segment with the caudal spines in contact with the walls; sometimes the cephalothorax and the first one or two abdominal segments are in direct contact with the wall of the pot, while the rest of the abdomen is swung from side to side; in other cases the cephalothorax and the abdomen except the first two abdominal segments are free of contact, the pupa being anchored to the wall by means of the ventral spines of the first two abdominal segments; sometimes the pupa swings all its body

being anchored to the wall by the caudal spines only. It is interesting to note that the adults emerged successfully from such pupae; and it appears that a very moist substratum is not essential for the welfare of the pupae.

When water is added to the surface of the culture medium, the pupae are to be seen struggling to leave their tunnels or the surface of the medium, when they are lying on it, to keep away from the water. In buried pupae, the cephalothorax with the breathing trumpets are seen swinging, then the abdomen is seen moving in every direction mostly in a weak wriggling fashion till the pupa frees itself from its tunnel. The movements of the abdomen continue as the pupa struggles through the layer of water. Every now and then the breathing trumpets penetrate the surface of the water for breathing. As the wriggling movement is not so vigorous as to allow rapid movement, some pupae may lay on their sides on the surface of the culture medium, and by forward contractions and expansions of the abdomen aided by the abdominal spines they contrive to move. Other pupae, while in the water, curve the abdomen till the caudal segment reaches nearly the middle of the venter of the abdomen, then release it suddenly so as to push themselves forwards

in a fashion like that of springtails.

If the pupa reaches an uninundated spot or one with a very thin layer of water, it attaches itself to this spot and begins to excavate a tunnel to bury itself within. This is achieved by working the caudal spines by means of the wriggling movements of the abdomen, and then pushing itself within the excavation step by step. The action of the caudal spines is undoubtedly supplemented by the action of the abdominal spines, as the pupa is to be seen boring the medium by side to side movements so as to have the tunnel the proper size. On the other hand, if the pupa reaches the wall of the pot it climbs it, aided by the abdominal spines and by the movements of the abdomen, till it attaches itself in a suitable position. Patton (1913) states that the pupa of C. kiefferi anchors itself by two prominent terminal spines, or it may float on the surface. Carter et al. (ibid.) state that the pupae of C. accraensis are aquatic and float in a vertical position with the body extended and the trumpets in contact with the surface. If they are stranded on the sides of a glass vessel by tilting, they are quite capable of wriggling back to the water over a distance of at least one inch. Such movements as they do make are affected by antero-

posterior and lateral motions of the abdomen. None of the pupae observed employed the processes at the posterior end of the abdomen as hooks to anchor themselves.

As I did not observe in any culture pot any pupae floating on the surface of the water, it was of interest to know if this is a normal habit or not. Two pupae, pre-:sumably less than one day old, were transferred on 6. VIII. 1952 to a specimen tube 2 x ½ inch containing ½ inch of water. The two pupae floated on the surface beside the wall of the tube, with the breathing trumpets penetrating the water surface and the body axis either parallel to the water surface or with an inclination from it. When the tube was inverted, the two pupae rose to the surface again. To see if emergence would occur successfully, the two pupae were left in the tube. On the morning of 10. VIII. 1952 two females were seen, one of them clinging to the plug and the other was striving to climb the wall of the tube; the latter female fell on its back on to water, but when the plug was removed in the collecting-cage, both females took the wing safely.

Initially the practice was to stop watering the pots at the time of pupation. But as the larvae of any given

batch of eggs do not reach pupation at the same time, and as the lagging larvae most probably need water till they pupate, the cessation of watering the pots must have been unfavourable to the lagging larvae. On the other hand, while the addition of much water to the culture pots is not harmful to the existing pupae though disturbing them, it does hamper new pupations. To overcome both disadvantages, the addition by means of a pipette of an amount of water sufficient to form a reasonably thin layer was found satisfactory.

SECTION 11.

ANATOMY AND HISTOLOGY OF THE ALIMENTARY TRACT.

ANATOMY AND HISTOLOGY OF THE ALIMENTARY TRACT.

Technique

The flies were dissected in 0.65 per cent. saline solution, and by means of a pair of fine sewing-needles mounted in pin-holders. The method used in dissecting out the alimentary tract is as follows.

1 - Etherize the specimen just before the operation, and transfer to a clean slide.

2 - Remove the wings and legs. Place the trimmed fly on its back in a drop of saline solution, with its head pointing away.

3 - Hold the fly in position by transfixing the thorax with one needle, and with the other needle nick the integument on both sides of the 6th or 7th abdominal segment. Exert a gentle traction to release the hind-gut and a portion of the mid-gut.

4 - Place the fly on its side and remove the dorsal scler-:ites of the thorax piecemeal. Remove the thoracic muscles carefully to expose the fore-gut. Amputate the head.

5 - Return the fly to its first position, ventral side up-:wards. Use one needle to hold the fly in place, and

insert the other needle between the integument and the posterior portion of the mid-gut, and move it forwards tearing the integument open. Free the gut from its tracheal attachments. Exert a gentle traction on the separated posterior abdominal segments and the whole gut will slip out. Remove any remaining bits of cuticle, and also the sclerites of the posterior abdominal segments piecemeal.

For dissecting out the salivary glands, place the trimmed fly on its side in a drop of saline solution on a clean slide. Remove the dorsal wall of the thorax, and remove the thoracic muscles carefully. The bent salivary glands proper will be seen as refractile bodies, and they are easily differentiated from the thoracic muscles. Cut off the head together with the anterior portion of the thorax, which contains the glands. Transfix the head with one needle, and by means of the other needle remove the tissues surrounding the glands. The utmost care is necessary especially when trying to free the accessory glands from the neck, otherwise one or more of them will be detached.

Serial Sections

Whole insects were used in sectioning because of their small size. A cytological study was not intended, and it was only necessary to use a fixative which gives a good histological picture. Picric acid fixatives were used, but they caused the specimens to shrink and symmetrical sections were found difficult to obtain. This disadvantage was overcome by killing and preserving the specimens in warm Pampel's fluid previous to the use of the picric acid fixative, as is recommended by Abul-Nasr (1950). Ester-wax was used as an embedding medium. Sagittal, horizontal, and transverse sections were cut at 6μ thick. Ehrlich's haematoxylin, Heidenhain's haematoxylin, and Mayer's haemalum as nuclear stains, and eosin in absolute alcohol, orange G, and Van Gieson as counterstains were tried. Ehrlich's haematoxylin and eosin in absolute alcohol gave satisfactory results and were used throughout the work.

Fixation and Embedding

1 - Kill and preserve the specimens in warm Pampel's fluid until needed.

2 - Remove the wings and legs, and put the trimmed insect

in Duboscq-Brazil for 24 hours.

3 - Wash with 70% alcohol. Leave the specimens in 70% alcohol for 24 hours (2 - 3 changes).

4 - Transfer to a 1 : 1 mixture of 70% alcohol and cellosolve and leave overnight.

5 - Transfer to pure cellosolve for 1 - 2 days (2 - 3 changes).

6 - Transfer to a 1 : 1 mixture of cellosolve and ester-wax for 2½ hours in an oven at 48°C.

7 - Give the specimens two baths of pure ester-wax for 1½ hours each in the oven.

8 - Embed in ester-wax in a watch-glass.

Flattening and fixing the ribbons

Float the ribbons on distilled water on a clean slide smeared with Mayer's albumen. Flatten on a hot-tray main-:tained at 42°C. When the ribbons flatten, blot out the water, leaving just a trace of it to aid the final flatten-:ing. Adjust the ribbons in the right position. Leave the slides on the hot-tray overnight to dry up. A trace of diacetin added to the albumen is advantageous for aiding the flattening of the ribbons especially when blood-fed speci-:mens are used.

Staining

1 - Xylene for removing the wax and clearing the sections; 2 baths for 2 minutes each.

2 - Absolute, 90%, 70%, and 50% alcohols; 1 minute each.

3 - Ehrlich's haematoxylin; 13 minutes.

4 - 50% alcohol to remove the excess of the stain; 2 baths for ½ a minute each.

5 - Acid alcohol for differentiation; 1 minute.

5 - Alkaline alcohol for blueing; 2 minutes.

7 - 70% alcohol; 2 baths for 2 minutes each.

8 - 90% alcohol, and absolute alcohol; 1 minute each.

9 - Counterstain in eosin in absolute alcohol for 5 minutes.

10 - Absolute alcohol to remove the excess of the stain; 2 baths for ½ a minute each.

11 - Clear in xylene; 6 minutes or more.

12 - Mount in Canada balsam.

Culicoides nubeculosus Mg. - (female).

Fig. 1 Showing the alimentary tract - diagrammatic. (The sucking apparatus and the salivary apparatus are not shown).

Fig. 2 Showing tumours at the base of the stomach, and abnormal features in the Malpighian tubes.

amp. : ampulla.

ant.m.g. : anterior segment of the mid-gut.

d.oes.d. : duct of the oesophageal diverticulum.

Mal.t. : Malpighian tube.

oes. : oesophagus.

oes.d. : oesophageal diverticulum.

rec. : rectum.

rec.p. : rectal papilla.

s.int. : small intestine.

st. : stomach.

tu. : tumours.

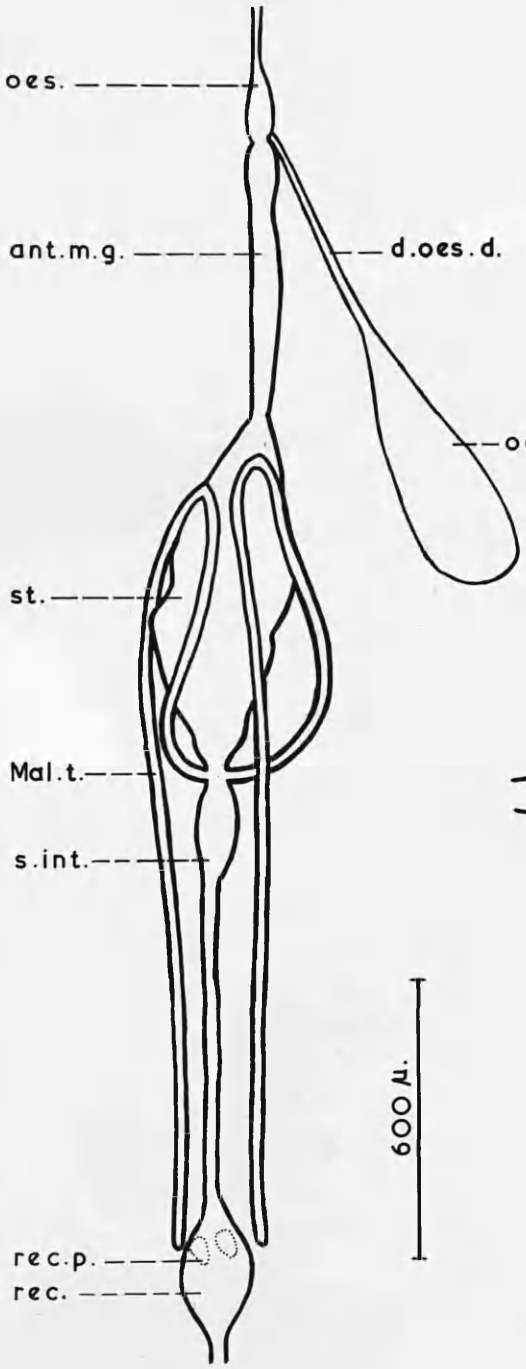

oes.
ant.m.g.
d.oes.d.
oes.d.
st.
Mal.t.
s.int.
rec.p.
rec.
600 µ.

Fig. 1

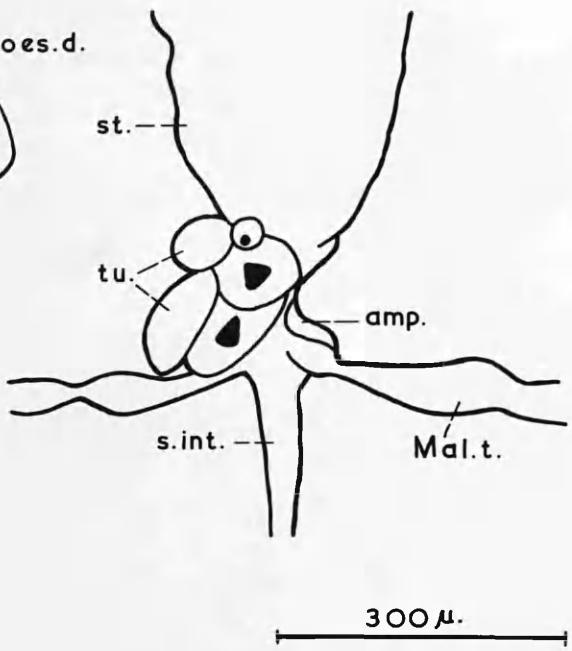

st.
tu.
amp.
s.int.
Mal.t.
300 µ.

Fig. 2

C. pulicaris L. - (female)

Fig. 3 Showing a median longitudinal section in the head. (after Jobling, 1928)

a.d.d. : precerebral dorsal dilator of the pharyngeal pump.

c.sl.d. : common salivary duct.

d.ph. : dilator of the cibarial pump.

e. : epipharynx.

f.g. : frontal ganglion.

h. : hypopharynx.

oe. : oesophagus.

lre. : labrum - epiharynx.

p.d.d. : postcerebral dorsal dilator of the pharyngeal pump.

p.l.d. : posterior lateral dilator of the pharyngeal pump.

p.s.oe.p. : posterior sphincter of the pharyngeal pump.

pr.ph. : protractor of the cibarial pump.

r.n. : recurrent nerve.

r.ph. : retractor of the cibarial pump.

sb.g. : suboesophageal ganglion.

sl.p. : salivary pump.

sl.p.m. : dilator of the salivary pump.

sp.g. : supraoesophageal ganglion.

(for other letterings see Jobling, 1928)

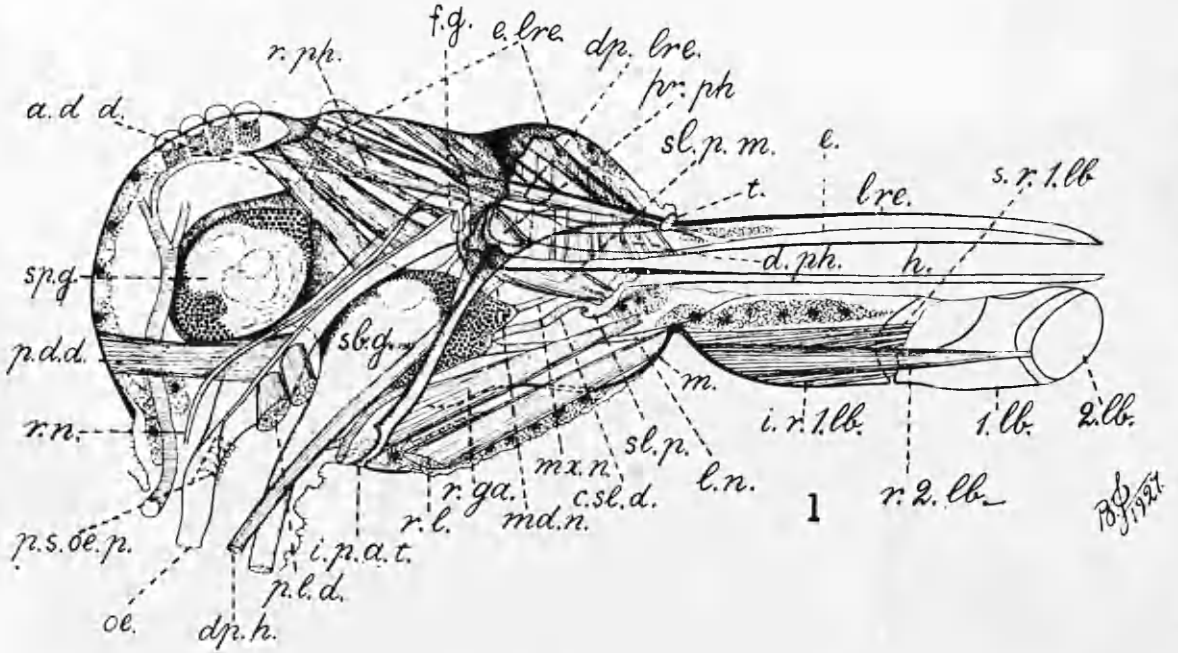

f.g.
e.lre.
dp. lre.
r. ph.
pr. ph
a.d d.
sl.p. m.
e.
t.
lre.
s. r. 1.lb
sp.g.
d.ph.
h.
p.d.d.
sb.g.
m.
r.n.
i. r. 1.lb.
1.lb.
2.lb.
sl.p.
mx.n.
l.n.
r. ga.
c.sl.d.
r. l.
md.n.
1
r. 2. lb.
p.s.oe.p.
i.p.a.t.
p.l.d.
oe.
dp.h.

Fig. 3

C. nubeculosus Mg. - (female).

Fig. 4 Photograph of the head-capsule showing the approximate level of text-figures 5 - 13. (Notice that it is dichoptic; also notice the stem of the epicranial suture).

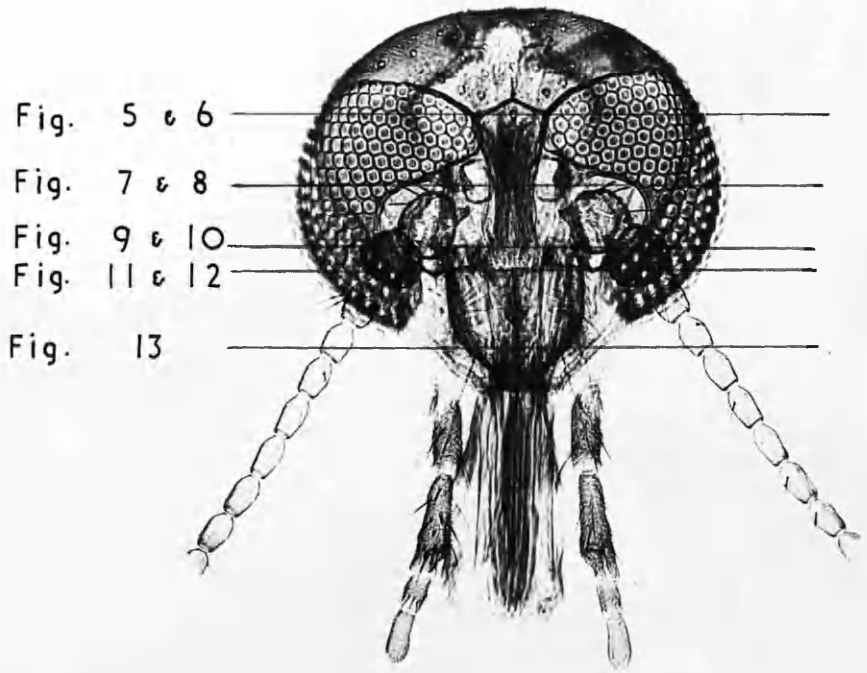


Fig. 4

C. nubeculosus Mg. - (female).

Fig. 5 T.S. through the posterior extremity of the frontal region of the head-capsule, showing the position and muscular attachment of the posterior region of the pharyngeal pump.

Fig. 6 T.S. through the posterior region of the pharyngeal pump as seen in Fig. 5 - enlarged.

Fig. 7 T.S. through the middle of the frontal region of the head-capsule, showing the position and muscular attachment of the anterior region of the pharyngeal pump.

Fig. 8 T.S. through the anterior region of the pharyngeal pump as seen in Fig. 7 - enlarged.

(For the approximate level of the sections see Fig. 4)

a.ph.p. : anterior region of the pharyngeal pump.

c.sl.d. : common salivary duct.

epi.c. : epithelial cells.

fr. : frons.

l.d.ph. : lateral dilator of the pharyngeal pump.

oc. : ocellus.

p.d.d.ph. : posterior dorsal dilator of the pharyngeal pump.

p.ph.p. : posterior region of the pharyngeal pump.

pr.d.d.ph. : precerebral dorsal dilator of the pharyngeal pump.

r.n. : recurrent nerve.

sb.g. : suboesophageal ganglion.

sl.d. : salivary duct.

sp.g. : supraoesophageal ganglion.

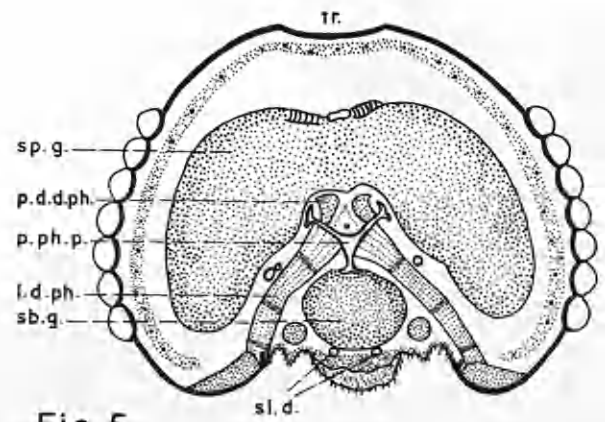


Fig. 5

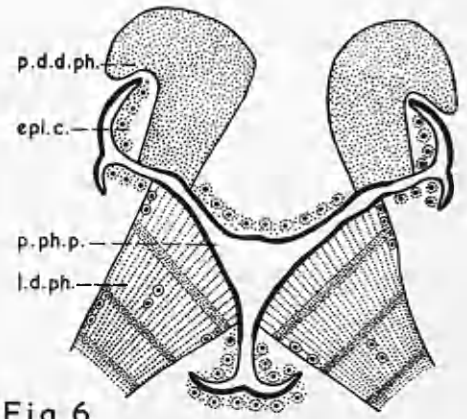


Fig. 6

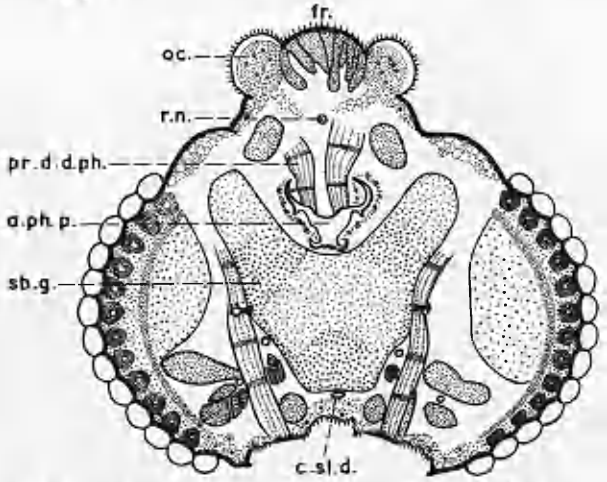


Fig. 7

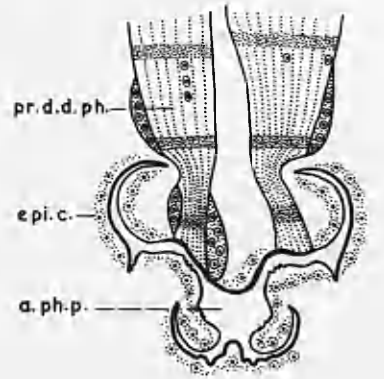


Fig. 8

C. nubeculosus Mg. - (female)

Fig. 9 T.S. through the anterior end of the frontal region of the head-capsule, showing the position of the anterior sphincter of the pharyngeal pump.

Fig. 10 T.S. through the anterior sphincter of the pharyngeal pump as seen in Fig. 9 - enlarged.

Fig. 11 T.S. through the posterior end of the clypeal region of the head-capsule, showing the posterior end of the cibarial pump.

Fig. 12 T.S. through the posterior end of the cibarial pump as seen in Fig. 11 - enlarged.

(For the approximate level of the sections see Feg. 4).

ant. : antenna.

a.s.ph. : anterior sphincter of the pharyngeal pump.

cib.p. : cibarial pump.

cl. : clypeus.

co. : cornu.

c.sl.d. : common salivary duct.

d.sl.p. : dilator of the salivary pump.

f.g. : frontal ganglion.

fr. : frons.

ph.p. : pharyngeal pump.

pr.cib. : protractor of the cibarial pump.

sb.g. : suboesophageal ganglion.

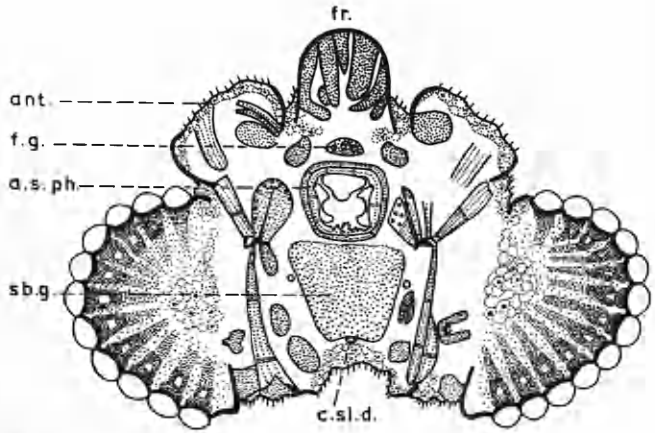


Fig. 9

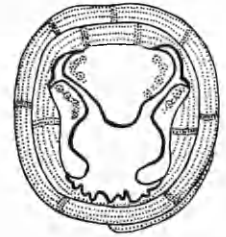


Fig. 10

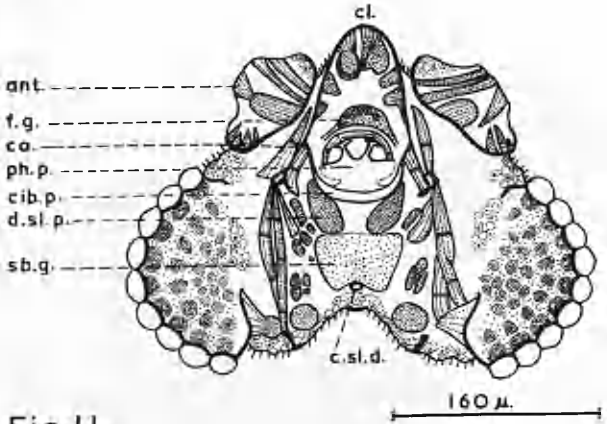


Fig. 11

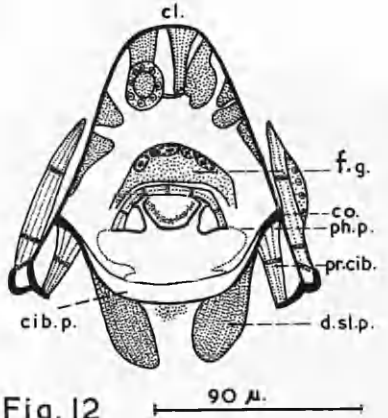


Fig. 12

C. nubeculosus Mg. - (female)

Fig. 13 T.S. through the anterior end of the clypeal region of the head-capsule, showing the position of the cibarial pump and the sali-:vary pump, and their muscles. (For the appro-:ximate level of the section see Fig. 4).

Fig. 14 H.S. through the venter of the head-capsule, showing the salivary ducts and pump.

cib.p. : cibarial pump.

cl. : clypeus.

c.sl.d. : common salivary duct.

d.cib.p. : dilator of the cibarial pump.

d.sl.p. : dilator of the salivary pump.

epi.c. : epithelial cells.

sb.g. : suboesophageal ganglion.

sl.d. : salivary duct.

sl.p. : salivary pump.

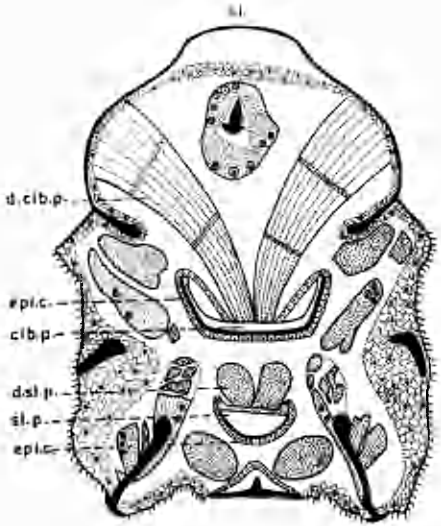


Fig. 13

75 μ.

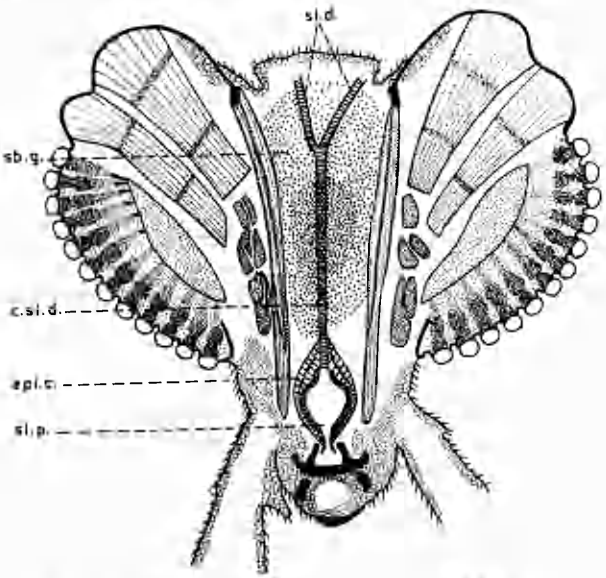


Fig. 14

130 μ.

C. nubeculosus Mg. - (female)

Fig. 15 A diagrammatic median longitudinal section through the oesophagus and the anterior segment of the mid-gut.

Fig. 16 A diagram of a distended oesophageal diverticu-:lum showing one of the various shapes taken by it during peristalsis.

Fig. 17 A diagram of a distended oesophageal diverticu-:lum showing the arrangement of its muscles.

Fig. 18 A photograph of fungal hyphae seen in some sections in the lumen of the oesophageal diverticulum.

c.m. : circular muscle.

cut.int. : cuticular intima.

d.oes.d. : duct of the oesophageal diverticulum.

epi.c. : epithelial cells.

l.m. : longitudinal muscle.

m.g. : mid-gut.

oes. : oesophagus.

oes.v. : oesophageal valve.

sph.m. : sphincter muscles.

st.b. : striated border.

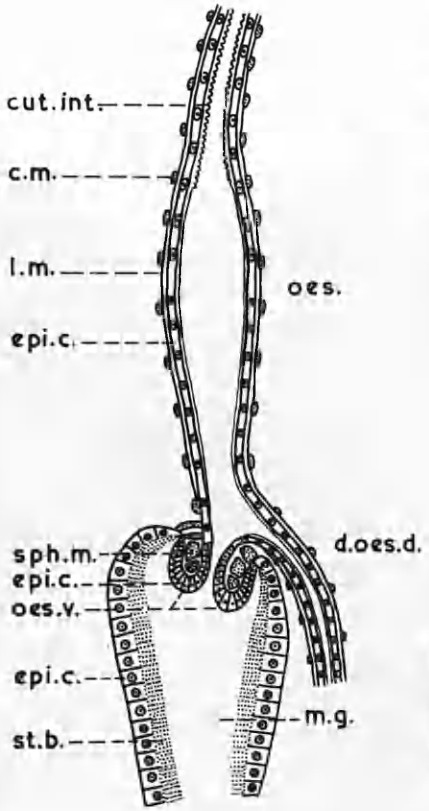


Fig. 15

Fig. 16

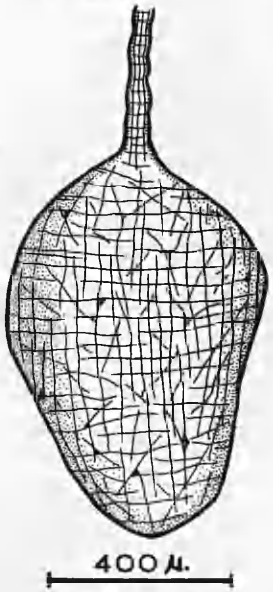


Fig. 17

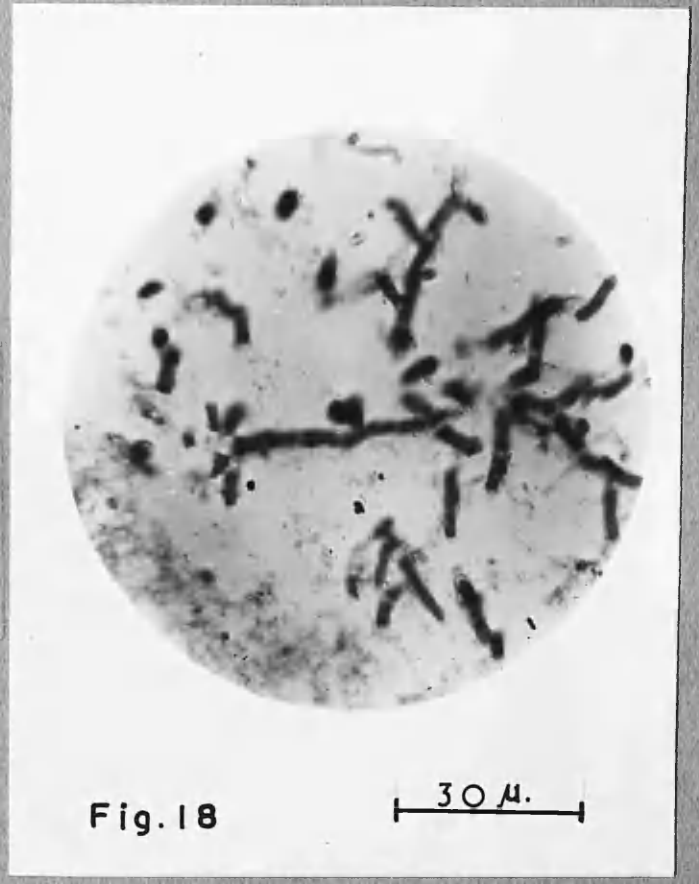


Fig. 18

C. nubeculosus Mg. - (female).

Fig. 19 Photograph of a horizontal section through the abdomen of a fly fixed directly after a small meal of blood - showing the hour-glass shape of the stomach due to peristalsis.

st. : stomach.

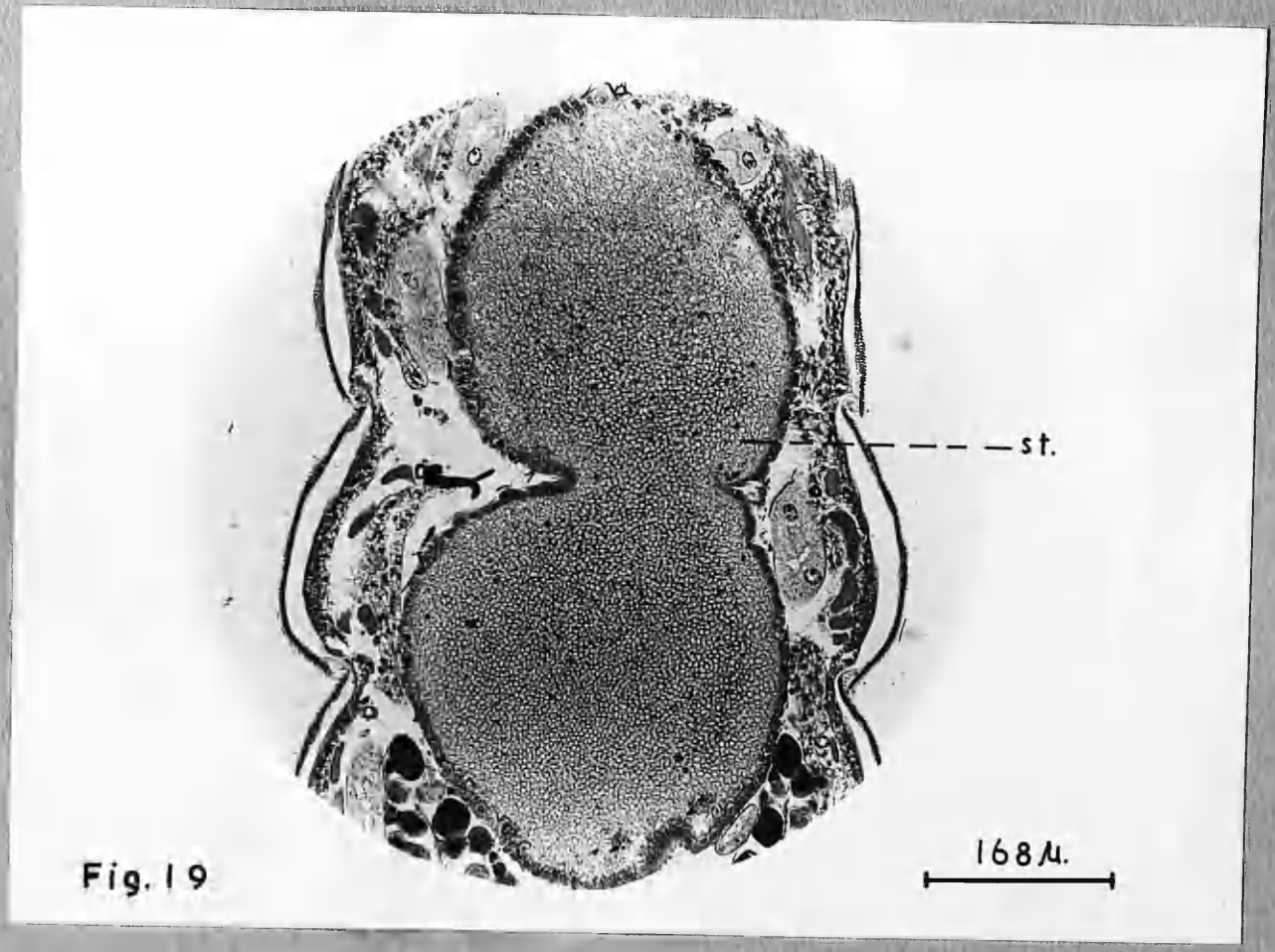


Fig. 19

C. nubeculosus Mg. - (female).

Fig. 20 T.S. through the anterior segment of the mid-gut in an unfed fly less than 24 hours old.

Fig. 21 T.S. through the stomach in the same fly.

Fig. 22 T.S. through the distended stomach of a fly 5 hours after a full meal of blood - showing the peritrophic membrane.

b. : blood.

b.m. : basement membrane.

c.m. : circular muscle.

epi.c. : epithelial cells.

h. : haematin.

l.m. : longitudinal muscle.

n. : nucleus.

pt.m. : peritrophic membrane.

pt.s. : peritrophic space.

rg.c. : regenerative cells.

s.pt.m. : droplets of secretion - the fluid precursor of the peritrophic membrane - secreted by the flattened epithelium of the stomach.

st.b. : stiated border.

v. : vacuoles.

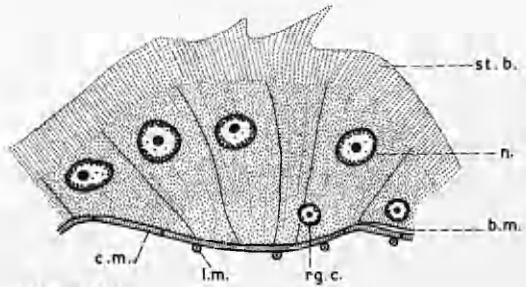


Fig. 20

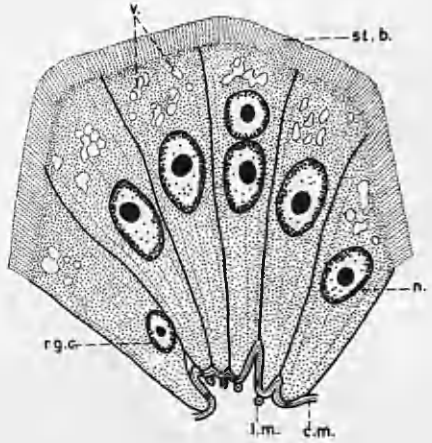


Fig. 21

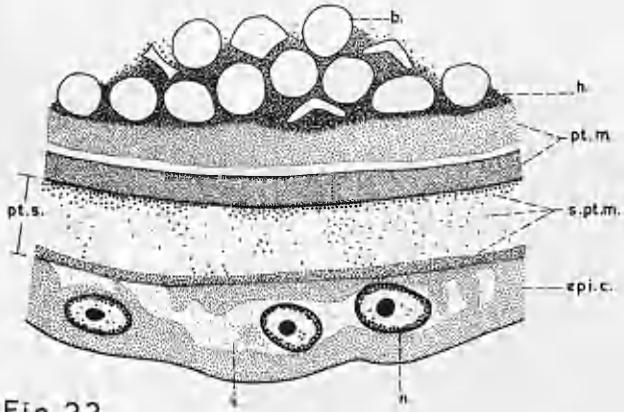


Fig. 22

15 μ.

C. nubeculosus Mg. - (female).

Fig. 23 Photograph of a horizontal section through the abdomen of a fly fixed 24 hours after a full meal of blood - showing basal perforation of the peritrophic membrane.

b. : blood.

epi.c. : epithelial cells.

f. : faecal matter.

h. : haematin.

mes.sph. : mesenteric sphincter.

pt.m. : peritrophic membrane.

st. : stomach.

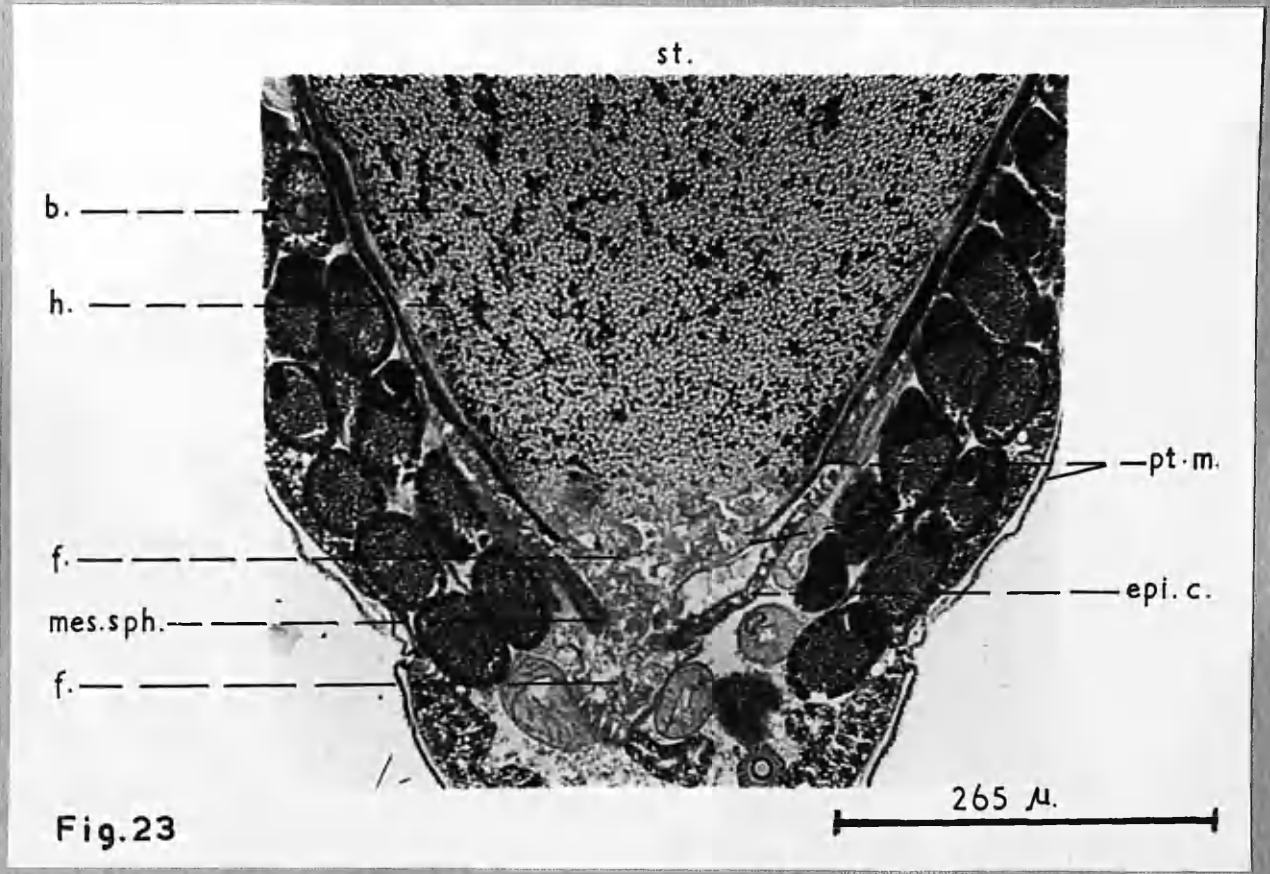


Fig.23

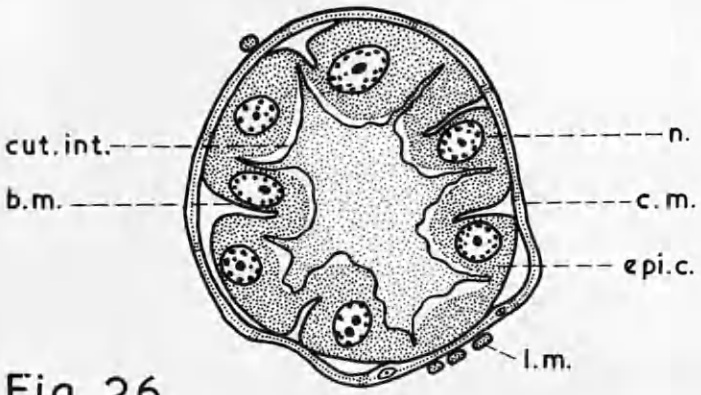
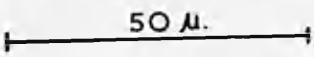


Fig. 26



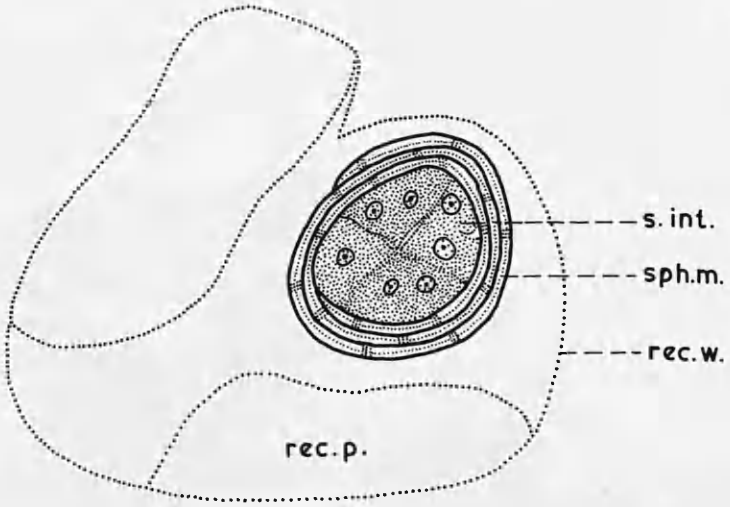


Fig. 27

C. nubeculosus Mg. - (female).

Fig. 28 H.S. through the posterior end of the small intestine, and the anterior part of the rectal sac.

Fig. 29 T.S. through the rectal sac.

c.m. : circular muscle.

cut.int. : cuticular intima.

epi.c. : epithelial cells.

l.m. : longitudinal muscle.

rec.p. : rectal papilla.

rec.s. : rectal sac.

rec.v. : rectal valve.

s.int. : small intestine.

sph.m. : sphincter muscle.

tr. : trachea.

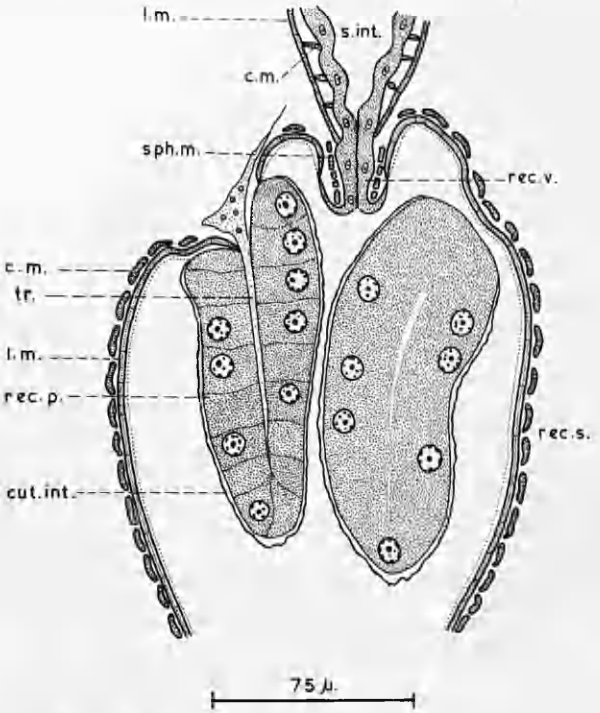

l.m.
s.int.
c.m.
sph.m.
rec.v.
c.m.
tr.
l.m.
rec.p.
rec.s.
cut.int.
75 µ.

Fig. 28

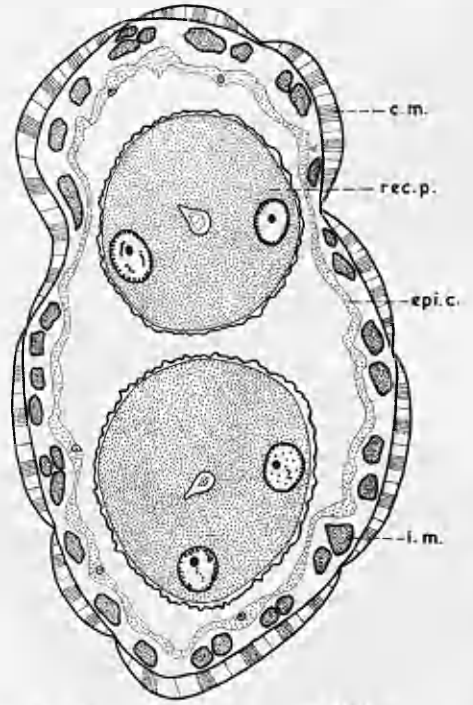

c.m.
rec.p.
epi.c.
i.m.
50 µ

Fig. 29

C. nubeculosus Mg. - (female).

Fig. 30 Diagram of a salivary gland and its accessory glands.

Fig. 31 H.S. through an accessory gland.

Fig. 32 T.S. through the posterior end of the salivary gland.

Fig. 33 L.S. through the anterior end of the salivary gland.

Fig. 34 T.S. through the anterior end of the thorax of an unfed fly - showing the salivary and accessory glands.

acc.g. : accessory gland.

b.m. : basement membrane.

cyt.s. : cytoplasmic strands.

epi.c. : epithelial cells.

m.g. : mid-gut.

n. : nucleus.

sec. : secretion in the lumen of gland.

sl.d. : salivary duct.

sl.g. : salivary gland.

thx.m. : thoracic muscles.

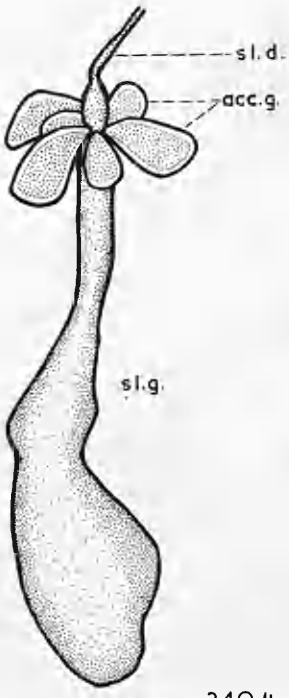


Fig. 30

240 µ

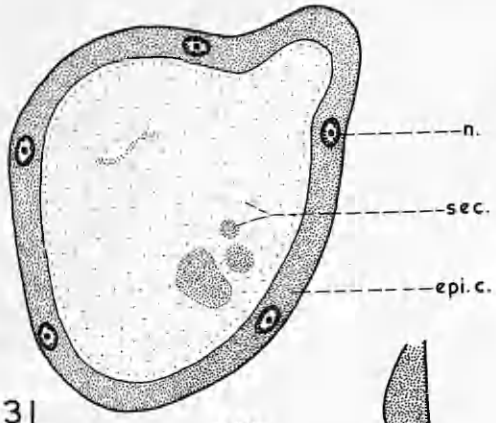


Fig. 31

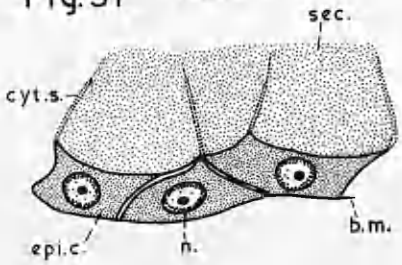


Fig. 32

50 µ

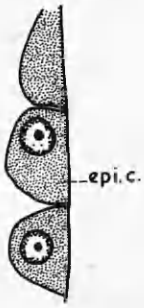


Fig. 33

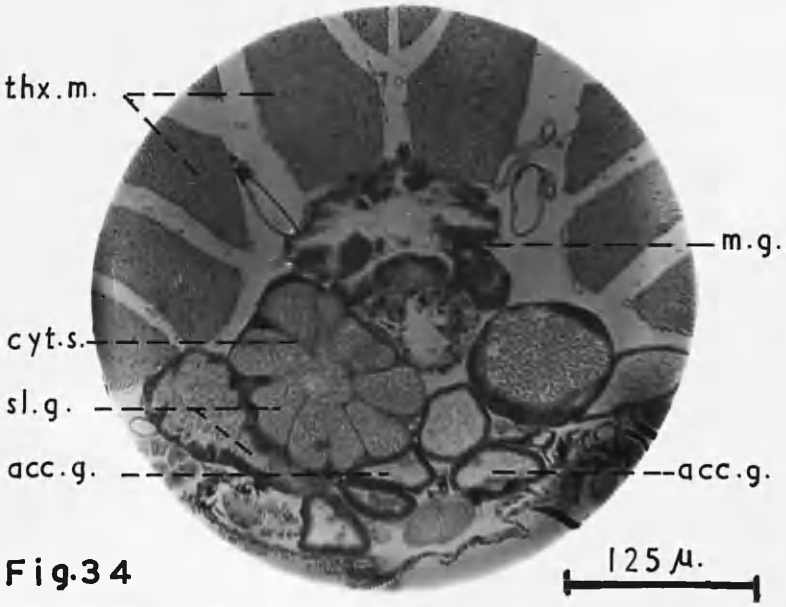


Fig. 34

General

The alimentary tract of female C. nubeculosus (Fig. 1) is a simple tube. The fore-gut consists of the sucking apparatus (in part), the oesophagus (oes.), the oesophageal valve (oes.v.), and the oesophageal diverticulum (oes.d.). The mid-gut consists of two portions, an anterior narrow portion (ant.m.g.) and a posterior dilated one (st.). The hind-gut consists of an anterior tubular portion which cannot be differentiated into parts (s.int.), and a posterior dilated rectum (rec.) which contains two rectal papillae (rec.p.).

In addition, there are two sets of accessory structures, namely, the salivary apparatus and the two Malpighian tubes (Mal.t.).

The alimentary tract lies in a median position, below the dorsal vessel and above the ventral nerve cord. It is held in position by the tracheae; also by the muscles of the sucking apparatus anteriorly, and the dilators and the sphincter of the anus posteriorly

THE FORE-GUT

The fore-gut of female C. nubeculosus consists of the sucking apparatus (in part), the oesophagus, the oesophageal valve, and the oesophageal diverticulum.

The Sucking Appartus

In Nematocera, the sucking apparatus consists of two chambers or pumps contained within the head-capsule. The first pump lies in the clypeal region, and the second lies in the back of the head. The nomenclature of these two pumps has been the subject of much controversy. Snodgrass (1943), however, shows clearly that the first pump is the homologue of the preoral cibarial pocket of the orthopteroid insects, and that the second pump is a modification of the pharynx. Thus, they are termed the "cibarial pump" and the "pharyngeal pump" respectively.

In C. nubeculosus, the cibarial pump (cib.p.) is a broad tube which occupies a median and rather vertical position within the head-capsule. It is continuous anterior-:ly with the epipharyngeal wall of the labrum and the anterior wall of the hypopharynx (see Fig. 3, e. & h.). In transverse sections (Figs. 11 - 13), it assumes the form of a basin-like trough. The dorsal wall of this pump

is thin and flexible and is seen collapsed against the lateral and ventral walls which are strongly sclerotized. Paired dilator muscles which originate on the clypeus are inserted in the midline of the dorsal wall (Fig. 13, d.cib.p.; see also Fig. 3, d.ph.). At the posterior end of the cibarial pump, the dorso-lateral parts are produced into two strong projections or cornua (Figs. 11 & 12, co.) which curve dorsally and serve for the attachment of the protractor (Fig. 12, pr.cib.) and the retractor muscles of the cibarial pump (see Fig. 3, pr.ph. & r.ph.). The cibar-:ial pump is covered with a compact layer of small epithelial cells (Fig. 13, epi.c.) which is in intimate con-:tact with the lateral and ventral walls but almost separated from the dorsal wall.

The pharyngeal pump (ph.p.) is shaped somewhat like a gourd being constricted in the middle and much broader at the posterior than at the anterior end. It lies between the suboesophageal (sb.g.) and the supraoesophageal (sp.g.) ganglia of the head (Fig. 5). The frontal ganglion (f.g.) lies immediately above its anterior end (Figs. 11 & 12). The posterior end of the pharyngeal pump joins the thin-walled oesophagus immediately behind the occipital foramen

(see Fig. 3, oe.).

In transverse section (Figs. 5 - 8), the pharyngeal pump is seen to be roughly triangular in shape, but the lumen varies at different levels (cf. Figs. 5 & 6, p.ph.p. and Figs. 7 & 8, a.ph.p.). It is composed of three plates, one dorsal and horizontal, and two latero-ventral, arranged in the form of a triangle, the base being superior, and all the three sides convex internally. Each plate is thicker in the middle than towards the edges. The three plates are hinged to each other through three small E-shaped sclerot-:ized bands. Two sets of dilator muscles are inserted into the dorsal plate : the precerebral dorsal dilators (pr.d.d.ph., Figs. 7 & 8; also see Fig. 3, a.d.d.) which arise on the apodemes of the frontal region and are inserted into the anterior part, and the postcerebral dorsal dilators (p.d.d.ph., Figs. 5 & 6; see also Fig. 3, p.d.d.) which arise on the occi-:put and are inserted into the posterior part. The lateral dilator muscles of the pharyngeal pump (l.d.ph., Figs. 5 & 6; also see Fig. 3, p.l.d.) arise on the edge of the postgenae and are inserted into the posterior part of the lateral plates.

The pharyngeal pump is a part of the fore-gut but it lacks the usual stomodeal sheath of circular muscle fibres,

except at the anterior and posterior ends; the anterior sphincter of the pharyngeal pump (a.s.ph., Figs. 9 & 10), and the posterior sphincter of the pharyngeal pump (see Fig. 3, p.s.oe.p.). The pharyngeal pump is covered with a layer of extremely flat epithelial cells (epi.c., Figs. 6 & 8).

The cibarial and the pharyngeal pumps meet at an angle just behind the posterior end of the clypeus.

The sucking apparatus of C. nubeculosus resembles that of C. pulicaris (Jobling, 1928), and that of C. impunctatus (Gad, 1951). But Jobling termed the cibar-:ial pump the "pharynx" and the pharyngeal pump the "oesophageal pump", and Gad did not mention the anterior and posterior sphincters of the pharyngeal pump.

According to Snodgrass (1943), no information is at present available as to the functional relations of the two pumps, but it may be supposed that their respective expansions and contractions have opposite rhythms, one contracting as the other expands, so as to give a contin-:uous flow to the stream of liquid food. The sphincter muscles present at the junction of the cibarial and pharyn-:geal pumps and behind the second pump constitute a

regulatory system.

The Oesophagus

The oesophagus (oes., Fig. 15) is a thin-walled, narrow, and short tube. It is about 225μ long from its beginning at the posterior end of the pharyngeal pump to the anterior limit of the mid-gut. It is very narrow anteriorly but it expands gradually to become about 36μ wide in its widest portion. It proceeds from its point of origin just behind the occipital foramen backwards through the cervix and joins the mid-gut about the level of the fore-legs.

The wall of the oesophagus consists of a layer of ill-defined epithelial cells (epi.c.) which differ in shape from low cubical to flat, according to the degree of con-:traction or expansion of the oesophagus. In the anterior portion of the oesophagus the nuclei of the epithelial cells are somewhat big and are oval in shape, but posteriorly they are round and rather small. The epithelial layer rests on a delicate basement membrane. The oesophagus is surrounded by an internal layer of longitudinal muscle fibres (l.m.) and an external layer of circular muscles (c.m.). The cuticular intima (cut.int.) which lines the inner surface of the epithelial cells is thin. It is easily detected anteriorly

where it is of a dark colour and has a serrated appearance, but it becomes so thin and colourless posteriorly that it becomes difficult to detect in most sections.

The Oesophageal Valve

The posterior end of the oesophagus is invaginated into the anterior portion of the mid-gut to form a regulat-:ory valve - the oesophageal valve (oes.v., Fig. 15). The oesophageal wall extends for a short length (about 27μ) into the lumen of the mid-gut (m.g.), then is reflected forwards to join the anterior end of the latter. It consists of a single layer of small but well-defined cubical cells (epi.c.) with small round nuclei. The stomodaeal circular muscles around the invagination are highly developed to form the sphincter muscles (sph.m.), occluding the lumen by their contraction. It is very difficult to detect a cuticular lining, but the cells of the valve, however, can be easily differentiated from those of the mid-gut. They are obvious-:ly smaller and narrower, and they lack the striated border (st.b.) which is characteristic of the cells of the mid-gut.

The oesophageal valve of C. nubeculosus plays no role in the formation of the peritropic membrane, and seems to serve only to regulate the influx of the ingested fluid into

the mid-gut, and to prevent its regurgitation.

The Oesophageal Diverticulum

Just in front of the point of invagination of the oesophagus into the mid-gut, the ventral wall of the oesophagus is evaginated to form a single capacious dumb-bell-shaped diverticulum (oes,d., Fig. 1) which lies ventral to the mid-gut, with some inclination to the left when distended with fluid (in some specimens at least). The oesophageal diverticulum consists of a dilated sac with a long narrow duct (d.oes.d., Fig. 1) : the sac is capable of much distension, while the duct is far less capable of distension. When empty, the diverticulum is collapsed and lies in the thorax or, sometimes, may extend into the anterior end of the abdomen. When distended with water or sugar solutions or raisin sap, it extends into the abdomen to a level which varies according to the degree of its distension and may reach the 4th or 5th abdominal segment. When the diverticulum contains a small amount of fluid, this collects at the bottom of the diverticulum while the rest of its length becomes very contracted and collapsed. On the other hand, when the diverticulum is highly distended the contracted portion becomes relatively short.

When dissected out in saline solution, the diverticulum is seen to undergo vigorous peristaltic movements which are best demonstrated in the distended diverticulum. In the latter case, the movements are seen to follow one another from one side to the other and from the fundus upwards, and vice versa (thus ensuring for some reason a thorough churn-:ing of the contained fluid). During peristalsis, however, the outline of the distended diverticulum becomes very variable and takes on many curious shapes (Fig. 16).

The oesophageal diverticulum is a very thin-walled structure. In sections, when the diverticulum is empty, it is so collapsed that its wall only appears as a wrinkled membrane with a few scattered small nuclei. On the other hand, when it is distended only a very thin outline can be seen with a still thinner cuticular intima. In favourable sections of the anterior portion of the duct of the divert-:iculum (d.oes.d., Fig. 15), however, it is seen to be of the same structure as that of the oesophagus. The muscular-:is of the dilated part of the diverticulum was studied by using the method of Nuttall & Shipley (1903), i.e. by simply allowing the dissected diverticulum (distended) to dry outwardly on a slide and then staining with aniline or

other stain; eosin in water was found satisfactory. It consists of a network of muscle fibres, the longitudinal and the circular fibres are incomplete. There are also diagonal interlacing fibres. These muscle fibres are seen to be branches of small muscle bands (Fig. 17). Such an arrangement of the muscle fibres is responsible for the vigorous peristaltic movements and the variable appearance of the distended diverticulum.

Adler & Theodor (1926) state that in female Phlebotomus papatasii there is a very narrow sphincter at the junction of the oesophagus and the diverticulum. I was unable to detect such a sphincter in C. nubeculosus, and it seems that the diverticulum opens into the oesophagus by means of a simple pore as is the case with the ventral diverticulum in mosquitoes (Nuttall & Shipley, 1903). It was observed, however, that when the dissected diverticulum is distended with fluid, the upper undistended part (sometimes long, sometimes short, according to the degree of distension) is extremely contracted, and when the contracted part of the diverticulum is excised anywhere above this level the contained fluid does not flow out.

It is noteworthy that in sections of some females the

lumen of the oesophageal diverticulum was seen to contain fungal hyphae (Fig. 18). The spores of the fungus must have been ingested with the raisin sap and have developed within the diverticulum.

THE MID-GUT

The mid-gut, or ventriculus, of female C. nubeculosus is a simple straight tube of varying diameter. It extends from the base of the outer fold of the oesophageal valve in the anterior part of the thorax, about the level of the posterior limit of the bases of the fore-legs, to the point of origin of the Malpighian tubes about the middle of the 5th abdominal segment. The relative position with regard to the abdominal segments, however, varies according to the degree of distension of the gut with food. It consists of two portions which merge into one another; an anterior narrow portion, and a posterior portion which is several times wider. At its posterior end the gut narrows abruptly, to form the mesenteric sphincter (ventricular valve), before it joins the hind-gut.

The anterior portion of the mid-gut, which in related insects is termed the "cardia" by some authors (e.g., Cragg, 1920; Adler & Theodor, 1926), is here termed the anterior

segment (ant.m.g., Fig. 1). The term cardia should be restricted to the anterior end of the ventriculus (Snod-:grass, 1935, p. 361). According to the same author (p. 360), nearly all the students of the alimentary canal of Diptera have called the cardia the "proventriculus", but its true nature is shown by the fact that the stomodaeal valve is invaginated into its anterior end. In female C. nubeculosus, however, the cardia is less differentiated and is clearly the anterior part of the mid-gut. The posterior segment of the mid-gut, as in related insects, (e.g., mosquitoes, Phlebotomus papatasii, Simulium, Tabanus, etc.), is here termed the stomach (st., Fig. 1). When empty, it has a tubular shape but more usually it takes on an ovoid shape due partly to traction during dissection and partly to its containing some fluid.

The anterior segment is widest anteriorly especially at the cardia. It extends to the posterior end of the thorax (or the anterior end of the abdomen) where it merges into the stomach. The anterior segment does not become distended with blood, in marked contrast with the stomach. In sections of flies fixed directly after a full meal of blood, the anterior segment is seen to contain varying

amounts of blood but is never distended with it. When the gut is dissected out after such a meal, usually no more than a trace of blood can be seen in the anterior segment. This is due partly to traction during dissection and partly to the release of the skeletal pressure against the stomach resulting in the blood being driven from the anterior segment into the stomach.

The stomach is the chamber into which almost all the ingested blood is received, and it is capable of great distension. In non-blood-fed flies it reaches the middle of the 5th abdominal segment, but when fully distended with blood it fills the greater portion of the abdomen, the rest of the viscera being pushed into the last two or three segments. Horizontal sections of the engorged flies show the stomach to be ovoid in shape. In dissections, the distended stomach assumes a globular shape, but this, of course, is due to the release of the skeletal pressure against it.

Normally, the stomach lies about the middle-line of the abdomen, but its position varies greatly according to the condition of the oesophageal diverticulum and the ovaries. When the diverticulum is distended and the stomach

is empty, the portion of the latter situated above the diverticulum is pushed towards the dorsal vessel, and the rest of the stomach curves down and lies against the ventral nerve cord. When the stomach is distended with blood, the distension of the diverticulum apparently does not matter; the distension of the abdomen seems to cope with both of them. Lewis (1953) observed that in Simulium damnosum, ½ - 1 hour after a blood meal, the tubular part of the mid-gut though devoid of blood contained some gritty particles similar to those found in the oesophageal divert-:iculum. He suggests that the diverticulum under pressure from the expanding mid-gut may disgorge some of its contents into the oesophagus from which they pass into the mid-gut. I was unable to detect this in C. nubeculosus, though it very probably occurs. When the ovaries are developed, they press the stomach against the ventral nerve cord.

The mid-gut shows peristaltic movements when dissected out in saline solution. This is most marked in the stomach especially when it contains syrup or a small amount of blood. In horizontal sections of some flies fixed after a small meal of blood, the stomach was seen contracted about the centre, assuming the form of an hour-glass (Fig. 19, st.);

a similar appearance was observed in the stomach of mosquitoes by Nuttall & Shipley (1903).

When empty, the surface of the mid-gut, and particu-:larly that of the stomach, has a roughened and irregular appearance, due, as will be seen presently, to the heaping up of the epithelium into folds or villi.

The mid-gut differs from both the fore - and the hind-gut in histological details. The main thickness of its wall (Figs. 20 & 21) consists of a single layer of epith-:elial cells which rests upon a basement membrane (b.m.). Following the basement membrane there is a double layer of inner circular (c.m.) and outer longitudinal muscles (l.m.).

The ventricular epithelium varies greatly according to functional activity. When the gut is empty, the cells are heaped together into various folds or villi which project into the lumen and give it a stellate appearance in trans-:verse sections. Each villus carries with it the basement membrane and the muscularis. The depth of these villi, and consequently the width of the lumen, is very variable among different specimens, but in general they are deeper in the stomach than in the anterior segment. After a full meal of blood, the greater part of the length of the wall of the

stomach suffers much distension, and the epithelial cells flatten and the villi disappear (Fig. 22). When the fly is allowed a smaller meal, the villi become reduced or disappear totally, and the epithelial cells become more or less low cubical in shape. In the anterior segment, however, the villi do not disappear nor the cells flatten after a blood meal; the amount of blood in the lumen is always small and does not cause any distension of the anterior segment.

In the unfed flies the epithelial cells are large, columnar or high cubical in shape, but, at any rate, their height and shape differ according to their situation. The cytoplasm is granular with a conspicuous fibrillar arrangement along the long axis of the cell. The nucleus (n.) is large, oval or round in shape, with a conspicuous nucleolus, and the chromatin granules are arranged mostly at the periphery. Each cell has one nucleus, but some cells, especially in the stomach, have two nuclei. The nucleus is usually situated towards the inner end of the cell, but in some cells it is central.

The striated border (st.b.) is evident in all the cells of the mid-gut. It stains pink with eosin. In the stomach

the striated border may disappear or become homogeneous due to the enormous vacuolation or flattening of the cells. The variable character of the striated border was observed in Glossina by Wigglesworth (1929, Footnote pp. 291, 308) who states that it agrees with the observations of van Gehuchten upon the epithelium in the larva of Ptychoptera (Ptychopteridae, syn. Liriopeidae).

The basement membrane on which the cells rest is elastic. Its elasticity is indicated by two facts. The first is that it is carried with the cells when they are thrown into villi. The second is that it stretches when the stomach is distended and the cells are flattened.

There are, however, some differences between the epithelium of the anterior segment and that of the stomach (cf. Figs. 20 & 21). The cells of the anterior segment do not stain as well with haematoxylin as those of the stomach, and are relatively small. Their nuclei are also smaller than those of the cells of the stomach, and are usually round in shape, while those of the stomach are usually oval. The most marked difference, however, rests in the striated border. In the anterior segment the striated border is almost always thick, sometimes homogeneous but never disappears except in

occasional cells which are about to discharge vacuolated masses of cytoplasm. In the stomach the striated border is always thin and may become homogeneous or totally dis-:appears. Smart (1935) noticed that in Simulium ornatum the striated border is thicker in the anterior segment than in the stomach. Adler & Theodor (1926) describe a thick striated border in the cells of the anterior segment in Phlebotomus papatasii, and state that the cells of the stomach lack the striated border. Cragg (1920) states that the striated border (intima) of the anterior segment in Tabanus is remarkably of a great thickness. The cells of both segments show vacuoles. In the epithelium of the anterior segment vacuoles are relatively few and are general-:ly small, and are located at the bases of the cells, though vacuoles at or towards the inner ends are not uncommon. In the epithelium of the stomach vacuoles are usually large and are located at the inner ends of the cells but they may be found at or towards the base (cf. Figs. 35 & 36 and Figs. 37 & 38, v.).

Scattered throughout the ventricular epithelium, there are small cells - the regenerative cells (rg.c., Figs. 20 & 21) - which occur singly. They are generally few in

number and are more easily demonstrated in the epithelium of the anterior segment. They are usually seen near the basement membrane or wedged between the bases of the columnar cells. What can be readily seen of these cells is the nucleus, which is small. Their cytoplasm cannot be differentiated from that of the epithelial cells; in some instances, however, it can be seen as a clear area.

The muscular coat of the mid-gut is thin. It consists of an inner layer of circular fibres, which are in close contact with the basement membrane, and an outer layer of longitudinal fibres. This double layer of muscle fibres forms a network which adapts itself to the irregularities of the surface of the mid-gut dipping into the hollows at the bases of the villi and also between the bases of adjacent cells (Fig. 21).

At its posterior end, just above the openings of the Malpighian tubes, the stomach narrows abruptly to form the mesenteric sphincter (mes.sph., Fig. 24). The circular muscles of this region are exaggerated to form the sphincter muscles (sph.m.). The cells of the mesenteric sphincter seem to lack a striated border, but it is common to see them vacuolated, eg., when the stomach is highly vacuolated. As

in the tsetse flies (Lester & Lloyd, 1928), this sphincter serves a double purpose. It holds back the blood meal, allowing only waste products to pass, and in the act of opening it closes the openings of the Malpighian tubes so that faecal matter cannot enter them.

The mid-gut, and especially the stomach, is richly supplied with trachea and tracheoles which rest on the outer surface of the organ. Many transition-cells are also seen on the surface of the mid-gut.

The Peritrophic Membrane

The peritrophic membrane (pt.m., Fig. 22) is a very thin capsule which envelops the ingested blood. It does not exist in the unfed females, and only forms in the stomach after a meal of blood. When the blood is digested the membrane is evacuated with the excreta.

In females fixed directly after a full meal of blood, the amount of blood in the anterior segment is seen in direct contact with the striated border of the cells. For a short length of the anterior end of the stomach the blood is also seen in direct contact with the striated border or the inner end of the cells when the former is absent. Following this region of the stomach, the blood is separated from

the flattened epithelium by a thin layer of pink fine granules (droplets) - the fluid precursor of the peritro-:phic membrane - which condenses later around the ingested blood. This layer is rather thick at the posterior end of the stomach.

One hour after a full meal, the blood recedes from the anterior segment; the blood plasma with a few corpuscles embedded in it forms a pink mass which occupies the lumen of the base of the anterior segment and that of the anterior end of the stomach. The peritrophic membrane is still re-:presented by a layer of pink granules which is thickest anteriorly between adjacent villi and posteriorly above the mesenteric sphincter.

Five hours after a full meal, the blood mass is seen separated from the epithelium, and the peritrophic membrane, which stains pink, attains a definite outline though not uniform in thickness or, in some places, in texture. The blood plasma by now has receded from the anterior segment and forms a plug above the blood mass in the stomach. This plug is pink anteriorly, brown posteriorly, and of both colours in between.

In the villated anterior end of the stomach, the space

between the peritrophic membrane and the epithelium - the peritrophic space (pt.s.) - is considerable. The outline of the peritrophic membrane is irregular and shows the impression of the villi. Following this region - in the distended part of the stomach - the peritrophic membrane becomes thinner and the peritrophic space narrower. In the region above the mesenteric sphincter, the peritrophic membrane thickens again and the peritrophic space widens.

In some sections the membrane is seen to be composed of successive layers, the innermost of which is the oldest and is rather thick and brown in colour. In favourable places the epithelial cells were seen secreting droplets of various sizes (s.pt.m., Fig. 22) which stain deep pink with eosin. These droplets stream towards the peritrophic membrane and add to its substance. It may be assumed that these droplets, or some of them, are digestive secretions. But the fact that they do not appear twenty-four hours after the meal, while the stomach still contains many intact corpuscles does not favour this assumption. In some places the outer surface of the peritrophic membrane is seen to be indented. This indentation has been noticed to be due to the agglomeration of the droplets secreted by the epithelial

cells on the surface of the membrane.

These observations indicate that the peritrophic membrane of female C. nubeculosus originates as a secretion of the cells of the stomach, which condenses around the ingested blood. Also, that it is composed of successive layers or lamellae.

Twenty-four hours after a full meal, the laminated peritrophic membrane is still seen surrounding the blood mass except above the mesenteric sphincter (mes.sph.), where it is perforated in the centre, and faecal matter (f.) is seen passing down to the hind-gut through the relaxed sphincter (Fig. 23). The membrane by now is mainly brown in colour (light to deep) but faint pink portions may also be seen. Myriads of dark-brown granules and rods - haematin or other derivatives of the blood pigment - are to be seen immersed in its substance (this may be an artifact). One or more layers of the peritrophic membrane may show partial disintegration, but as a capsule enveloping the blood the membrane remains intact; it is only at its posterior end that the membrane becomes wholly perforated.

Forty-eight hours after the full meal, the blood is practically digested and the haematin residue makes the

identification of the peritrophic membrane very difficult. In some places, however, it can be traced.

Seventy-two hours after the full meal, the stomach becomes empty and crumpled, and the peritrophic membrane has been discharged with the faeces. In one female dissect-:ed after laying her eggs, during the fourth day after a full meal, the peritrophic membrane was seen gripped by the mesenteric sphincter and projecting into the hind-gut, as is recorded in some females of Simulium damnosum by Lewis (1953).

In females which took an incomplete meal of blood, an evident peritrophic membrane was seen in specimens fixed five hours after the meal. It is composed of one layer which is of considerable thickness, a feature which was ob-:served in Simulium damnosum by Lewis (1953).

It was found difficult to get a reaction for chitin from this membrane. Sections stained with Bethe's stain gave a negative result. Professor V.B. Wigglesworth (personal communication) kindly informed me that Bethe's stain is of almost no value as a test for chitin, and that the only satisfactory test is the chitosan test. Guts of flies 1 - 2 days after a full meal were used; these are less

distended with blood and can be dissected out without the least injury to them. The chitosan test also gave a negative result; it should be noted, however, that the chitosan test gave also a negative result with the peri-:trophic membrane of the larva of Drosophila, which is known to be chitinous. The membrane, however, can be isolated by macerating the gut in lactic acid in an oven at 48°C. for a few hours. It was left in the lactic acid in the oven for several days without any change. It appears as a very thin and transparent membranous capsule sprinkled with haematin granules. It stains well with acid fuchsin.

A peritrophic membrane has been known to exist in some blood-sucking Diptera. In Anopheles maculipennis, a chitin-:ous peritrophic membrane is formed in the mid-gut each time the latter is filled with blood and on the completion of digestion it is evacuated with the excreta. It envelops the blood as a thin capsule, and in the early stages of digestion it is frequently open at the posterior end, and when the digestion is completed the membrane closes up. Apparently, a new membrane can be added to, or formed over the old one, which partly degenerates in the process of digestion (Yaguzhinskaya, 1940). A peritrophic membrane

forms in Anopheles culicifacies, A stephensi, A. subpictus, and Culex fatigans when the gut is distended with blood (Pal, 1943). Adler & Theodor (1926) state that in Phlebotomus papatasii (and also in P. minutus and P. perniciosus) a very definite peritrophic membrane is produced. It is a thin white amorphous structure which contains the mass of red cells, and may be likened to a sealed tube closed anteriorly and posteriorly; anteriorly it extends into the "cardia" and posteriorly into the hind-gut. Dolmatova (1942) states that a peritrophic membrane similar to that of Anopheles maculipennis forms round the ingested blood in P. papatasii. In Simulium damnosum and S. griseicolla, a peritrophic membrane envelops the ingested blood (Lewis, 1950). In S. damnosum (Lewis, 1953), the epithelium of the distended part of the mid-gut (i.e., stomach) secretes the peritrophic membrane which encloses the ingested blood. This membrane is chitinous, tough and inelastic, and is laid down in a series of laminae which probably continue to be added to for a short time. It usually remains complete for over 24 hours and part of it is seen when the blood has disappeared. A peritrophic membrane also forms in tabanids (Olsufev, after Yaguzhinskaya, ibid.). In Glossina, the

the peritrophic membrane is secreted by a group of specialized cells at the anterior limit of the mid-gut, and its secretion is enhanced by a blood meal (Wigglesworth, 1929). In Stomoxys calcitrans and Haematobia stimulans a peritrophic membrane is present in the females, whether they had fed or not (Kuzina, 1942); it forms 3 - 4 hours after emergence, and remains after the blood has all been digested.

There is much similarity between the peritrophic membrane of C. nubeculosus, Anopheles maculipennis (Yaguzhinskaya, ibid.) and Simulium damnosum (Lewis, 1953). They are secreted by the epithelium of the stomach only after a blood meal, are composed of several laminae, and rupture posteriorly some time after the meal. It is evident that the peritrophic membrane of these insects is different from the two types reviewed in textbooks (e.g., Wigglesworth, 1950,b, p. 313; Day & Waterhouse, 1953, p. 284) ; the first type is made up of concentric lamellae seperated from the surface of the cells throughout the mid-gut (not agreed upon universally), and the second type is a single uniform layer secreted by a group of cells at the anterior limit of the mid-gut. This difference may be the reason that Day &

Waterhouse (ibid., p. 288) state that "there is some doubt whether these are true membranes". But what is a true peritrophic membrane? Snodgrass (1935, p. 378) identifies the peritrophic membrane as "a cylindrical membranous envelope surrounding the food in the ventriculus, and sometimes extending into the proctodaeum; generated from the ventricular epithelium, either from all or a part of the length of the latter or from a ring of specialized cells at the anterior end". This generalized identification applies to the membrane which envelops the ingested blood in these nematoceran blood-sucking flies. Waterhouse (1953), however, found that the membrane of such insects as well as those of a variety of insects hitherto known to lack a peritrophic membrane, are real peritrophic membranes. He repudiates, what he says is, a rather widely held belief that the endodermal mid-gut is unable to produce a chitinous membrane. He also raises two interesting points : 1- the generalization that the fluid-feeding forms lack a peri-:trophic membrane must be modified, and 2- the embryonic origin of the cells at the anterior end of the mid-gut (said by him to have been reported as being ectodermal in origin) which secrete the peritrophic membrane of the second

type now requires re-examination.

It is generally agreed upon that the peritrophic membrane protects the cells of the mid-gut from damage by hard or sharp particles of food. Also, a number of dye-feeding experiments suggest that the peritrophic membrane acts as an ultra-filter with a maximum pore size varying somewhat from insect to insect (Day & Waterhouse, 1953, p. 306). The first view certainly does not apply to blood-sucking insects. Yaguzhinskaya (1940) suggests that in Anopheles maculipennis, it may have a bearing on the penetration of parasites into the wall of the mid-gut, and that the transmission of causal agents of disease by blood-sucking insects may depend partly on the character of the peritrophic membrane. Lewis (1953) states that the peritrophic membrane of Simulium damnosum plays an important part in limiting the number of microfilariae that survive to reach the sausage stage.

THE HIND-GUT

The hind-gut is composed of two regions; an anterior simple tube which shows no differentiation into parts - the small intestine, and a posterior rectum (s.int. & rec., Fig. 1).

The small intestine is a narrow tube of moderate length

which forms one or two loops in the abdominal cavity. The anterior portion of the small intestine is capable of much distension during the excretory activity of the mid-gut and the Malpighian tubes. The wall of the small intestine (Fig. 26) consists of a single layer of epithelial cells (epi.c.) which rests on a basement membrane (b.m.) followed by a sparsely arranged double layer of inner circular (c.m.) and outer longitudinal muscles (l.m.); the inner end of the cells is covered with a thin cuticular intima (cut.int.). The epithelial cells are fairly large and cubical in shape, the long axis of the cell being parallel to the basement membrane, and the intercellular walls absent. The cytoplasm does not stain well with haematoxylin. The nucleus is conspicuous (n.) and has an open arrangement of chromatin. The wall of the small intestine is thrown into small folds; but when the anterior portion of the small intestine is distended with excrement, the folds disappear and the epithelium flattens to a degree proportional to the degree of distension, and the nuclei become elongated and compressed.

The opening from the small intestine into the rectum is guarded by a simple valve - the rectal valve (rec.v., Figs. 27 & 28). This valve resembles the oesophageal valve

in that it is formed by a slight protrusion of the hinder portion of the small intestine into the lumen of the anterior end of the rectum, and its reflexion forwards to join the rectal wall (Fig. 28); the circular muscles of this region are exaggerated to form the sphincter muscles (sph.m., Figs. 27 & 28). The rectal valve has not been mentioned in the works of Adler & Theodor (1926) on Phlebotomus papatasii, Cox (1938) and Smart (1935) on Simulium spp., and Nuttall & Shipley (1903) and Christophers (1901) on mosquitoes. Graham-Smith (1934) described the rectal valve in Calliphora erythrocephala. He suggests that the circular muscle bands immediately proximal and distal to the valve seem to act as sphincters which, except when open, prevent the passage of the contents of the hind-gut forwards or of the contents of the rectum forwards to the valve itself. Wigglesworth (1932) found that in Lucilia sericata the rectal contents are separated from the rest of the hind-gut by a valve which obviates any reflux. Lester & Lloyd (1928) also described in the tsetse flies a valve which occurs between what they term the protorectum and the mesorectum. It has been observed in female C. nubeculosus that the contents of the small intestine do not pass directly

into the rectum. These contents keep on moving to and fro in the lumen of the small intestine for some time, as a result of the peristaltic and antiperistaltic move-:ments of the small intestine, before the rectal valve re-:laxes and they are allowed into the rectum; Graham-Smith (1934) described a somewhat similar phenomenon in Calliphora erythrocephala. This observation suggests that, apart from preventing any reflux, the rectal valve in female C. nubeculosus for some reason (probably the ab-:sorption of some nutrient by the cells of the small in-:testine) prevents the rapid and untimely entry of the excrement into the rectum.

The rectum is dilated anteriorly into a capacious sac - the rectal sac, and narrows posteriorly to form a short straight tube - the rectum proper - which proceeds direct to the anus. The rectal sac (rec.s., Fig. 28) is a capacious oval chamber which contains two rectal papillae (rec.p.) projecting into the lumen, and usually visible through the membranous wall of the rectal sac.

The rectum is thin-walled (Figs. 28 & 29), and the epithelium (epi.c.) is greatly reduced except where it forms the rectal papillae. A cuticular intima is very

difficult to detect except around the rectal papillae. The muscular coat of the rectum is well developed and its arrangement is the reverse of that of the small intestine, i.e., an inner longitudinal (l.m.) and an outer circular layer of muscles. In transverse sections of the rectal sac (Fig. 29), the circular muscles are seen to be composed of several crescent-shaped bands with obvious striation. The rectal papillae (rec.p., Fig. 28) are composed of large cells with very big nuclei. Each papilla is covered with a thin cuticular intima (cut.int.), and its lumen contains a tracheal branch.

Posteriorly, the rectum ends in the anus in the last abdominal segment. The anus is furnished with a strong sphincter muscle.

THE MALPIGHIAN TUBES

At the point of junction of the mid- and the hind-gut two Malpighian tubes (Mal.t., Figs. 1 & 24) of a white and light yellowish colour arise, one on each side of the gut. They are cylindrical, of considerable length, and of practically uniform diameter throughout. They take a convoluted course in the abdominal cavity (in transverse sections the same tube is infrequently cut twice), extending

forwards to the anterior end of the abdomen, then recurve and extend backwards to lie beside the rectum. They are bound to the gut with numerous tracheal branches.

At the entrance of the tubes into the gut (Fig. 24), they are seen to be lined for a short distance by relative-:ly small low cubical cells (a.c.) which stain blue with haematoxylin. These cells are continuous with and similar to the cells of the mesenteric sphincter. Very large cells (p.c.) soon appear. These cells contain many granules and droplets which conspicuously increase both in number and size after a meal of blood. The nucleus (n.) is very large and oval in shape. The inner border is markedly striated (st.b.). They are arranged alternately in a double row and surround a central lumen. The alternate arrangement of the cells gives the tube a wavy appearance; as a result of this appearance only one nucleus at most is cut in transverse section. The lumen of the tube is somewhat flattened but during excretory activity it dilates and contains many excretory granules and spheres. The cells rest on a basement membrane. The tubes are richly supplied with tracheae and tracheal end-cells.

The Malpighian tubes of C. nubeculosus were found to

give attachment to muscular branches of different sources. At the anterior region of the abdomen (Fig. 25) a branch of the alary muscles of the pericardial septum ($m._1$) is seen attached to them. At their basal end (Fig. 24), the tubes were found to receive longitudinal muscle fibres from the posterior end of the mid-gut ($m._2$). At the same place, the tubes receive a branch of the longitudinal muscle fibres of the small intestine ($m._3$) (this may be a small tracheal branch).

Eastham (1924) found that the Malpighian tubes of Drosophila funebris give attachment to a branch of the alary muscles of the pericardial septum, and also to circular and longitudinal muscle fibres continuous with the muscles of the mid-gut. In Rhodnius prolixus, Wigglesworth (1931, c) found only a few muscle fibres running out from the mid-gut over the ampullae to the lower end of the Malpighian tubes.

THE SALIVARY APPARATUS

The salivary apparatus of female C. nubeculosus consists of a pair of salivary glands, six accessory glands arranged around the mouth of each gland proper, two salivary ducts, and a common salivary duct, a salivary pump,

and the hypopharyngeal salivary duct.

The Salivary Glands

The two salivary glands (sl.g.) lie in the low anterior part of the thorax, ventral to the huge thoracic muscles (thx.m., Fig. 34), at both sides of the fore-gut and a little below the anterior segment of the mid-gut. Each gland (Fig. 30, sl.g.) is a tube which dilates gradually from before backwards, and bends upon itself in the form of a U. It is not uncommon, however, for one or both glands to lie straight and extend backwards into the anterior portion of the abdomen. In one case only, one of the two glands (with its corresponding accessory glands of which one only could be seen) was seen to extend forwards into the head-capsule; the salivary duct of this gland was obviously shorter than that of the other gland. This seems to be an abnormal case.

The salivary gland consists of a single layer of cubical cells (epi.c., Figs. 32 & 33) surrounding a central lumen which contains a granular eosinophil secretion (sec.). The intercellular walls are evident, and the cells rest on an obvious basement membrane (b.m.). The nucleus (n.) is large and conspicuous, oval or round in shape, and is

centrally situated. The cytoplasm is granular, stains blue with haematoxylin, and no vacuoles can be detected in it. The height of the secretory cells differs according to the degree of distension of the gland with secretion. When the salivary gland is highly distended, the cytoplasm thins out except around the nucleus which never becomes compressed.

In different sections of the lower dilated part of the salivary gland, cytoplasmic strands (cyt.s., Fig. 32 & 34) which stain blue are to be seen protruding into the lumen. This is not the case with the cells of the anterior portion of the gland (Fig. 33). The actual relation of these strands was difficult to ascertain. In the first sections of this part of the gland, these strands are seen to come together in a network with the granular eosinophil secretion filling its meshes except in one or two positions where complete polygonal cells are seen. In sections of flies killed after a full meal of blood, these strands are also seen. These observations may suggest that these strands are the remains of cells removed in previous sections. But, at any rate, it is also probable that the free end of the cells forms a pocket in which the secretion accumulates,

and when the pocket becomes fully distended it eventually bursts open to discharge the secretion into the lumen, leaving its sides as such protrusions. In the anterior part of the gland, though the lumen is also to be seen more or less full of the granular eosinophil secretion, the cells were sometimes seen discharging small cytoplasmic globules which stain blue.

Directly after a full meal of blood, the salivary glands contain only a trace or, sometimes, a very little amount of secretion. This is different from what occurs in the salivary glands of mosquitoes which, according to Christophers (1901), for the most part are still quite full of secretion after feeding. In a series of females section-:ed after one hour, five hours, and twenty-four hours after a full meal of blood, the salivary glands contained increas-:ing amounts of secretion. The method of secretion is not known.

After a small meal of blood the salivary glands contain considerable amounts of secretion; more or less inversely proportional to the amount of the ingested blood. This observation suggests that the saliva is not forced into the wound at once, but is discharged throughout the period

of feeding.

The Accessory Glands

The accessory glands (acc.g., Fig. 30) are six small saccular structures, three small and oval and three rather big and rectangular, arranged alternately in a rosette around the mouth of each of the salivary glands proper, each sac opening into it by means of a very tiny duct. They lie partly in the thorax and partly in the neck; the dissection of the salivary glands with the whole set of accessory glands is a very delicate job, and much concentration is required otherwise some of the sacs will be torn out. Each sac (Fig. 31) consists of a single layer of low or flat cells (epi.c.) which rests on a basement membrane, and surrounds a relatively wide lumen. The intercellular walls are absent. The nucleus (n.) is small and oval in shape. The cytoplasm is granular and stains blue with haematoxylin. In many of these sacs the lumen appears to be empty or contains a little quantity of a homogeneous secretion (sec.) which very faintly stains with eosin. In some others, traces of secretion similar to that found in the salivary glands proper, a little amount of a granular secretion which stains blue, and a few glistening, colourless, small droplets may

be seen in the lumen. In only one specimen, a fly fixed two days after a full meal of blood, two sacs only were found full of a granular eosinophil secretion similar to that seen in the salivary glands proper.

Patton & Evans (1929, p. 140) mentioned and figured only four sacs to each salivary gland of female Culicoides varius (?). They suggested that these sacs probably serve as temporary reservoirs for the secretion of the salivary glands. In C. nubeculosus, these sacs have been shown to be active secretory glands, the secretion of which is of a different nature from that of the salivary glands proper, and in rare cases some of them may serve as reservoirs for the secretion of the salivary glands proper. This case is somewhat similar to what has been described in some of the Hemiptera-Heteroptera, i.e., Notonecta. According to Baptist (1941), the accessory gland of Notonecta is vesi-:cular, the glandular epithelium is thin and flattened with small round nuclei, and it secretes a watery fluid but may serve as a reservoir of the principal gland under special circumstances.

The Salivary Ducts

At the point of junction between the salivary gland and its accessory glands emerges the salivary duct which is wide to begin with but soon narrows down (sl.d., Fig. 30). The salivary duct of each side passes through the neck into the head-capsule, and, a little distance in front of the occipi-:tal foramen and beneath and in contact with the suboeso-:phageal ganglion, the two ducts converge to the middle line (Fig. 14) where they unite to form the common salivary duct (c.sl.d.). The common salivary duct passes forwards till it opens into the salivary pump (sl.p.) underneath the cibarial pump (see also Fig. 3, c.sl.d. & sl.p.).

The salivary ducts and the common salivary duct are annulated tubes with chitinous walls covered with a thin layer of flat epithelium.

The Salivary Pump

The salivary pump of C. nubeculosus resembles that of C. pulicaris (Jobling, 1928) and C. impunctatus (Gad, 1951). It is a small capsule situated beneath the ventral wall of the cibarial pump. In transverse sections (sl.p., Fig. 13), the ventral wall of the salivary pump is cup-shaped, strongly sclerotized, and covered with a layer of small epithelial

cells (epi.c.). The dorsal wall is elastic. The dilator muscles of the salivary pump (d.sl.p., Fig. 13; see also Fig. 3, sl.p.m.), which arise on the ventral wall of the cibarial pump, are inserted into the middle of the elastic dorsal wall. The anterior end of the salivary pump continues as the hypopharyngeal salivary duct.

The expulsive power of the pump results from the elasticity of its dorsal wall. According to Jobling (1928), when the dilators contract, the elastic dorsal wall becomes convex, the resulting negative pressure drawing the saliva into the lumen of the salivary pump. When these muscles relax, the dorsal wall invaginates into the lumen of the pump, pushing the saliva through the hypopharyngeal salivary duct.

A NOTE ON TUMOURS IN THE ALIMENTARY TRACT, AND ABNORMAL FEATURES IN THE MALPIGHIAN TUBES.

In the course of this study of the alimentary tract of female C. nubeculosus, tumours were observed in one specimen only. These (tu., Fig. 2) were several protruding, well defined, saccular swellings of different sizes situated at the base of the stomach (st.). Two of them had a triangular dark-brown area, and one had a small round spot of the same

colour. The cause and pathogenicity of these tumours are not known. It would be interesting to know if these tumours occur in the developing stages and, if they do occur, to what extent they affect these stages.

Very few insects are known to develop tumours. In Drosophila melanogaster (Russell, 1940; 1942) both non-genetic and mutiple genetic factors influence the produc-:tion of tumours. None of these tumours is malignant and all of them may be transplanted successfully without hampering the development of the host. It is suggested that pupation may restrict these abnormal growths and pre-:vent their becoming malignant. In the cockroach Leucophaea maderae (Scharrer, 1945; 1948), severence of the recurrent nerve causes the development of tumours in organs supplied by this nerve. The sites of experimentally induced neo-:plastic growths are the fore-gut, the anterior mid-gut, the salivary glands, and the salivary reservoirs. These tumours are well defined conspicuous tissue masses which in advanced stages can be easily noticed with the naked eye. Features indicating the malignant character of these tumours are their tendency to invasive growth and their transplantability. A few specimens not operated upon showed changes in the

alimentary canal which, although of milder degree, were comparable to the tumours developing after the section of the recurrent nerve. According to Scharrer (1945), a brain tumour in an ant was described by Brun, and allatectomy of young nymphs of the orthopteran Dixippus was shown by Pflugfelder to be followed by tissue growth and degeneration. The European spruce sawfly Gilpinia hercyniae (Bird, 1949) develops non-malignant tumours after infection with the virus causing a "virus polyhedral disease". In the diseased larva the virus affects only the digestive cells of the mid-gut epithelium. Most of the proliferations project into the body cavity, but infiltration into the cytoplasm of the digestive cells does occur. Large tumours are formed only when the larva becomes infected just prior to the last larval moult. The tumours rapidly mature during an early stage of pupation. In the adult some of the tumours may be pushed out into the body cavity during pupation, where they remain as non-living, hard, dark-brown pellets throughout the life of the adult.

Another abnormality in the same specimen was seen in the Malpighian tubes (Mal.t., Fig. 2). In normal females the two Malpighian tubes are cylindrical and of a whitish

or yellowish colour. They extend forwards to the anterior end of the abdomen then recurve and extend backwards to lie beside the rectum (Fig. 1). In this specimen they were of a deep orange colour. The base of each tube was swollen and ampulla-shaped (amp., Fig. 2) followed by a small narrow area after which the tube was flattened for a considerable length before it took its usual cylindrical shape. Each tube was coiled in a mass, intermingled with many tracheae and tracheal branches, and lay beside the hind-gut. It is not known if these abnormal features in the Malpighian tubes are related to the presence of the tumours in the base of the stomach.

SECTION 111.

THE CHANGES IN THE MID-GUT EPITHELIUM FOLLOWING A BLOOD MEAL.

THE CHANGES IN THE MID-GUT EPITHELIUM FOLLOWING A BLOOD MEAL.

The flies used in this study were fed on the writer's arm. The unfed flies were given a daily opportunity to take a blood meal. All the flies, except those used during the first day after emergence, had access to water and raisin before and after the blood meal. All the flies were kept at 20°C. and 80 - 90 per cent. relative humidity.

The mid-gut epithelium in the females which refused to feed, and mainly that of the stomach, showed differences in specimens of different ages. In flies of different ages fixed directly after a meal of blood, the mid-gut epithelium, except for some insignificant variations in the anterior segment, was almost the same. Accordingly, the blood-fed females used were of different ages.

Flies which were given a small meal of blood were fixed directly (within 1 minute), 1 hour, 5 hours, or 24 hours after the meal. The term "small meal" implies that the fly had been given about half of the full blood meal. It was not possible to standardise a time-limit so as to obtain a standard small meal. This end was only achieved by judging by sight. The flies were fed individually. To remove the feeding fly from the skin, a tube containing a trace of

ether was inverted on it; this causes the fly to withdraw spontaneously but does not narcotize it.

Flies which were allowed a full meal of blood were fixed directly (within 1 minute), 1 hour, 5 hours, 24 hours, 48 hours, 72 hours, 96 hours after the meal, or after oviposition. The females used in the latter case were those which laid their eggs during the fourth day after a full meal.

The technique used in preparing the sections is that described in Section 11. No chemical or enzymologi-:cal studies were intended. As the substances discharged by the cells are fluid in nature, it should be noted that their appearance in preparations as globules or granules is largely due to their precipitation and coalgulation by means of the reagents used. For convenience, the brown material - derivative of the blood pigment - which occurs during the digestion of the blood is termed haematin throughout.

C. nubeculosus Mg. - (female).

Fig. 35 T.S. through the anterior segment in a fly less than 24 hours old, which refused to take a blood meal.

Fig. 36 T.S. through the anterior segment in the same fly (another section).

Fig. 37 T.S. through the stomach in the same fly.

Fig. 38 T.S. through the stomach in a fly 72 hours after a full meal of blood, showing highly vacuolated cells heaped into a very high villus (striated border homogeneous).

b.m. : basement membrane.

c.m. : circular muscle.

dg.n. : degenerate nucleus.

l.m. : longitudinal muscle.

n. : nucleus.

st.b. : striated border.

v. : vacuole.

vs. : cytoplasmic globule.

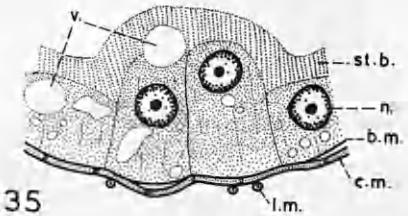


Fig.35

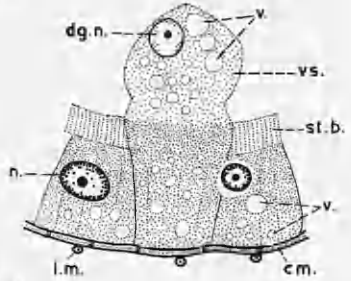


Fig.36

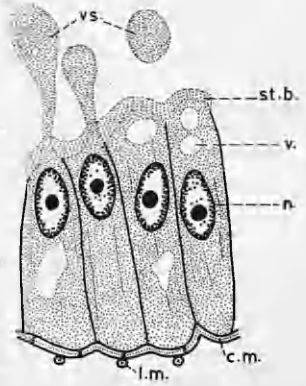


Fig.37

15 µ.

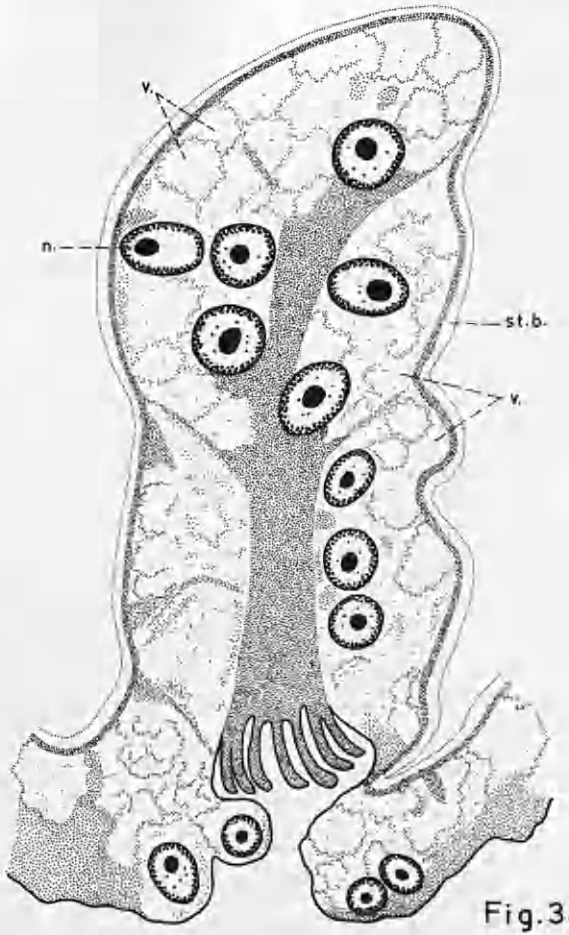


Fig.38

C. nubeculosus Mg. - (female).

Fig. 39 Photograph of a horizontal section through the anterior end of the stomach 24 hours after a full blood meal - showing the long vacuolated cells and the discharge of cytoplasmic globules. (Same fly as in Fig. 23).

epi.c. : epithelial cells.

h. : haematin.

v. : vacuole.

vs. : cytoplasmic globule.

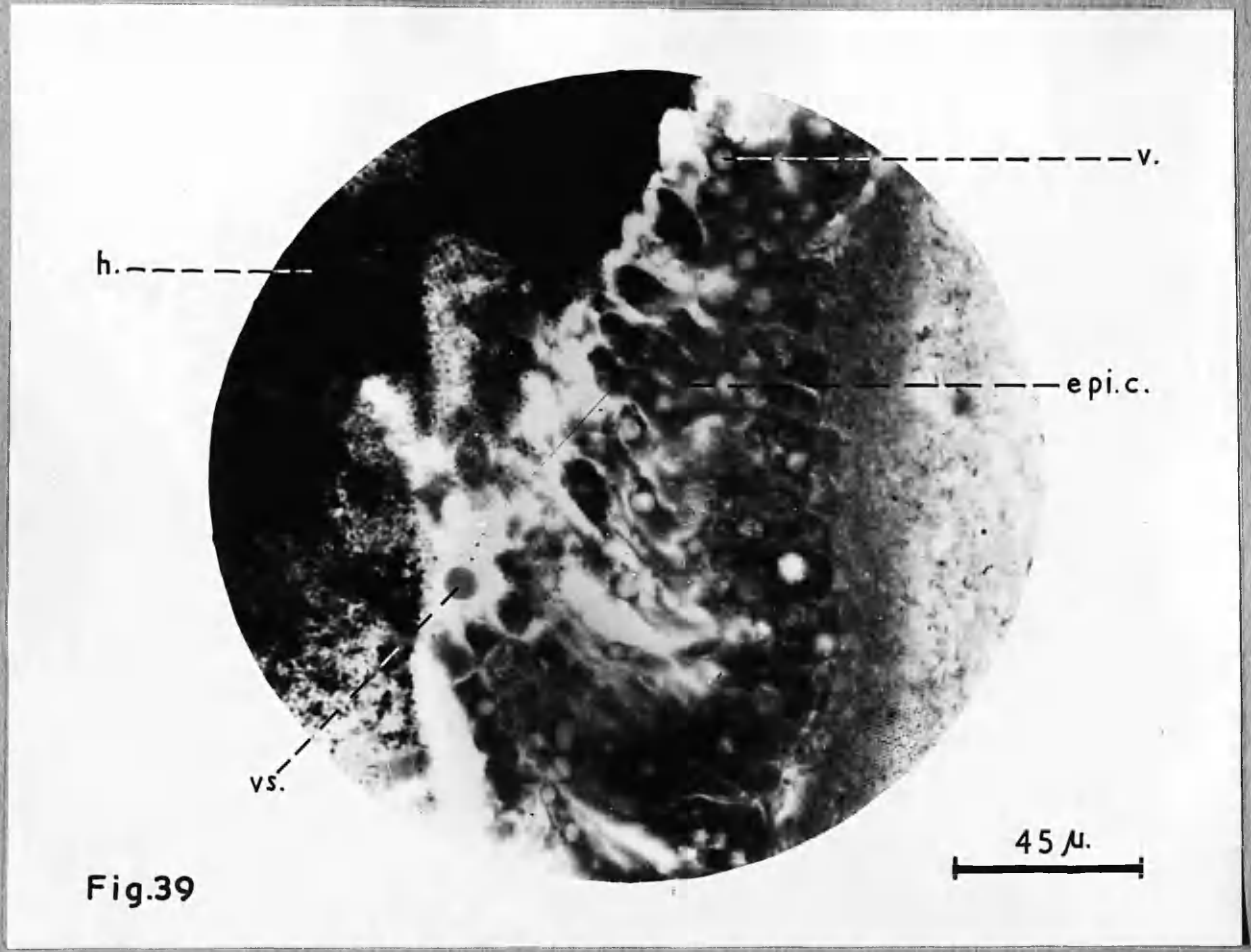


Fig.39

C. nubeculosus Mg. - (female).

Fig. 40 Photograph of a horizontal section through the posterior end of the stomach 24 hours after a full blood meal - showing digested matter streaming towards the cells of this region (Same fly as in Figs. 23 & 39).

b. : blood.

dig.m. : digested matter.

epi.c. : epithelial cells.

h. : haematin.

s.int. : small intestine.

(The peritrophic membrane which is visible in Fig. 23 cannot be seen here because it is obscured by haematin).

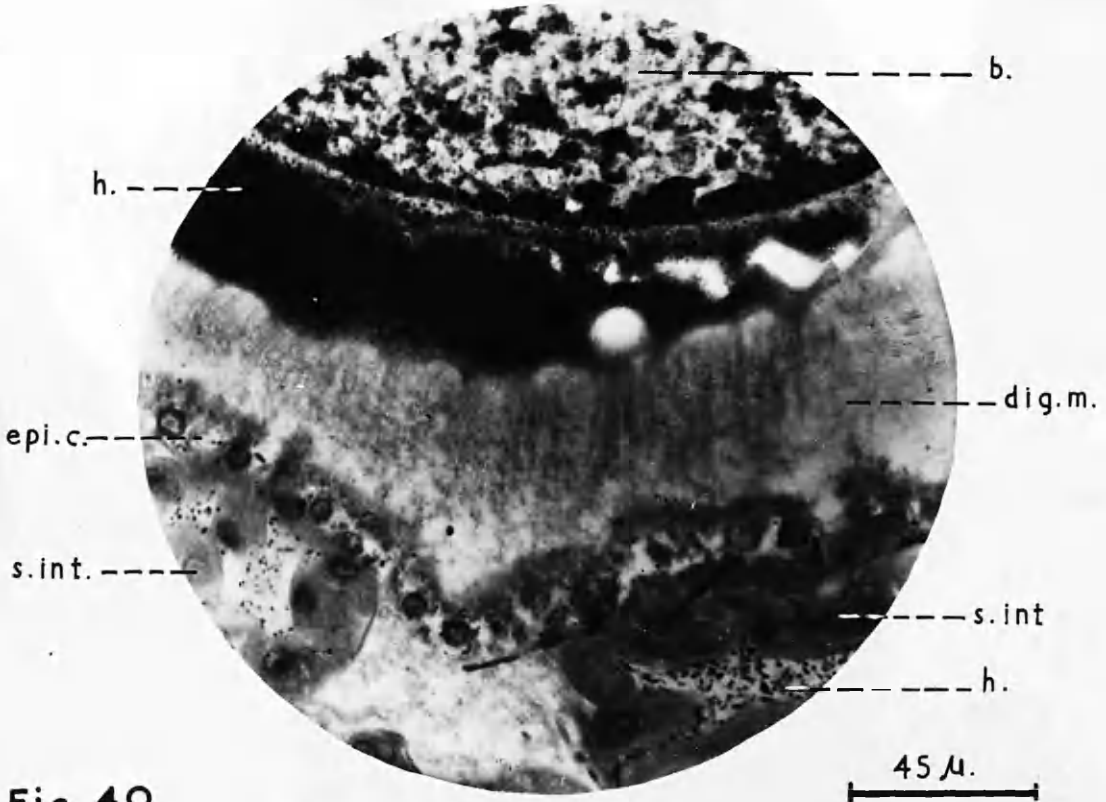


Fig. 40

C. nubeculosus Mg. - (female).

Fig. 41 Photograph of a transverse section through the stomach in a fly killed directly after starting to feed - showing massive secretion of cytoplasmic globules.

Fig. 42 Photograph of a longitudinal section through the abdomen, 48 hours after a full blood meal - showing the presence of haematin outside the stomach (artifact).

d.v. : dorsal vessel.

epi.c. : epithelial cells.

f.b. : fat body.

h. : haematin.

Mal.t. : Malpighian tube.

ov. : ovary.

st. : stomach.

v. : vacuoles (vacuolated inner end of the cell).

vs. : cytoplasmic globules.

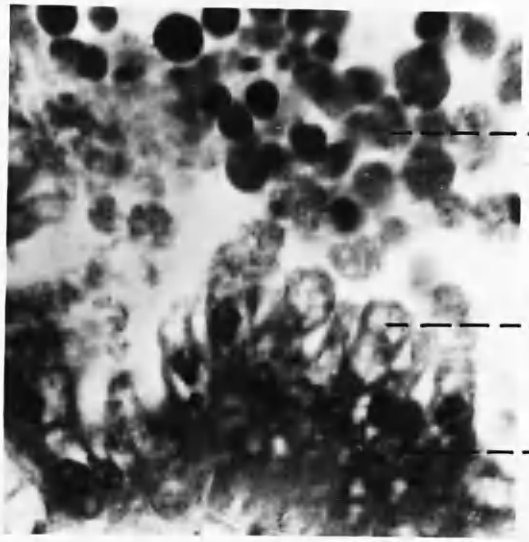


Fig. 41

30 μ

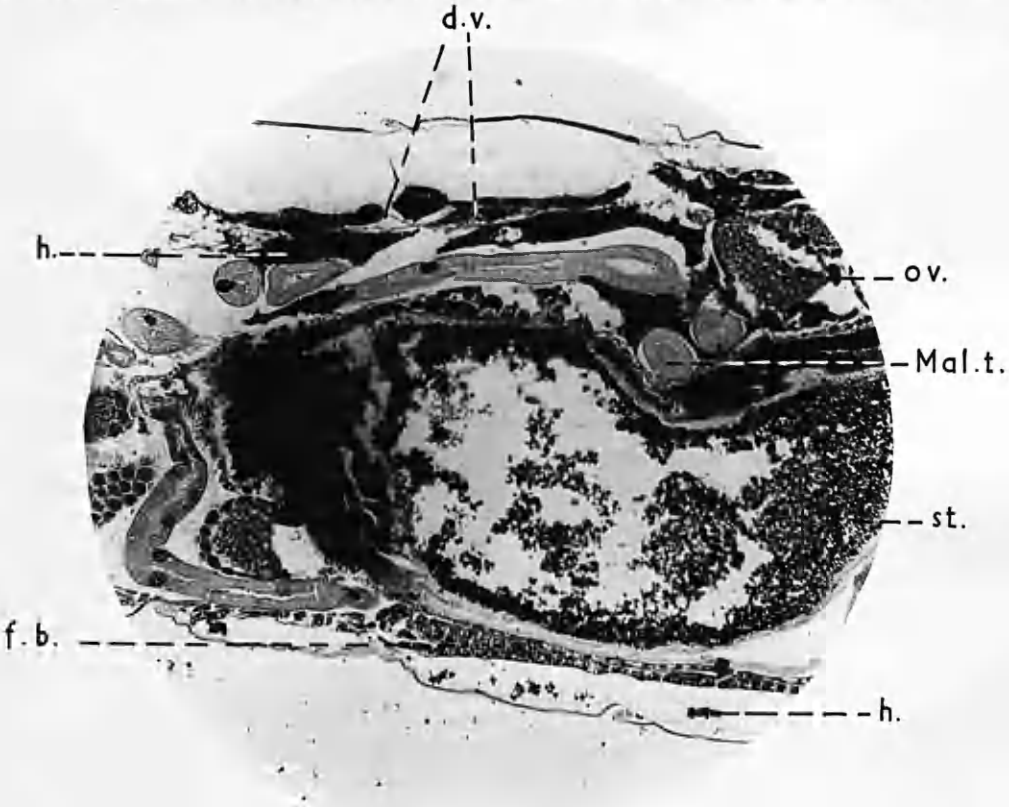


Fig. 42

150 μ.

Condition Of The Mid-Gut Epithelium In Females Which Refused To Take A Blood Meal.

A- The Anterior Segment

The epithelial cells of the anterior segment are low columnar or cubical in shape, with the intercellular walls never well marked. The cells are thrown into irregular folds or villi. These villi are rather high at the anterior end though of a lesser degree than those of the stomach. At the posterior end it is not unusual to see the epithelium unvillated.

In the females which refused to feed during the first day after emergence, a few are seen to have the epithelial cells with an even cytoplasm which contains no vacuoles (Fig. 20); these flies were probably the youngest ones. A brownish material - probably a residue of larval metabolism - is seen in the lumen.

In other specimens, several small vacuoles are seen at the outer end, and sometimes at the inner end, of the cells. In favourable sections, some vacuoles are to be seen pushing themselves through the striated border (Fig. 35, v.). Occasionally, the inner end of one or two cells is seen swollen and bulging into the lumen (Fig. 36, vs.); the striated border in such cells disappears. The bulging mass

contains several small vacuoles (v.) and sometimes also a degenerate nucleus (dg.n.). These masses are eventually nipped off, and a few of them are sometimes seen in the lumen, in which they ultimately break down. The lumen con-:tains a small amount of a granular secretion which stains pink with eosin; in some instances this secretion is a considerable amount and forms a homogeneous mass in the lumen. The striated border is clearly striated, but in some sections of the same specimen it may look homogeneous. It is very broad and of an irregular outline; it is rarely thin. Many granules of secretion similar to those in the lumen are commonly seen attached to its inner end.

In flies which refused to feed during the second and the third days after emergence, the histological appearance is almost the same. There are small vacuoles at the outer end of the cells and also a few vacuoles at the inner end. Also, occasional cells may be seen while about to discharge cyto-:plasmic globules. In favourable sections, a few cytoplasmic globules mixed with some pink granules are seen in the lumen. The striated border is strikingly broad and irregular in outline, and it reduces the lumen in many sections to a narrow branched channel. Many granules of secretion are also seen stuck to its inner end.

B- The Stomach

In flies which refused to feed during the first day after emergence, the epithelial cells of the stomach are from long columnar to cubical in shape, according to their position, and they are heaped up into villi. The villi differ in height in different specimens, but they are generally much higher than those of the anterior segment. The cytoplasm is dense and stains deeply with haematoxylin except at the locations of the vacuoles. The intercellular walls and also the striated border are clear. A few relatively small vacuoles appear at the inner part of the cells (Fig. 21, v.). Similar vacuoles may also be present at the inner end of the cells.

In some specimens, the cells of the anterior and the posterior parts of the stomach are seen to contain vacuoles, while the cells of the middle part do not. In others, the cells in a whole section (or sections) of the stomach may be devoid of vacuoles, while in an adjacent section (or sections) they are vacuolated. Also, some of the cells in a given section (or sections) may contain a few minute vacuoles, while the rest of the cells contain several larger vacuoles. In all these specimens the lumen of the stomach

contains a small amount of blue granules; similar granules are usually seen stuck to the inner end of the striated border. The difference in the presence and the size of vacuoles in different parts, or the same part of the stomach, reasonably suggests that the initial development of the vacuoles is not simultaneous throughout the organ, and that their degree of development is not the same even in adjacent cells.

In one specimen, a good number of the epithelial cells had given off round cytoplasmic masses or globules which are of considerable size. They stain blue with haematoxylin, and are seen in the lumen in different stages of disintegration. Some of them contain tiny vacuoles and a few are nucleated. In cells fixed in the act of secretion, the striated border is absent and the cytoplasmic globule is seen attached to the cytoplasm of the secreting cell by a long narrow neck. Cells which have already discharged their secretion have regained their columnar shape and also the striated border. In occasional places (Fig. 37), the neck connecting the globule (vs.) to the cytoplasm of the secreting cell is seen penetrating the striated border (st.b.); it is probable that these cells were fixed while about to regain their normal appearance.

This specimen was the only unfed fly in which the discharge of cytoplasmic globules was observed. However, Cox (1938) states that sections taken through the stomach of an unfed Simulium nigroparvum show a very active secretion taking place; the inner ends of the cells bud off and there is no rupturing of the cell wall.

In the flies which refused to feed during the second day after emergence, the epithelial cells are generally greatly vacuolated, and the intercellular walls tend to disappear. They are heaped up into villi. The inner part of the cells becomes converted into a mass of large vacuoles. The vacuolated tips of the cells stain faintly, if at all, with haematoxylin, except for a narrow area immediately below the striated border which stains deeply. Deeply-staining granules are also seen in the matrix between the vacuoles (see Fig. 38, v.); the extent of vacuolation, however, varies in different specimens. The striated border (st.b.) is commonly homogeneous, but it may disappear in some places. The lumen of the stomach contains a few blue granules which are sometimes mixed with pinkish ones. Similar granules may be seen attached to the inner end of the striated border.

In flies which refused to feed during the third day after emergence, the histological picture is quite different. The number and the size of vacuoles are excessively reduced. In the posterior two-thirds of the length of the stomach there appear gaps between adjacent cells, but the cells re-:main attached to each other at the inner and the outer ends. The striated border is clearly striated. Many cells have either lost their nuclei or contain diffusely-staining nuclei - which are symptoms of degeneration. The lumen con-:tains a small amount of the blue granules; similar granules are also seen stuck to the inner end of the striated border. This appearance was constant in the five specimens studied. As these features were confined to this group of flies, they are not likely to be artifacts. However, even if they were artifacts they would reasonably denote a significant change in the properties of the epithelium in this group of flies. These changes, however, may be compared with the findings of Hobson (1931) in the larva of the blow fly. According to this author, "In a larva starved for 48 hours, the histologi-:cal appearance is very different in the anterior and poster-:ior segments. Vacuolation is almost absent ...".

The Changes In The Mid-Gut Epithelium At The Start Of Feeding.

If a fly is killed a few seconds after inserting its proboscis in the skin, a small amount of blood is seen in the oesophagus, and only a trace of blood is seen in the mid-gut; in the stomach, a few corpuscles are seen at the anterior end of the lumen, but otherwise the lumen is devoid of blood. Nevertheless, the epithelium of the stomach demonstrates striking changes.

A- The Anterior Segment

A negligible number of blood corpuscles is seen in the lumen. The epithelial cells remain low columnar or cubical in shape, and are grouped into villi. The striated border is clearly striated. Vacuoles are few.

B- The Stomach

The epithelial cells of the stomach (epi.c.) are high columnar in shape and are heaped up into villi, but the lumen is rather wide. The inner end of the cells is converted into a mass of large vacuoles (v.) which come together. The striated border is absent except in a few places. At the anterior end of the lumen a few blood corpuscles are seen

mixed with a few cytoplasmic globules. Proceeding backwards, the lumen contains no blood, but it contains a large accumulation of round cytoplasmic masses or globules (Fig. 41, vs.). Many of these cytoplasmic globules are vacuolated and stain faintly with haematoxylin, while the others are not vacuolated and stain deeply with haematoxylin. In some sections pink granules are also seen mixed with the globules. Many cells are still engaged in nipping more globules off their inner ends. These features suggest that the act of probing the skin, or the taste of blood, or both induce the cells of the stomach to discharge these numerous globules.

The Changes In The Mid-Gut Epithelium Following A Small Blood Meal.

A- The Anterior Segment

1- Directly after the meal

In flies fixed directly after having a small blood meal, a very small amount of blood is seen in the lumen. The blood corpuscles are in direct contact with the striated border. The epithelial cells remain low columnar or cubical in shape, and are heaped up into villi. The striated border is clearly striated. Vacuoles are scarce.

2- 1 hour after the meal

There is no blood in the lumen, except for a few scattered corpuscles at the anterior end. The epithelial cells are grouped into villi. The striated border is clearly striated, and in some places it occludes the lumen. A very few vacuoles are seen at the posterior end of the epithelium.

3- 5 hours after the meal

The epithelial cells are thrown into villi, but the lumen may be rather wide. The striated border is homogene-:ous. Vacuoles are extremely rare. A small amount of a pink granular secretion is seen in the lumen.

4- 24 hours after the meal

The epithelial cells are thrown into villi; at the posterior end, however, the villi may be absent. The striat-:ed border is clearly striated. A few vacuoles are seen at the posterior end of the epithelium. The lumen is empty and narrow.

B- The Stomach

It is clear, of course, that the ingested blood accumulates first of all at the fundus of the stomach, and that the level of the ingested blood in the lumen of the stomach after a partial meal is proportionate to the volume ingested. The amount of the ingested blood varied from specimen to specimen, but the histological picture is al-:most the same except for the difference in the length of any area concerned.

1- Directly after the meal

The blood in the lumen is in direct contact with the inner end of the cells. A few scattered granules of haematin are seen. The cells of the anterior portion of the stomach are still columnar in shape and are grouped into villi. A few vacuoles are seen in the posterior end of this area. Following this area, the villi have disappeared and the epithelium is reduced to low cubical cells; the height of the cells varies according to the amount of the ingested blood. Scattered vacuoles are seen. Many cells are about to bud off. A few cytoplasmic globules are seen in the blood mass. The striated border is absent in some places

and is evident in others. At the posterior end of the stomach several batches of a pink homogeneous secretion (or plasma) are seen in the lumen.

2- 1 hour after the meal

The cells of the anterior end of the stomach are long columnar, highly vacuolated, and thrown into villi. They are discharging many cytoplasmic globules which stain blue; some of these globules are nucleated. The lumen in this area is narrow and does not contain blood. Following this area, the blood appears in the lumen - scanty in the first few sections, and in plenty in later sections. The shape of the epithelial cells differs accordingly. In the first few sections, the cells are from low columnar to cubical in shape, and some of them are grouped into low villi. They contain many vacuoles. Many of these cells are discharging cytoplasmic globules, some of which may be nucleated. In later sections, the cells are very low (almost flat) and contain a few vacuoles. Cytoplasmic globules are rarely seen. At the base of the stomach, however, high cells are seen, and some of them are in the course of budding off. The striated border is generally homogeneous, but in some places it is absent.

The blood is separated from the epithelial cells by a thin layer of pink fine granules of secretion; in some places, however, it is in direct contact with the cells. A few haematin granules are seen scattered within and on top of the blood mass.

3- 5 hours after the meal

The blood in the lumen of the stomach is, by now, enclosed in the peritrophic membrane. This membrane is rather thick, single-layered, granular in appearance and stains pink with eosin; it is excessively thick at the anterior end of the stomach (where the lumen contains not more than a trace, if any, of blood) where it fills the lumen for a considerable length. A large number of the blood corpuscles are still intact. A small number of scattered granules of haematin is seen.

The cells of the anterior end of the stomach are cubical or columnar, and they contain many vacuoles at their inner ends. They are more or less grouped into small villi. The major part of the length of the epithelium is composed of vacuolated cells which vary in shape from low cubical to almost flat; this variation in shape can be seen in a single section, and it is attributed to peris-

:talsis. The cells at the base of the stomach are low cubical and are highly vacuolated. The striated border is generally homogeneous.

4- 24 hours after the meal

The stomach is empty and crumpled. The cells are high columnar and are heaped up into deep villi. They are highly vacuolated, the intercellular walls absent, and the striated border homogeneous (see Fig. 38, v. & st.b.). In general, the histological picture resembles that of unfed flies fixed during the second day after emergence. (The ovaries remain undeveloped).

The Changes In The Mid-Gut Epithelium Following A Full Blood Meal.

A- The Anterior Segment

1- Directly after the meal

In flies fixed directly after taking a full blood meal, the lumen contains varying, but generally small, amounts of blood. The blood corpuscles are in direct contact with the striated border. The anterior segment never becomes dis-:tended with blood, and the epithelial cells remain low columnar or cubical in shape and heaped up into villi. The

striated border is clear. A few vacuoles are seen at the posterior end of the epithelium.

2- 1 hour after the meal

The blood has receded from the organ. A few scattered corpuscles, however, are seen at the posterior end of the lumen, and are in direct contact with the stiated border. The blood plasma occupies the very posterior end of the lumen (and the anterior end of the stomach). It stains pink, and a few blood corpuscles may be seen embedded in its substance. The epithelial cells are grouped into villi. The striated border is clearly striated, but in some places it is homogeneous. Some cells contain a few small vacuoles. Occasionally, some rather big vacuoles are seen at the inner end of the cells or within the striated border. Secretion of granules seems to take place through the striated border; the striae are driven apart, apparently to permit the dis-:charge of the secretion. Here and there, a cytoplasmic globule may be seen in the lumen or budding off the cell.

3- 5 hours after the meal

The epithelial cells are thrown into villi, but the lumen of the segment is rather wide. A minute amount of the

pink granular secretion is seen at the anterior end of the lumen. Vacuoles are absent except at the posterior end of the segment, where a few vacuoles are seen. The striated border is broad and clearly striated, but towards the posterior end of the segment it becomes homogeneous. In one specimen the striated border was rather thin.

4- 24 hours after the meal

The epithelial cells are thrown into villi. Vacuoles are very few. The striated border is clearly striated, and in some places it almost occludes the lumen. The lumen is empty except for an occasional cytoplasmic globule.

5- 48 hours after the meal

The epithelial cells are thrown into villi. A very few vacuoles are present. Occasionally, one or two vacuoles may be seen within the striated border. The striated border is clearly striated, and in some places it almost occludes the lumen. The lumen is generally empty, but in some specimens it contains a few granules of secretion. Also, one or two nucleated cytoplasmic globules may be seen.

6- 72 hours after the meal

The epithelial cells are thrown into villi. A few

scattered vacuoles are present. The lumen contains some granules of secretion, and sometimes one or two nucleated cytoplasmic globules. The striated border is clearly striated.

7- 96 hours after the meal

The epithelial cells are thrown into villi. A few vacuoles are seen. In one specimen a few rather big vacuoles are seen at the inner end of the cells. A few granules of secretion are seen in the lumen. The striated border is clearly striated; in one specimen it is rather thin.

8- After oviposition

The cells are thrown into villi. A few scattered vacuoles are present. The striated border is clearly striated. Some granules of secretion may be seen in the lumen.

B- The Stomach

1- Directly after the meal

Practically speaking, almost all of the ingested blood is received in the stomach, which consequently becomes highly distended with it. Except for a short area anteriorly, and in some instances also posteriorly, the epithelium of the

stomach becomes greatly reduced in height. The villi have totally disappeared, and the cells are quite flat; the cells may become almost as thin as the short diameter of the nucleus. The nuclei become compressed and elongated, the striated border and the intercellular walls disappear, and vacuoles are almost absent. In the short area at the anterior end of the stomach, the villi are still seen though they are comparatively low. The cells are columnar in shape, and the vacuoles are not uncommon. The striated border is clear in some places. In a short area at the posterior end of the stomach, the cells are sometimes low cubical in shape.

The blood stains pink with eosin. A few granules of haematin are seen scattered within the blood mass. Some cytoplasmic globules (blue) are also seen. In the anterior portion of the stomach, the blood corpuscles are in direct contact with the cells. Posteriorly, the blood corpuscles are separated from the flattened epithelium by a thin layer of fine pink granules - the fluid precursor of the peritro-:phic membrane which condenses later around the ingested blood. This layer is rather thick at the posterior end of the stomach.

2- 1 hour after the meal

By this time, the blood in the anterior segment has been inspired into the stomach. The blood plasma forms a pink homogeneous mass on top of the blood mass, and occupies a short area of the lumen in the anterior end of the stomach (and the very posterior end of the anterior segment). A few blood corpuscles are sparsely embedded in the mass. A great number of corpuscles are still intact, and only a few scattered granules of haematin are seen. The peritrophic membrane is not yet evident as a solid structure. The blood in the lumen is separated from the cells of the stomach by a thin layer of pink granules, which is thickest at both the anterior and posterior ends of the stomach.

The epithelial cells of the short area at the anterior end of the stomach are still columnar and are grouped into low villi, except at the posterior end of this area where the villi have disappeared but the cells remain cubical in shape. A few vacuoles are seen in some cells, and occasionally a cytoplasmic globule or a degenerate nucleus is seen in the lumen. The striated border is homogeneous. The rest of the epithelium is composed of flat cells. The stiated border and the intercellular walls are absent.

Vacuoles are extremely rare, and when present they are small.

3- 5 hours after the meal

The epithelial cells in the short area at the anterior end of the stomach are still columnar in shape and grouped in rather low villi. The cells at the posterior end of this area are cubical and are not grouped in villi. Vacuoles are present in some cells, and a few cytoplasmic globules are rounded off. The striated border is absent in most places, and is homogeneous in others. Also, in a very short area at the posterior end of the stomach, the cells are sometimes columnar and vacuolated; some of these cells are budding off, and a few cytoplasmic globules are seen lying against the peritrophic membrane. The rest of the epithelium is still flat, the striated border is homogeneous or absent, and vacuoles are scarce except at the posterior end where they are numerous.

The blood plasma has receded by now from the anterior segment, and it becomes contaminated with haematin so as to be pink and brown in colour. The peritrophic membrane is clear. It is lamellated, stains pink, and wholly encloses the blood mass. In favourable sections (Fig. 22), the

distended epithelium is seen discharging droplets of secretion which add to the substance of the peritrophic membrane; these droplets are eosinophil and take on a bright pink colour. The blood mass has somewhat decreased in size; it is considerably separated from both the anterior and posterior ends of the stomach, but the peritrophic space remains narrow elsewhere. The blood mass seems to be held in place through the contraction of the anterior end of the stomach and the mesenteric sphincter. There is some increase in the amount of haematin granules, but, however, the number of intact corpuscles is still great.

4- 24 hours after the meal

The blood mass has decreased considerably in size. The space between each of its two poles and the respective pole of the stomach has consequently increased. The peritrophic membrane becomes mainly brown in colour due to the incor-:poration of haematin in its substance, which may be an artifact; in some places pink portions may be seen. The membrane is perforated at its posterior end to allow the passage of faecal matter down to the hind-gut. The faecal matter is composed of spherules and granules which stain blue. The amount of haematin granules and rods has increased

considerably, but, however, a large number of corpuscles are still intact (Fig. 23). The haematin is scattered within the blood mass, and lines the peritrophic membrane, but it is densely accumulated at the anterior end of the stomach (Fig. 39, h.). The blood plasma has almost dis-:appeared. (The presence of rods and granules of haematin in the epithelial cells of the stomach and elsewhere will be considered later).

The cells of the short area at the anterior end of the stomach show a strikingly different appearance. The villi have almost disappeared, and the cells become elongate (epi.c.) and contain many scattered round vacuoles (v.). The striated border and the intercellular walls have dis-:appeared. The cells are discharging a considerable amount of cytoplasmic globules (vs.), some of which are nucleated (Fig. 39). The posterior end of this area is still com-:posed of cubical cells ungrouped in villi, which contain a few small vacuoles; cytoplasmic globules are occasionally discharged.

At the base of the stomach the cells are low cubical, and contain many vacuoles (Fig. 40, epi.c.). In this region, a stream of a blue-staining fluid - presumably of digested

matter (dig.m.) - is seen passing from the blood mass to the epithelial cells (Fig. 40). The main length of the epithelium, however, is still composed of flat cells. A few small vacuoles appear in many cells.

5- 48 hours after the meal

The blood by now is almost completely digested. In seven out of nine specimens, the stomach contains much haematin and, in the centre, there is a small amount of a pinkish fluid mixed with haematin. In one specimen, the stomach contains only haematin. In all these specimens it is difficult to differentiate the peritrophic membrane from the dark-brown or blackish contents of the stomach. The epithelial cells of the anterior and posterior ends of the stomach have become cubical or low cubical, while the rest of the epithelium is still flat despite the considerable decrease in the size of the stomach. All the cells contain varying, but generally small, numbers of small scattered vacuoles. Many cells throughout the stomach are showing features of degeneration; they either have lost their nuclei or contain diffusely-staining ones.

One specimen, however, was odd. A very big number of intact blood corpuscles is seen in the lumen, and only a

small amount of haematin is present. The histological picture is almost the same as that of flies fixed 5 hours after the full meal. The cause of the delay of digestion of the meal is not clear. (In this specimen the ovaries are undeveloped, while in the other specimens they were in an advanced stage of development).

6- 72 hours after the meal

The stomach is empty and crumpled. The cells are high columnar and are heaped up into deep villi. They are highly vacuolated, the intercellular walls absent, and the striated border is homogeneous (Fig. 38, v. & st.b.). In general, the histological picture resembles that of flies fixed 24 hours after a small meal, and that of unfed flies fixed during the second day after emergence.

7- 96 hours after the meal

As in (6).

8- After oviposition

As in (6).

The Occurrence Of Haematin Outside The Alimentary Tract During The Digestion Of Blood.

In flies fixed directly after a blood meal (both full

and partial), and at different times during the process of digestion, many of the tracheae and tracheoles in the abdomen, and particularly those attached to the mid-gut, take on a dark-brown colour. Some small tracheae may show only a portion of their length which is dark-brown. In the unfed flies, as well as in flies which have completely digested the blood meal and have excreted the residue of the blood, the tracheae are of a yellowish-brown colour and the tracheoles are almost colourless.

In flies fixed 24 hours after a full meal, a few haematin granules and rods are seen in many cells in the stomach. Also, a few batches of haematin may be seen in the fat-body.

In flies fixed 48 hours after a full meal, excessive amounts of haematin in batches of different sizes (Fig. 42, h.) are seen in the fat-body (f.b.), inside and outside the dorsal vessel (d.v.), around the Malpighian tubes (Mal.t.) and around the ovaries (ov.).

In flies fixed 72 hours after a full meal, the stomach is empty and crumpled. The residue of the blood has been excreted, and no haematin is seen anywhere.

These curious features are undoubtedly artifacts which

occur during fixation. They are not present in fresh dissections. Wigglesworth described somewhat similar features in Glossina (1929), and to a lesser degree in Chrysops silacea (1931, a). He (1929) believed he had evidence that intercellular tracheoles were present in the mid-gut, and that this was confirmed in Calliphora. He sug-:gested that the haematin rods were casts of these inter-:cellular tracheoles, which had been ruptured during the extreme distension, filled with blood pigment from the lumen of the gut and had been subsequently discharged again mould-:ed to the form observed. He states that sometimes only the intercellular tracheoles of the epithelium are filled with blood and most of this is discharged into the lumen as the epithelium becomes more and more compressed; very frequently the pigment may spread to the tracheae and tracheoles of adjacent organs. Since pigmented tracheoles were not present in the fasting fly, he states that it follows that the blood pigment which enters the tracheal system may be disposed of in some way though the ultimate fate of this blood pigment could not be determined. Applying his ingenious injecting method, Wigglesworth (1950, a) states that " .... the tracheoles of the mid-gut

in Calliphora are confined to the perivisceral membrane in which they form an immensely rich tangled plexus. At many points this membrane with its tracheoles is invaginated deeply between the cells and the tracheoles may then some-:times have the appearance of being intercellular". He (1950, a) attributes the presence of haematin in the tracheal system of Glossina to fixation. My own findings confirm this.

Regeneration Of The Mid-Gut Epithelium

The regenerative cells which are scattered throughout the mid-gut epithelium serve to replace the succumbing cells. The process of regeneration seems to be a rapid one, and it was not possible to follow it. However, cells intermediate between the regenerative and the columnar cells were fre-:quently seen pushing their way up towards the inner end of the epithelium.

SECTION lV.

THE FUNCTION OF THE OESOPHAGEAL DIVERTICULUM.

THE FUNCTION OF THE OESOPHAGEAL DIVERTICULUM.

The females used in this study were fed as follows: blood was taken in the normal way by allowing the female to bite, water and cane-sugar solutions were given on wet cotton-wool.

Condition of the diverticulum in females collected from the rearing-cages:

The flies were collected at daily intervals and thus were between 0 and 24 hours old. It was of interest, before starting the feeding experiments, to study the condition of the diverticulum in those females which had no access to food except, probably, the water which was regularly added to the mud in the rearing pots. Out of 25 such females which were dissected directly after being collected, 17 had the diverticulum empty and crumpled, 7 had it distended with an amber-coloured fluid, and 1 had it distended with a colourless fluid. (TABLE 1).

The nature of the amber-coloured and the colourless fluids was not known. There were, however, two alternatives, viz., either water imbibed from the dirty drainage water in the basins surrounding the rearing pots (amber-coloured) and from the walls of the pots (colourless), or

saliva or gastric secretions forced into the diverticulum as is claimed to be the case in some other blood-sucking insects, i.e., Simulium damnosum (Wanson & Lebied, 1948); Tabanus (Cragg, 1920).

To elucidate this point 40 pupae were transferred from the rearing pots to vials furnished with cotton-wool wet with eosin-stained water, the excess of the stained water being blotted out and the walls of the vials lined with filter paper, which subsequently becomes stained, to absorb the condensation water. Each vial was plugged with a bored cork lined with muslin. It was thought that if any colourless or amber-coloured fluid was found in the diverticulum in any of the emerging females, the fluid should be of internal origin, while if the stained water was found instead it would indicate that these fluids were imbibed water. The vials were inspected at daily intervals, and any females found were collected, etherized at once and dissected.

Out of 26 females thus treated, none showed the least trace of the stained water in either the gut or the divert-:iculum. The diverticulum in all of them was empty and crumpled. (TABLE 1).

TABLE 1. The condition of the diverticulum in females of C. nubeculosus collected at daily intervals being 0 - 24 hours old.

Condition of the females.	Number of the females.	Condition of the diverticulum.
A. Collected from the rearing-cages.	17	Empty and crumpled.
	7	Distended with an amber-coloured fluid.
	1	Distended with a colourless fluid.
Total	25	
B. Reared from pupae in tubes fitted with cotton-wool wet with eosin-stained water.	26	Empty and crumpled.

This result was indecisive, but if the amber-coloured or the colourless fluids were secretions there would be no reason for their absence from all these females. In fact, the occurrence of females less than 24 hours old, i.e., females collected from the rearing-cages, which have the diverticulum distended with the colourless or the amber-coloured fluid was lower than is recorded here; in scores of unrecorded dissections the occurrence of such females

was very low. Serial sections, and also the coming experi-:ments, however, do not favour the idea of the diverticulum being a store of saliva or gastric secretion. As none of these females ingested the stained water, it is probable that either the stained water was unpalatable, or they were not thirsty at all.

A. Destination Of Blood In The Alimentary Tract:

Females kept in the collecting-cage were offered individually a full blood meal. Immediately after finish-:ing its meal, the engorged fly was etherized and dissected. Out of 25 females thus treated, all had the stomach full of blood, 19 of them had the diverticulum distended with water, 4 had the diverticulum distended with raisin juice, and 2 had the diverticulum empty and crumpled.

Though 2 of the females had the diverticulum empty and crumpled without the least trace of blood in it, it was thought possible that the absence of blood from the diverticulum in all, or at least some of the rest was due to its being distended with fluid. So, 10 females, after being collected from the rearing-cages, were left without water and raisin in vials plugged with bored cork lined with muslin, and kept for one or more days in a desiccator

at a relative humidity of 80 per cent. These females were fed individually to repletion and dissected directly. All of them had the stomach full of blood and the diverticulum empty and crumpled without any trace of blood in it. (TABLE 11). (This also shows that the diverticulum is not a store of saliva or gastric secretion. It cannot be said, of course, that the diverticulum was emptied during the blood meal: in the previous experiment 23 females had the diverticulum distended with fluid).

TABLE 11. The condition of the stomach and the oesophageal diverticulum in females of C. nubeculosus after a full meal of blood.

Condition of the females.	Number of females.	Stomach.	Diverticulum.
A. Kept before being fed on blood in a cage with water & raisin available.	19	Full of blood.	Distended with water.
	4	" " "	Distended with raisin juice.
	2	" " "	Empty and crumpled.
Total	25		
B. No water or raisin available.	10	" " "	Empty and crumpled.

In addition to the above results, it occurred that

one of the females destined to egg-laying in routine culture work fed for no less than half an hour, and became so extra-:ordinarily engorged that it could not move freely. By dissecting it at once, the diverticulum was found empty and crumpled without the least trace of blood in it. In serial sections of flies fixed at different times after a full and an interrupted meal, the diverticulum was never found to contain blood corpuscles.

These results indicate definitely that the blood does not enter the diverticulum, and that the diverticulum does not function as a reservoir for blood. The destination of blood is the stomach (the excess of blood is to be found in the anterior segment of the mid-gut). It is also clear that the distension of the diverticulum with fluid does not interfere with the size of the blood meal.

Observations on blood-feeding females:

It has been observed that when the females are blood-thirsty they bite immediately they come into contact with the skin. Others with less appetite usually roam about for a while till they decide to have their meal. The female tries to puncture the skin at the chosen site; this being achieved, the proboscis is thrust into the wound. The

proboscis - except for the labium, of course, - is sometimes wholly buried in the skin, other times it is buried to from one-third to one-half of its length. I am of the opinion that the difference in the extent of the proboscis within the puncture is due to the difference in the availability of the blood in the different sites, i.e., it depends upon the vascularity of the point of attack.

According to Lutz (1912), in consequence of the shortness of the lancet in the blood-sucking heleids this must be buried deep in the skin close up to the head. Steward (1933) states that C. nubeculosus thrusts its proboscis till "it was buried up to the base of the antennae". This statement is an exaggeration as it means that the part of the head from the base of the proboscis to the base of the antennae, and also the labium, are thrust through the skin. The above description, in agreement with the findings of Gad (1951), and Jobling (1928), makes it clear that this is not the case.

It was noticed that a small number of females, especially during the first day, try unsuccessfully to obtain a blood meal. They succeed, however, in inserting the proboscis and remain with it inserted for a considerable time

then withdraw suddenly and insert it again in another spot and so on for several times. When these females were dis-:sected directly, no trace of blood could be detected in the alimentary tract. Hill (1947) observed the same phenomenon in C. impunctatus and C. obsoletus. Lutz (ibid.) states that the first attempt of Brazilian blood-sucking heleids to insert the proboscis does not always succeed, and the process is more or less slow, but the quantity of blood taken relative to the size of the insect is large. A similar phenomenon was observed in mosquitoes. Marshall and Staley (1932) state that a mosquito may penetrate the skin at as many as eight different points, producing that number of wheals, but not drawing blood.

When the meal is finished, if the proboscis is buried deep in the skin the engorged fly withdraws by pulling its head suddenly and vigorously, this being aided by the bracing action of the legs. If, on the other hand, the proboscis is inserted relatively superficially it is with-:drawn comparatively quietly. Hill (ibid.) dealing with C. impunctatus, states that the majority of the flies appeared to have difficulty in withdrawing their proboscis after a meal. C. impunctatus is a notorious man-biter, and

the suggestion of Hill sounds as if this species is not well adapted to its mode of feeding. Steward (ibid.) also mentions that in C. nubeculosus occasionally the withdrawal appeared difficult to the fly which seemed to press down with its feet and to pull its head back vigorously in an endeavour to extricate the porboscis.

I have never observed what could be termed difficulty in withdrawing the proboscis in C. nubeculosus. The sudden and vigorous pulling of the head, when the proboscis is inserted deep in the skin, does not give the impression of any difficulty. The piercing stylets in Culicoides spp. are toothed (Gad, 1951; Snodgrass, 1943; Jobling, 1928), so when they are inserted deep in the skin it is reasonable that some effort is needed to pull them out; difficulty means to me a sort of struggle to withdraw the proboscis, a struggle which does not occur in C. nubeculosus at least.

According to Gad (ibid.), the whole process of feeding takes from 3 - 4 minutes in C. obsoletus and C. Chiopterus. Hill (ibid.) recorded that the time taken for individuals to become fully engorged varied from 3 - 20 minutes in C. impunctatus and up to 40 minutes in C. obsoletus. Steward (ibid.) found that the time of engorgement in C. nubeculosus

varied from 3 - 8 minutes. In this work, the time of engorgement in C. nubeculosus, apart from individual variation, was found to vary considerably according to the condition of the females. In females which had access to water and raisin before having the blood meal, it varied from 1.54 - 9.40 minutes (average 3.59 minutes). In females which had no access to these materials before the blood meal, it varied from 3.58 - 22.35 minutes (average 8.37 minutes). (TABLE 111).

TABLE 111. Time taken for the engorgement of the females of C. nubeculosus.

Condition of the females.	Number of females.	Time in minutes and seconds.
Had access to water and raisin before the blood meal; the diverticulum almost always distended.	25	1.45 - 1.58 - 2.17 - 2.28 - 2.30 - 2.35 - 2.40 - 2.42 - 2.47 - 2.52 - 2.55 - 3.01 - 3.02 - 3.14 - 3.20 - 3.40 - 3.41 - 3.54 - 4.23 - 4.33 - 6.58 - 7.02 - 7.21 - 8.20 - 9.40 - (Average 3.59)
Had no access to water or raisin before the blood meal; diverticulum empty and crumpled.	10	3.55 - 3.58 - 5.20 - 6.20 - 6.34 - 6.54 - 7.50 - 9.25 -13.20 - 22.35 - (Average 8.37)

From this considerable variation between the two groups, it seems that the complete fasting of the females before having the blood meal either impairs their power of sucking, or the stomach becomes so crumpled that it needs a longer time for distension. There was, however, no apparent difference in the distension of the abdomen in the females of both groups.

A few seconds after the start of the blood meal, the fly dejects drops of a clear colourless fluid which accumulate on the skin near the tip of the fly's abdomen or may be shot several millimetres away. The discharge of this fluid continues for some time after the finishing of the meal. Occasionally, it was noticed that some of the drops were retained at the tip of the abdomen in the form of a bubble, being protruded and withdrawn several times before being discharged. I wonder if this observa-:tion suggests that air enters the gut, sometimes at least, during or directly after the finishing of the blood meal and is eliminated via the anus. It is extremely rare to detect air-bubbles in the diverticulum or the mid-gut. In very occasional specimens (non-blood-fed), however, a few big air-bubbles were seen in the distended diverticulum,

or in the stomach, or in both. The common absence of air-bubbles from the diverticulum of the female C. nubeculosus is in accord with what is found in (Phlebotomus papatasii (Adler & Theodor, 1926), but differs from what is found in the mosquito (Nuttall & Shipley, 1903; MacGregor, 1930; Marshall & Staley, 1932; Pawan, 1937), and the tsetes fly (Lester & Lloyd, 1928).

According to Wigglesworth (1931, b), the discharge of much clear fluid soon after feeding is characteristic of nearly all blood-sucking insects and there has been much speculation as to its nature: it has often been re-:garded as "serum" separated in the intestine from the blood corpuscles. In Glossina, Lester & Lloyd (1928) found that it is produced by the Malpighian tubes. Wiggles-:worth (ibid.) dealing with Rhodnius prolixus, states that it is a salt solution, more or less isotonic with the in-:gested blood, which serves for the rapid elimination of the unwanted salts in the diet; he also thinks that this is probably true of other blood-sucking insects. About 75 per cent. of the water in the meal is got rid of in this way, and it can be shown by calculation that this volume of isotonic salt solution will contain nearly all

the salts in the blood.

Steward (ibid.) states that in C. nubeculosus this fluid sometimes appeared to be tinged with blood either from the previous meal of blood, or from one end of the alimentary tract to the other, during the meal which was being observed. I have never observed this phenomenon though I have fed hundreds of females on my arm. In one case, however, a female took a second blood meal some days after the first meal, and during feeding it dejected some brown viscous drops which were undoubtedly faeces. This was an abnormal case, and this female died the next day. The suggestion of Steward that the fluid might have been tinged with blood from the previous meal does not seem probable. The females of C. nubeculosus take only one meal of blood in every gonotrophic cycle, and usually lay their eggs 3 - 4 days after a full meal, by which time there is no trace of blood in the alimentary tract.

While feeding, the females of C. nubeculosus are not easily disturbed. If the tube is removed while the female is engaged in its meal, the latter remains feed-:ing: neither shaking the hand, nor scratching the skin, nor blowing smoothly on the fly, nor even blowing some

whiffs of tobacco smoke, will oblige the feeding female to leave its feeding place. On the other hand, if a hot needle or a piece of filter paper wet with ether approaches the feeding female, it instantaneously leaves the feeding site and may try to bite again in another site. In its persistence the female C. nubeculosus differs from that of Phlebotomus papatasii which, according to Waterson (1922), is easily disturbed, the slightest move-:ment of the skin being sufficient to put the fly to flight.

When the distended stomach is incised, the contained blood escapes in granular lumps - an appearance which is due to the agglomeration of the corpuscles. Wigglesworth (1929) describes a similar appearance for the blood found in the anterior segment of the mid-gut in Glossina; as true agglutinins are absent, he states that this pseudo-agglutination is merely the result of the absorption of fluid.

To the writer, the bites and the red spots which form around the sites of puncture are quite painless and, apart from a slight inclination to scratch the skin after feeding a lot of females, never caused any disagreeable sensations. Gad (1950) mentioned that he was not affected by the bites

of C. obsoletus and C. chiopterus, while Jobling (1928) described the intense irritation caused to him by the bites of C. obsoletus and C. vexans, though to him the irritation and swelling caused by the bite of C. pulicaris was of a very short duration. Actually, as stated by Gad, this confirms the general conception that some people are more susceptible subjects to the bites of midges (or mosquitoes).

B. Destination Of Water And Sugar Solutions In The Alimentary Tract:

The females used in this study were given water and cane-sugar solutions stained with eosin for easy detection. Two concentrations of the sugar solution were used. The first was approximately 66.6 grams of sugar per 100 c.c. of solution, and is here termed "thick sugar solution". The second was approximately 4.18 grams of sugar per 100 c.c. of solution, and is here termed "thin sugar solution".

A piece of cotton-wool, soaked in the stained liquid was inserted in the bottom of a tube 4 x 1¼ inch, and the excess of the liquid blotted out by a piece of filter paper. The females were introduced, one by one, into the prepared tube. Each female was allowed to imbibe the

liquid for a minimum time so as to take only as much as might be easily detected, and thus show which part of the gut first receives it. For this purpose nearly half a minute was found satisfactory. The fly was then removed, etherized at once and dissected.

1- Destination of water in the alimentary tract:

The process of sucking of water, at least by the described method, is not continuous. The female dips its proboscis in the wet cotton-wool for a very short while, then withdraws it, rubs it several times with the fore-legs, then re-inserts it in the same, or another, place. Because of this discontinuity of sucking, each female was allowed to dip its proboscis for several times within the time limit of half a minute.

During the first day, very few of the females could be induced to ingest the stained water. When the females were introduced to the "water" tube they climbed its walls towards the light, paying no attention to the wet cotton-wool. It was necessary to shake them from the walls and to evert the tube to oblige them to cling to the cotton-wool. Al-:though they appeared to be sucking, it was found by dis-:secting them, that the great majority of them did not

ingest the stained water. Out of 13 females dissected after such trials, only 3 females were found to have ingested the stained water which was seen in the mid-gut; the diverticulum was empty and crumpled in 1 female, distended with plain water in the second, and distended with dirty water in the third. The other 10 females had the gut empty and the diverticulum empty and crumpled. (TABLE 1V). The low number of females which ingested the stained water during the first day of adult life confirms my previous statement concerning the occurrence of females 0 - 24 hours old which had the diverticulum distended with plain or dirty water.

By starving the collected females for one day at a relative humidity of 80 per cent., and then introduc-:ing them individually to the "water" tube, almost all of them ingested the stained water readily. Out of 9 females thus treated, 8 females ingested the stained water, and 1 female only did not; the female which did not ingest the stained water had the mid-gut empty and the diverticu-:lum empty and crumpled. Out of the 8 females which in-:gested the stained water, 4 had the diverticulum empty

and crumpled, 3 females had a trace of the stained water in the diverticulum, and 1 female had the diverticulum half distended with it: in all of them the stained water was seen in the mid-gut. (TABLE iV).

It is interesting to note that when 3 starved females were left to imbibe the stained water to satiety, none of them had the diverticulum distended with it; the diverticulum either contained a trace or none at all. This is strikingly different from what was seen in the females 0 - 24 hours old, which had the diverticulum distended with a colourless or an amber-coloured fluid which are suggested to be plain and dirty water respective-:ly. It was necessary, therefore, to apply plain water instead of the stained water, as it was very possible that the stain might have rendered the water less pala-:table to the flies so that these only ingested little of it.

Four flies were starved as usual, and each of them was given plain water, by the same method, to satiety. Each fly was dissected directly after finishing the ingestion of water. It was found that the oesophageal diverticulum was much distended with the plain water in 3 flies, and

little distended with it in the fourth fly; this proves the lesser palatability of the stained water. (TABLE lV).

It is evident from these results that the ingested water is dispatched first to the mid-gut and, when the urgent need for water is gratified, the excess of the ingested water is directed to the diverticulum. It is also clear that the oesophageal diverticulum serves as a reservoir for water.

TABLE 1V. The destination of water in the alimentary tract of female *C. nubeculosus*.

Condition of the females	Directly from the rearing-cages. (½ minute)	Starved for one day at 80% R.H. (½ minute)	Starved for one day at 80% R.H. (To satisfaction)
Total number of females used.	13	9	3
Number of females which refused the stained water.	10	1	-
Number of females which ingested the stained water.	3	8	3
Number of females with the stained water in mid-gut.	3	8	3
Number of females with stained water in diverticulum.	-	4	-

Appendix : 4 females starved for one day at 80% R.H., were allowed plain water to satiety. The diverticulum was much distended in 3 and little in 1.

2- Destination of cane-sugar solutions in the alimentary tract

It was observed that the females of C. nubeculosus commonly ingested the stained sugar solutions more readily than the stained water; a far lesser number did not ingest it. Although the process of sucking the stained sugar solutions is also discontinuous, as is the case with the stained water, the fly remains sucking for a longer period before it withdraws its proboscis. The amount of the ingested solution is also greater, in the allowed time, than in the case of the stained water.

a- Thick sugar solution:

Four females, after being collected from the rearing-cages, were allowed individually to imbibe the solution for about one-half-minute. Each of them was then removed, etherized at once and dissected. They were all found to have the diverticulum more or less distended with the stained solution, while the mid-gut contained at most a trace of it. (TABLE V).

Four females, after being collected from the rearing-cages, were starved for one day at 80 per cent. relative humidity, then were allowed, one by one, to imbibe the solution, and then treated as usual. They were all found

to have the diverticulum more or less distended with the solution, while the mid-gut contained not a trace of it. (TABLE V).

Each of 4 other starved flies was allowed to imbibe the solution to satiety. They were all found to have the diverticulum fully distended with the solution, while the mid-gut had at most a trace of it. (TABLE V).

These results show that the thick sugar solution, i.e., concentrated sugar solution, is dispatched directly to the diverticulum, and that the mid-gut, during the time of ingestion, gets at most a trace of it. It is interest-:ing, however, to note that in some unrecorded females which happened to have the diverticulum distended with water taken up from the rearing pots, the thick sugar solution was dispatched to the mid-gut. It is also evident that the oesophageal diverticulum serves as a reservoir for the thick sugar solution.

b- Thin sugar solution:

In this experiment, all the females used were starved for one day at 80 per cent. relative humidity, after being collected from the rearing-cages. Each of 6 females was allowed to imbibe the solution for about a half a minute and

then dissected directly. They were all found to have the mid-gut more or less distended with the solution, while the diverticulum was empty and crumpled. (TABLE V).

3 other females were allowed, one by one, to imbibe the solution to satiety. They were all found to have both the mid-gut and the diverticulum more or less dis-:tended with it. (TABLE V). This result is in marked contrast to what was shown in the cases of the stained water and the stained thick sugar solution.

These results indicate that the thin sugar solution, i.e., dilute sugar solution, is dispatched first of all to the mid-gut, and that when the latter has received a sufficient amount, the surplus of the ingested solution is directed to the diverticulum. It is also clear that the oesophageal diverticulum serves as a reservoir for the thin sugar solution.

TABLE V. The destination of cane-sugar solutions in the alimentary tract of female C. nubeculosus.

Condition of the females.	Number of females.	Nature of solution.	Mid-gut.	Diverticulum.
Directly from the rearing-cages. (½ minute)	4	Thick.	Nil or trace.	Distended.
Starved for one day at 80% R.H. (½ minute)	4	Thick.	Nil or trace.	Distended.
Starved for one day at 80% R.H. (To satiety)	4	Thick.	Nil or trace.	Fully distended
Starved for one day at 80% R.H. (½ minute)	6	Thin.	Distended.	Nil.
Starved for one day at 80% R.H. (To satiety)	3	Thin.	Distended.	Distended.

DISCUSSION & CONCLUSIONS.

Discussion & Conclusions.

1- Culture Method and the Biology of C. nuculosus Mg. in the Laboratory.

As expressed by Leeson (1932) and Bates (1941), when the object is to obtain an adequate supply of adults, the method employed should be devised so as to achieve its object with a minimum of effort and the least expenditure of time. There is no particular need for standardization in such methods except in so far as may be necessary to insure a supply of vigorous and "normal" adults. For a "routine" culture method for C. nubeculosus, a simple and satisfactory method has been developed. A mixture of powdered mud which fills a depth of about 4 mms. of the used pot (about 50 grams), 2 moderate teaspoonfuls of dried autolyzed yeast (about 6 grams) and 1 moderate teaspoon-:ful of powdered charcoal (about 4 grams), was found adequate for maintaining the larvae. The pots used were 6 x 1 inch unglazed earthenware pots, without a drainage hole. The culture pots should be prepared about 3 weeks beforehand, and should be kept well watered. The recently hatched larvae are turned to the prepared pots and maintained at 20°C.

The "ripe" medium provides the larvae with a variety

of living and non-living organic matter, on which the larvae feed without discrimination; however, it was not possible to see them feeding on fungal spores or on the protozoan organ-:isms which usually occur in great numbers, but it is possible to assume that at least the dead bodies of these organisms contribute to the nourishment of the larvae. The bacterial film on the water surface is especially eaten through by the larvae, and it appears to contribute much to their nourish-:ment. Rodina (1949) states that the role of bacteria in the nutrition of tendipedid larvae is very great. Atkin & Bacot (1917) and Rozeboom (1935) point out the importance of bacteria for the normal development of mosquito larvae. In their indiscriminate feeding habits, the larvae of C. nubecu-:losus resemble the anopheline larvae (Coggeshall, 1926; Senior-White, 1928).

Up to 220 larvae were introduced into each pot, and apparently the larval density does not affect the develop-:ment or the percentage of emergence. In anopheline larvae, Bates (1941) states that in an unfavourable medium the re-:sults may be greatly influenced by the concentration of the larvae, but in favourable media the larval density does not seem to be so important.

The number of adults which emerge from a given culture pot varies from 17 - 91 per cent. of the larvae (from 23 - 120 adults), with an average of 45 per cent. Occasionally, some pots may yield only a few adults or none at all. Apparently, the inherent differences in the viability of the larvae belonging to different batches, or the same batch, play an important part. Barber (1927) expressed the same opinion concerning anopheline larvae; he states that such differences in the viability in larvae of the same age are often seen in open laboratory cultures and doubtless exist under normal conditions.

It is interesting to note that two salt-march species of Culicoides could be reared successfully by this method. Recently hatched larvae of C. halophilus, obtained from eggs laid in the laboratory by wild gravid females, thrived well in the described medium, and many of them reached the adult stage. Similar results were obtained by my colleague Mr. P. Becker with first instar larvae of C. circumscriptus obtained in the same way, and with advanced larvae obtained from the field. It is probable, therefore, that the described medium can maintain other species of Culicoides; and if the difficulty in inducing mating and feeding in the laboratory

can be overcome, more species can be established as laboratory cultures. Another possible practical use of this medium is to facilitate the study of the morphology, anatomy and systematics of the early stages of Culicoides spp. Undoubtedly, it is easier and more certain to rear larvae obtained from eggs laid in the laboratory by wild gravid females of known identity, than to collect samples of the natural habitat and sieve the larvae out for study.

The life-cycle of individual adults belonging to the same batch of eggs varies considerably. This is due to the larvae developing at different rates from each other, and, therefore, a proportion of them always lags behind in development. Hill (1947) also found that in C. impunctatus a proportion of the larvae from the same batch of eggs always considerably lagged behind in development. In the period January - October, the earliest adults to emerge complete their life-cycle in 3 - 6 weeks, while the latest adults take $4\frac{1}{2}$ - $8\frac{1}{2}$ weeks; the period of emergence varies from 1 - 5 weeks. Larvae obtained during October usually take a longer time for their transformation; a few adults may emerge in the normal time, but usually emergence takes place in late December, January and February. Thus the earliest adults to

emerge from such larvae complete their life-cycle in 6 - 11 weeks, while the latest adults take 13 - 18 weeks; the period of emergence occupies 2 - 12 weeks. However, it is possible to obtain 6 - 8 generations each year, the last of which - obtained from the October larvae - occupies a period of several months. Roberts (1950) mentioned that the duration of the life-cycle is 4 - 5 weeks, and Downes (1950) mentioned that the complete life-cycle may be as short as 3 weeks; these figures undoubtedly apply only to the earliest adults to emerge in the period January - October. Hill (ibid.) stated that the first flies of C. impunctatus and C. obsoletus to hatch from a given batch of eggs were all males. This is not always the case with C. nubeculosus.

Mating takes place readily in 3 x 1 inch tubes, and rarely in the cage. As observed by Pomerantsev (1932) and Downes (ibid.), mating also takes place while the females are engaged in feeding. It is certain that mating takes place independent of a blood meal; Hill (ibid.) suggests that fertilization in C. impunctatus and C. obsoletus appears to be independent of a blood meal.

A full blood meal is necessary for the development of the ovaries. A small meal does not cause any development

of the ovaries. In rare cases, however, it was observed that the ovaries of flies which fed to satiety remained undeveloped, and it can only be suggested that the size of the meal taken was inadequate. The female C. nubeculosus takes only one full blood meal in each gonotrophic cycle. Contrary to what is suggested by Gad (1951), sugars are not essential for ovulation. According to Hill (ibid.), individual females of C. impunctatus have been recorded as taking up to seven blood meals, and individual females of C. obsoletus have been recorded as taking five meals; however, she found that similar numbers of eggs are laid after one meal as after more than one. Sharp (1928) suggests that it is possible that C. austeni requires two blood meals in order to complete ovulation. Downes (ibid.) states that the females of C. nubeculosus will not bite until two to three days old, and Roberts (ibid.) states that they will take their first blood meal three days after emergence. In my experience, the females may feed during the first as well as the second or the third day after emergence, and occasional females may refuse to feed at all.

The females may lay their eggs 3 - 16 days after feeding, with an average of 3 - 4 days. In sections, the oocytes

appear to be fully developed in flies fixed 3 days after feeding. Downes (ibid.) found that egg-laying took place 4 - 5 days after the blood meal. On the other hand, Roberts (ibid.) states that oviposition takes place 2 - 3 days after the meal. However, I have never encountered any female which laid its eggs 2 days after the meal; in sections of females fixed 2 days after feeding, the oocytes are always seen to be oval in shape and never banana-shaped as in flies fixed 3 days after the meal. As found by Downes (ibid.), single females (each with a male) often refuse to lay their eggs, and it was the practice to put two females and two males in each egg-laying tube. It should be emphasized that a moist surface is essential for oviposition.

The number of eggs laid varies from 40 - 206 eggs per batch, with an average of 135 eggs. Roberts (ibid.) states that females fed on man laid an average of 380 eggs, but she did not mention whether this number was per batch or was the total number of eggs laid in several batches; in my experi-:ence, such a number is too big to belong to one batch. A good proportion of the gravid females frequently refuse to oviposit. Sometimes, white or grey eggs which fail to be-:come pigmented, are laid. Usually, oviposition is complete,

but in several cases from 1 - 2 eggs up to 70 eggs may be retained. According to Hill (ibid.), C. impunctatus may on rare occasions retain 1 - 2 eggs. In Simulium damnosum, according to Lewis (1953), after oviposition some old eggs often remain behind, sometimes 2 or 3 as in mosquitoes, and sometimes up to 20 or more.

The eggs hatch 3 - 6 days after oviposition with an average of 3 - 4 days; the minimum time as calculated by Lawson (1950) is more than 48 hours but less than 65 hours. The number of eggs which hatch varies from 26 - 96 per cent., with an average of 55 per cent.; Downes (ibid.) found that more than 80 per cent. of the eggs hatch. Sometimes, whole batches of eggs fail to hatch, sometimes only a few eggs hatch. All the viable eggs usually hatch within a short period, but occasionally a period of 1 - 2 days is required. As remarked by Parker (1950), possibly some artificiality in the laboratory environment acting, either directly on the eggs, or indirectly through the parent, is responsible for the high mortality sometimes experienced.

2- Anatomy and Histology of the Alimentary Tract

The alimentary tract of female C. nubeculosus is a simple tube. The fore-gut consists of the sucking apparatus (in part), the oesophagus, the oesophageal valve, and the oesophageal diverticulum. The mid-gut consists of an anterior narrow tubular part - the anterior segment, and a posterior dilated chamber - the stomach. The hind-gut consists of an anterior tubular portion which cannot be differentiated into parts - the small intestine, and a posterior dilated rectum which contains two rectal papillae. In addition, there are two Malpighian tubes and the salivary apparatus.

The sucking apparatus consists of two chambers or pumps contained within the head-capsule. In Nematocera, the nomenclature of these two pumps has been the subject of much controversy. Snodgrass (1943), however, shows clearly that the first pump is the homologue of the preoral cibarial pocket of the orthopteroid insects and terms it the "cibarial pump", and that the second pump, which he terms the "pharyngeal pump", is a modification of the pharynx. In particular, the sucking apparatus of C. nubeculosus resembles that of C. pulicaris (Jobling, 1928) and C. impunc-

:tatus (Gad, 1951). But Jobling termed the cibarial pump the "pharynx" and the pharyngeal pump the "oesophageal pump", and Gad did not mention the anterior and posterior sphincters of the pharyngeal pump.

According to Snodgrass (ibid.), the cibarial pump is present in all the Diptera. The Brachycera also have a pharyngeal pump, but it is formed from the anterior part of the pharynx and is activated by the precerebral dilator muscles; the Cyclorrhapha have only the cibarial pump.

The oesophagus is a thin-walled, narrow and short tube. It is very narrow anteriorly but it expands gradually. Its walls consist of a single layer of thin cells which rests on a basement membrane, and it is surrounded by a double layer of internal longitudinal muscle fibres and external circular muscles. The inner ends of the cells are lined by a thin cuticular intima, which becomes so thin and colourless posteriorly that it becomes difficult to detect in most sections. In Simulium nigroparvum, Cox (1938) describes only a layer of circular muscle fibres. In Phlebotomus papatasii, Adler & Theodor (1926) state that the oesophagus is attached for a considerable distance to the sides of the pharyngeal pump and thus a pouch is formed between the ex-

:ternal wall of the pharyngeal pump and the oesophagus; this pouch has been found to contain Herpetomonas. In the mosquito, Nuttall & Shipley (1903) stated that the oesophagus is plentifully supplied with bands of muscles, and that the average outline of the cells is roughly cubical. In Tabanus, Cragg (1920) states that the cuticular intima is well developed.

The oesophageal wall extends slightly into the lumen of the mid-gut, then is reflected forwards to join the latter. The stomodaeal circular muscles around the invagina-:tion are highly developed to form the sphincter muscles. No cuticular intima can be detected. The whole structure forms a regulatory valve - the oesophageal valve. The oesophageal valve has almost the same structure as in the nematoceran and brachyceran blood-sucking flies. In these flies the oesophageal valve has no role in the formation of the peri-:trophic membrane, and only functions as a regulatory valve. On the other hand, in the cyclorrhaphan blood-sucking flies, e.g., Glossina (Wigglesworth, 1929), in which the peritrophic membrane is produced by a group of specialized cells at the anterior end of the mid-gut, the oesophageal invagination is funnel-shaped and is composed of large tense cells forming

a solid plug, and serves to press the precursor fluid of the peritrophic membrane into a solid homogeneous tube. Smart (1935) states that in Simulium ornatum, the component tissues of the oesophageal valve, except for some ill-defined differences in the shape and size of the component cells and the complete absence of a striated margin in the cells, are similar to those of the anterior portion of the mid-gut. In S. nigroparvum, Cox (ibid.) states that the inability to distinguish a cuticular intima made it difficult to determine the limits of the fore- and the mid-gut, i.e., of the ectodermal and mesodermal tissue. In C. nubeculosus, however, the cells of the valve can be easily differentiated from those of the anterior segment. They are obviously smaller and narrower, their nuclei are smaller and they lack the characteristic striated border. In Phlebotomus papatasii, Adler & Theodor (ibid.) state that a small pouch forms between the sides of the anterior segment and the oesophageal valve. Four small elliptical oesophageal glands, which contain large yellow granules, lie round the commencement of the mid-gut and each gland opens by a minute duct into the pouch between the oesophageal valve and the mid-gut.

Just in front of the point of invagination of the oesophagus into the mid-gut, the ventral wall of the

oesophagus is evaginated to form a single capacious diverti-:culum, which lies ventral to the mid-gut. It is composed of a dilated sac with a long narrow duct. It is capable of much distension, and when fully distended it may reach the 4th or 5th abdominal segment. It shows vigorous peri-:staltic movements. As in the ventral diverticulum in mosquitoes (Nuttall & Shipley, ibid.), the oesophageal diverticulum in C. nubeculosus seems to open into the oesophagus by means of a simple pore. The duct of the diverti-:culum is of the same structure as that of the oesophagus; the walls of the sac are extremely thin and are coated with an irregular network of muscle fibres.

All the blood-sucking flies, except mosquitoes, have only one oesophageal diverticulum, which is more or less similar to that of C. nubeculosus. In Phlebotomus papatasii (Adler & Theordor, ibid.), there is a very narrow sphincter at the junction of the oesophagus and the diverticulum; the diverticulum opens into the oesophagus at a varying distance from the posterior end of the latter and exceptionally it opens into the pharyngeal pump together with the oesophagus. In Tabanus (Cragg, ibid.), it is bi-lobed. In mosquitoes (Nuttall & Shipley, ibid.), there are three diverticula:

one large and ventral, which is similar to that of C. nubeculosus, and two small latero-dorsally situated ones which open laterally into the oesophagus. In serial sections, the oesophageal diverticulum of C. nubeculosus was sometimes seen to contain fungal hyphae.

The mid-gut is a simple straight tube of varying diameter. It is composed of two portions which merge into one another; the anterior portion - the anterior segment, is narrow and lies in the thorax, the posterior portion - the stomach, is several times wider and lies in the abdomen. The stomach is the chamber which becomes greatly distended with blood after a feed, while the anterior segment contains only a small amount and never becomes distended. The mid-gut undergoes peristaltic movements which are most marked in the stomach.

The wall of the mid-gut consists of a single layer of epithelial cells which rests on a basement membrane, and is surrounded by a network of inner circular and outer longi-:tudinal muscles. The ventricular epithelium varies greatly according to functional activity. When the gut is empty, the cells are columnar or cubical and are grouped into folds or villi which project into the lumen and give it a stellate

appearance in cross sections; the villi are far higher in the stomach than in the anterior segment. After a full blood meal, the greater part of the length of the epithelium of the stomach becomes reduced to flat cells. Scattered throughout the epithelium of the mid-gut are small regenera-:tive cells, which occur singly, and are usually seen at the outer end of the epithelium. The posterior end of the stomach - just above the openings of the Malpighian tubes - narrows abruptly, and the circular muscles become exaggerated to form the mesenteric sphincter. The mid-gut is well supplied with tracheae, tracheoles and transition cells, which rest on the outer surface of the gut. No peritrophic membrane exists in the unfed flies.

There are, however, some differences between the epi-:thelium of the anterior segment and that of the stomach. The cells of the anterior segment do not stain deeply with haematoxylin as do those of the stomach, and are relatively small. Their nuclei are also smaller than those of the cells of the stomach, and are usually round in shape, while those of the cells of the stomach are usually oval. The striated border, which is characteristic of the cells of the mid-gut, is strikingly broad in the anterior segment, and is always

evident though it may become homogeneous; in occasional cells, in which a vacuolated cytoplasmic mass is about to be nipped off the inner end, it disappears. In the stomach, the striated border, when evident, is always thin and it may become homogeneous or totally disappears. Vacuoles are usually present in the cells of both segments but they are relatively big and in greater numbers in the stomach.

There seems to be but a little difference - probably specific - in the morphology and histology of the mid-gut between C. nubeculosus and the nematoceran and brachyceran blood-sucking flies. In Simulium ornatum (Smart, ibid.), the epithelium of the stomach, when undistended, may be as thick as that of the anterior segment; the striated border is thicker in the anterior segment. In Phlebotomus papatasii (Adler & Theodor, ibid.), the two segments of the mid-gut differ histologically; the cells of the stomach lack the striated border, while the cells of the anterior segment have a conspicuous striated border with the striae (rods) each 7.5 - 10 μ long. In the mosquito (Christophers, ibid.), there is little structural difference between the anterior segment and the stomach; Nuttall & Shipley (ibid.), however, state that the mid-gut has a similar structure throughout.

In Tabanus (Cragg, ibid.), the layer of cells in the anterior segment is folded into innumerable small caeca; the striated border is remarkably well developed in the anterior segment, while it is delicate in the stomach. Cragg did not mention the mesenteric sphincter, but Wigglesworth (1931, a) described it in Chrysops silacea. On the other hand, in the cyclorrhaphan Glossina (Wigglesworth, 1929), the mid-gut is long and coiled, and is divided into three regions. The anterior segment is composed of an irregularly columnar epithelium which does not stain deeply with haematoxylin. The base of each cell is invaginated to receive the circular muscle fibres, a peculiar feature which is doubtless associat-:ed with the great distension to which this region of the gut is subjected; in this segment there is a zone of giant cells which contain symbionts. In the middle segment, the epi-:thelial cells are heaped together so that in the contracted state the lining of the gut appears to be composed of many layers of cells packed with deeply staining granules and pro-:ceeding as a free border, sometimes hyaline, sometimes striated, which stains pink with eosin. The change from the middle to the posterior segment is less abrupt. After a few secondary turns the diameter of the gut diminishes, the epithelium gradually becomes less heaped and eventually

gives way to a regular cubical or low columnar of rather deeply staining cells which continues unchanged until the entry of the Malpighian tubes marks the termination of the mid-gut.

In the blood-fed flies, a peritrophic membrane forms in the stomach as a thin capsule which envelops the ingest-:ed blood, and when the blood is digested the membrane is evacuated with the excreta. It is very evident in flies fixed 5 hours after the meal. In such flies droplets of an eosinophil secretion can be seen streaming from the cells of the stomach towards the membrane, thus adding to its substance. The membrane stains pink, but later it becomes almost brown due to the incorporation of haematin in its substance, which may be an artifact. In flies fixed 24 hours after the meal, the posterior end of the membrane is seen perforated to allow the faecal matter to pass down to the hind-gut. In the full-fed flies, the peritrophic mem-:brane is composed of a few lamellae, but in flies which took a partial meal it is single-layered and is relatively thick. Lewis (1953) observed the same phenomenon in Simulium damnosum; in this insect, however, up to 30 laminae may be seen. He suggests that it is possible that the mid-

gut epithelium secretes as much membrane material after a small meal as after a large one and that the blood mass after a small meal, therefore, becomes enclosed by a thicker membrane.

A peritrophic membrane has been known to exist in other blood-sucking flies after a feed. In Nematocera, it has been found in mosquitoes (Yaguzhinskaya, 1940; Pal, 1943), Phlebotomus (Adler & Theodor, 1926; Dolmatova, 1942) and Simulium (Lewis, 1950; 1953). In Brachycera, it has been found in tabanids (Olsufev, after Yaguzhinskaya, 1940). In Cyclorrhapha, it has been found in Glossina (Wigglesworth, 1929), Stomoxys calcitrans and Haematobia stiumlans (Kuzina, 1942). The peritrophic membrane of Anopheles maculipennis (Yaguzhinskaya, ibid.) and of Simulium damnosum (Lewis, 1953) have much in common with that of C. nubeculosus. They are secreted by the epithelium of the stomach after a blood meal, are laminated, and they rupture posteriorly some time after the meal. It appears, however, that the nematoceran and the brachyceran blood-sucking flies agree in that the substance of the peritrophic membrane is secreted by the cells of the stomach, and only after a blood meal. On the other hand, in the cyclorrhaphan Glossina (Wigglesworth, ibid.), it is secreted by a group of specialized cells at the anterior limit

of the mid-gut, and its secretion is enhanced by a blood meal; in the freshly emerged fly the peritrophic membrane is ragged and discontinuous, but immediately after the first meal it is present and formed as perfectly as in the mature insect and extends to the limit of the ingested blood. In Stomoxys calcitrans and Haematobia stimulans (Kuzina, ibid.), it is also present in fed and unfed flies; it forms 3 - 4 hours after emergence and remains after the blood has all been digested.

It has been generally agreed upon that the peritrophic membrane in insects serves to protect the cells of the mid-gut from damage by hard or sharp particles - a view which certainly does not apply to blood-sucking flies. Also, it is suggested that it functions as an ultra-filter (Day & Waterhouse, 1953, p. 306). However, it seems that the presence of the peritrophic membrane in the blood-sucking flies is of special significance. Yaguzhinskaya (ibid.) suggests that in Anopheles maculipennis, it may have a bear-:ing on the penetration of parasites into the wall of the mid-gut, and that the transmission of causal agents of disease by blood-sucking insects may depend partly on the character of the peritrophic membrane. Lewis (1953) states

that in Simulium damnosum, the peritrophic membrane plays an important part in limiting the number of microfilariae that survive to reach the sausage stage.

The hind-gut is composed of the small intestine and the rectum. The small intestine is a narrow tube of moderate length, which forms one or two loops in the abdominal cavity. Its anterior part is capable of much distension during the excretory activity of the stomach and the Malpighian tubes. The wall of the small intestine is composed of a single layer of cubical cells which rest on a basement membrane, are lined with a thin cuticular intima, and are coated by a sparsely arranged double layer of inner circular and outer longitudinal muscles. The cells are fairly large - their long axes being parallel to the basement membrane. The wall of the small intestine is thrown into small folds; in the anterior end of the small intestine, when it is distended with excrement, the folds disappear and the cells flatten. The opening from the small intestine into the rectum is guarded by a simple valve - the rectal valve. This valve is formed by a slight protrusion of the hinder portion of the small intestine into the lumen of the anterior end of the rectum, and its reflexion forwards to join the rectal wall;

the circular muscles of this region are exaggerated to form the sphincter muscles. The rectal valve, apart from pre-:venting any reflux, prevents the rapid and untimely entry of the excrement into the rectum. The rectum is dilated anteriorly into a capacious sac - the rectal sac, and narrows posteriorly to form a short tube - the rectum proper - which proceeds direct to the anus. The rectal sac contains two rectal papillae projecting into the lumen, and usually visible through the membranous wall of the rectal sac. The rectum is thin-walled, and the epithelium is greatly reduced except where it forms the rectal papillae. A cuticular intima is difficult to detect, except around the rectal papillae. The musculature of the rectum is well developed and is composed of an inner layer of longitudinal and an outer layer of circu-:lar muscles. The rectal papillae are composed of large cells with big nuclei. Each papilla is covered with a thin cuticu-:lar intima, and its lumen contains a tracheal branch. The anus is furnished with a strong sphincter muscle.

In Simulium nigroparvum (Cox, ibid.), the hind-gut is divided into the distal intestine, the rectal pouch and the rectum. The epithelium of the distal intestine consists of flattened cells, which are raised into folds in the posterior

part. Covering the epithelium is a layer of circular muscles which forms a continuous layer at the posterior end. The intima is very thin. The walls of the rectal pouch are ex-:tremely thin and the epithelium is greatly reduced except where it forms the rectal papillae. Both muscle layers are very thin. In the rectum, both muscle layers as well as the epithelium are well developed and the cuticular intima can be easily detected. In Phlebotomus papatasii (Adler & Theordor, ibid.), the wall of the hind-gut consists of two layers of muscles, one longitudinal and external and the other oblique and internal. The lumen is lined by a single layer of cubi-:cal epithelium, the inner aspect of which is covered with an exceedingly thin glistening chitinous layer. In the mosquito (Nuttall & Shipley, ibid.), the hind-gut is divided into the ileum, the colon and the rectum, and ends with the anus. The ileum is very short and transparent, and may be somewhat dilated near the mid-gut; it is lined with flattened epi-:thelium covered with a cuticular intima. The colon succeeds the ileum, without there being any line of demarcation; it is lined by a single layer of cubical cells, and the muscular coat is well developed, showing a well marked fenestration or crossing of fibres. The rectum forms a spacious oval

chamber into which the colon suddenly opens; it is lined with flattened epithelium. In Tabanus (Cragg, ibid.), the hind-gut may be divided into an ileum, which forms a single loop in the posterior part of the abdomen, a colon, of slightly greater diameter running straight backwards to open into the rectum. In the tsetse flies (Lester & Lloyd, 1928), the hind-gut is divided into three portions. The first portion or "protorectum" is a narrow powerful tube, the pores of the Malpighian tubes entering it at a small dilatation immediately behind the mesenteric sphincter. At its hinder end is a simple valve formed by a reflexion of the gut wall inwards and backwards to form a nipple-like projection, which protrudes into the next region, the "mesorectum". This region is form-:ed by a sausage-shaped distensible tube, which communicates with the globular "metarectum" by a small pore on the antero-dorsal wall of the latter. The rectal papillae are of almost the same structure in all adult Diptera, but in the blood-sucking flies, they differ in their number: in C. nubeculosus and Phlebotomus papatasii (Adler & Theodor, ibid.) they are two; in Simulium spp. (Smart, ibid; Cox, ibid.), mosquitoes (Christophers, ibid.; Nuttall & Shipley, ibid.), and Tabanus (Cragg, ibid.) they are six; in the tsetse flies (Lester &

Lloyd, ibid.) they are four. According to Engel (1924), the usual number of rectal papillae in the Diptera is four or six, the larger number being confined to the Orthorrhapha; in Culex pipiens there are four in the male and six in the female.

Except in the tsetse flies, the rectal valve was not mentioned by any of the afore-cited authors, and it is very likely that it was overlooked. Among the non-blood-sucking Diptera in which a rectal valve was described is Lucilia sericata (Wigglesworth, 1932) and Calliphora erythrocephala (Graham-Smith, 1934).

At the point of junction of the mid-and the hind-gut two Malpighian tubes of a white yellowish colour arise, one on each side of the gut. They are cylindrical tubes of consider-:able length and of practically uniform diameter throughout. They take a convoluted course in the abdominal cavity, extend-:ing forwards to the anterior end of the abdomen, then re-:curve and extend backwards to lie beside the rectum. They are bound to the gut with numerous tracheal branches. At the entrance of the tubes in the gut, they are seen to be lined for a short distance by relatively small cubical cells, which are continuous with and similar to the cells of the mesenteric sphincter. The very large cells, characteristic of the Mal-

:pighian tubes, soon appear. The Malpighian tubes of C. nubeculosus give attachment to muscular branches of different sources. At the anterior region of the abdomen, a branch of the alary muscles of the pericardial septum is seen attached to them. At their basal ends, the tubes receive longitudinal muscle fibres from the posterior end of the mid-gut. At the same place, the tubes receive a branch of the longitudinal muscle fibres of the small intestine (but this may be a small tracheal branch). In Drosophila funebris (Eastham, 1929), the Malpighian tubes give attachment to a branch of the alary muscles of the pericardial septum, and also to circular and longitudinal muscle fibres continuous with the muscles of the mid-gut. In Rhodnius prolixus (Wigglesworth, 1931, c), only a few muscle fibres, running out from the mid-gut over the ampullae to the lower end of the Mapighian tubes, can be seen.

C. nubeculosus has two Malpighian tubes, and that is an exceptional number. The usual number in Diptera is four (Snodgrass, 1935, p. 380). However, according to Saunders (1924), it appears that the heleids (larvae at least) are divided fairly evenly in possessing two or three Malpighian tubes, but Forcipomyia is the only genus in which both con-

:ditions occur. He states that the possession of three is unique among insects; Berlese quotes Culex and Psychoda with five as the only known examples of "oligonephrous" insects with odd number of Malpighian tubes, to which may be added Anopheles, Ptychoptera and the Blepharoceridae, all with the same number. In these the odd one is dorsal, but in the heleids it is ventral.

The salivary apparatus consists of a pair of salivary glands, six accessory glands arranged around the mouth of each gland proper, two salivary ducts and a common salivary duct, a salivary pump, and the hypopharyngeal salivary duct.

Each salivary gland is a tube which dilates gradually from before backwards, and bends upon itself in the form of a U. They lie in the anterior part of the thorax, but it is common for one or both glands to lie straight and extend back-:wards into the anterior end of the abdomen; abnormally, one gland was seen to extend forwards into the head-capsule. The salivary gland consists of a single layer of cubical cells which rest on a basement membrane, and surround a central lumen which contains a granular eosinophil secretion. The lumen of the salivary glands becomes almost empty of secretion after a full meal, in contrast with mosquitoes (Christopher,

ibid.) in which the salivary glands remain quite full of secretion. After a small meal, the lumen contains amounts of secretion more or less inversely proportional to the amount of ingested blood; this suggests that the saliva is not forced into the wound at once, but is discharged throughout the period of feeding.

The accessory glands are six small saccular structures, three small and oval and three rather big and rectangular, arranged alternately in a rosette around the mouth of each salivary gland proper, each sac opening into it by means of a minute duct. They lie partly in the thorax and partly in the neck, Patton and Evans (1929, p. 140) suggested that these sacs probably serve as temporary reservoirs for the secretion of the salivary glands. This would have been true if their contents were similar to those of the salivary glands proper. On the contrary, they rarely contain more than a trace of secretion similar to that in the salivary glands, though the latter are full of it; rarely, the lumen of one or two sacs may be seen filled with it. They frequently look empty or may contain a little amount of a homogeneous secretion which stains faintly with eosin, a little amount of a granular secretion which stains with haematoxylin, and a

few small, glistening and colourless droplets. As in Notonecta (Baptist, 1941), each accessory gland is vesicular, the glandular epithelium is thin and flattened, and it secretes a watery fluid but may sometimes serve as a reservoir of the principal gland. The presence of accessory glands in C. nubeculosus is very interesting as, so far as I am aware, these have not been described in any other blood-sucking fly.

At the point of junction between the salivary glands and its accessory glands emerges the annulated, cuticular salivary duct which is wide to begin with but soon narrows down. The two ducts unite in the head-capsule, beneath and in contact with the suboesophageal ganglion, to form the common salivary duct which proceeds forwards and opens into the salivary pump.

The salivary pump is a small capsule situated beneath the ventral wall of the cibarial pump. Its anterior end con-:tinues as the hypopharyngeal salivary duct. Its dilator muscles which arise on the ventral wall of the cibarial pump are inserted into the middle of its elastic dorsal wall. The salivary pump of C. nubeculosus resembles that of C. pulicaris (Jobling, ibid.) and C. impunctatus (Gad, ibid.). According to Snodgrass (1943), the salivary pump is present in all the Diptera.

There are, however, some specific differences between the salivary glands in the blood-sucking flies. In Simulium (Smart, ibid.; Cox, ibid.), the two salivary glands are tubular and U-shaped, each with a round reservoir from which passes the salivary duct. In Phlebotomus papatasii (Adler & Theodor, ibid.), they are almost spherical. In mosquitoes (Christophers, ibid.; Nuttall & Shipley, ibid.), each salivary gland is composed of three tubular acini lying one above the other in the long axis of the body. Each acinus has an intra-glandular or intra-acinar duct; shortly after leaving the acinus the three intra-acinar ducts unite to form a single duct. A common abnormality is a small accessory acinus near the proximal end of an acinus (Christopher, ibid.); Roy & Mayne (1931) observed supernumerary acini in the salivary glands of several anopheline mosquitoes. The middle acinus differs from both the two lateral ones. Christopher mentions that the middle acinus is of the clear or colloid-like type and, in preparations, its secretion is clear and homogeneous, and stains readily, sometimes quite deeply, with haematin; the other two acini are of the granular type, and their secretion is granular and stains faintly with haematin. Nuttall & Shipley, on the other hand, state that the secretion

of the lateral acini does not stain with eosin as does that of the middle one.

It is interesting to note that in one case, tumours were observed at the base of the stomach. These were several pro-:truding saccular swellings of different sizes; two of them had a triangular dark-brown area, and one had a small round spot of the same colour. In the same specimen, the Mal-:pighian tubes were of a deep orange colour, the base being swollen and ampulla-shaped followed by a small narrow area after which the tube was flattened for a considerable length before it took its usual cylindrical shape. Each tube was coiled in a mass, intermingled with many tracheae and tracheal branches, and lay beside the hind-gut. Insects known to develop tumours are few i.e., Drosophila melanogaster (Russell, 1940; 1942), Leucophaea maderae (Scharrer, 1945; 1948), Dixippus and ants (Vide, Scharrer, 1945), and Gilpinia hercyniae (Bird, 1949).

3- The Changes in the Mid-Gut Epithelium following a Blood Meal

To get a reasonable account of the changes which occur in the mid-gut epithelium in C. nubeculosus following a blood meal, it was found necessary to study the changes which occur in flies which did not feed as well as the changes which occur at the start of feeding and after taking small and full meals. All the flies used, except those used during the first day after emergence, were supplied regularly with water and raisin. They were kept at 20°C and 80 - 90 per cent. relative humidity. The flies which did not feed were of known ages. As the histological appearance of the epithelium directly after a blood meal was almost the same in flies of different ages, the age factor was not observed in all the fed flies.

There are no apparent changes in the epithelium of the anterior segment, but the changes in the epithelium of the stomach are remarkable. Both segments were shown to have structural differences, and they appear to differ functionally as well. Structural and functional differences in the two segments of the mid-gut are recorded by Cragg (1920) in Tabanus and by Wigglesworth (1931, a) in Chrysops silacea. According to Wigglesworth (ibid.), the absence of enzymes

from the anterior segment (cardia) of Chrysops is interesting, for, as found by Cragg in Tabanus, and confirmed in Chrysops, there is a continuous secretion on the part of the cells of the anterior segment; the nature of this secretion is unknown. Wigglesworth (1929) also describes the structural and functional differences in the three segments of the midgut of Glossina.

A- The anterior segment

The cells of the anterior segment are low columnar or cubical in shape, and are thrown into rather small villi. In flies which refused to feed during the first day after emergence, some are seen to have the cells with a uniform cytoplasm which contains no vacuoles; the lumen is empty of secretions. These specimens are probably the youngest in the group. In the rest of the specimens, a few small vacuoles are seen at the outer half, and sometimes at the inner half, of the cells. Occasionally, one or two vacuoles are seen pushing themselves against, or within, the striated border. Also, one or two vacuolated cytoplasmic masses or globules, which may be nucleated, are sometimes seen while about to be nipped off the inner end of the cells; in such cells the striated border disappears. In flies which refused to feed

during the second and the third days after emergence, the histological picture is almost the same. In all the unfed flies, however, the striated border is almost always thick, clearly striated or sometimes homogeneous. The lumen of the segment contains varying amounts of a granular secretion which stains pink; similar granules are usually seen stuck to the inner end of the striated border.

Cragg (1920) states that in the unfed Tabanus, vacuoles in the cells of the anterior segment are rare, and when pre-:sent are small and are situated in the inner half of the cell. In the unfed C. nubeculosus, however, though they are usually few and may be absent in several cells, vacuoles can hardly be described as rare. They are generally small, and are seen at either half of the cell or scattered; they are more common, however, at the outer half of the cell.

At the start of feeding, a negligible amount of blood may be seen in the lumen. The epithelial cells remain low columnar or cubical in shape, and are thrown into villi. The striated border is clearly striated. Vacuoles are few.

Directly after a small meal, a very little amount of blood is seen in the lumen of the anterior segment, and the corpuscles are in direct contact with the striated border.

One hour later, no blood is seen in the lumen. From the time of ingestion till the mid-gut becomes emptied - 24 hours after the meal - the epithelial cells of the anterior segment remain columnar or cubical in shape, thrown into small villi, and the striated border remains clearly striated except in flies fixed 5 hours after the meal, in which it is homogeneous. Vacuoles are generally scarce, but they usually appear in the cells at the posterior end of the segment. The lumen contains a little amount of the pink granular secretion, except in flies fixed 24 hours after the meal in which it is empty; similar granules are usually seen stuck to the inner end of the striated border.

Directly after a full meal, the lumen of the anterior segment is seen to contain varying - generally small - amounts of blood, which cause no distension to the segment. The blood corpuscles are in direct contact with the striated border. A few vacuoles are seen at the posterior end of the epithelium. One hour later, only a few corpuscles may be seen at the posterior end of the lumen, in direct contact with the striated border or embedded in the blood plasma which occupies the very posterior end of the lumen (and also the anterior end of the lumen of the stomach). A few vacuoles are seen in some cells.

Occasionally, some rather big vacuoles are seen at the inner end of the cell or within the striated border. Here and there, a cytoplasmic mass may be seen while about to separate itself from the cell, or contained in the lumen. In some favourable places, it appears that secretion of granules is taking place through the striated border; the striae are driven apart in two sets, apparently to allow the granular secretion to pour out.

In flies fixed 5 hours and also 24, 48, 72, 96 hours after the meal and after oviposition, the lumen contains, more or less, a little amount of the pink granular secretion, and rarely a few cytoplasmic globules. Vacuoles are few and scattered. In the groups studied, the cells remain low columnar or cubical in shape and are thrown into small villi. The striated border remains conspicuously broad, clearly striated or homogeneous; in one fly fixed 5 hours after the meal it was thin.

It is clear, therefore, that the cells of the anterior segment do not demonstrate any significant change following a blood meal. Their secretions are apparently discharged as free granules and clear unstainable vacuoles which pass to the lumen through the striated border, and as vacuolated

cytoplasmic masses which are nipped off the inner end of the cells, which eventually break down in the lumen. In Tabanus (Cragg, ibid.) and similarly in Chrysops (Wigglesworth, ibid.), the cells of the anterior segment discharge spherical masses of secretion which break up and become reduced to an eosinophil matter; also secreting cells in which small clear droplets of an unstainable substance emerge between the striae of the striated border, are occasionally seen. In C. nubeculosus, somewhat as in Tabanus, the secreting cells in the anterior segment are most numerous in the unfed fly. Also, the discharge of the secretions does not depend upon feeding, and usually, only a small proportion of the epithelium is secreting at any one time. In Culex pipiens, De Boissezon (1930) states that the cells of the anterior segment have two sorts of secretion: a hyaline secretion of nucleolar origin and a secretion made up of cytoplasmic granules. He also suggests that their secretory activity seems to be exercised also in the state of starvation.

B- The stomach

In the unfed flies, the epithelial cells of the stomach are from long columnar to cubical in shape according to their position. They are grouped into villi which, though differing

in height in different specimens, are generally far higher than those of the anterior segment. The striated border is obviously thinner than that of the cells of the anterior segment. The cytoplasm stains deeply with haematoxylin ex-:cept at the locations of vacuoles.

In flies which refused to feed during the first day after emergence, a few vacuoles are seen. These are generally small and are usually located at the inner part of the cell, though they may also be present at the outer end. These vacuoles, however, differ in size and in number in the different cells, and may be absent in many cells even in the same section. These features reasonably suggest that vacuoles do not develop simultaneously in all the cells of the stomach, and that their degree of development is not the same even in adjacent cells. In these specimens, the lumen contains a little amount of a blue granular secretion, and similar granules are usually seen stuck to the inner end of the striated border. In Tabanus (loc.cit.), no such granules are described. In one specimen, an active secretion is taking place. In cells fixed in the act of secretion the striated border is usually absent, and a mass of cytoplasm is passing towards the lumen of the stomach, the mass being attached to

the cytoplasm of the secreting cell by a long narrow neck; in some places the neck is seen penetrating the striated border. Eventually, the cytoplasmic globule is nipped off and set free in the lumen. Several such globules are seen accumulating in the lumen in different stages of disintegration. Cells which had already discharged their secretion regained their normal shape. This specimen was the only one in all the groups of unfed flies which showed this secretory activity.

In flies which refused to feed during the second day after emergence, the cells are generally highly vacuolated, and the intercellular walls tend to disappear. The inner part of the cells usually becomes converted into a mass of large vacuoles which come together. The vacuolated tips of the cells stain faintly, if at all, with haematoxylin, but the narrow area just below the striated border and also the granules in the matrix between the vacuoles stain deeply. The striated border becomes homogeneous, but it commonly disappears in some places. The lumen contains a little amount of the blue granular secretion, which is sometimes mixed with pinkish granules.

In flies which refused to feed during the third day

after emergence, the cells demonstrate striking changes. Vacuoles are excessively reduced in number and in size; only a few small vacuoles are seen. A somewhat similar case has been reported in the larva of the blow fly. According to Hobson (1931), vacuolation is almost absent in larvae starved for 48 hours. Gaps between adjacent cells are also seen, and many cells are showing symptoms of degeneration, i.e., either have lost their nuclei or contain diffusely-staining ones. The striated border is clear, and some blue granules, similar to those in the lumen, are seen stuck to its inner end.

It is clear, therefore, that in the unfed C. nubeculosus, the histological appearance changes according to the age of the fly. In flies less than 24 hours old, vacuoles are usually few and small, then they increase considerably in flies of about 48 hours old, and then become excessively reduced in flies of about 72 hours old. In the latter group of flies, many cells undergo degeneration. In all the groups, however, the cells are grouped into villi. The lumen contains a small amount of a blue granular secretion; similar granules are usually seen stuck to the inner end of the striated border. This granular substance is either secreted as such, or it may be the product of the break-down of some cytoplasmic masses.

The discharge of cytoplasmic globules or masses was observed in one specimen less than 24 hours old only. In the unfed Simulium nigroparvum (Cox, 1938), the inner ends of the cells bud off and there is no rupturing of the cell wall. On the other hand, Cragg (ibid.) states that in the hungry Tabanus, the cytoplasm of the epithelial cells of the stomach is compact, containing at most one or two large vacuoles and frequently none at all.

On the other hand, if a fly willing to feed is killed a few seconds after inserting its proboscis, the cells of the stomach show a very pronounced secretory activity, though only a trace of blood is seen in the anterior end of the lumen. The cells are still high columnar and grouped into villi; their inner parts are highly vacuolated and the striated border is absent in most places. They are actively discharg-:ing large amounts of cytoplasmic masses or globules which accumulate in the lumen of the stomach. These masses are round and of varying, but considerable, diameters; a few of them are nucleated. They stain blue with haematoxylin, but the vacuolated masses stain lightly. This activity of the cells suggests that either the probing of the skin, or the taste of the blood, or both stimulate the cells of the stomach

to discharge their secretion; it is also possible that approaching the host acts as a stimulus.

Following a blood meal, the epithelium of the stomach undergoes striking changes, which differ in flies which take a small meal and those which take a full meal. It has already been pointed out that it is the stomach which receives the ingested blood, and that the amount of blood seen in the anterior segment directly after a blood meal is very small and does not cause any distension to it. After a blood meal, the fore- and the hind-gut become shut off by the closure of the oesophageal valve anteriorly and the mesenteric sphincter posteriorly. In the stomach, the ingested blood is digested and the assimilable materials are absorbed. A full meal of blood usually takes about 48 hours (at 20°C. and 80 - 90% R.H.) to be completely digested, though the haematin contained in the stomach passes to the hind-gut some time afterwards. A small meal is usually digested, and the stomach emptied, within 24 hours.

Directly after a small meal, the epithelial cells of the anterior part of the stomach remain columnar in shape, contain but a few vacuoles, and are grouped into villi. In the rest of the epithelium the villi have disappeared and the

cells become reduced to a more or less low cubical shape; the height of these cells, however, depends on the amount of the ingested blood. Vacuoles are few and scattered, and some cells are still seen budding off. The striated border is absent in several places. The ingested blood is seen in direct contact with the cells, and a few haematin granules and an occasional cytoplasmic globule are seen mixed with it. In Tabanus (Cragg, ibid.) and similarly in Chrysops (Wigglesworth, ibid.), when the fly is fixed within 5 minutes after having about half the full meal, the least alterated cells, though still columnar in shape and arranged in villi, have the inner parts converted into a mass of large vacuoles, and the striated border which becomes homogeneous at first eventually disappears. The inner ends of the cells are eventually cast off; a certain proportion of the discharged masses contain degenerate nuclei. In C. nubeculosus, these features - except that the nucleated cytoplasmic masses are few - are only seen when the feeding fly is killed a few seconds after starting to feed. In flies killed within 1 minute after having a small meal, only a relatively small number of cells is seen in the act of secretion - the majority of the cells have already discharged their secretions. Vacuoles are generally few, and only a few globules are discharged. Similar features were

observed in Tabanus and similarly in Chrysops when the flies were fixed within 1 hour after the meal. In Glossina, Wigglesworth (1929) states that if the middle segment of the mid-gut be examined in sections 1 hour after a small meal, the changes in the epithelium (and the contained blood) are seen to be very similar to those described in Tabanus by Cragg, except that the epithelium does not seem to undergo such extreme disruption as in Tabanus and that the nuclei in the epithelial cells are rarely discharged in the process.

In flies killed 1 hour after the meal, the cells at the anterior end of the stomach are still columnar in shape and are grouped into villi. They become highly vacuolated. Many cytoplasmic globules, some of which are nucleated, are discharged. In this area, the lumen is devoid of blood, or at most contains a little amount of it. At the posterior end of the stomach, high cells are also seen, and some of them are budding off. The major part of the length is composed of almost flat cells which contain but a few vacuoles; cytoplasmic globules are very rarely met with. The striated border is generally homogeneous, but it disappears in some places. The blood becomes separated from the epithelial cells by a thin layer of fine pink granules - the precursor fluid

of the peritrophic membrane. Five hours after the meal, the villi at the anterior end of the stomach become remarkably reduced in height, and the cells become low columnar or cubical in shape, and highly vacuolated. The rest of the epithelium is composed of cells which vary in shape from low cubical to almost flat, and contain some vacuoles; as stated by Cragg (ibid.), the variation in the shape of the cells is largely due to peristaltic movements which cause one part of the stomach to be stretched and the other to contract. The blood in the lumen is enclosed in a well-defined, single-layered peritrophic membrane, which stains pink with eosin. It is granular in appearance and is rather thick. The number of intact corpuscles is still great, and only a few scattered haematin granules are seen. The striated border is homogeneous. Twenty-four hours after the small meal, the stomach is seen empty and crumpled; the meal has been digested, the assimilable materials absorbed, and the wastes dejected. The epithelial cells are high columnar or cubical in shape, according to their position, and are highly vacuolated. They are heaped up into high villi. Generally, the histological appearance is more or less the same as that of flies which did not feed during the second day after emergence.

The histological changes in the epithelium of the stomach after a full blood meal are generally different from those which occur after a small meal. Except for a short area anteriorly, and frequently also posteriorly, the epi-:thelium becomes greatly stretched and the columnar cells become flat. The reduction of the columnar epithelium to flat cells has been noted in the mosquito by Christophers (1901) and in Tabanus by Cragg (ibid.). It is interesting to note that in Glossina (Wigglesworth, ibid.), it is the anterior segment which becomes greatly distended with blood, while the middle segment is never so pronouncedly distended. For convenience, the changes in the different parts of the stomach will be discussed separately.

Directly after a full meal, the cells of the anterior part of the stomach remain columnar in shape and grouped into rather low villi. They contain some vacuoles, and some of the cells are budding off. The striated border is clear in several places. The villi at the posterior end of this area flatten out within 1 hour after the meal and the cells become cubical in shape. In flies fixed 1 hour and 5 hours after the meal, the striated border is homogeneous, vacuoles are few, and only a few cytoplasmic globules are nipped off.

Striking changes occur 24 hours after the meal. The villi almost flatten out, the cells frequently become highly elongated and contain a few scattered round vacuoles, and the striated border and the intercellular walls disappear. A considerable number of buds of cytoplasm, some of which are nucleated, is rounded off. The cells at the posterior end of this region, however, remain cubical; some vacuoles are seen and only a few cytoplasmic globules are discharged. Forty-eight hours after the meal, the cells of the whole area become cubical or low cubical in shape. Cragg (ibid.) states that in Tabanus, and this is also true of Chrysops (Wigglesworth, ibid.), the process of secretion begins in the upper part of the stomach and extends downwards as the blood flows in; he also states that the discharge of the secretion is in fact simultaneous with the flattening of the villi and the stretching of the columnar cells to a flattened epithelium. These statements of Cragg suggest that the first part of the stomach to become stretched is the anterior part, which is not the case in C. nubeculosus.

At the posterior end of the stomach, directly after a full meal, some of the cells may sometimes remain cubical in shape. In flies fixed 5 hours after the meal, they are some-

:times columnar and vacuolated, and some of them are budding off. In flies fixed 24 hours after the meal, they become low cubical and highly vacuolated. In these flies, a stream of a blue-staining fluid is seen passing from the blood mass to the cells of the posterior region of the stomach. It seems that this fluid is digested material, and subsequently, the cells of the posterior region of the stomach probably play an important part in absorption. De Boissezon (1930) states that in Culex pipiens the absorptive cells are localized in the posterior end of the stomach. Wigglesworth (1929) suggests that the cells of the posterior segment of the mid-gut of Glossina probably play an important part in absorption. Forty-eight hours after the meal, the cells become cubical or low cubical in shape.

The major part of the length of the epithelium, however, becomes reduced to flat cells. The villi and the intercellu-:lar walls disappear, and the striated border is either ab-:sent or homogeneous. Vacuoles become scarce, and when present they are small. The appearance of the epithelium remains as such - though a few vacuoles appear - up to the final stages of digestion of the contained blood. No cytoplasmic globules are discharged, but in flies fixed 5 hours after the meal,

small round eosinophil droplets - the fluid precursor of the peritrophic membrane - are secreted by the distended epithelium. In flies fixed 48 hours after the meal, the cells are still flat despite the obvious decrease in the size of the stomach. In Tabanus, Cragg (ibid.) states that the reformation of the columnar epithelium from the stretched and flattened cells begins soon after the ingestion of blood has ceased; as the stomach is gradually emptied of its contents the cells are re-grouped into villi. This is in contrast with what is seen in full-fed C. nubeculosus. The females of Tabanus studied by Cragg took a partial meal of blood, and judging by what has been observed in females of C. nubeculosus which took a small meal, no ovarian development took place. It follows that the digested matter instead of passing to the ovaries was directly utilized by the cells of the stomach for re-forming the epithelium. It is reasonable to assume, therefore, that in the females of C. nubeculosus which take a full meal, the assimilable materials are used first of all for the nourishment of the developing oocytes, and when the latter have obtained their requirements - apparently at a late stage of digestion of the meal - the absorbed material is used for the re-formation of

the epithelium.

Cragg also states that when the fly is undisturbed and takes a full meal under natural conditions, practically the whole of the cells have discharged their secretion and become flattened out by the time the ingestion of blood is completed; occasionally a few cells at the lower end of the organ retain their secretion for a short time afterwards, but only very rarely one finds secreting cells later than an hour after the meal. In C. nubeculosus, though in small numbers, cells showing secretory activity (i.e., budding off) may be seen in the lower end of the stomach 5 hours after the meal, and in the anterior end of the stomach up to 24 hours. Also, the distended epithelium can be seen secreting droplets which add to the substance of the peritrophic membrane in flies fixed 5 hours after the meal. Cragg also states that at a period subsequent to the re-formation of the columnar epithelium, and before the stomach is completely emptied, many of the cells, especially in preparations made from flies one day after the meal, show at their border minute and clear spherical droplets which emerge through the striae of the striated border to the lumen. No such cells were observed in C. nubeculosus.

In flies fixed 48 hours after the meal, a few small vacuoles are seen in almost all the cells of the stomach. Also, many cells throughout the epithelium show features of degeneration; they have either lost their nuclei or contain diffusely-staining ones. In flies fixed 72 hours after the meal, the stomach is empty and the cells are high columnar and are grouped into deep villi. The cells are highly vacuolated, the intercellular walls are absent, and the striated border is generally homogeneous. In general, the histological appearance resembles that seen in flies fixed 24 hours after a small meal, and in flies which did not feed during the second day after emergence.

The ingested blood becomes enclosed in a peritrophic membrane, which is more obvious in flies fixed 5 hours after the meal. Wigglesworth (1929) states that the blood shows an abrupt change on reaching the middle segment of the mid-gut in Glossina; it turns black where it is in contact with the epithelium and amorphous masses of altered blood pigment are deposited. No such abrupt change of the blood was observed in the stomach of C. nubeculosus. In flies fixed 1 hour and 5 hours after a full meal, the amount of haematin is small and is scattered throughout the blood mass. In flies fixed 24

hours after the meal, the amount of haematin is increased considerably though a large number of intact corpuscles is still seen; the haematin lines the peritrophic membrane, is scattered throughout the blood mass, and also accumulates in a considerable amount at the anterior end of the lumen. In flies fixed 48 hours after the meal, no corpuscles are seen; in one specimen, for unknown reasons, the blood was in a very early stage of digestion. The stomach contains a considerable amount of haematin, and frequently a little amount of a pink-:ish fluid. In Tabanus (Cragg, ibid.), the pigment is at first in small scattered granules of a dense black colour, but later, as a result of the peristaltic movement of the wall of the stomach, it becomes collected into a densely packed layer which runs around the outer part of the lumen. The effect of the peristaltic contraction of the wall is to drive the pigment - presumably the heaviest part of the stomach contents - towards the middle and lowest part of the lumen, and here it is found in a dense mass in some prepara-:tions. The pigment does not pass into the hind-gut at this stage. Wigglesworth states that the changes in the ingested blood in Chrysops (1931, a) and in the middle segment of the mid-gut in Glossina (1929) resemble those described by Cragg

in Tabanus. In C. nubeculosus, the changes in the ingested blood were studied in flies which took a full meal, and they somewhat resemble those described in Tabanus, except that the collection of the haematin in a dense mass at the middle and lowest part of the lumen was seen when the blood was completely digested, i.e., in flies 48 hours after the meal (at any rate, more than 24 hours after the meal). However, the blood corpuscles do not suffer the rapid distortion and reduction which, according to Cragg, occur in Tabanus within a short time. In Glossina, Wigglesworth (ibid.) states that even when the digestion is far advanced, if the cheesy mass of blood be removed from the middle segment its central part will still be found to be red in colour. According to Adler & Theodor (1926), digestion of blood in Phlebotomus papatasii is a relatively slow process, and haemolysis does not take place as a rule until the third or fourth day after a feed; it is not uncommon to find erythrocytes in a good state of preservation four days after a feed, and in one instance unimpaired erythrocytes were observed eight days after a feed. These facts added to the fact that sandflies will feed when the red cells from the previous feed are still present in the stomach, led these authors to suggest that the

essential food element in the blood is the plasma and not the red cells. (Yoeli & Mer (1938), however, state that neither haemoglobin alone nor serum alone is capable of inducing egg maturation in Anopheles elutus).

In flies fixed 1 hour after a full meal, the blood plasma is seen forming a homogeneous mass on top of the blood mass, occupying a short area of the lumen in the anterior end of the stomach and the very posterior end of the anterior segment. This mass decreases in size till it almost disappears 24 hours after the meal. In Anopheles maculipennis, Yaguzhin-:skaya (1945) finds that the blood plasma separates directly after the meal and surrounds the clump of erythrocytes on every side, and subsequently moves forwards and accumulates at the anterior end of the stomach; it extends into the first 1 - 2 abdominal segments. Later it is resorbed under the influence of digestive enzymes, and in the later stages re-:mains a minor amount in the extreme front of the peritrophic capsule. Weitz & Buxton (1953), dealing with females of a laboratory strain of C. nubeculosus fed on man and kept at 25°C. and 80 per cent. relative humidity, state that the stomach contents (test for plasma) invariably yield positive precipitin test up to 12 - 16 hours, and a high proportion

up to 24 hours, after which the proportion of positive results fell rapidly; they got the same results with mosquitoes.

In this work, the discharge of granules, vacuoles, and vacuolated masses of cytoplasm by the cells of the anterior segment, and of vacuolated and non-vacuolated buds of cytoplasm and of droplets from the cells of the stomach, are considered as secretory activities of these cells. Similar features have been observed in many insects, but their physiology is a subject of much controversy. Many authors consider these discharged materials as secretions, while several others consider them as degenerative changes of the cells. For instance, Yung-Tai (1929), from a study on the mid-gut epithelium of Galleria mellonella larvae, points out that secretions are always in the form of a diffusible liquid, and that the granular contents of the buds and globules given off from the digestive cells have all the aspects of cytoplasmic degeneration. Henson (1929), from a study on the mid-gut epithelium of Vanessa urticae larvae, favours the view that the formation of "secretion" vesicles is really a process of cell disintegration. Snodgrass (1935, p. 370) regards these processes as anatomical degenerative changes and describes them in this category; but he suggests that the sub-

:ject must be studied from a physiological standpoint before conclusions can be justified. Day & Powning (1949), from concurrent quantitative enzyme estimations and cytological investigations on Blattella germanica, state that the ex-:trusions of cytoplasm are in no way connected with secretion and that they are more probably signs of cell breakdown; they are not associated with an increase in enzyme concentration in the gut contents, while the greatest enzyme concentrations are found when the cytoplasm is cytologically uniform. On the other hand, Wigglesworth (1950, b, p. 318) agrees that there is no doubt that secretion in the mid-gut can take place with-:out the cells showing any alteration, either in the living state or in fixed preparations. But he is of the opinion that, though vesicular secretion can be produced artificially, it is possible that the technical procedures cause the cell contents to protrude in a manner resembling the normal secretory changes. Sutton (1951), dealing with the Corixidae, got information which, she says, to some extent obviates the criticism that pictures seen in fixed and stained material are not necessarily due to secretory activities but may be pro-:duced by the reactions of the fixatives in the cells. She does not agree with Snodgrass (ibid.) in considering them as

disintegrating processes; according to her, this is only in part correct, as it applies to the formation of nucleate but not to non-nucleate vesicles. In a blood-sucking fly as C. nubeculosus, if these processes are really degenerative processes, why then do they appear at the time of feeding? It is convenient, at any rate, to consider the materials discharged by the cells of the mid-gut in C. nubeculosus as secretions for the sake of comparing it with other blood-sucking flies.

4- The Function of the Oesophageal Diverticulum

Females of C. nubeculosus which were given blood by means of a bite, and eosin-stained water and cane-sugar solutions by means of wet cotton-wool, gave consistent results.

In fully gorged females (and also females which got an interrupted meal), the blood was always found in the mid-gut but never in the diverticulum. When these females were allow-:ed access to water and raisin before they took the blood meal, the diverticulum was almost always found distended with the water or raisin sap, and in very few cases it was found empty and crumpled. If, on the other hand, they were starved before they took the blood meal, the diverticulum was always

found empty and crumpled. In no case was the diverticulum found to contain even the least trace of blood. This indicates clearly that the oesophageal diverticulum in female C. nubeculosus does not serve as a reservoir for the ingested blood.

The absence of the blood from the oesophageal diverticulum in the fully gorged (and also unreplete) females of C. nubeculosus is in accord with what was found in Simulium nigroparvum, S. damnosum, Phlebotomus papatasii, Tabanus, Chrysops silacea, and Stomoxys calcitrans, but it differs from what was found in Haematopota pluvialis, tsetse flies, and also in mosquitoes by some of the mosquito students.

Cox (1938) found no blood in the crop of females of Simulium nigroparvum which had fed on turkey. Wanson & Lebied (1948), and also Lewis (1953), state that blood does not enter the diverticulum in the females of S. damnosum. In Phlebotomus papatasii, Adler & Theodor (1926) state that it is unusual to find blood in the oesophageal diverticulum at any time, and that in the few cases where blood is found it is present only in negligible quantities as compared with the amount found in the mid-gut, and that even when the female is fully engorged and the stomach is distended to its full capacity, the

oesophageal diverticulum contains very few red cells or none at all. In Tabanus, Cragg (1920) states that the crop in the recently fed fly is never distended with blood. Wigglesworth (1931, a) states that in Chrysops silacea the newly ingested blood goes straight into the stomach and that the diverticulum certainly does not serve as a reservoir for blood. Kuzina (1942) states that in Stomoxys calcitrans the blood passes directly into the mid-gut.

On the other hand, Cameron (1934) states that in Haematopota pluvialis the blood proceeds directly to the mid-gut and when the latter becomes filled to capacity, and the fly still continues to feed, the excess is found to enter the crop. Lester & Lloyd (1928) state that the diverticulum in the tsetse flies becomes filled with blood after the stomach is full of it. In mosquitoes, discrepant results were obtain-:ed. Nuttall & Shipley (1903) state, "That blood is also taken up into the sacs has already been stated by Grassi, and de Grandpre' and Charmoy". Patton & Cragg (1913, p. 110) state that in mosquitoes killed during the act of feeding, the diverticula were always found to be full of blood. Philip (1930) states that in Aedes aegypti a small portion of the blood meal seems to pass through the diverticula during feed-

:ing, but is probably never detained there very long. He also mentions that interrupted flies showed noticeable amounts in the diverticula. Marshall & Staley (1932) state that when mosquitoes feed on blood, all the blood goes direct into the mid-gut; traces of blood, negligible in quantity but sufficient to cause visible colouration, are occasionally to be observed in the diverticula. Lumsden (1947) states that in the majority of fed Ae. aegypti mosquitoes, blood was found in the stomach alone, but partly fed flies showed a higher proportion with blood in the diverticula than did those fully gorged; this is attributed to probable regurgitation from the stomach when feeding is interrupted before its normal completion. Fisk (1950), who worked also with Ae. aegypti, states that the blood passes directly to the mid-gut. It is interesting to note here that he undertook experiments to discover some relatively simple property or properties of blood which enables it to pass directly to the mid-gut. The blood properties simulated were: pH, temperature, peptone content, amino acid content, viscosity, osmotic pressure, and the particulate nature of the erythrocytes. None of these experiments, however, was successful. Wright (1924) working with the British mosquitoes, states that although mosquitoes

which had recently fed on blood were fairly common, he never encountered any mosquito which had the least trace of blood in the oesophageal divertiula. (Nevertheless, he has given tables which show that 2 females had much blood in the diverti-:cula, and that 21 had traces). Roy (1927) dealing with Anopheles subpictus and A. stephensi, found that blood was never present in any sac before the stomach was full, and he states that it is the surplus quantity, therefore, that is stored in the diverticula. MacGregor & Lee (1929) and MacGregor (1930) state that blood withdrawn by puncturing the skin passes directly into the stomach and not into the diverti-:cula. Pawan (1937) claims that there is a difference between Aedes (Stegomyia) aegypti and Anopheles tarsimaculatus; in Aedes, immediately after a blood meal the diverticula become distended and filled with blood cells, but in Anopheles they are never distended, or appreciably filled, with blood immediately after a full meal and even with an engorged and distended stomach. Roy & Ghosh (1940), however, do not agree with Pawan's view concerning Anopheles. Robinson (1939, p. 231) mentioned that the function of the anticoagulin in the saliva of mosquitoes seems necessary "because the blood is stored in the ventral reservoir for a considerable time

before it is received into the stomach for digestion". Bishop & Gilchrist (1946) state that blood and blood fractions pass to the stomach. Trembley (1952) states that under all conditions, blood was dispatched primarily to the stomach, and with few exceptions, in small amounts to the diverticula. He suggests that the proportion of mosquitoes showing blood in the diverticula appear to be a distinguishing physiological characteristic of the species.

When the flies were given eosin-stained water and cane-sugar solutions by means of wet cotton-wool for about half a minute each, the water and the thin sugar solution were found to have been dispatched to the mid-gut, while the thick sugar solution was dispatched to the diverticulum. It appears from these results that the nature of the ingested fluid (other than blood at least) seems to influence its first destination in the alimentary tract. Nevertheless, when it happened that the diverticulum was already distended with water (taken up from the pots) at the time of the experiment, the ingested thick sugar solution was dispatched to the mid-gut.

On the other hand, when the females were allowed to imbibe the stained fluids to satiety, the results were

peculiar. The stained water was found in the mid-gut, while the diverticulum contained only a trace or none at all; the thin sugar solution was found in both the mid-gut and the diverticulum; the thick sugar solution was found in the diverticulum, while the mid-gut contained only a trace.

When the females were given plain water instead of the stained water, to satiety, the diverticulum was found to be distended with it. We have already seen that the diverticulum in some females less than 24 hours old, and also in many females taken from the collecting-cage, i.e., supplied with water and raisin, was distended with water. Why the diverticu-:lum did not become distended with the stained water may be attributed to some effect of the stain. It is probable, as it was observed that the quantity of the ingested stained water was far less than that of the sugar solutions, that the stain made the water less palatable so that the females ingested very little of it - an effect which the sugar conceals.

These results show clearly that the first destination of water and dilute sugar solution is the mid-gut, while that of the concentrated sugar solution is the diverticulum. They also show that the oesophageal diverticulum serves as a reservoir for water and sugar solutions.

Experiments with water and sugar solutions were performed on several blood-sucking Diptera, especially mosquitoes. Wright (1924) found that mosquitoes fed on sugar solution or fruit juice had the diverticula full of the fluid, while the mid-gut was empty. MacGregor (1930) states that the normal destination of water, fruit saps and sugar-containing fluids taken up by the mosquito is the diverticula. Roy & Ghosh (1940) state that when a mosquito is allowed to imbibe fluid from wet cotton-wool, water and sugary solutions are invariably found in the diverticula. Bishop & Gilchrist (1946), however, state that sweet solutions containing glucose or honey pass to the stomach or the diverticula but only the diverticula become distended. Fisk (1950) found that in Aedes aegypti a 5% sucrose solution went to the crop but not to the stomach. Trembley (1952) states that glucose solutions, regardless of the species or manner of feeding, were found primarily but not exclusively in the diverticula. In Stomoxys calcitrans, according to Kuzina (1942), coloured sugar syrups pass directly into the crop. In Simulium damnosum, Wanson & Lebied (1948) found that the ingested sugar solutions and fruit juices passed into the crop.

We have seen that MacGregor (1930) and Roy & Ghosh (1940)

state that water imbibed by mosquitoes is transported to the diverticula. This is quite different from what is found in C. nubeculosus, in which the imbibed water is dispatched first of all to the mid-gut. On the other hand, with the exception of Bishop & Gilchrist (1946) who found that sweet solutions containing glucose or honey pass to the stomach or the diverticula, and Trembley (1952) who found that glucose solutions may pass to the stomach, the other authors (i.e., Wright, 1924; MacGregor, 1930; Roy & Ghosh, 1940; Fisk, 1950) state that sugar solutions, sugary fluids, and fruit juices taken up by the mosquitoes pass directly into the diverticula. Sugar solutions are also said to pass directly into the diverticulum in Stomoxys calcitrans (Kuzina, 1942) and in Simulium damnosum (Wanson & Lebied, 1948). Roughly speaking, these results agree only with those obtained when the females of C. nubeculosus were fed on the thick sugar solution, and differ from those obtained when they were fed on the thin sugar solution. However, as the concentration of sugar in the fluids was not given (except Fisk, 1950), the difficulty of making a thorough comparison is quite apparent. There is one thing in common, however, and that is, the oesophageal diverticulum serves as a reservoir for water and sugar

solutions.

Denisova (1943; 1949) pointed out the importance of the function of the oesophageal diverticulum in the blood-sucking Diptera as a reservoir for water and sugar solutions. This author has shown that the injection into the body cavity of Anopheles maculipennis and of tabanids, of amounts of water greater than 50% of the body weight produces an osmotic shock; tabanids are more susceptible than Anopheles. He con-:cludes that the crop, having a water-impermeable wall, re-:presents a valuable adaptation which controls the expenditure of the consumed water and the variation of the blood concen-:tration. The function of the crop, therefore, enables the blood-sucking Diptera to consume hypotonic solutions, namely of water and of plant juices, notwithstanding the rapid absorption of fluids through the mid-intestine. He also quotes Biklemishev to the effect that this double nutrition is typical of all blood-sucking Diptera, and is lost only in the much specialized forms Glossina and Pupipara.

It is noteworthy that peculiar functions were attributed to the oesophageal diverticulum in some of the blood-sucking Diptera. Cragg (1920) observed a clear fluid in the crop of Tabanus, and he suggested that some of the secretion from the

mid-gut had been regurgitated into it. But Wigglesworth (1931, a) who at first thought that the fluid in the crop of Chrysops silacea was saliva and that the crop served as a reservoir for saliva, suggests that it is possible that the crop contents represent free fluid obtained from the surface of plants and that as in mosquitoes, when the insect feeds on such substances the crop does function as a temporary reservoir.

Wanson & Lebied (1948) found that the fluid from the crop of Simulium damnosum when injected in man produced the same reactions as the insect saliva, and concluded that the crop is a reservoir for saliva. But they also showed that the ingested sugar solutions and fruit juices passed into the crop, and Lewis (1953) recorded that chromatographic analysis detected glucose, fructose, and sometimes sucrose in the fluid contained in the oesophageal diverticulum of wild females of S. damnosum. The reactions produced, which were the same as those produced by the insect saliva, however, might have been due to bacteria or fungi (or to their products) contaminating the injected crop contents. (In serial sections of C. nubeculosus, fungal hyphae were sometimes seen in the oesophageal diverticulum). Another possible reason for

these reactions is the saliva which probably becomes mixed with the imbibed fluids at the time of ingestion.

It is interesting to note that Hocking (1953) reviewed the published records on biting flies visiting flowers, which included several species of tabanids and simuliids, and published original ones for females of 4 northern species of mosquito, 4 tabanids, and 2 simuliids. He found sugar concentrations of up to 76% in the crops of the insects studied. He concludes that the northern biting flies obtain the energy for flight almost exclusively from floral nectar, of which some flies may carry in their crops quantities ranging up to 217% of their basic weight.

Summary

1- A simple method for culturing C. nubeculosus in the laboratory is described. A mixture of powdered mud, dried autolized yeast and powdered charcoal forms an adequate medium for rearing the larvae. The number of adults obtain-:ed from each culture pot varies, but it averages 45% of the larvae. Brief accounts of the biology of the species in the laboratory are also given.

2- The alimentary tract of female C. nubeculosus is simple. There is much similarity between it and that of other nematoceran and brachyceran blood-sucking flies. Among the interesting features observed are the following:

a- The mid-gut is composed of two portions, a tubular anterior segment and a dilated stomach, which show structural differences.

b- The peritrophic membrane, which is absent in the unfed fly, forms around the ingested blood. Its substance is secreted by the epithelium of the stomach.

c- A simple rectal valve guards the opening of the small intestine into the rectum.

d- The Malpighian tubes are two in number, and they give attachment to muscular branches from different sources.

e- There are six accessory glands arranged in a rosette around the mouth of each salivary gland. Some of them may sometimes function as reservoirs for the secretion of the salivary glands proper.

In one specimen, tumours at the base of the stomach, and abnormal features in the Malpighian tubes were observed.

3- A full account is given of the changes which occur in the mid-gut epithelium at the start of feeding, after a small blood meal and after a full blood meal, as well as the changes which occur in the mid-gut epithelium of flies which refused to feed during the first three days of adult life. The epithelium of the anterior segment does not show any significant change, but that of the stomach shows striking changes. The two segments, which show structural differences, seem to differ functionally as well.

The changes in the ingested blood are also described. At 20°C and 80 - 90% relative humidity, a small meal is digested and the stomach is emptied within 24 hours. A full meal takes about 48 hours, but the stomach becomes empty some time afterwards.

4- The oesophageal diverticulum in female C. nubeculosus functions as a reservoir for water and sugar solutions but

not for blood. The first destination of the ingested water and the dilute (4.18%) cane-sugar solution is the mid-gut, and that of the concentrated (66.6%) cane-sugar solution is the oesophageal diverticulum. The nature of the ingested fluid (other than blood at least) seems to influence its first destination in the alimentary tract.

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