

STUDIES ON THE DIGESTION OF WOOL BY INSECTS

IX. SOME FEATURES OF DIGESTION IN CHEWING LICE (MALLOPHAGA) FROM BIRD AND MAMMALIAN HOSTS

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Summary

Some aspects of digestion in eight species of Mallophaga infesting birds and mammals have been examined.

The anatomy and histology of the alimentary canal are described and the relatively poor tracheation of the crop and midgut recorded.

The bird-infesting species all regularly ingest feather particles and the poultry body louse in addition obtains fresh blood from its host. In these species the food in the crop is subjected to the action of digestive juices passed forwards at regular intervals from the midgut and, as a consequence, the pH (about 8.0) and oxidation-reduction potential (about —200 mV.) of both of these regions is the same. The presence of a comb of teeth at the posterior end of the crop in *Amblycera* and the arrangement of the inlet and outlet of the crop in *Ischnocera* prevent the passage of most intact feather particles into the midgut, which receives partially digested material with disorganized structure. Free sulphydryl groups can be detected in the crop and midgut, and the ingestion of cobalt-impregnated feathers results in the formation of a characteristic brown, cobaltic-cysteine complex. Metallic sulphides are not produced, indicating that, as in dermestid larvae, the cystine of feather keratin is reduced to cysteine, but that further breakdown to hydrogen sulphide (which is characteristic of the clothes moth larva, *Tineola*) does not occur.

Neither hair nor wool could be detected regularly in the digestive tract of the mammal-infesting species examined, which instead appear to ingest mainly epithelial debris and skin secretions. In the sheep louse *Damalinia ovis*, the slightly acid (pH 6.5), oxidizing (greater than +77 mV.) midgut digestive juices are not passed regularly into the crop. Free sulphydryl groups could not be detected and it is doubtful if wool would be digested during passage through the alimentary canal.

I. INTRODUCTION

It is now well established that larvae of the clothes moth *Tineola* and of at least three species of carpet beetle are able to digest wool keratin, and something is known about the mechanism of this process (Waterhouse 1952a, 1952b, 1952c). Although there is good circumstantial evidence that the chewing lice of birds are able to digest feather keratin (Eichler 1937), additional proof is necessary. Furthermore, it is not known whether the chewing lice of mammals can or do digest the hair (or wool) of their host, although it is often stated in

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textbooks that species infesting sheep and goats cause damage by partially cutting and weakening the wool or hair fibres.

The evidence that bird Mallophaga digest feather keratin is firstly that the feather barbs and barbules are consumed, secondly that there are partly digested feathers in the crop and midgut where sulphhydryl groups can also be demonstrated and conditions are at least mildly reducing (Duspiva 1936), and thirdly that some species can be reared for a period *in vitro* on host feathers (Barber 1921; Martin 1934; Wilson 1934, 1939). It is clear, however, that this diet is not pure feather keratin and that the feathers must have been contaminated with various accessory growth factors unless, perhaps, these were supplied by contaminating microorganisms or by symbionts. It is well known, for example, that many Mallophaga harbour rickettsiae, although their relation to the metabolism of the lice is not known. It is also well known that Mallophaga have a varied diet when living on their avian hosts. For example, Waterson (1926) records that the protecting sheaths of growing feathers, feather fibre, down, skin scurf, scabs, blood, their own egg shells and cast skins, and perhaps other Mallophaga as well are ingested. An examination of the crop contents further indicated the presence of small particles of various minerals and other fortuitous feather contaminants. There is no doubt that blood regularly forms portion of the diet of some species (Strindberg 1917; Kotlan 1923; Wilson 1933; Eichler 1937; Crutchfield and Hixson 1942; Bouvier 1945), but, for others, blood alone is not an adequate diet. Thus, although the poultry body louse (*Eomenacanthus stramineus*) is recorded as actively feeding on the blood of its host (Wilson 1933), the poultry head louse (*Lipeurus heterographus*) will ingest only dried blood and then lives only 3 days on this diet alone, whereas it thrives on dried blood plus feathers. Silk and blood is an inadequate diet for this species (Wilson 1934). Further, the slight pigeon louse (*Columbicola columbae*) will not feed on a blood clot, although it can be induced to feed on blood-stained feathers (Martin 1934).

Very little is known about the digestion of those Mallophaga which live on mammals. The cattle biting louse (*Damalinia bovis*) can be reared *in vitro* on fresh cattle hair sprinkled with dried brewer's yeast (Matthysse 1944) and the biting louse of sheep (*Damalinia ovis*) on greasy wool, skin scurf, and powdered yeast (Scott 1952), but it is not known whether these species digest the keratin components of these diets.

It will be shown in this paper that the three species of bird Mallophaga examined regularly digest feather keratin, and some features of this process are described. The biting louse of sheep, however, seldom if ever ingests wool and probably cannot digest fibres which may be fortuitously ingested.

II. METHODS

The following species of Mallophaga were examined:

Sub-order AMBLYCERA

Family Menoponidae *Eomenacanthus stramineus* (Nitzsch), the poultry body louse.

Boopidae	<i>Heterodoxus</i> sp. (from the red wallaby, <i>Macropus ruficollis</i>).
Gyropidae	<i>Gyropus ovalis</i> Nitzsch, the oval guinea pig louse.
Sub-order ISCHNOCERA	
Philopteridae	<i>Columbicola columbae</i> (L.), the slight pigeon louse.
Trichodectidae	<i>Lipeurus caponis</i> (L.), the poultry wing louse.
	<i>Damalinea ovis</i> (L.), the sheep body louse.
	<i>D. bovis</i> (L.), the cattle biting louse.
	<i>D. caprae</i> (Gurlt), the goat body louse.

It can be seen that the species are representative of both bird- and mammal-infesting families of each of the two sub-orders of Mallophaga.

All species (except *Heterodoxus* and *Gyropus*) were collected from their respective hosts as required and maintained in an incubator at 36-37°C. and 74 per cent. relative humidity, physical conditions roughly similar to those employed by the authors, cited above, who bred lice *in vitro*. Only a small number of *Heterodoxus* (about 50) were available. This species died in a few hours at 36°C., although it lived for several days at 18°C. Similarly, *Gyropus* lived far better off its host at 18-20°C. than at 36°C.

In most experiments indicators or metal salts were added to the diets fed, which consisted of freshly obtained white breast or neck feathers, unwashed hair, or greasy, scurfy wool from the respective hosts. The addition of materials to these diets was carried out by dipping the source of keratin in 70 per cent. alcohol (to ensure wetting), rinsing in distilled water, immersing in the test solution, and drying. No attempt was made to remove contaminating debris from the keratin before treatment. On most of the diets lice survived the duration of a test (1-2 days) and often for a longer period, particularly the ischnoceran species. It was not possible to induce *Heterodoxus* to feed on any of the diets offered and *Gyropus* did not ingest the treated diets at all readily unless the chemicals were introduced into the hair of the living guinea pig and the lice allowed to feed *in situ*.

Because of their relatively transparent cuticle it was generally possible to select lice for dissection which had fed on the treated diet and also to follow certain of the colour changes of indicators in living lice. It was also possible to observe peristalsis and antiperistalsis in the crop and midgut, particularly if the lice were examined with dark-field illumination.

III. RESULTS

(a) Morphology of the Digestive System

(i) *Alimentary Canal*.—The following are the more important papers which contain information on the general morphology of the digestive system of Mallophaga, although most of the species used in the present investigations have apparently never been adequately dealt with: Nitzsch 1818; Kramer 1869;

Grosse 1885; Snodgrass 1899; Cummins 1913; Strindberg 1916; Ewing 1924; Blagoveshchensky 1931; Risler 1951; Haug 1952.

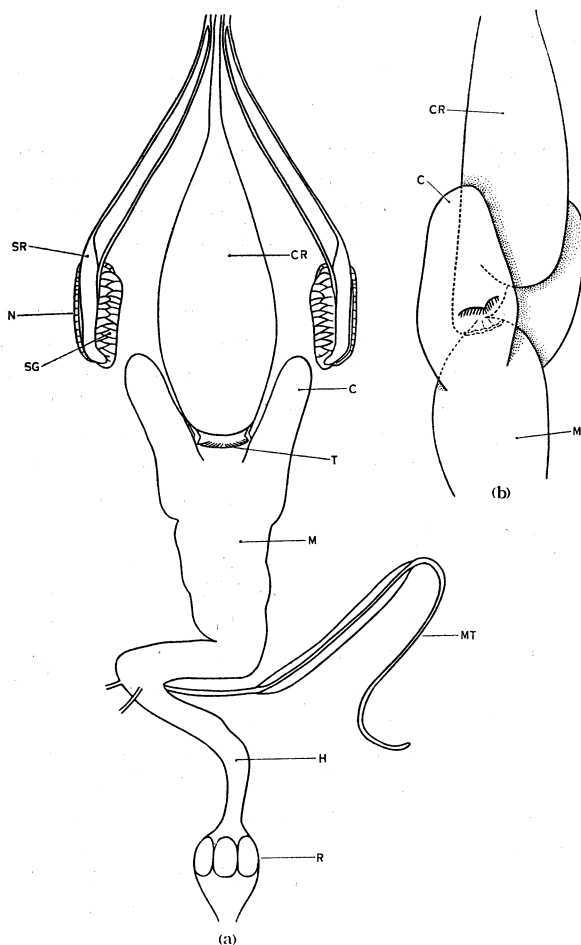


Fig. 1.—(a) Alimentary canal of *E. stramineus*, dorsal view.

(b) Diagram showing relationship of crop teeth and oesophageal invagination.

C, caeca; CR, crop; H, hindgut; M, midgut; MT, malpighian tubules; N, perioesophageal nephrocytes; R, rectal pads; SG, salivary gland; SR, salivary reservoir; T, comb of teeth.

The general morphology of the alimentary canal of Mallophaga conforms to three basic patterns (Snodgrass 1899). In the sub-order Amblycera the oesophagus expands into a crop before leading directly into the midgut (Figs. 1, 2, and 3). On the other hand in most families of Ischnocera the oesophagus leads into a large, expanded crop diverticulum interrupting the direct passage

of food to the midgut. This diverticulum extends anteriorly only for a short distance, but posteriorly almost to the end of the body (Fig. 4). Nearly opposite the entrance of the oesophagus into the crop the foregut leads out of the crop and, soon after, into the midgut. The ischnoceran family Trichodectidae is an exception to this arrangement, for here the crop is a simple diverticulum which leads off by means of a constricted neck from the lower end of the oesophagus, the latter continuing on directly to the midgut (Fig. 5).

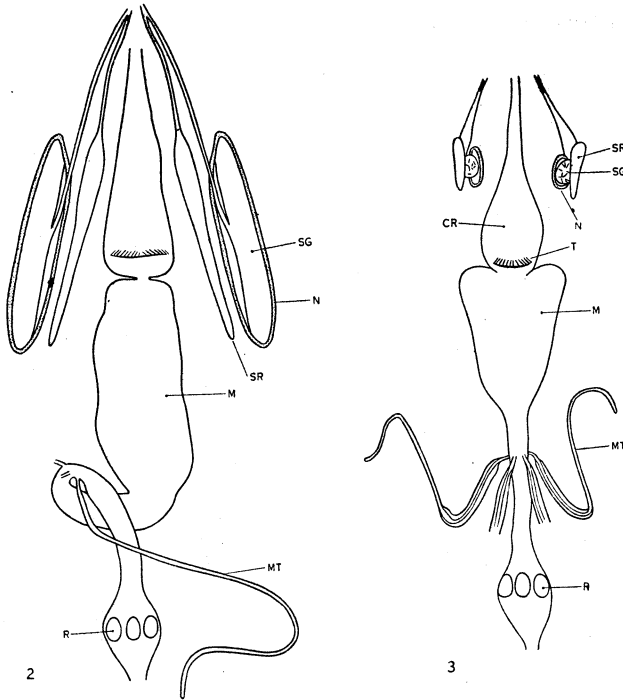


Fig. 2.—Alimentary canal of *Heterodoxus* sp.

Fig. 3.—Alimentary canal of *G. ovalis*. Details as in Figure 1.

Except in the Trichodectidae the crop always possesses teeth of characteristic shape, grouping, and restricted distribution (Cummins 1913; Waterson 1926). In the Amblycera the teeth are situated in a circular or semicircular fashion near the posterior end of the crop. Thus in *Eomenacanthus* (Plate 1, Fig. 1) the teeth are arranged in two contiguous arcs arising in the dorsal wall of the crop and projecting ventrally. Each tooth is C-shaped. The free anterior arm of the C is long, slightly curved, and may reach $50\ \mu$ in length, whereas the posterior arm is short (up to about $10\ \mu$ in length). The long anterior arms each have a very fine tip. These crop teeth form an effective comb-like sieve which prevents any large particles of food from entering the midgut. A somewhat similar arrangement of teeth occurs in *Heterodoxus* and *Gyropus*. In *Eomenacanthus* the poorly developed oesophageal invagination (see also *Heterodoxus*, Plate 2, Fig. 2) leads out slightly in front of the blind posterior

end of the crop and is situated where its anterior end receives maximum protection from the comb of teeth. It scarcely protrudes at all into the midgut, having the form of a short, thin-walled, funnel-shaped structure (Fig. 1*b*). In the Ischnocera the teeth occur singly or in small rows in the anterior diverticulum of the crop and mainly in the dorsal wall. In *C. columbae* and *L. caponis* (Fig. 4; Plate 1, Fig. 2) the groups may contain up to six teeth, and in *L. caponis* (considerably the larger of these two species), they may reach a length of about 7 μ . In the Ischnocera, as in the Amblycera, the oesophageal invagination is poorly developed.

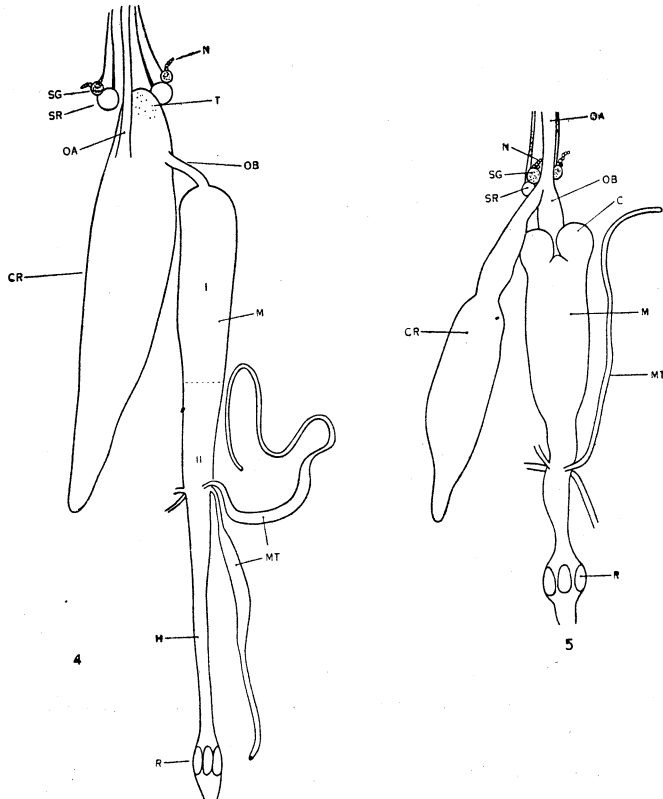


Fig. 4.—Alimentary canal of *C. columbae*, with crop rotated and moved to the left.

Fig. 5.—Alimentary canal of *D. ovis*.

C, caeca; CR, crop; H, hindgut; M, midgut; MT, malpighian tubules; N, perioesophageal nephrocytes; OA, oesophagus region A; OB, oesophagus region B; R, rectal pads; SG, salivary gland; SR, salivary reservoir; T, position of teeth.

The mallophagan midgut is simple and is either fairly straight (*C. columbae*, *D. ovis*) or possesses a single bend or loop at its posterior end (*E. stramineus*, *Heterodoxus*). There are sometimes two anterior caeca (*E. stramineus*, *D. ovis*), but these may be absent (*Heterodoxus*, *C. columbae*). The anterior

two-thirds or three-quarters of the midgut has a greater diameter than the posterior region and may be differentiated histologically (see later).

There are always four malpighian tubules (one pair often longer than the other), which generally have in their basal half a differentiated zone of greater opacity than the rest. This zone, which is generally of greater diameter also, may be very restricted or it may occupy almost half of the tubule. In some species (e.g. *Heterodoxus*, *E. stramineus*) portion of the tubule may be pigmented.

The hindgut narrows shortly after the entrance of the tubules, but expands again into a conspicuous rectum supplied with six rectal pads.

(ii) *Salivary Glands*.—Lying on either side of the crop in the vicinity of the anterior end of the midgut there are two salivary organs which probably represent a relatively opaque gland and a more transparent reservoir (Figs. 1-5). In the Amblycera the gland, and more particularly the reservoir, are relatively elongate, whereas in the Ischnocera both gland and reservoir are more or less spherical (Snodgrass 1899; Stowe 1943; Risler 1951; Haug 1952). The ducts often arise near, or at, the anterior ends of the salivary organs (*C. columbae*, *D. ovis*) but, in some species (e.g. *E. stramineus*, *Heterodoxus*) the duct of the salivary gland (but not that of the reservoir) arises near the centre of the gland. The ducts of gland and reservoir unite in the head and the resultant common ducts of each side fuse shortly before discharging into the salivarium.

(iii) *Perioesophageal Nephrocytes*.—Associated with each pair of salivary organs in Ischnocera is a group of some 7-24 spherical or polygonal cells (the number varies with the species), which were regarded by Snodgrass (1899) as constituting glands whose ducts discharged into the anterior end of the crop. Kramer (1869) observed these cells, which are generally binucleate and, although he was unable to see any efferent duct, he regarded them as bearing some relation to salivary secretion because of their position. Grosse (1885), however, discounted their salivary function, since he found similar cells associated with the fat body in other parts of the body. Nuttall and Keilin (1921) examined similar cells in the sucking louse *Pediculus* (Order Anoplura) and demonstrated that they were nephrocytes. They suggested that Snodgrass may have taken for a "duct" leading from the nephrocytes either tracheae, nerves, muscle fibres, or real salivary ducts with which these particular nephrocytes are intimately associated.

These two groups of cells (each about a dozen in number) are clearly visible in *C. columbae* and *D. ovis* (Figs. 4, 5). Injection into the haemocoel of a saturated solution of ammonia carmine in saline demonstrated clearly that they were indeed nephrocytes, since they (and small groups of cells distributed throughout the fat body) readily took up this dye.

Associated with the salivary glands in the Amblycera (*E. stramineus*, *Heterodoxus*, and *Gyropus*) there is a garland of cells forming an ellipse and lying close to the surface of the salivary organs (Figs. 1, 2, 3). The affinity of these cells in *E. stramineus* for injected ammonia carmine indicates that they

too are nephrocytes and it appears that they are the counterparts of the salivary or perioesophageal nephrocytes of the Ischnocera.

(b) *Histology of the Digestive System*

(i) *Alimentary Canal*.—There is little published information on the histology of the alimentary canal, although some regions have been incidentally figured (but with little detail) by authors such as Snodgrass (1899) and Strindberg (1916). A more detailed account of the midgut histology of *Bovicola* (= *Damalinia*) *caprae* and *E. stramineus* is about to appear (Haug 1952).

There appear to be no unusual features in the histology of the foregut (except the presence of teeth, mentioned previously) and there is no indication of any secretory function of the epithelium even in species (see later) in which food breakdown proceeds rapidly in the crop. The crop is very well supplied with muscles, especially circular muscles, and the latter are a particularly noticeable feature of the philopterid crop.

The bulk of the midgut epithelium is composed of mature cells with some immature cells and many small regenerative cells (Plate 1, Figs. 3, 4, 7; Plate 2, Figs. 1, 3). The mature cells usually have a well-defined striated border, and are at times completely separated from the basement membrane by regenerative cells. Their nuclei (each with a fuchsinophilic nucleolus) therefore tend to lie towards the gut lumen. On the other hand, the immature cells (and their nuclei) lie closer to the basement membrane and expose far less striated border to the lumen (Plate 1, Fig. 3). Their cytoplasm is denser than that of the mature cells. Haug (1952) interprets what are here regarded as mature and immature cells as two distinct cell types, each producing a characteristic secretion. The regenerative nuclei are very numerous and are slightly smaller than those of the mature cells. In most species they form an almost continuous layer adjoining the basement membrane. Although the difference is not always marked, the epithelium of the anterior two-thirds of the midgut is generally higher (Plate 1, Fig. 5) and has the histological appearance of greater activity than the relatively lower epithelium of the posterior third (Plate 1, Fig. 6). The midgut epithelium is generally higher and more "active" in appearance in *E. stramineus* (Plate 1, Figs. 5-7) than in the other species examined. However, this may have been due to greater difficulties in obtaining satisfactorily fixed preparations of this species, since poor fixation was found to produce more vacuolated, elongated, and "active" cells than good fixation. Granules which stain heavily with Bodian's protargol method occur in some individuals (especially *Heterodoxus*, Plate 1, Fig. 8) near the lumen of the mature cells. No structure resembling a peritrophic membrane of the type arising at the anterior end of the midgut was seen. However, in several species (e.g. *E. stramineus*) the food in the posterior half of the midgut was loosely compacted into a cylindrical shape. A membrane envelops these food masses and can be shown by van Wisseling's test to contain chitin. In *Lipeurus* the cylinder of food material is regularly of smaller diameter than the lumen and first becomes visible about one-third of the way down the midgut. There is no doubt that a peritrophic membrane is produced by the midgut epithelium in some species.

There are few unusual features in the hindgut. The anterior quarter often possesses a higher or more convoluted epithelium than the remainder (e.g. *Lipeurus*, Plate 2, Fig. 4) but the six well-developed rectal pads, each with a number of centrally placed nuclei, are the most conspicuous feature (Plate 2, Figs. 5, 7).

(ii) *Salivary Glands*.—The epithelium of the salivary glands is high and fuchsinophilic, the nuclei large and oval, and there are frequently granules which stain with Bodian (Plate 2, Figs. 8, 9). The saliva is discharged into a relatively small central lumen. By contrast the epithelium of the reservoir is flat, the nuclei elongate, and the central lumen very large (Plate 2, Fig. 8). Histological details for other species are given by Strindberg (1916), Stowe (1943), and Risler (1951).

(iii) *Perioesophageal Nephrocytes*.—These have a structure typical of other nephrocytes. The cytoplasm is not very dense, nor is it differentiated and there are frequently two nuclei per cell (Plate 2, Fig. 6). They have been figured by Risler (1951) for *D. caprae*.

(c) *Tracheation of the Alimentary Canal*

Harrison (1915) published the only detailed study of the respiratory system of Mallophaga, but paid little attention to the tracheation of the alimentary canal, which he reported very briefly as being well developed. This is incorrect as far as the crop and midgut are concerned in any of the species used in the present study.

There is no important difference in the degree of tracheation of the alimentary canal in *Eomenacanthus*, *Columbicola*, *Lipeurus*, or *Damalinia*. In each the crop does not appear to be tracheated, except occasionally and very sparsely at its posterior end in *Eomenacanthus*. The midgut is almost devoid of tracheae except for the anterior quarter, which is poorly supplied with a few small tracheae. Even these tracheae branch into relatively few tracheoles which supply air directly to only a small proportion of epithelial cells. In *Eomenacanthus*, tracheae are generally restricted to the region of the caeca. The anterior quarter of the hindgut is well tracheated and the rectal pads are richly supplied. The salivary glands are also supplied with several tracheal branches.

As in other species, the crop of *Heterodoxus* and *Gyropus* is poorly tracheated. However, the anterior midgut is supplied with more tracheae than observed in other Mallophaga examined, although the tracheoles reach a far smaller proportion of the epithelium than observed in many other insects (Day 1951). The posterior portion of the midgut and the hindgut are tracheated as in the other species.

(d) *Movement and Microscopic Examination of Food Undergoing Digestion*

In *Eomenacanthus* the crop is customarily kept full of blood (obtained fresh from the host) and feather particles up to 600-800 μ in length. Under polarized light it is evident, from widely differing degrees of birefringence, that the feather barbs are in various stages of digestion.

The crop contracts at frequent intervals, driving its contents alternately backwards against the comb of teeth at its posterior end and forwards up into the oesophagus almost as far as the head. At the same time the anterior two-thirds of the midgut undergoes waves of contraction and relaxation. During contraction (which is mainly antiperistaltic in nature) its semi-fluid contents are forced up into the tips of the large, sac-like caeca and at times also forwards into the crop, the comb of teeth being simultaneously displaced forwards. Fluid and semi-solid material are then returned to the midgut, most feather particles being strained out in this process by the comb of teeth, although occasionally small particles are not withheld.

In the posterior third of the midgut (which is flexed to the left) there is comparatively little contraction and its contents are in a far less violent state of movement. It will be seen later that the contents of this region are more acid and less reducing than those of the remainder of the midgut.

Stained food was observed to enter the rectum 6 hr. after feeding commenced at 37°C.

In the wallaby louse (*Heterodoxus*), collected from the fur of its host, which had been skinned some hours earlier, the crop, midgut, and rectum had red to dark brown contents and blood proved to be present. In addition there was a good deal of solid irregularly shaped birefringent material in the crop, which appeared to be skin and epithelial debris from the host. There was no evidence that hair had been ingested. The midgut contained much fluid, together with a small number of weakly birefringent solid masses. Both the crop and midgut showed rather regular and vigorous peristaltic and antiperistaltic movements. In these the food was driven back and forth in the crop and, at intervals, fluid and smaller pieces of solid material passed through the crop teeth into the midgut, the larger solids being retained. Shortly afterwards an antiperistaltic wave would return some of the midgut contents to the crop.

Forward movement of contents from the midgut into the crop was not observed in *G. ovalis*. The food of this species consists largely of epithelial debris, skin secretions, and perhaps skin from its host. Neither blood nor hair was ever observed in the digestive tract and no damage could be detected by microscopical examination of host hair from heavily infested areas. This confirms the report of Ewing (1924) for two guinea pig lice (*G. ovalis* and *Gliricola porcelli*). He believed, furthermore, that the cutting and piercing nature of the *Gliricola* hypopharynx indicated that serum was also ingested by this species, although blood was never observed in the digestive tract. He reported that the skin of a guinea pig heavily infested with *G. porcelli* usually did not show any sign of abrasion but that the hair tended to become brittle. He observed that this species thrusts its head into the opening of a hair follicle while holding the hair and believed that the products of the oil glands, which open through the mouths of the hair follicles, probably also constitute an important item of diet.

In the pigeon louse, *C. columbae*, as in the poultry wing louse, *L. caponis*, the food passes rapidly down the oesophagus into the crop, which, when full, extends from the mid line of the mesothorax into the beginning of

the fifth abdominal segment, where it lies to the left of the mid line. The most conspicuous elements of the food which packs the crop are the feather barbules, many of which may be a length almost equal to that of the crop, although shorter pieces are also present, particularly when no food has been ingested for some time. In addition to barbules, a small number of hard mineral particles are present and also material which is apparently skin scurf. Blood was not seen in lice obtained directly from the host, although lice kept on feathers on which blood had been dried were found to have ingested it along with feather particles. These observations confirm the report of Waterson (1926) who found small grains of quartz, mica, olivine, feldspar, a lepidopterous scale, a seed coat, and fungal spores in the philopterid crop. These feather contaminants were present in addition to various portions of the feather itself and also of skin scurf, scales, blood (when available), cast egg shells, and cast skins of lice.

The oesophagus enters the right side of the crop and leads out at about the same level ventrally (Fig. 4). It is clear, therefore, that the long, slender barbules have little or no chance of passing directly through the crop into the midgut. Furthermore, once the barbules have passed into the crop there is equally little chance of ends being oriented so that long, intact barbules can pass out of the crop towards the midgut. Examination under polarized light indicates that the longer barbules in the crop are generally highly birefringent, whereas the shorter pieces are frequently less birefringent, suggesting that some disorganization of structure has already occurred in the latter.

In living, resting lice the crop undergoes regular antiperistaltic movements of considerable magnitude, each movement arising at the posterior end of the crop and being followed immediately by a peristaltic movement in the opposite direction. Crop movements in Philopteridae have also been recorded by Waterson (1926). The vigorous antiperistaltic movements drive the crop contents against the groups of teeth in the blind anterior end of crop, although these teeth are so small that it is not certain that they play an important part in triturating the food. The vigorous contractions also cause fluid and solid material (which shows little or no birefringence) to pass into the region of the foregut between the crop and the midgut. The contents of this latter region are then, as a general rule, immediately returned to the crop, but at intervals are passed on to the midgut. The anterior region of the midgut is also involved in both anti- and peristaltic movements. In these processes the midgut and hindgut are drawn forwards and then allowed to relax posteriorly by associated contraction and relaxation of muscles at the anterior end of the midgut. In addition to the mass forward and backward movement, the hindgut itself contracts and relaxes, coiling and uncoiling as it does so.

On no occasion was there visible in the *Columbicola* midgut any material which exhibited either the form or the birefringence of even partially degraded feather, although amorphous material and dyes ingested with the feathers were clearly visible therein. This confirms the findings of Waterson (1926), who was unable to detect unaltered feather fibres in the midgut of a philopterid. There are evidently exceptions in the group, however, since small, partially digested feather particles were often visible in the midgut of the poultry wing

louse, *L. caponis*. Careful observation demonstrated that, at intervals, some of the contents of the anterior midgut were passed forwards into the crop. This observation explains the data (see later) demonstrating that in *Columbicola* and *Lipeurus*, as in *Eomenacanthus*, the contents of the crop and the anterior region of the midgut have the same pH, oxidation-reduction potential, and reactions to the feeding of metals.

In the sheep body louse, *D. ovis*, the crop leads off dorsally from the oesophagus through a slightly constricted tube and extends nearly to the posterior end of the abdomen, often lying slightly to the right of the mid line. It contains a good deal of poorly birefringent material which is apparently mainly skin or skin debris. There are also occasionally present granules and small, highly birefringent, needle-shaped bodies, the former being mineral in nature. On two occasions only of many individuals examined was wool (a single short length) observed in the digestive tract (midgut) and it therefore appears most unlikely that *D. ovis* ever actively ingests wool fibres. These small pieces of wool showed no signs of digestion. The crop undergoes relatively weak and infrequent anti- and peristaltic movements and, in common with the crop of other members of the Trichodectidae, possesses no teeth. There is a good deal of evidence to indicate that it is mainly a temporary storage organ and it appears that, unlike the philopterid crop, it can be bypassed by food travelling directly down the oesophagus into the midgut. Not infrequently is it empty or almost empty when the midgut contains food after recent feeding. Shortly after feeding on dyed, scurfy wool the midgut was filled with dyed fluid with either very little solid material or with a certain amount of almost non-birefringent, finely divided material of no apparent structure. This further suggests that wool is not readily ingested, for even if pieces were chewed off the sides of fibres they should retain their birefringent properties for some time after ingestion. Stained food passed completely through the alimentary canal in less than 16 hr. at 37°C.

(e) The pH of the Digestive Tract

The pH records obtained by adding sulphonphthalein indicators to the food of three representative species of Mallophaga are summarized in Table 1.

In *Eomenacanthus* the contents of the crop and the anterior two-thirds of the midgut have the same alkaline pH (8.0-8.4). However, except when food has just passed on, the contents of the posterior midgut are, like the hindgut contents, slightly acid (6.4-7.2).

In *Columbicola* (and in *Lipeurus*) the pH of the contents of the first part of the oesophagus (OA, Fig. 4) appeared to be the same as that of the food although this region seldom contained sufficient food for accurate records to be obtained. Although the feathers fed were acid to both phenol red and cresol red, the food in the crop, in the short region of the oesophagus leading into the midgut (O), and in the anterior two-thirds of the midgut was alkaline to phenol red and within the colour change range of cresol red, indicating a pH of 7.8-8.0. Bromthymol blue gave slightly lower values (6.8-7.1) than those quoted for crop and midgut, but experience has shown that this indicator is

not as reliable in this type of experiment as phenol red or cresol red. The contents of the posterior third of the midgut have a pH of about 6.5 and those of the hindgut about 5.8, although both may occasionally record slightly higher values, presumably due to recent passage of food.

In *D. ovis* the pH of the crop contents was greatly influenced by that of the food. When recently fed on greasy, scurfy wool exhibiting the coloration of either acid or alkaline cresol red, the crop contents were correspondingly coloured. They did, however, tend to be influenced towards a pH of about 6.5, the value of oesophagus region *B* and of the entire midgut.

TABLE 1
pH OF THE CONTENTS OF THE ALIMENTARY TRACT OF THREE SPECIES OF MALLOPHAGA

Species	Foregut			Midgut		Hindgut	
	Oeso- phagus <i>A</i>	Crop	Oeso- phagus <i>B</i>	Anterior	Posterior	Anterior	Rectum
<i>E. stramineus</i>	8.0-8.4			8.0- 8.4	6.4- 7.2	6.4-7.2	
<i>C. columbae</i>	Same as food	7.8- 8.0	7.8- 8.0	7.8- 8.0	About 6.5	About 5.8	
<i>D. ovis</i>	Same as food	Similar to food (about 6.5)	About 6.5	About 6.5		5.8- 6.0	5.0- 5.5

(f) *Oxidation-reduction Potential of the Digestive Tract*

The oxidation-reduction potential of various regions of the digestive tract (Table 2) was determined by feeding the range of indicators employed in earlier studies (Waterhouse 1952*b*, 1952*c*).

In *Eomenacanthus*, *Columbicola*, and *Lipeurus* very similar results were obtained. The contents of both crop and anterior two-thirds of the midgut are very reducing, having a potential in the range —170 to —220 mV. (indigo disulphonate was reduced, whereas brilliant alizarine blue remained oxidized). The posterior third of the midgut was coloured a greenish yellow by indigo disulphonate, indicating partial reduction and an Eh of about —100 to —115 mV. In the hindgut indigo tetrasulphonate and galloxyaniline exhibited their oxidized colours, indicating an oxidizing potential. Blue inclusions were observed in the epithelium of the anterior portion of the *Columbicola* midgut after feeding on thionine and indigo disulphonate.

In *Damalinea* the picture was quite different. Reducing conditions were not encountered in any region of the gut. Thus, in the crop and midgut the

potential was greater than +77 mV. and in the anterior and posterior regions of the hindgut even more oxidizing. (Thionine was not reduced in any region, an observation supported by feeding a range of indicators which change at more negative potentials.) Furthermore, tetrazolium blue was not reduced during passage through the gut, indicating that reducing conditions were not encountered at any stage (Rutenburg, Gofstein, and Seligman 1950; Waterhouse 1952c). Both toluylene blue and methylene blue were absorbed from the digestive tract and appeared in the fat body and other internal organs.

Gyropus was not examined in the same detail as *Damalinia*, but it is relevant to record that indigo tetrasulphonate exhibited its oxidized colour throughout the entire alimentary canal. In this louse the crop and hindgut were acid, and the midgut alkaline, to phenol red.

TABLE 2
OXIDATION-REDUCTION POTENTIAL (mV.) OF THE CONTENTS OF THE ALIMENTARY TRACT
OF THREE SPECIES OF MALLOPHAGA

Species	Crop	Midgut		Hindgut	
		Anterior	Posterior	Anterior	Rectum
<i>E. stramineus</i>	-175 to -220	-175 to -220	About -115	> +30	
<i>C. columbae</i>	-170 to -215	-170 to -215	About -100	> +70	
<i>D. ovis</i>	> +77	> +77		> +97	> +124

(g) Metal Feeding Experiments

Lice were fed on feathers, wool, or hair which had been immersed in 5 per cent. solutions of nickel or copper sulphates, cobalt, cadmium or ferric chlorides, silver nitrate, lead acetate, or in saturated sodium tellurate (about 4 per cent.).

With none of the species did any of the elements (except cobalt and tellurium) produce any visible colour in the food undergoing digestion or in the faeces, although microchemical tests indicated that treated food had been ingested. It is clear, therefore, that metal sulphides are not formed during digestion.

In *Eomenacanthus*, *Columbicola*, and *Lipeurus* feeding on cobalt-enriched feathers the crop, midgut, and anterior region of the hindgut contained light brown food, whereas the contents of the rectum and faeces were a darker brown. Furthermore, when sodium tellurate was ingested the crop, midgut, and hindgut contained black material. The nitroprusside reaction indicated that free —SH groups were present in the crop and midgut. It is clear, therefore, that the disulphide bonds of feather keratin are reduced with the production of cysteine or cysteine peptides and, in the presence of cobalt, of a brown cobalt-cysteine complex (see Waterhouse 1952c).

Application of the ferrocyanide test to *Columbicola* fed on iron-enriched feathers indicated that ferric iron was present throughout the midgut epithelium. In this louse, and more particularly in *Lipeurus*, there was generally a far higher concentration of iron in the posterior midgut than elsewhere. Low concentrations of ferric iron could only be detected in this region in *Lipeurus* obtained directly from its host. In *Eomenacanthus*, whose iron intake is high because of the regular presence of blood in its diet, the entire midgut (and particularly the caeca) stained relatively heavily, but there was no preferential accumulation in the posterior region of the midgut. The salivary glands, perioesophageal nephrocytes, and malpighian tubules also stained lightly.

In *D. ovis* it is clear that the course of digestion is different from that in bird-infesting species. Thus, no deviation from normal coloration occurred when cobalt-impregnated food was ingested and, furthermore, no region of the alimentary canal gave a positive nitroprusside reaction. However, when sodium tellurate was fed the contents of the midgut were blackened, although the colour of the crop contents was unaffected. The faeces contained black material which was visible also in the midgut epithelium. This is an apparently anomalous result, since it was tentatively suggested for dermestid larvae that the dark colour was caused by reduction to metallic tellurium (Waterhouse 1952c). Whatever the mechanism of the reaction and the nature of the dark material, it is evident that this reaction cannot be taken to indicate intensely reducing conditions.

After *D. ovis* had fed on iron-enriched food, ferric iron was detected throughout the midgut epithelium and after feeding on barium-enriched food, barium was present throughout the midgut epithelium and in the enlarged regions of the malpighian tubules.

IV. DISCUSSION

In the Mallophaga examined there are no anatomical features of the alimentary canal which have not been broadly covered by previous work (Snodgrass 1899; Cummins 1913). It is perhaps worth emphasizing, however, that the pair of glands described by Snodgrass (1899) as discharging into the crop are, in fact, groups of nephrocytes. The midgut histology is somewhat unusual in that there are numerous regenerative nuclei, which form an almost continuous layer adjoining the basement membrane, but are not aggregated to form nidi. This might be taken to indicate an active, but rather unspecialized, type of epithelium. The tracheal supply of the crop and midgut is poorly developed, as in other insects having the capacity to digest keratin (Day 1951; Waterhouse 1952c). It is, however, also poorly developed in mammal-infesting Mallophaga which, if one can generalize from *Damalinea*, do not maintain reducing conditions in their digestive tract. Thus it appears that, whereas effective maintenance of highly reducing conditions may demand poor tracheation, this feature alone will not produce a negative redox potential.

A very striking feature of digestion in the bird-infesting Mallophaga examined is its dynamic nature. The continuous and vigorous circulation of

digestive juices from the midgut into the crop and back again, carrying with them partly degraded food, results in the crop being a far more important site for digestion than it is usually held to be in most other insects. The regular flushing of the crop contents with digestive juices would appear to be more important in this process than mechanical breakdown, for the crop teeth in the Amblycera act mainly as a sieve and in the Ischnocera the teeth are small and sparse. The mineral particles in the crop may play some part in mechanical breakdown, even if far less important than those in the crop of their avian host. It is probable that the small mineral grains are ingested as fortuitous feather contaminants from the habit of many birds of taking dust baths. The oesophageal invagination of Mallophaga is poorly developed and funnel-shaped and this region would appear to function mainly as a sphincter during the periods when the contents of the crop and midgut are subjected independently to their respective contractions and relaxations. There is no evidence that the oesophageal invagination functions in aspirating digestive enzymes from the midgut into the crop, as has been suggested for the well-developed invagination of *Periplaneta* (Day and Powning 1949). Indeed, there is no need to postulate such a function in these Mallophaga in view of the vigorous anti-peristaltic waves of the midgut and the shape of the invagination.

Regurgitation of fluid from the midgut to the crop occurs to a very much smaller extent in the mammal-infesting Mallophaga examined. It was observed in *Heterodoxus*, but could not be detected in *Gliricola* or *Damalinea*. The fact that the pH of the contents of the *D. ovis* crop is similar to that of the food ingested and does not rapidly approximate that of the midgut supports the belief that there is little forward passage of fluid from the midgut in this species.

There is clear evidence that bird-infesting Mallophaga are capable of digesting feather keratin by a process similar to that in dermestid larvae (Waterhouse 1952c) and that they do not carry the degradation as far as hydrogen sulphide, as in larvae of the clothes moth *Tineola* (Waterhouse 1952a). The pH of the crop and midgut is slightly alkaline (about 8.0) and the oxidation-reduction potential very reducing (about -200 mV.). The cystine of the keratin molecule is reduced to cysteine and the free $-SH$ groups of the latter can be detected. When cobalt salts are added to the feather diet the characteristic brown, soluble, cobaltic-cysteine reaction product is formed in the crop and midgut. Metallic sulphides are not produced. It is interesting to note that feathers have only about two-thirds of the cystine content of wool or hair (Graham, Waitkoff, and Hier 1949), which may render them more readily digested, and that skin has an even lower cystine content (Block 1951). Fresh blood is a normal part of the diet in some species (e.g. *E. stramineus*) whereas others only ingest dried blood when this occurs as a feather contaminant. In relation to their dietary requirements it is of interest that a lower population of *E. stramineus* is carried on poultry suffering from several types of avitaminoses than on well-nourished poultry (Kartman 1949). Absorption evidently occurs throughout the midgut, but evidence from iron absorption suggests that the posterior midgut may be specially concerned in this process. This latter region is less involved than the rest of the midgut in continuous exchange of fluid with the crop.

The mammal-infesting Mallophaga examined do not appear to utilize keratin. In *D. ovis* the midgut pH is slightly acid (about pH 6.5), the oxidation-reduction potential is oxidizing, and no —SH groups can be detected, data which alone would throw doubt upon the ability of this species to digest keratin. In addition, wool is seldom ingested and, when occasionally observed in the digestive tract, is not visibly degraded. Its food appears to consist mainly of skin, skin scurf, wool wax, and any other materials contaminating the fibres, which suggests that its food is similar to that of other mammal-infesting Mallophaga (Eichler 1937). It is clear that the presence of *D. ovis* causes irritation to its host, for heavily infested sheep can often be detected by the rolls of rubbed wool on the surface of the fleece and a general "plucked" appearance. Whether the irritation and the greatly increased scurfiness of the lousy sheep is due merely to the crawling of lice on the tender skin, or whether, as suspected, the lice also chew the surface layer of skin has not been determined. It is relevant to mention, however, that as blood does not form a regular part of their food any attack on the skin must be confined to its outermost layers, which may result in tissue fluids, but not blood, being available also.

It may be unsafe, however, to generalize on the basis of the mammal-infesting species examined that this group is unable to digest keratin. Thus there are reports in textbooks (e.g. Essig 1942) that the goat louse, *Bovicola* (= *Damalinia*) *caprae*, damages the hair of its host, although Haug (personal communication) states that hair particles are only rarely observed in the digestive tract of this species and, when present, are scarcely, if at all, digested. His observations are supported by an examination of about a dozen specimens of *D. caprae* which were dissected several days after removal from their host and left in a tube with goat's hair. No hair was detected in the gut, although some epithelial debris was present. If the reports are correct that hair is both ingested and digested, there must be important differences in the digestive physiology of related species. If some mammal-infesting species do regularly ingest the hair of their host it is possible, from the relative diameters of the hair and the midgut lumen, that attack would take the form of chewing pieces off the side of a hair. Even pieces of this nature should, however, be readily distinguishable by their high degree of birefringence, although small, extensively damaged pieces of hair would almost certainly be more rapidly attacked than lengths of intact hair.

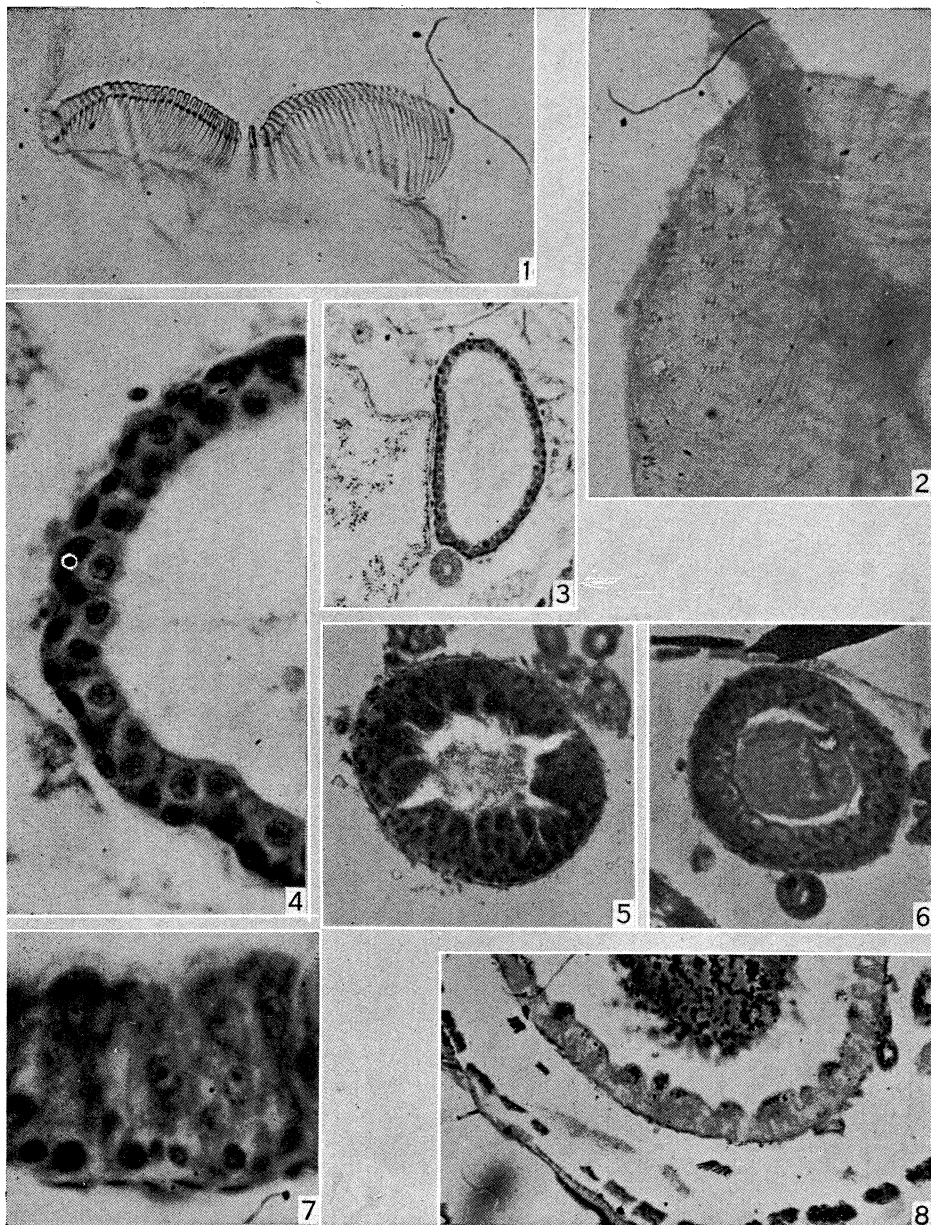
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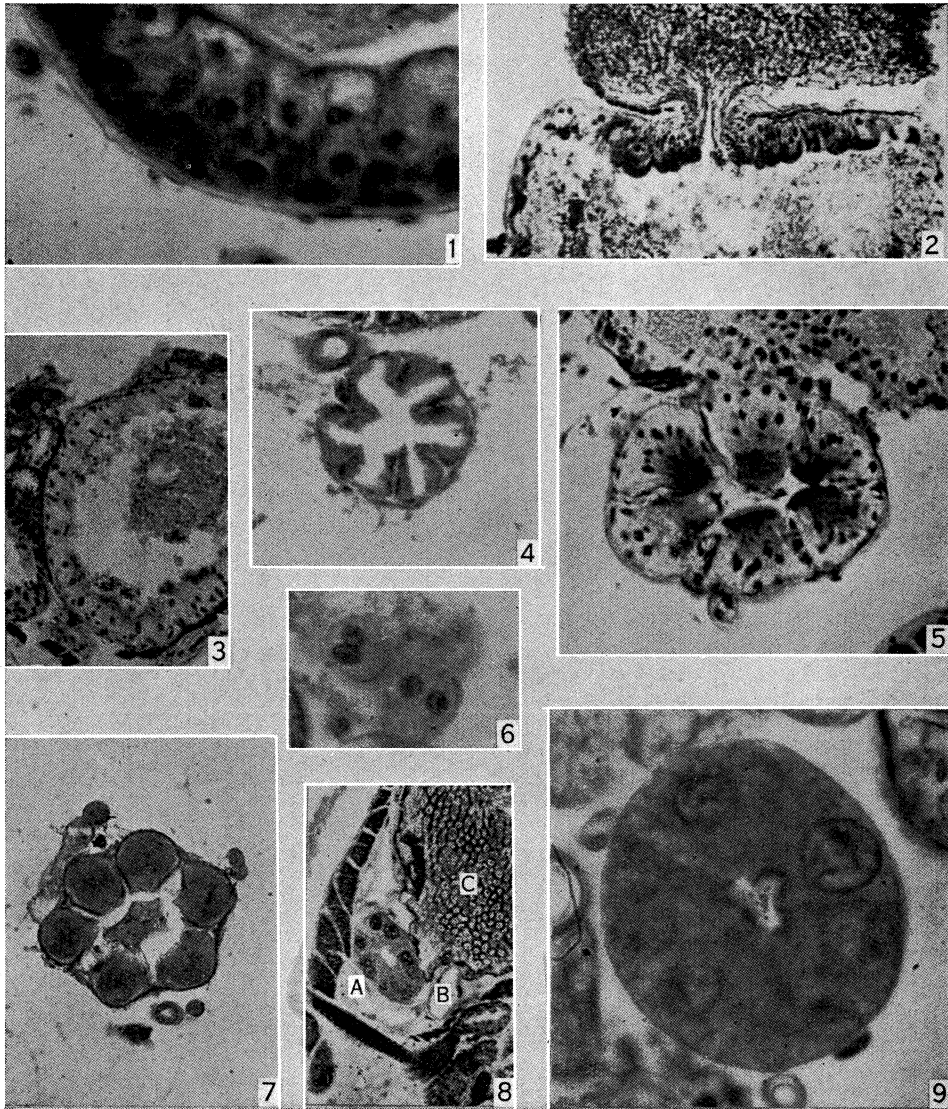
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DIGESTION OF WOOL BY INSECTS. IX



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EXPLANATION OF PLATES 1 AND 2

PLATE 1

- Fig. 1.—Comb-like crop teeth of *E. stramineus*.
- Fig. 2.—Small rows of crop teeth of *L. caponis*.
- Fig. 3.—T.S. midgut of *D. ovis* showing low epithelium with many regenerative nuclei. Bodian.
- Fig. 4.—T.S. midgut of *D. ovis*. Bodian.
- Fig. 5.—T.S. anterior midgut of *E. stramineus*, showing relatively high epithelium. Bodian.
- Fig. 6.—T.S. posterior midgut of *E. stramineus*, showing relatively low epithelium. Bodian.
- Fig. 7.—Anterior midgut of *E. stramineus*. Bodian.
- Fig. 8.—T.S. midgut of *Heterodoxus* showing granular inclusions in cells. Bodian.

PLATE 2

- Fig. 1.—T.S. midgut of *L. caponis*. Bodian.
- Fig. 2.—L.S. crop and midgut of *Heterodoxus* illustrating poorly developed oesophageal invagination. Bodian.
- Fig. 3.—T. S. midgut of *Heterodoxus*. Bodian.
- Fig. 4.—T. S. anterior hindgut of *L. caponis*. Bodian.
- Fig. 5.—T.S. rectal pads of *C. columbae*. Mallory.
- Fig. 6.—Perioesophageal nephrocytes of *D. ovis*. Bodian.
- Fig. 7.—T.S. rectal pads of *D. ovis*. Bodian.
- Fig. 8.—T.S. *E. stramineus*, showing salivary gland (A), salivary reservoir (B), and crop (C) containing blood. Bodian.
- Fig. 9.—T.S. salivary gland of *D. ovis*. Bodian.