# STUDIES ON THE DIGESTION OF WOOL BY INSECTS

## III. A COMPARISON BETWEEN THE TRACHEATION OF THE MIDGUT OF *TINEOLA* LARVAE AND THAT OF OTHER INSECT TISSUES

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*Summary*

A study of the tracheation of nine tissues and organs of five species of insects reveals that the arrangement of tracheae and especially of tracheoles is determined by the structure of the tissue supplied. Adaptations are found in the ovary permitting the supply of the oocyte during its rapid enlargement. There is a correlation between the abundance of the tracheal supply of a tissue and its probable oxygen requirements.

The tracheal supply of the *Tineola* larval midgut is less well developed than that of many other insects, but a number of insects have an even less well developed supply. The poor tracheal supply of the midgut of *Tineola* larvae probably contributes to their ability to digest keratin, but other insects, which do not digest keratin, also have poor tracheation of the midgut.

Tracheolar anastomoses could not be found in any insect examined, although anastomoses of tracheae are frequent in some organs. Tracheal end cells are found in most organs and tissues, but differ in form in different tissues and in the same tissue in different species. They are absent from the crop and midgut caeca of *Periplaneta* and from wing muscles. Tracheae in the *Periplaneta* wing do not respond to injury and are fairly static. Nor is there proliferation of surrounding tracheae in a detracheated area.

## I. INTRODUCTION

Very low oxidation-reduction potentials are maintained in the alimentary tract of certain insects, for example in the midgut contents of larvae of the clothes moth, *Tineola,* where the potential approximates $-0.30$ volts (Linderstrom-Lang and Duspiva 1936). The mechanism of the maintenance of this low potential has never been explained.

Uric acid forms a high proportion of *Tineola* faeces and, in view of the results of Leifert (1935) on *Antheraea* larvae, it seems likely that some of it must be produced from hypoxanthine by xanthine oxidase. This hypothesis is confirmed by qualitative tests which reveal the presence of xanthine oxidase in the gut of *Tineola.* The hypoxanthine-uric acid reaction has one of the lowest redox potentials recorded in a biological system (Green 1934), and may well be a factor contributing to the maintenance of the gut potential. Now, xanthine oxidase is inhibited by oxygen (Stadie and Hangaard 1945), which suggests that, if this enzyme is important, the tracheation of the midgut must be such as to restrict the supply of oxygen.

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A preliminary study of the tracheation of the midgut of larval *Tineola* showed that it was far less highly developed than that of *Blattella* (cf. Day and Powning 1949). It seemed desirable, therefore, to extend the comparison to other insects. With the exception of certain organs like the silk gland, surprisingly little collated information about the tracheation of insect tissues is to be found in the literature. Those reports which do exist deal mainly with the possible intracellular nature of tracheoles (see Keister 1948), the presence of liquid in the tracheoles (see Wigglesworth 1931), and the existence of tracheolar anastomoses.

It was apparent that further comparative anatomical information was required, and some data on this subject are presented in this paper. These anatomical considerations suggested related problems of tracheation and the data reported have provided some information on the function of the tracheal system.

It seemed likely that a thorough study of many tissues of a few species would provide useful preliminary data, and so nine organs (heart, nerve, foregut, midgut, hindgut, rectum, malpighian tubules, fat body, and muscle (wing and body muscle, when possible) ) were studied in five species, namely *Ctenolepisma longicaudata* Esch., *Periplaneta americana* (L.), larvae and adults of *Tenebrio molitor* L., larvae of *Tineola bisselliella* Humm., and larvae and adults of *Lucilia cuprina* Wied. Other species used in later comparisons will be referred to below. All species were terrestrial and none would be expected to have a peculiar tracheal system correlated with an unusual habitat.

## II. Methods

This work necessitated a detailed investigation of the relative effectiveness of several methods for making the tracheoles visible. It was found that no one method was satisfactory for all species, and all of the following methods were finally used with each species:

(1) Dark field observation of spreads of living tissue mounted in Toisson's solution. This was useful for all species, especially for larvae of *Lucilia,* which could not be studied by methods (3) and (4) below. Toisson's solution has the following composition: glycerine 30 ml., sodium sulphate 8 g., sodium chloride 1 g., methyl violet 0.025 g., distilled water 160 ml.

(2) Observation with the phase contrast microscope. Cooke, Troughton, and Simms phase contrast equipment was employed, and excellent differentiation of tracheoles was observed at magnifications up to 1425x. Both this and method (1) required the use of thin spreads, a condition not easily met in all tissues, especially of the larger species.

(3) Trypan blue injections by the method of Hagmann (1940) had the advantage over methods (1) and (2) of permanence. Spreads, rather than sections, provided the most generally useful preparations.

(4) The osmic acid method for studying tracheal end cells. This very useful method consisted of suspending the insects in a small cage over a 2 per cent. solution of osmic acid for periods of 1 to 4 hours, depending on the species,

washing in water, dehydrating, and clearing, followed by the preparation of spreads. Sections of osmic acid-impregnated material were also used but were less useful than the tissue spreads.

(5) The silver nitrate method as used for the demonstration of Buck's (1948) "possible ultratracheolar network." Buck (personal communication) has described this method as follows: "Soak the fresh tissue in pieces of about 1 to 2 $mm.^3$ in 1 per cent. silver nitrate overnight in the dark, then remove tissue to a drop of glycerine on a slide, tease out, and place in the sunlight."

(6) Acetic orcein, on fresh or Carnoy-fixed material. This was useful for studying the tracheal epithelium.

Many attempts were made to use the osmic acid and trypan blue injection methods on *Lucilia* larvae. The osmic acid was administered under vacuum, under $CO_2$ or ether anaesthesia, and after the anterior and posterior spiracular closing mechanism had been destroyed; and the injections were attempted on anaesthetized larvae, larvae killed by hot water, and larvae with their spiracular closing mechanisms punctured. None was successful. Nerves were stained by the methylene blue method outlined by Day and Powning (1949).

Photomicrographs or scale drawings were made of all preparations for ease of comparison.

## III. Observations

### (*a*) *Arrangement of Tracheae in Tissues*

A comparison between similar tissues of different species shows clearly that the arrangement of tracheae in tissues, though typically arborescent, is determined by the fine structure of the tissue supplied. Thus, the tracheation of abdominal skeletal muscles in all species studied is characterized by main trunks with which the branches make approximate right angles and from these many fine branches run parallel with the muscle fibres. Similarly, the tracheation of ganglia of the ventral nerve cord follows a very regular pattern in all species (Plate 1, Fig. 1). Large trunks enter the ganglion from each side, branch fairly regularly and send long, fine branches down the length of the nerve cord. Again, the distribution of tracheae in the fat body is characteristic in all species studied (Plate 1, Fig. 2). Large trunks run among the large fat body cells, branching infrequently; branches usually form an acute angle between them and finer branches are given off to individual cells.

It is clear that the statement sometimes still quoted in textbooks that every cell in the body of an insect is tracheolated is inaccurate. Every cell of certain tissues, e.g. muscle, may be tracheolated, but many epithelial cells are well removed from the nearest tracheole. Keister (1948) mentions that the digestive tract and certain other organs of *Sciara* larvae are never tracheated.

The arrangement of tracheae in tissues is always such as to permit flexion and considerable movement of viscera. In muscle the finer branches are very sinuous, especially when the muscle is in the contracted condition. Modifications of tracheal patterns are encountered infrequently. An interesting example

is found in the insect ovary, where there is an unusual degree of tracheal folding on the surface of small oocytes (Plate 1, Fig. 3). This permits tracheae to supply rapidly developing oocytes without extensive growth.

### (*b*) *Relative Abundance of Tracheae in Tissues*

Even though tissue spreads are not of uniform thickness, observations at a restricted focal plane under high magnification permit a close approximation to the "quantitative anatomy" which Krogh (1929) considers essential to the proper understanding of the details of physical respiration. Spreads are much superior to sections for such observations. In general, trypan blue injections showed more and finer tracheoles than the other methods. A comparison of many such preparations demonstrates a relationship between the degree of tracheation of organs and their probable oxygen requirements. Until the metabolism of various insect tissues has been studied such comparisons must be tentative, but the relationship is shown clearly in a comparison of, for example, wing muscle, abdominal skeletal muscle, brain, and fat body. The only organ with a high metabolic rate (as indicated by its tracheation) that might not have been expected is the rectum, but rectal pads are well known to be unusually richly tracheated in all species in which they have been examined. The malpighian tubules, normally poorly tracheated, are also well supplied with tracheae where they are associated with the rectum.

There are in most species differences in the degree of tracheation in different regions of the midgut, but the significance of such variations is not known. The midgut of *Tineola* larvae is better tracheated at its anterior end than at its posterior end, but is well supplied with tracheae throughout its length (Plate 1, Fig. 4, and Plate 2, Fig. 11). A comparison between the tracheation of several larval Microlepidoptera (*Gnorimoschema* (Plate 1, Fig. 5), *Sitotroga, Ephestia,* and *Plutella* (Plate 1, Fig. 6) ) has shown that that of *Tineola* is less well developed than those of the other species of comparable size. In the anthelid, *Pterolocera amplicornis* Wlk., which is a much larger species whose mature larvae weigh about 0.7 g., almost every midgut cell is tracheated. The midgut is of the order of 0.5 cm. in diameter, so that thorough tracheation would be necessary to supply oxygen to the centre of the food mass. Yet the tracheal pattern is quite comparable to that of *Tineola* and there are no anastomoses as are found in *Blattella* (Day and Powning 1949) or *Periplaneta* (Plate 2, Fig. 7). Nor do tracheal vesicles occur, such as Metalnikov (1908) and Gäbler (1936) have described in larvae of *Galleria mellonella.* There is, at present, no reasonable explanation for the unusually rich tracheation of the midgut of *Galleria.*

It has been demonstrated from these comparisons that the tracheation of the larval midgut of *Tineola* is less well developed than in most other insects (Plate 2, Figs. 7 and 9) and in some other lepidopterous species of comparable size (Plate 1, Figs. 4, 5, and 6). However, it is better developed than that of many other species also examined, for example *Anthrenus* and *Attagenus* larvae (cf. also *Sciara* larva (Keister 1948) ).

### (*c*) *Tracheal End Cells*

The most significant feature of a tracheal system is its ability to transfer oxygen to tissues in the body. The sites of transfer are considered to be demonstrated by the reduction of osmic acid to the metal, which produces a darkening at the site of transfer. This has been known for 75 years (see Wigglesworth 1931), but no comparative study of the sites of osmic acid reduction is available. Study of the tracheal end cells in nine organs of *Periplaneta,* larval and adult *Tenebrio,* larval *Tineola,* and adult *Lucilia* resulted in the following conclusions:

(i) Osmic acid is not normally reduced in the walls of large tracheal trunks or in tracheal branches of most insects (Plate 2, Figs. 8 and 10).

(ii) The microlepidopterous larvae (Plate 2, Fig. 12) examined and *Ctenolepisma* constitute an exception to this, however, and blackening of tracheal trunks usually occurs in these species right up to the spiracles.

(iii) Wing muscle is also exceptional. The entire muscle is darkened during exposure to osmic acid.

(iv) In the majority of tissues, reduction occurs at well-defined regions along the trachea or tracheole, frequently at the tracheal end cell. The latter has been observed in almost all tissues, except wing muscles and the crop or midgut caeca of *Periplaneta.*

(v) Tracheal end cells differ considerably in form in different tissues, and in the same tissue in different species. Frequently observed types are shown in Plate 2, Figures 8, 10, and 12. Sometimes the unstained tracheole can be discerned distal to the osmophile region. This does not seem to be due to the fact that this section is normally fluid-filled, for two reasons:

(*a*) The position of the osmophile region does not change if the insect is treated so that the amount of fluid in the tracheoles is reduced (e.g. exposure to $CO_2$) immediately before or during the exposure to osmic acid;

(*b*) Instances have been found of two separated osmophile regions along the length of a single tracheole.

(vi) Within a species there appears to be a relationship between the oxygen requirements of a tissue and its supply of tracheal end cells. Thus, in *Periplaneta* the order of decreasing abundance of tracheal end cells is roughly: muscle, rectum, midgut, hindgut, nerve, heart, malpighian tubules, and fat body. A comparison between the tracheal end cells of the *Tineola* larval midgut and those of the other species examined shows that they are well developed in *Tineola* but fewer in number, supporting the hypothesis that the oxygen supply to the midgut of *Tineola* is less abundant than that of many other insects of comparable size.

### (*d*) *The Silver Nitrate "Network"*

Cajal (1890) observed, with the Golgi technique, a network binding tracheoles together in insect muscles, and Buck (1948) has observed with silver

nitrate what he tentatively describes as an ultratracheolar network in the light organ of lampyrids. It has been found that many tissues show characteristic patterns of deposited silver when treated by the method described above. Thus, muscles show a beautiful pattern of granules, frequently paired, regularly arranged both longitudinally along the fibril and transversely across the striations. The details of the pattern vary from muscle to muscle and from species to species. The relation of the granules to the striations has not been determined, but it seems certain that they represent silver deposits related to the fine structure of the muscle fibre. They do not appear to be connected with the numerous tracheoles which ramify between the fibrils.

On the surface of the male accessory glands of *Periplaneta* a complex network of fine granules outlines the cell boundaries, whereas the cells of the malpighian tubules are covered uniformly with granules and the cell walls are not outlined by them. The same is true of the salivary glands of larval *Lucilia.*

On the surface of the larval *Tineola* midgut an irregular network of deposited silver appears to be related to the tracheoles, but it is much coarser than that found by Buck (1948) on the cells of the light organ.

Summarizing these and similar observations on many tissues of *Periplaneta, Tenebrio* larvae and adults, *Tineola* larvae, and *Lucilia* larvae and adults, the generalization seems warranted that silver granules are deposited on tissues in a manner dependent upon the fine surface structure of the tissue; but no evidence was obtained to substantiate the existence of an ultratracheolar network in any of the tissues examined.

### (*e*) *Histological Structure of Tracheae and Tracheoles*

Tracheae are remarkably uniform in structure along their length. Careful study of acetic orcein preparations has failed to reveal any structures which might be involved in constricting the lumen. There is apparently no "physiological reserve" as there is of arterioles and capillaries of vertebrates. Similarly, there are in the species studied no valvular mechanisms providing a unidirectional flow, or any internal mechanism for increasing the intratracheal pressure, although this can be accomplished by spiracular and body movements (McCutcheon 1940). A peculiar intratracheal valve has been described by Webb (1945) in *Melophagus,* demonstrating that the above generalizations are not without exceptions among insects.

Tracheae are not innervated. This was shown by a detailed examination of methylene blue preparations of the tissues of *Periplaneta* by the method of Kuwana as outlined by Day and Powning (1949). Many nerves in many organs were traced, but none was ever observed to terminate either on a large tracheal trunk or at a tracheolar ending. Frequently, nerves run along side tracheal trunks and from the latter small tracheae or tracheoles run to the nerve at irregular intervals. Frequently the nerves and tracheae branch together, but the tracheae become smaller and end, whereas the nerve may continue undiminished in diameter. The best demonstration of the relationship between the

two systems is seen in the rectum, which is both well tracheated and well innervated. It is clear that the nervous system of this organ is tracheated but that the tracheal system is not innervated. The number of fine nerve branches is only a small fraction of the number of tracheoles.

It has been claimed that the "dark staining sheath" of Dahlgren (1917), which surrounds the tracheoles, provides a mechanism for controlling the oxygen supply to tissues, especially to the light organ of fire flies. Such control could only be exercised over the whole organ by a nervous or a humoral mechanism, and neither seems likely. Available data indicate that control of the tracheal system occurs only at the spiracles (the muscles of which are, of course, innervated) and at the tracheoles by movements of their fluid contents. Indirect control is also produced by the movements of ventilation.

The fine structure of the tracheal epithelium was studied by the acetic-orcein technique. The general belief that the tracheal epithelium of different insects is not markedly different is without foundation. It has been found that:

(1) The tracheal epithelium of the larvae studied is more conspicuous than that of their respective adults.

(2) The epithelium of the smaller trunks is relatively thicker than that of the larger trunks.

(3) Mitoses are not infrequent in the tracheal epithelia of larvae, and aberrant nuclei are frequently found in both larvae and adults.

(4) Cell walls vary in conspicuousness, but could always be demonstrated by appropriate techniques, methylene blue being useful for this purpose.

The observations made in the course of this work suggest, as certainly as possible by the use of light microscopy alone, that, in the majority of insect tissues, the tracheoles end blindly without anastomoses. This is in contradiction to the opinion of Snodgrass (1935, p. 450) who says that in tissues that have been studied the ultimate branches of the tracheoles "have been found to anastomose in a fine capillary network over the tissue cells . . . ." In the light organ of lampyrids, which is particularly thoroughly tracheated, anastomosing tracheoles have been described by many authors and may occur (Buck, personal communication), even though electron microscopy has shown that many tracheoles end blindly (Buck 1948). In view of the specialization of the light organ the presence of anastomoses in that tissue would not invalidate the generalization that they do not ordinarily occur (cf. also Keister 1948; Richards and Korda 1950). Tracheal anastomoses, on the other hand, may be found not infrequently in tissues that are well supplied with tracheae, as for example the midgut of *Periplaneta* or *Blattella.*

### (*f*) *Static Nature of Tracheae*

An organ implanted into a larval insect becomes tracheated as does a normal organ of the host (Meisenheimer 1907). The mechanism by which this occurs has not been investigated. In vertebrates, the blood vessels are broken down and rebuilt continually. Although such dynamic changes are not characteristic of insect tracheae, the occurrence of mitoses in the epithelium

suggested that some growth of tracheae may go on in the adult insect. An attempt was made to study tracheal changes in the cockroach wing after wounding. Cuts were made in the tegumen, and drawings of the surrounding regions were made immediately and at intervals of about a week thereafter for six weeks. In some insects the tracheae distal to the cuts became invisible within one week, probably being filled with liquid. No other changes were visible. In other specimens practically no change could be seen in the tracheae even after six weeks. Special attention was paid to possible changes in fine branches proximal to the cut and to the adjacent intact tracheae to determine any growth or anastomoses. No such changes were observed.

These observations indicate that the tracheae in the adult have lost the capacity to repair damage to an area deprived of its normal oxygen supply, and that, as far as can be observed at the magnifications that could be employed (100x), tracheae in the *Periplaneta* tegumen do not have the ability to undergo structural alteration once the insect is adult. If it is objected that the tegumen is not a favourable site for such a study because of the probable ease of aeration even without tracheae, corroborative evidence for the static nature of tracheae can be found in the midgut of termites (*Coptotermes, Nasutitermes*). In workers the midgut is relatively poorly tracheated, but that of the comparably sized alates is extraordinarily well developed. In the event of these alates becoming physogastric, growth of the alimentary canal can occur and it can be adequately tracheated without extensive changes in the tracheae already present.

### (*g*) *Effects of Pressure Changes*

The above observations showed the static nature of the tracheae in the cockroach tegumen and suggested a study of their reaction to decreases and increases in environmental pressure. *Periplaneta* adults were lightly anaesthetized with carbon dioxide and fixed by plasticine in a clear plastic chamber in which the pressure could be increased or decreased. A tegumen was held flat against the upper surface by a coverslip held in position by stopcock grease. Changes in tegumen tracheae could be observed with magnifications of 100x. Higher magnifications could not be used because of the technical difficulty of obtaining a window thin enough but still strong enough.

The tracheae are practically unaffected by a vacuum of 8 in. of mercury. At 27 in. of mercury they collapse in some specimens, particularly those that have recently moulted. In others, however, even at this pressure they are practically unaffected. On sudden return to atmospheric pressure they regain their normal shape within a few seconds and the insect is quite unaffected. This can be repeated many times with similar results. Since this pressure is equivalent to a height of about 50,000 feet above sea level it is readily appreciated that the high altitudes at which insects have been taken are unlikely to affect their physical respiration adversely.

At increased pressures of 4 lb. per sq. in. (equivalent to a depth of only about 8 ft. under water) the tracheae collapse, but regain their normal condi-

tion when the pressure is released. Again the insects suffer only very temporarily from the effects, and can undergo repeated compressions and sudden decompressions without apparent physiological embarrassment.

## IV. Discussion

Leaving aside special adaptations for parasitic and aquatic respiration, and respiration of unusual types, the tracheal system is probably the simplest system, anatomically and histologically, in insects. This simplicity is correlated with its uncomplicated function. Thus, the system responsible for gas transport (including the gases of respiration) in vertebrates also functions in the transport of food materials and hormones, in temperature control, and in the maintenance of the internal environment of the organism; the tracheal system of insects functions only in the transport of gases. In vertebrates the "arrangement of the capillaries is determined by the architecture of the particular organ or tissue whose life lines they constitute" (Cowdry 1938, p. 121), and this is similarly evident for the small tracheae and tracheoles. The similarity between the tracheation of an insect muscle and the capillaries of a vertebrate (e.g. Fig. 1 of Krogh 1929) is most striking. But, although the arrangement of tracheae in tissues is dependent upon the tissue supplied, the abundance of the tracheation seems to be dependent upon the oxygen requirements of that tissue.

All detailed anatomical studies, except that of Webb (1945), have failed to demonstrate any complexities in the structure of the tracheae. They provide a pathway for gaseous diffusion, a function generally requiring no active participation of the organ system. Correlated with its single function the organ system is one of unusual simplicity. There is no evidence that, when adequate ventilation occurs, the tracheal system is, in any respect, limiting in the development of increased size. Similarly, the tracheal system is suitable for physical respiration in an unusual variety of terrestrial habitats.

## V. Acknowledgments

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## EXPLANATION OF PLATES 1 AND 2

### PLATE 1

Tracheation of insect tissues

Fig. 1.—*Periplaneta* abdominal ganglion, trypan blue injection, showing type of tracheation in nervous tissue.

Fig. 2.—*Periplaneta* fat body, phase contrast spread, showing characteristic type of tracheal distribution and branching in this tissue.

Fig. 3.—*Tenebrio* adult ovary, trypan blue injection, showing sinuous tracheae on small oocytes, and straighter tracheae on larger oocytes.

Figs. 4, 5, and 6.—Spreads of midgut under dark field of *Gnorimoschema, Plutella,* and *Tineola,* respectively, illustrating comparative tracheation.

### PLATE 2

Tracheation of insect midgut

Figures 7, 9, and 11 with 8x ocular and 10x objective

Figures 8, 10, and 12 with 8x ocular and 33x objective

Fig. 7.—*Periplaneta* midgut, spread of trypan blue injection, showing thorough tracheation and anastomosing tracheae.

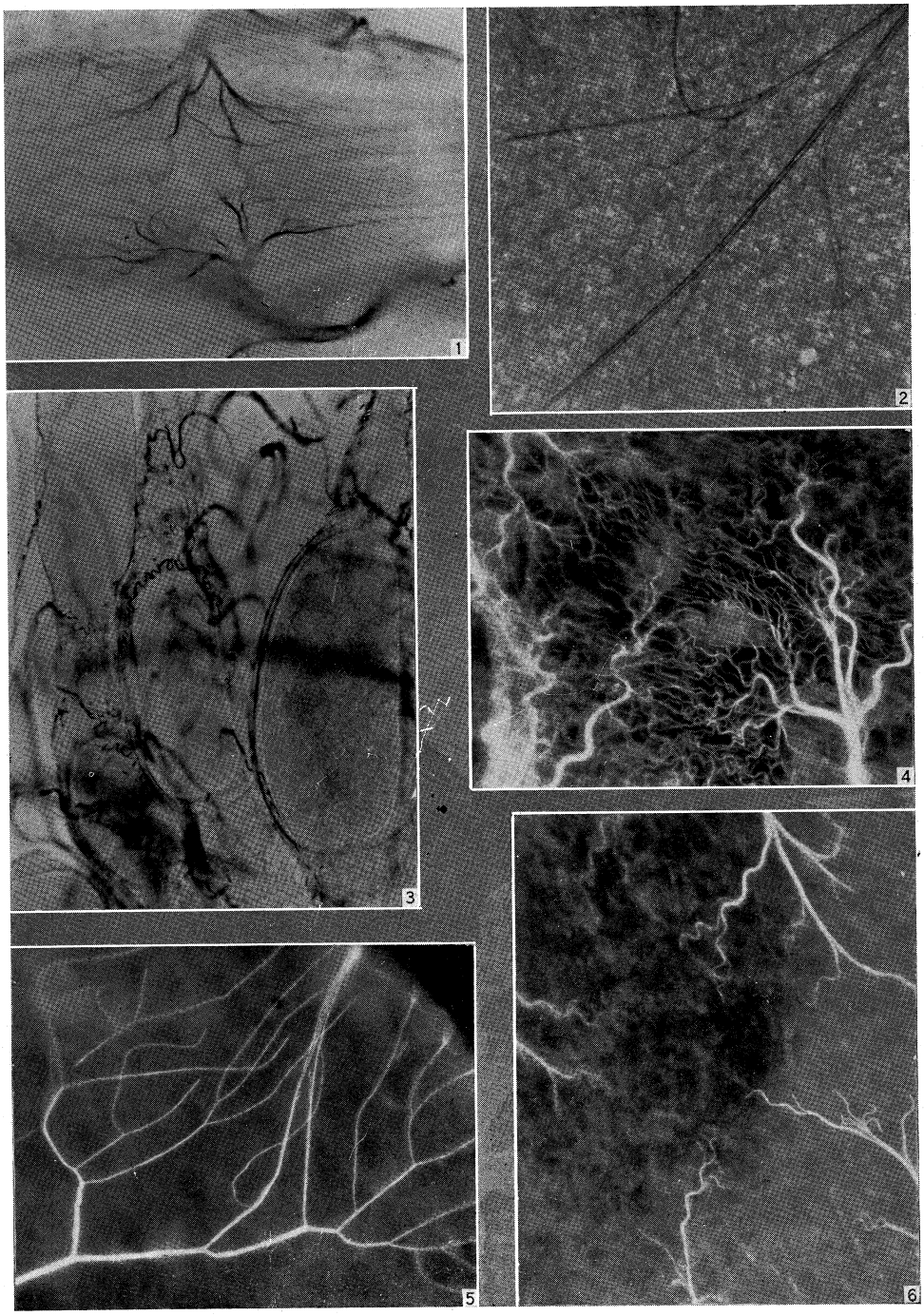
Fig. 8.—*Periplaneta* midgut, osmic acid preparation showing tracheal end cells surrounding every nidus.

Fig. 9.—*Tenebrio* larval midgut, spread of trypan blue injection, showing thorough tracheation but absence of anastomoses.

Fig. 10.—*Tenebrio* larval midgut, osmic acid preparation showing isolated tracheal end cells uniformly scattered over the muscularis.

Fig. 11.—*Tineola* larval midgut showing relatively sparse tracheation.

Fig. 12.—*Tineola* larval midgut, osmic acid preparation, showing characteristic form of tracheal end cells and staining of tracheoles and of connecting tracheae.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DIGESTION OF WOOL BY INSECTS. III