The Structure of Insect Tracheae

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With two plates (figs. 1 and 2)

Summary

Tracheae of Rhodnius prolixus Stål have been studied with the light and electron microscopes. The tracheae have three cuticular components: a two-layered membrane lining the tube and the taenidia between it and the epithelium. The layer upon the lumen face is similar in appearance and properties to the cuticulin layer over the abdomen. The other layer is of chitin with the micelles axially oriented and protein with a stability suggesting tanning. A comparable but slightly thicker layer not penetrated by pore canals exists over the abdomen where it is sclerotized and lipid impregnated. The taenidia also contain chitin and protein but the micelles are arranged tangentially. A tube constructed in this way is well adapted to resist lateral compression while allowing changes in length.

The structure of tracheae has been described by Weber (1933), and Richards and his collaborators (1942 a, b, 1948, 1950, 1951, 1953). While studying the development of tracheae in Rhodnius it became apparent that the structure described in the textbooks is not that most commonly found. The account which follows is concerned solely with the cuticular layers. The pattern formed by the taenidia and the epithelium will be reserved for a future paper.

Material and Methods

The tracheae in 5th instar larvae and 5th instar exuviae of Rhodnius prolixus Stål were used as the standard test material, but many tests were repeated upon larvae of Tenebrio molitor L. and Periplaneta americana L. Tracheae were fixed in neutral 10% formalin for histochemistry. For the polarizing microscope tracheae were extracted with 10% potassium hydroxide for several days at 60° C. This slowly dissolved tissues, tanned protein, sclerotin, &c., leaving chitin only slightly deacetylated. This method of preparing chitin proved much more sensitive than the cruder methods commonly used which employ saturated potash and high temperatures. Chitin was found in all tracheae studied. For the electron microscope tracheae were fixed in 1% osmium tetroxide buffered at pH 7.4, dehydrated, and embedded in 1:1 methyl : butyl methacrylate. Polymerization was induced in 48 h at 50° C. Sections were cut with a glass knife on a rotary microtome modified from the design of Hodge, Huxley, and Spiro (1954). For the comparison between tracheal and body cuticle 3rd instar larvae were sectioned. Larger insects proved difficult to cut without extensive tearing. Whole tracheae were

mounted direct upon collodion-covered grids. The photographs were taken at the Cavendish laboratory using a Siemens Elmiscop I electron microscope.

RESULTS

Structure

With the light microscope a trachea is seen in longitudinal section to be composed of a corrugated membrane lining the tube with the taenidia lying in the folds between it and the tracheal epithelium. Both the taenidia and the membrane contain chitin and are Millon positive. No other cuticular layers can be distinguished. With the electron microscope thin sections show the lining membrane to be about 450 Å thick with an inner layer (160–200 Å) more opaque to electrons (fig. 1, e, j). The membrane is approximately uniform in thickness over and between the taenidia and in tracheae of different diameter. It is not smooth but raised in small tubercles everywhere except over the inner face of the taenidia (fig. 1, i). The taenidia are attached to the membrane only upon this inner flattened face, their sides are free. This arrangement allows the trachea to be freely extensible. There is no dense structure between the taenidia and the epithelium. All the tracheae exposed to the blood are invested by a basement membrane about 600 to 1,100 Å thick, continuous with connective tissue elsewhere. This has an inner and outer membrane enclosing finely granular material perhaps the result of poor fixation. It is strongly positive to the PAS test for polysaccharides. According to Wigglesworth (1956) it is secreted by the haemocytes. The appearance and dimensions of the tracheal cuticle in the cockroach and the mealworm (fig. 1, f) are very similar to that in Rhodnius.

Composition

The lining membranes can be isolated in an almost pure form from the tracheal exuviae. The taenidia are almost completely dissolved by the moulting fluid, leaving only traces attached to the membrane. The membrane can also be prepared by dissolving the taenidia from fresh tracheae in dilute potassium hydroxide. When cockroach tracheae are treated in this way careful teasing reveals the two components. Only the outer one of these survives the treatment for chitin purification and the inner one prolonged acid hydrolysis. Some experiments show the double nature of the membrane in other insects. Tracheae or exuviae in Schulze’s reagent give the characteristic sudanophil droplets on heating on the lumen side only, the rest of the membrane being temporarily unaffected. Droplets similar in appearance and position also appear during treatment with potassium hydroxide. Later they dissolve leaving the purified chitin membrane. The layer upon the lumen face may be separated by digesting exuviae in 2 N hydrochloric acid at 50° C for a week to remove the chitin and protein component. Under the electron microscope it appears as a very thin structureless membrane without trace of the tubercles. Its lipophilic nature is shown by the lack of penetration of aqueous dyes. Brom-phenol blue in saturated aqueous mercuric chloride can be used
as a combined fixative and protein stain (Mazia, Brewer, and Alfert, 1953). When freshly dissected *Rhodnius* and *Tenebrio* larvae are immersed in it, all the trachea appears to stain. Tracheal exuviae also take up the dye. But when the dye is injected into the tracheal system using the method of Wigglesworth (1950), the trachea remains unstained, although the mercuric chloride can later be detected in the tissues. Mercuric chloride is appreciably oil soluble. This barrier to aqueous dyes is not a simple lipid monolayer for there is still no penetration when dilute detergents (1% 'Teepol', 1% cetyl alcohol) are injected. Water does not penetrate the interstices of exuviae even after extraction with chloroform—further evidence against the presence of a labile lipid layer. The barrier is presumably the non-chitinous layer which resists acid hydrolysis.

Thus the layer upon the lumen face of the trachea has all the properties of cuticulin, the innermost layer of the epicuticle, characterized by Wigglesworth (1947, 1948). It is lipophilic, non-chitinous, resistant to acids including cold concentrated sulphuric acid, and gives sudanophil droplets with Schulze's reagent. The tracheal membrane then is made up of two layers, an ultra-thin inner layer which is a barrier to water soluble dyes, and an outer layer of protein and chitin.

Although in fresh tracheae the taenidia dissolve in dilute potassium hydroxide (showing that the protein-chitin association differs in the taenidia and lining membrane), chitin is readily demonstrated in fixed material. Whole tracheae show strong form birefringence after chitin purification. The chitin component of the lining membrane is positively birefringent with respect to the axis of the tube (fig. 1, c, d), while the taenidia are positive with respect to the circumference (fig. 1, a, b).
tions confirm these results. In whole mounts of potassium-hydroxide-treated exuviae the chitin appears as long threads (diameter about 250 Å) arranged in the axis of the tube. In the taenidia the threads are arranged tangentially.

These results are summarized in the diagram of tracheal structure presented in fig. 2, B.

Relation to the body cuticle

Figs. 2, A and 1, G, H, L, show the structure of an abdominal tergum. From Wigglesworth's papers (1948) this may be interpreted as follows.

On the outside is an irregular layer of cement. This is separated from the cuticle below by a clear area where the wax is presumed to have dissolved during embedding. Next comes a very thin electron opaque layer which is presumably the cuticulin layer. Below this is a homogeneous layer not penetrated by pore canals. In fresh material and exuviae this is amber coloured, tanned (the positive Millon reaction is only eliminated after drastic treatment with hot 10% potassium hydroxide which leaves a thin chitin membrane), and lipid impregnated (it is unaffected by aqueous stains and the oily droplets from Schultze's reagent come mainly from this layer). The lamellate endocuticle with pore canals lies between this and the epidermis. The pore canals, about 1,500 to 2,000 Å in diameter and about 3 to $4 \times 10^6$ per mm$^2$ over the abdomen, are probably helical as Richards and Anderson (1942c) found in the cockroach (fig. 1, G, H). They have some contents but no obvious cell membranes. The subcuticular layer of Schmidt (1956) appears as a series of membranes next to the epidermis. The cuticle from other parts of the body may differ considerably from this. In the tibia of a fore leg (fig. 1, K) the cement cannot be distinguished. The outer region including part of the lamellate layer with pore canals is opaque and probably corresponds to the amber sclerotized region seen with the light microscope. Darker layers may appear in the endocuticle in addition to the fine lamellae. Other features in the cuticle may be discerned but for the present it is of interest to distinguish the unvarying presence of an ultra-thin outer layer, probably cuticulin, and a rather thicker homogeneous layer below it which is not penetrated by pore canals.

It is of interest to determine to which layers of the abdominal cuticle the tracheal cuticle may correspond. Dermal glands are absent so that it is not surprising that the cement layer is missing. There is no evidence for an ultra-thin lipid layer although such a layer may be present (Wigglesworth, 1953). The thin electron-dense layer on the exposed face is strikingly similar in all cuticles examined, whether tracheal or body surface, differing only in thickness (250–300 Å over the abdomen, 120–200 Å in tracheae). All give the characteristic reactions for cuticulin. The nature of this material is obscure but tracheal

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**Fig. 2 (plate).** A, section through a small part of the 3rd abdominal tergum of a 3rd instar *Rhodnius* larva. The section is slightly oblique to emphasize the cuticular components. There is a part of a plaque upon each side.

B, diagram of tracheal structure in *Rhodnius*. 

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inner face smooth and flattened over the taenidia

non chitinous resistant layer 120-200\(\AA\) thick

protein/chitin layer with axially oriented micelles, 240-370\(\AA\) thick

taenidia with chitin micelles tangentially oriented

tracheal epithelium

basement membrane

FIG. 2

M. LOCKE
exuviae promise to be better for its preparation than the more complex pigmented cuticles studied hitherto. The protein-chitin layer of the tracheal membrane would then correspond with the layer below the cuticulin which is not penetrated by the pore canals, differing only in its lack of marked sclerotization. The tracheal cuticle is not lipid impregnated for the whole membrane appears to stain when aqueous dyes are not impeded by the cuticulin. There is some evidence that the protein component is tanned. The layer appears to be more Millon positive than the taenidia and is resistant to 0.2 N potassium hydroxide, 6 M urea, saturated lithium iodide, and other reagents which attack electrovalent links and disperse the taenidia. Tanning of the layer subsequent to the formation of the taenidia would explain why the adjacent parts of the taenidia are left attached to the membrane unattacked by the moulting fluid or dilute potassium hydroxide. The absence of sclerotization and lipid impregnation would be expected to increase flexibility and permeability, two important characteristics of tracheal cuticle.

**DISCUSSION**

The diagram of tracheal structure most frequently met with in the textbooks derives from Weber’s (1933) picture of tracheae from *Deilephila* (Sphingidae). This portrays a lining epicuticle, the taenidia, and a continuous layer between the taenidia and the epithelium referred to as an endocuticle. Richards (1951, 1953) was presumably influenced by this work when he described the sheet of axially oriented chitin which he could see with the electron microscope in whole mounts of potassium-hydroxide-treated cockroach tracheae. He called this sheet a procuticle, and in his diagram placed it between the taenidia and the epithelium. No trace of such a layer has been found in the insects studied. Axially oriented chitin micelles certainly lie upon the lumen side of the taenidia, for potassium hydroxide treated tracheal exuviae from which the taenidia have been dissolved by the moulting fluid show strong form birefringence positive with respect to the axis of the tube. If Richards’s procuticle may be identified with the chitin-protein in the lining membrane this work confirms many of the details in his study.

It was difficult to reconcile the extensive endocuticle in tracheae described by Weber with the simple tracheae lacking this layer in *Rhodnius, Tenebrio*, and *Periplaneta*. *Deilephila* was not available for study but in larvae of the Sphingid *Phlegethontius* some of the large tracheae do have such a layer. In fresh material with phase contrast it shows up as a number of thin lamellae. This will have to await future study but it serves as a warning against undue generalization. Nevertheless, most tracheae must be freely extensible within the body cavity for which purpose the structure described in fig. 2, B seems well adapted.

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Locke—The Structure of Insect Tracheae

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