DISTRIBUTION OF LIPID IN THE LAMELLATE ENDOCUTICLE OF RHODNIUS PROLIXUS (HEMIPTERA)

V. B. WIGGLESWORTH

Department of Zoology, University of Cambridge, Downing Street, Cambridge, England

SUMMARY

The lamellate appearance of the cuticle in the abdomen of the Rhodnius larva conforms to the conception of Bouligand in being an optical artifact which results from the spiral arrangement of successive layers of oriented fibrils. But superimposed on this structure is an actual lamination of bound lipid with the same spacing. The relation of the lipid layers to the optical lamination changes with the aspect from which the system is viewed.

There must therefore be a cyclical secretion of lipid by the epidermal cells. Since the period of this cycle agrees with the cycle of rotation of the fibrous layers, which is supposedly inherent in the chemistry of the system, it is possible that it is the lipid which controls or initiates this helicoidal 'cholesteric crystallization'.

There is evidence of a cyclical change in the secretion of lipid by the microvilli; it is suggested that there may be alternating cycles of eccrine and apocrine secretion, and that the lipid laminae represent the apocrine phases.

The pore canals in Rhodnius are roughly cylindrical in cross-section, with lipid-impregnated walls. The contents of the lumen become slightly more electron opaque before the cuticle is stretched by feeding. There is probably some enzymic dissolution of the cuticle which precedes stretching; and this may concern particularly the lipid fraction. After the great distension and expansion of the cuticle which occur at feeding, lipid laminae can no longer be demonstrated in the old cuticle.

INTRODUCTION

The abdomen in the larva of Rhodnius has a cuticle of typical lamellate character as defined by Neville (1967). In the 4th-stage larva it consists of regular spirally oriented sheets of fibrils which give an apparent lamination, when viewed in vertical section (Bouligand, 1972), with a period of about 0.5 μm - but subject to variation as described in this paper.

The lamellate endocuticle is bounded externally by an epicuticle rich in lipids (Wigglesworth, 1973) which will be the subject of a separate study (Wigglesworth, 1975b). (Immediately below the epicuticle the lamellate cuticle may be sclerotized; notably below the plaques which surround the abdominal setae. Sclerotized lamellate cuticle, which occurs throughout the abdomen of the adult Rhodnius and is general in the thorax and appendages of all stages, will not be dealt with in this paper.) In certain areas, such as the walls of the setae, the sockets of the setae, and the points of insertion of the dorso-ventral muscles of the abdomen, there is substantial local
sclerotization which will be considered in connexion with the epicuticle (Wigglesworth, 1975b).

The lamellate endocuticle is bounded internally by the epidermal cells. This is the major site of cuticle formation and of the incorporation of lipids into the cuticle as described in the present paper. The lamellate cuticle is traversed by vertical pore canals, which arise from pits at the apex of the epidermal cells and extend to the epicuticle, which their finer extensions penetrate and gain access to the surface (Wigglesworth, 1947, 1975b).

**METHODS**

For the demonstration of lipid in the light microscope, the integument was fixed in glutaraldehyde and osmium tetroxide and embedded in agar and esterwax (Steedman, 1947 formula) and sections were cut, as already described (Wigglesworth, 1970) at 1–2 μm, floated on solutions of sodium hypochlorite at a range of concentrations and exposure times to render the lipid accessible to staining, washed in distilled water, dried on the coverslip, and stained with Sudan black B after brief immersion (5–6 s) in xylol to remove the esterwax. The Sudan stain is best dissolved at 0.25 % in 50 % pyridine and the sections differentiated for a good half minute in 70 % ethanol before mounting in Farrants' gum medium.

For the study of lipid distribution with the electron microscope, a newly described method (Wigglesworth 1975a) has been used. This consists in the partition of small pieces of tissue in a saturated solution of the monoterpene myrcene, plus 0.1 % ethyl gallate, in 70 % ethanol; followed by renewed osmium tetroxide to reveal the lipid sites at which myrcene has been incorporated. No further metal staining is employed; the increased electron density due to the uptake of osmium by the added myrcene indicates the sites rich in lipid. This method is used, according to requirements, after fixation in osmium tetroxide alone, in combined osmium tetroxide and glutaraldehyde (Hinde, 1971) or after glutaraldehyde, with or without graded exposure to sodium hypochlorite. The retention of lipid and of osmium in the thin sections is ensured by embedding in Spurr's (1969) medium and by avoiding the use of propylene oxide. The 'lipids' in question are doubtless mainly combined in the form of lipoprotein (or perhaps glycolipid); but hydrocarbons, free waxes and triglycerides are demonstrable in sections by these methods.

**RESULTS**

*The nature of lamination*

According to Bouligand (1972) the fine lamination of cuticle as viewed in vertical or oblique sections is an optical artifact. The cuticle is made up of superimposed sheets of fibrils whose orientation changes by a fixed angle in each successive layer, in a regular spiral fashion. Where the fibrils in these layers are seen in lateral view, they appear most dense; where they are viewed end on, they appear most pale; so that the cuticle as a whole shows an alternating graduation of pale and dark layers. The laminae do not represent differing degrees of condensation of protein, for example, as has been claimed in the past.

In larvae of *Rhodnius*, sections of the cuticle, cut at 5–10 μm, mounted in a fresh mixture of equal volumes glycerol and Millon's reagent (Wigglesworth, 1942) and warmed on the hot plate at 60 °C, show a uniform intense pink coloration which reaches a peak in about 5–10 min and then gradually fades. At no stage is there any layering in the intensity of colour. This is in accordance with the views of Bouligand.
Lipid in lamellate cuticle of *Rhodnius*

When the reaction is complete and the cuticle is colourless again, the fine lamination is faintly visible as a result of optical diffraction. The period of lamination is about 0.5 \( \mu \text{m} \).

In an earlier publication, however, it was claimed that fine layers of lipid occur in the lamellate pre-exuvial cuticle of adult *Rhodnius* (Wigglesworth, 1970). Sections cut at 5–10 \( \mu \text{m} \) were immersed in chlorated nitric acid for 30 s or so, washed in water and stained in Sudan black B. The results depend on arresting the violent oxidation at the right moment and are therefore variable; but in some preparations deeply staining lipid layers can be seen; in other preparations these layers are made up of numerous minute lipid droplets. When lipid was liberated by treatment of 5–10-\( \mu \text{m} \) sections with sodium hypochlorite, some coarse lipid laminae could be seen in the post-exuvial cuticle, but fine lipid laminae seemed to be absent (Wigglesworth, 1970). Subsequently, however, the hypochlorite method was adapted for 0.5–1-\( \mu \text{m} \) sections (Wigglesworth, 1971) and by using this procedure the fine lipid laminae become very evident.

Fig. 1 shows a vertical section of the cuticle of a 4th-stage larva shortly before ecdysis, after hypochlorite treatment, showing the lipid laminae. Figs. 3–5 show vertical sections of the cuticle of an experimentally produced '6th-stage' larva fixed several days after ecdysis; these sections have been treated with graded concentrations of sodium hypochlorite to reveal progressive stages in the unmasking and dissolution of bound lipids. The cuticle consists of an outer half or two thirds laid down before ecdysis, which shows a uniform fine lamination and an inner half or one third laid down after moulting which, in Fig. 3, shows the same fine lamination. More severe treatment with hypochlorite (Figs. 4, 5) reveals dense lipid staining below the epicuticle, along the boundary between pre-exuvial and post-exuvial cuticle, and in the form of extensive deposits between thick layers in the post-exuvial cuticle, each of which seems to represent the cuticle laid down in 24 h.

The lipid laminae also become apparent when, in the late stages before ecdysis, the cuticle is being digested by the moulting fluid. The lipid components are more resistant to solution and, after mild treatment with hypochlorite, they stain with Sudan B as a meshwork lying between the pore canals in the form of fine laminae: as the protein and chitin of the cuticle are digested and dissolved, the residual lipid-rich material condenses to form the multi-layered ecdysial membrane (Fig. 7). There seems no doubt that the lamellate cuticle does show corresponding lipid-rich lamellae.

**Pore canals and associated lipid**

When sections of adult cuticle of *Rhodnius* were plunged briefly into chlorated nitric acid, the pore canals sometimes persisted and resembled a brush border arising from the epicuticle. The slender processes were believed to be lipid-rich filaments from the lumen of the pore canals (Wigglesworth, 1970). Sections cut at 1–2 \( \mu \text{m} \) and treated with hypochlorite have confirmed the presence of lipid; but longitudinal and particularly tangential sections show that for the most part the lipid is concentrated in the walls of the pore canals.
V. B. Wigglesworth

Figs. 1 and 2 show vertical sections of the new cuticle in 4th-stage larvae about 2 days before ecdysis to the 5th instar, after exposure to sodium hypochlorite (10% solution, diluted 1:20; 4 min). The horizontal lipid lamellae are traversed by vertical columns which also show lipid staining. These columns (like the isolated filaments persisting after immersion in chlorated nitric acid) are the pore canals invested by lipid-rich walls.

Fig. 6 shows a tangential section of the cuticle in a newly moulted 5th-stage larva. At the base of the cuticle (to the left) the pore canals are seen as round structures with an empty lumen and deep-staining lipid-rich walls. As they run towards the surface of the cuticle the lumen contracts and near the epicuticle they often appear to contain dense lipid-staining contents (visible in this specimen but not in the photograph).

In Fig. 4 the fine lipid laminae are faintly visible only at the centre of the pre-exuvial cuticle, but the thin vertical lipid strands, or rows of granules, represent the pore canals. The same strands and granules are well seen in Fig. 5. They give the impression of coming from the lumen of the pore canals; but this cannot be firmly decided from these preparations. It is shown elsewhere (Wigglesworth, 1975a) that the pore canals are sometimes filled with lipid-rich material.

Sections cut for the electron microscope, after myrcene partition and osmium treatment, show lipid located mainly in the walls of the pore canals (Figs. 8, 9). Lipid laminae are not well seen in these electron-microscope preparations in the absence of hypochlorite treatment.

Lipid lamination and the Bouligand theory

The problem now is to reconcile the lipid lamination as here described with the Bouligand hypothesis that the appearance of laminae (in lamellate-type cuticle) is merely an optical artifact. According to the Bouligand interpretation as described by Neville & Luke (1969a, b) the pore canals take the form of 'twisted ribbons' compressed between the spirally deposited arrays of fibrils in such a way as to appear spindle-shaped when viewed in exact horizontal section in a single plane (see Locke, 1961, fig. 35). But most sections through the cuticle are oblique and the spindles then assume a crescentic form; and within this crescent the 'axial filament' and the 'wax canal filaments' (Locke, 1961) can be seen in cross-section.

In Rhodnius the pore canals, as seen with the light microscope, are approximately round in cross-section, with lipid-rich walls and a diameter of about 0.2 μm (Fig. 6). But in the electron microscope in oblique section the cylindrical pore canal proper with its lipid walls is seen to be enclosed within a crescentic space (Fig. 25, left of centre). The axial filament is conspicuous; other filaments are not evident. The crescent is bounded and its form is determined by the tangential lines of the cuticular fibrils which, in oblique sections, appear to run an arcuate or 'parabolic' course. The horns of the crescents indicate the orientation of the fibrillae at that level of the cuticle.

In ordinary electron-microscope sections of the cuticle (in my hands) the cuticular fibrillae are not easily seen. But in material fixed in glutaraldehyde alone, treated with dilute bromine water for 15 min before osmification, and the sections stained with
Lipid in lamellate cuticle of *Rhodnius*

lead citrate, the appearance of the cuticle is quite changed. The lipids no longer bind osmium, so that the lipid walls of the pore canals do not stain; but the cuticular fibrils are well stained with the lead. The appearance (Fig. 10) is as described by Bouligand (1972): where the fibrils run longitudinally the section appears dark; where they run transversely the section appears pale. The dark laminae resemble valleys and the pale laminae resemble crests.

What is the relation between the lipid lamination and the optical artifact which depends on the orientation of the cuticular fibrillae? If lipid material were uniformly associated with the cuticular fibrillae, then lipid staining would show a Bouligand effect which would coincide with that visible after lead staining. But such an arrangement could not account for the material deposition of lipid in the form of continuous laminae or as rows of droplets, as already described.

Moreover, where the apparent lamination is due to fibrillar orientation, the appearance will change with the aspect from which it is viewed: laminae which appear dark (i.e. with fibrils seen in side view) from one aspect, will appear pale if viewed after a 90° rotation, when the same fibrils will be seen end-on. If, therefore, the lipid laminae have a real existence they should change their relation to the fibrillar laminae as the aspect changes. If the lipid appears to lie in the ‘valley’ from one aspect, it will appear to lie in the ‘crest’ when viewed at right angles.

This has been tested by treating the cuticle of a 4th-stage larva at 13 days after feeding, when the new cuticle is well developed, after fixation in glutaraldehyde/osmium tetroxide, with sodium hypochlorite (10% solution diluted 1:20, for 2 min) before partition in the myrcene solution and renewed osmium treatment; and then cutting oblique sections of a small piece of cuticle (a) oriented in the long axis of the insect and (b) oriented in the transverse axis. Figs. 12 and 13 show the results. Although the cuticular fibrillae cannot be seen their orientation can be deduced from the form of the crescents around the pore canals. This orientation has been marked in the figures by means of linked dots. In a (Fig. 12) the dark lipid-rich bands lie predominantly in the valleys; in b (Fig. 13) they lie in the crests. If an oblique section is cut through the corner of a small square of cuticle the change in orientation of the section can be followed in a single preparation (as in Fig. 11) and the gradual displacement of the lipid bands from the crests (top left) to the valleys (bottom right) can be seen.

One must conclude that lipid-rich layers in the cuticle are superimposed upon an underlying spiral fibrillar orientation in which lamination is an optical artifact.

**Deposition of lamellate cuticle and formation of the pore canals**

The formation of the spirally oriented fibrillae in the cuticle is generally believed to be a spontaneous process such as occurs in liquid crystals of cholesteric substances (Bouligand, 1972; Neville & Caveney, 1969; Neville & Luke, 1971). It is not known which component of the cuticle is primarily responsible for initiating this crystallization, and which components are following this conformation secondarily. In view of the widespread distribution of lipid in the lamellate cuticle and the strong tendency for lipids to crystallize in this way, it is not unlikely that lipids of some kind are the initiators. Certainly lipids follow the arcuate pattern of fibrillae. Thus Fig. 14,
prepared by fixation of the cuticle with glutaraldehyde, followed by bromine water, myrcene partition and osmium tetroxide, shows the same pattern as lead staining (Fig. 10); but this pattern is produced by lipid staining between the fibrils. Beyond that there is no evidence.

In material for the electron microscope treated with bromine water, and lead citrate staining, it is evident that the spiral formation extends right up to the inner epicuticle (Fig. 16). Indeed, it may well be that the same structure exists within the inner epicuticle itself, although it cannot be seen (for the inner epicuticle sometimes appears laminated when it is first formed: Delachambre (1970) in *Tenebrio*; Noirot & Noirot-Timothee (1971) and Zacharuk (1972) in various insects; also in *Rhodnius* in the present work). At the inner limit of the cuticle the spiralling fibrillae are visible right up to the surface of the microvilli of the epidermal cells (Fig. 15) (cf. Mitchell, Weber-Tracy & Schaar (1971) on *Drosophila*).

The presence of laminae rich in lipid with the same periodicity as the spiral laminae means that there must be a cycle of secretion, one phase of which is characterized by lipid deposition. During the period of 6 days between apolysis and ecdysis (at 26 °C) approximately 30 lamellae are laid down, so that something like 4-5 lamellae are formed each 24 hours. An attempt has been made to observe changes in the epidermis and its microvilli which might be associated with different secretory phases.

Fig. 25 shows an almost tangential section of the apex of the epidermal cells and the base of the cuticle at 9 days after feeding, when only about 10 laminae of the endocuticle have been laid down. The microvilli are rich in lipid. Around the base of the pore canals is a ring of microvilli which presumably lay down the continuous cylindrical lipid wall of the pore canal. The cuticular fibrillae are not visible but the crescent-shaped spaces around each pore canal are pale and conspicuous at the base of the cuticle, and become smaller and more compact further out; to the right of the figure they have almost disappeared. This presumably indicates progressive incorporation of fibrillar material as the laminae leave the surface of the epidermal cells. Below the microvilli are numerous lipid-rich droplets.

The appearance of the microvilli in vertical sections of the integument is exceedingly variable, and it is not easy to represent these appearances as a regular sequence of secretory changes. With regard to the pore canals there is a constant feature: the slightly dilated proximal region of the canal arises from a pit extending some 0.5 μm into the apex of the cell (Figs. 17–20); the base of this pit, from which the axial filament arises (Fig. 18), is completely enveloped by a lipid-rich cytoplasm continuous with the microvilli which invest the pore canal as it emerges from the cell, and which, as suggested above, are probably responsible for secretion of the lipid-rich wall of the pore canal.

The other microvilli vary in appearance in different preparations (Figs. 21–24). Sometimes they show a reduced lipid content and are simply bounded by a lipid-containing plasma membrane (Fig. 24). Often the apex of each microvillus has a conspicuous lipid-rich cap (Fig. 23). This appearance has been illustrated by many authors including Locke (1961), Delachambre (1970), etc. Sometimes there is a sharply defined line, which is lipid-containing and separates the cuticle already deposited.
Lipid in lamellate cuticle of Rhodnius

from the microvilli below (Fig. 17). Very commonly, indeed in most preparations, the most recently formed lamina above the microvilli contains what look like 'ghosts' of the microvilli, which often agree closely in position with the microvilli below. This appearance may be seen again, but less distinctly, in the lamina above. But it becomes progressively less distinct and is no longer visible in the more remote laminae (Fig. 22). Sometimes a stage is seen in which new microvilli seem to be forming at the base of existing microvilli.

All this gives the impression that the microvilli, or portions of them, may be shed at some point in the cycle and incorporated in the cuticle. If that is so, it is natural to suggest that this may be the source of the lipid laminae in the endocuticle.

Expansion of lamellate cuticle during feeding

The 4th-stage larva of Rhodnius takes a single meal of blood which amounts to about tenfold its fasting body weight. This results in great expansion of the abdomen: the linear extension is about 75%; so that the increase in surface area is about three-fold and the thickness of the cuticle is reduced to about one third – from about 30 μm to about 10 μm. The associated changes have been studied in sections under the electron microscope.

The cuticle of the unfed larva is highly resistant to stretching; but very soon after feeding has begun it becomes readily extensible (Bennet-Clark, 1962). This change is brought about by a neurosecretory hormone from the fused abdominal ganglia acting upon the epidermis (Maddrell, 1966). Presumably, under the action of this stimulus the epidermal cells must discharge into the cuticle, probably by way of the pore canals, some enzyme which acts upon the substance of the cuticle. Whether such a postulated enzyme acts upon protein, carbohydrate or lipid components is not known. It was suggested (Wigglesworth, 1970) that the enzyme might be an esterase acting upon cuticular lipids. Locke (1961) demonstrated the presence in the lumen of the pore canals of an 'esterase' which hydrolysed 5-bromoindoxyl acetate; but this effect is given also by many proteases.

In the unfed 4th-stage larva, the cuticle (after glutaraldehyde/osmium fixation, followed by myrcene partition and renewed osmium tetroxide) shows the pore canals very clearly with dark lipid-staining walls and unstained lumen (Fig. 26). If the cuticle is similarly treated soon after the larva has begun to feed, perhaps about half distended, many of the pore canals appear to have vanished (Fig. 27). Close examination of the sections reveals that the canals are still present but the walls are not conspicuous, and the lumen now shows about the same electron density as the substance of the cuticle, or sometimes a greater density. This suggests that the pore canals are now filled with some proteinaceous or perhaps lipid-containing material.

In sections of other regions of the partially expanded cuticle examined at a higher magnification, the pore canals are again inconspicuous but the cuticular laminae show a curious change: horizontal fibrillar laminae alternate with rows of rounded processes, reminiscent of the microvilli (Figs. 28, 29). The whole gives the impression that as the cuticle is softened in preparation for expansion, the 'ghosts' of the microvilli again become visible.
In the fully distended cuticle similarly treated, the pore canals are to some extent evident again; the lumen may be clear and sometimes greatly enlarged (Fig. 30). Since the pore canals will now be shortened to a third of their original length, the axial filament will be much too long and it becomes highly convoluted. The cuticle is made up of thin but conspicuous dark and pale lamellae. By one day after feeding the cuticle has contracted down to a great extent; the pore canals are still distorted and their contents still somewhat dark (Fig. 31).

Fine structure of the fully stretched cuticle

It was of interest to see to what extent the fine fibrillar structure of the cuticle is affected by the great expansion during feeding. Fig. 32 shows a section of the cuticle in the newly gorged 4th-stage larva prepared in the same way as that of the unexpanded cuticle (see Fig. 10) (that is, fixation in glutaraldehyde followed by dilute bromine water and the sections stained with lead citrate). The lamellae are more closely packed than in the unstretched larva but the fibrillar structure is unchanged. Preparations made in the same way as that shown in Fig. 14 of the unstretched cuticle (that is, fixed in glutaraldehyde, followed by dilute bromine water, partition in myrcene, and osmium treatment) show some irregular deposits of lipid and a faint indication that these may follow the same arcuate pattern (Fig. 33); but this is not so well defined as in the unstretched cuticle (Fig. 14).

The same results have been obtained on the cuticle of larvae at one day after feeding, when the cuticle has contracted down considerably. On the other hand I have been unable to detect lipid laminae in the cuticle after feeding. Cuticle at one day after feeding, embedded in agar and esterwax and cut in sections of 1 and 2 µm, has been exposed to all grades of sodium hypochlorite treatment, followed by Sudan B staining (as illustrated in Figs. 1–5 in the developing cuticle and in the cuticle of unfed and unstretched larvae) but has shown no sign of lipid laminae. The observations have been repeated at 3 days, 4 days and 5 days after feeding and in no case have lipid laminae been found. It looks as though the lipid laminae may have been dispersed during distension and not reconstructed.

During the 6 days that follow the blood meal, and before deposition of the new cuticle begins, the epidermal cells add to the old cuticle, which may increase by more than half its thickness (Zwicky & Wigglesworth, 1956). This added cuticle is in the form of laminae, each about 0.6 µm in thickness, separated by thin layers rich in lipid. Roughly one of these thick layers is formed each day. They do not show the fine lamellation seen in the established cuticle above (Fig. 34). Examination at a higher magnification in the electron microscope suggests that they are composed of spirally oriented layers of fibrils, but that there is only one period of rotation in each thick layer. As can be seen in Fig. 34, the pore canals in the pre-feeding cuticle remain distorted. But the pore canals in the post-feeding cuticle below run a straight vertical course and are continuous with the distorted canals in the pre-feeding cuticle above.

During the deposition of this post-feeding cuticle the microvilli are often virtually absent and the surface of the epidermal cell is smooth or just slightly folded (as
Lipid in lamellate cuticle of Rhodnius described by Lai-Fook (1968) in *Rhodnius*. But in some preparations normal microvilli with corresponding 'ghosts' in the overlying cuticle can be seen.

DISCUSSION

In conformity with the theory of Bouligand (1972) the apparent lamination in the abdominal endocuticle of *Rhodnius* is an optical artifact, depending on the helicoidal disposition of superimposed layers of fibrils; and there is no periodic change in protein density at different levels. But Bouligand did not discuss the lipid lamination described in the cuticle of *Rhodnius* (Wigglesworth, 1970).

The existence of lipid laminae has now been confirmed in 1-μm sections stained with Sudan black B after sodium hypochlorite. The periodicity of these laminae agrees with that of the helicoidal lamellae, but the level in the spiral at which the lipid laminae appear to lie varies with the aspect from which they are viewed—a good indication that they really exist. This confirms the earlier observation that the lipid laminae may be broken down into rows of lipid droplets, which would not happen if the lipid were merely a component in the helicoidal system.

In the formation of the cuticle there must therefore be a cyclical secretion of lipid, presumably liberated by the microvilli. The microvilli are very variable in appearance. A clear correlation between these changes and the lipid phases of secretion was not established. But it is suggested that secretion may go through an eccrine phase when precursors of the components of the cuticle are secreted in solution, and an apocrine phase when the lipid-rich substance of the microvilli may be discharged.

Besides the lamellae which are rich in lipid, traces of lipid-staining material can be seen between the helicoidal fibrils at all levels of the lamellar system. Although the absolute quantity of lipid is small, it may yet be responsible for the initiation of cholesteric liquid crystallization to which the major components, protein and chitin, conform.

Apart from the lipid laminae, and a substantial deposit of bound lipid immediately below the epicuticle, bound lipid is prominent in the endocuticle in forming the walls of the pore canals. The system described by Neville & Luke (1969a, b, 1971), of pore canals compressed into the form of rotating ribbons by the helicoidal fibrillar structure of the cuticle, applies also to the cuticle of *Rhodnius*. But the crescentic sections of the spaces so formed are conspicuous only at the base of the cuticle where it is being actively laid down. Throughout the bulk of the cuticle the canal itself is circular in cross-section and completely invested by its lipid-rich wall. The crescentic spaces are inconspicuous.

The pore canal filament is very evident in sections, but it seems likely that its opacity in the preparations is due to intrinsic electron density rather than lipid staining. It is readily dispersed by dilute hypochlorite (Figs. 12, 13). The contents of the pore canals are normally translucent and lipid free. The distal parts become loaded with lipid-rich material in the late stages of moulting, as will be described elsewhere (Wigglesworth, 1975b); and then the axial filament appears pale by contrast in cross-section.
It seems likely that the axial filament serves a structural function, to keep the pore canals aligned (Figs. 8, 17). The canals are highly distorted during feeding, and the axial filaments become convoluted as the thickness of the cuticle is reduced to about one third. But during the days after feeding they gradually become aligned again.

At the time of feeding, when the cuticle becomes plasticized in preparation for stretching by the large meal of blood (Bennet-Clark, 1962) the contents of the pore canals become darker and the canals are inconspicuous. After feeding it is found that the lipid laminae, which before were easily demonstrable by Sudan staining after hypochlorite treatment of the sections, can no longer be seen. It is suggested that dispersal of the lipid laminae by enzymes from the epidermal cells may be responsible for the plasticization of the cuticle. Reynolds (1975) supports the suggestion of Bennet-Clark (1962) that a fall in pH is responsible for plasticization in Rhodnius.

If the dispersion of lipid as reported in the present paper is brought about by the action of a lipase or esterase that would lead to a rise in hydrogen ion concentration.

Although the cuticle of the fully gorged larva has decreased to one third of the original thickness, the helicoidal lamination appears essentially unchanged; although the small lipid component may have been partially dispersed.

This work has been supported by a grant from the Agricultural Research Council. I thank Professor T. Weis-Fogh and Dr D. A. Parry for research facilities, Dr J. E. Treherne and Dr Nancy J. Lane for the use of the electron microscope, and Miss L. S. Swales and Mr W. M. Lee for technical assistance.

REFERENCES


Lipid in lamellate cuticle of *Rhodnius* 449


(Received 29 May 1975)
Fig. 1. 1-μm section of cuticle shortly before ecdysis. NaOCl 1:20 dilution (1 min), Sudan B; showing lipid staining of laminae and of vertical pore canals. ×1000.

Fig. 2. New cuticle at 12 days after feeding. NaOCl 1:20 (4 min), Sudan B. Inner part of cuticle dispersed; residue shows lipid laminae and lipid-staining walls of pore canals. ×1200.

Fig. 3. Cuticle of 6th-stage larva about 6 days after moulting. NaOCl 1:20 (4 min), Sudan B. Fine lipid laminae throughout both the upper half (pre-exuvial cuticle) and the lower half (post-exuvial cuticle). ×1000.

Fig. 4. As Fig. 3, after longer treatment with NaOCl. In pre-exuvial cuticle the lipid laminae have almost disappeared and in post-exuvial cuticle coarse lipid laminae are present. Lipid from walls or contents of pore canals seen as vertical strands or rows of droplets. ×1000.

Fig. 5. As Fig. 4; note much lipid staining below epicuticle and at boundary between pre-exuvial and post-exuvial cuticle. ×1000.

Fig. 6. Tangential 2-μm section of cuticle of 5th-stage larva, newly moulted; NaOCl, Sudan B; showing pore canals round in cross-section with lipid-staining walls. ×1200.

Fig. 7. Old cuticle of 5th-stage larva in process of digestion, about 2 days before ecdysis. NaOCl, Sudan B. a, cement layer detached; b, endocuticle with pore canals in process of digestion; c, lipid-rich residue of cuticle condensing to form laminated 'ecdysial membrane'. ×750.
Lipid in lamellate cuticle of Rhodnius
Fig. 8. Vertical section of base of new cuticle at 11 days after feeding, showing pore canals in longitudinal section with axial filaments (a) and lipid-staining walls (b). Osmium with myrcene partition. x 20000.

Fig. 9. Horizontal section of cuticle at 9 days after feeding showing relatively pale axial filaments and strongly lipid-staining walls of the pore canals. x 20000.

Fig. 10. Oblique section of endocuticle. Lead staining after glutaraldehyde fixation and treatment with bromine water. Showing arcuate pattern of fibrils. x 40000.

Fig. 11. Oblique section of cuticle shortly before moulting. Osmium/glutaraldehyde, NaOCl treatment, myrcene partition and OTO treatment; showing the change in relation between lipid laminae and fibril pattern as the orientation of the section changes (see Figs. 12, 13). x 6000.

Fig. 12. Oblique section prepared as Fig. 11, orientated in the long axis of the abdomen. Apparent course of fibril pattern indicated by inked dots. Lipid-staining laminae lie mostly in the apparent valleys. Note absence of axial filaments in pore canals. x 14000.

Fig. 13. As Fig. 12, but section cut in the transverse axis of the abdomen. Lipid-staining laminae now lie mostly in the apparent crests. x 14000.

Fig. 14. Oblique section of cuticle of unfed 4th-stage larva. Glutaraldehyde fixation, bromine water, myrcene partition, osmium tetroxide, Spurr's medium. Showing lipid staining of the arcuate pattern. x 40000.

Fig. 15. As Fig. 10, showing lead-stained fibrils in immediate contact with microvilli (below). x 40000.

Fig. 16. As Fig. 15. Fibrils in immediate contact with epicuticle (above). x 40000.
Lipid in lamellate cuticle of Rhodnius
Figs. 17–20. Vertical sections of base of cuticle a day or so before moulting, showing the origin of the pore canals and axial filament (note arrow in Fig. 18). Osmium/glutaraldehyde and myrcene partition. × 20000.

Figs. 21–24. Sections at base of cuticle showing varied appearance of microvilli. × 20000.

Fig. 21. 10 days after feeding in 4th-stage larva; apparent detachment of tips of microvilli. Abundant lipid droplets below.

Fig. 22. 11 days after feeding; apparent ‘ghosts’ of microvilli in recently formed laminae above.

Fig. 23. 12 days after feeding; as Fig. 22.

Fig. 24. 10 days after moulting to 5th-stage larva.

Fig. 25. 4th-stage larva at 9 days after feeding; almost horizontal section at base of cuticle. To left, base of pore canals invested by microvilli with abundant lipid droplets in the cytoplasm below. Then, left of centre, a zone with pore canals lying in crescentic spaces. To right of centre, pore canals round in cross-section with lipid-rich walls; crescentic spaces barely visible. × 20000.
Lipid in lamellate cuticle of Rhodnius
Figs. 26–33 show changes in cuticle at the time of feeding.

Fig. 26. Cuticle of unfed 4th-stage larva, 10 weeks starved. Osmium/glutaraldehyde, myrcene, OTO. Pore canals conspicuous. $\times 14,000$.

Fig. 27. The same about half fed. Pore canals inconspicuous. The 3 canals to the left (arrows) are more electron dense than the general cuticle. $\times 14,000$.

Figs. 28, 29. As Fig. 27. $\times 20,000$.

Fig. 30. The same, fully gorged. $\times 14,000$.

Fig. 31. The same, at 1 day after feeding. Pore canals distorted (arrows). $\times 14,000$.

Fig. 32. The same, fully gorged. Lead staining after glutaraldehyde and bromine water. $\times 40,000$.

Fig. 33. The same, fully gorged. Myrcene partition and osmium staining after glutaraldehyde fixation and bromine water. Only faint traces of lipid staining of arcuate pattern (cf. Fig. 14). $\times 40,000$.

Fig. 34. Old cuticle of 4th-stage larva at 6 days after feeding. $a$, closely spaced laminae of pre-feeding cuticle, containing distorted pore canals; $b$, wide laminae of post-feeding cuticle, containing straight pore canals; $c$, epidermal cells in process of apolysis. $\times 20,000$.