The Cuticle and Wax Secretion in Calpodes ethlius
(Lepidoptera, Hesperidae)

By M. Locke

(From the Department of Zoology, University of Cambridge; on leave from the Department of Zoology, University College of the West Indies, Jamaica)

With three plates (figs. 1 to 3)

SUMMARY

Larvae of Calpodes ethlius have an area of integument specialized for the secretion of long hollow filaments of wax. The epicuticle in this region has numerous crater-shaped tubercles about 0.5 μ in diameter, from the rims of which the filaments are secreted. Rows of tubes 60 Å in diameter emerge round the rim of each crater. The wax is supposed to traverse these tubes and they have been termed 'wax canals'. On the inside the wax canals ramify irregularly. Tubes that appear to be similar occur elsewhere in the epicuticle, where they may be concerned with normal wax secretion. The epicuticle consists of cement, a cuticulin layer 60 to 100 Å thick, and an inner dense layer of variable thickness. Microfibres are described in the endocuticle.

INTRODUCTION

Larvae of many but not all of the family Hesperidae secrete paired patches of white powdery wax on the underside of the abdomen of segments 7 and 8 shortly before pupation. These are the 'glandes cirières' of Grassé (1951). They have been known since early in the last century (Stainton, 1857), but very little studied. In the Canna and arrowroot leaf roller, Calpodes ethlius Stoll, the glands are large and conspicuous (fig. 1, A) and provide convenient material for a study of the problem of how an insect wax is secreted through the cuticle. According to my own observations (Locke, 1959), the wax is synthesized in the epicuticle from water-soluble precursors, which can diffuse across the endocuticle from the epidermis. Part of the evidence for this is based on the absence of pore canals, which I failed to detect with the light microscope in the endocuticle below the wax. An electron-microscope study was begun to check this observation, for it might be that the wax is transported in pore canals below the resolution of the light microscope. A secondary object was to study the fine structure of the epicuticle itself, for if it is the...

Fig. 1 (plate). A, mature larva of Calpodes ethlius from the underside, showing the paired patches of white powdery wax at the posterior end. B, vertical section through one of the crater-shaped tubercles which secrete the wax. (Electron micrograph.) C, a group of wax canals cut obliquely and a group cut almost transversely. (Electron micrograph.) D, a pair of crater-shaped tubercles cut obliquely showing the wax canals passing into the tubule layer. (Electron micrograph.) E, transverse section through the base of a crater. Note the wax canals emerging into the clear space below the dense layer. (Electron micrograph.) F, two fragments of a secreted hollow wax filament. (Electron micrograph.)

site of esterases and wax synthesis it might be expected to show some structural 
modification adapted to this function. As an aid to this the structure of the 
cuticle in the wax-secreting area has been compared with the cuticle from 
other parts of the integument with normal wax secretion. The endocuticle 
was a third object of study, for if the lamellae are composed of dense sheets of 
chitin/protein they might provide a formidable barrier to the transport of 
large molecules.

MATERIAL AND METHODS

Larvae were fixed in ice-cold 2% osmium tetroxide solution at pH 7.2, and 
embedded in a mixture of equal volumes of methyl and butyl methacrylate. 
The cuticle is harder than methacrylate and tends to tear away along the outer 
edge but fairly good sections could still be cut at 200 to 300 Å on an A. 
Huxley type ultramicrotome. A number of stains were used on the sections 
but lead hydroxide prepared according to Watson (1958) revealed most detail 
in the cuticle. Many structures such as the microfibrils in the endocuticle are 
only dimly discernible without staining, but after lead hydroxide show greatly 
enhanced contrast. Photographs were taken at magnifications up to 120,000 
on a Metropolitan Vickers E.M. 6 or a Siemens Elmiskop 1 electron micro-
scope.

THE CUTICLE OVER THE WAX-SECRETING AREA, AND THE WAX

General remarks

In fresh material with the phase-contrast microscope and in routinely fixed 
and stained cuticle only two regions can be distinguished with certainty—a 
lamellated endocuticle without pore canals and an epicuticle appearing in 
sections as a thin refractile line. The epicuticle has a curious pattern. There 
are polygonal areas with raised edges approximately equal to the areas of the 
epidermal cells, and scattered all over—several hundred to each polygon—
are minute tubercles with depressed centres like miniature craters. These are 
at the limit of resolution and in surface view appear as dark circles under the 
highest powers of the phase-contrast microscope (fig. 2, A). The wax is secreted 
as fine filaments less than 1 μ in diameter from each of these craters. The 
filaments may grow to as long as 500 μ before being removed and wiped round 
the inside of the cocoon by the caterpillar.

The endocuticle

No trace of pore canals can be discerned with the electron microscope. The 
lamellae are variably spaced from 0.1 μ to 1 μ apart, and under high resolu-
tion are seen to be made up, at least in part, of microfibrils not more than 25 Å 
in diameter. The fibres tend to be densely packed parallel to one another in

FIG. 2 (plate). A, surface view of the cuticle which secretes the wax. The crater-shaped 
tubercles show up as dark circles. Phase contrast.
B, section tangential to the surface of the cuticle, cutting the craters in transverse section. 
Note the regular array of wax canals. (Electron micrograph.)
C, small part of B at a higher magnification.
the centre of a lamella, but they fan out at angles up to 90° into the less well-ordered region between lamellae (figs. 3, A, B). When the endocuticle is being laid down, the epithelium appears to be secreting material which only takes the form of lamellae about 2 μ away from the cell membrane.

The epicuticle

Cement. Even before wax secretion the cement rarely survives embedding as a distinct layer but fragments away from the surface. This is not surprising if the cement is a shellac-like substance, as Beament (1955) suggests. In fig. 3, A it appears as loose strands some way from the surface. During wax secretion it is pushed right away from the surface.

Cuticulin. The term 'cuticulin' has been retained for the very dense, heavily staining outer layer, about 60 to 100 Å thick, which follows even the finest sculpturing of the cuticle. It is probably not just a region of the layer below, secondarily differentiated by a chemical change at the surface, for it sometimes appears on its own above occasional granules in the dense layer below.

Dense layer. Below the cuticulin and including all the wall of the wax-secreting craters is a dense, homogeneous layer about 1,000 Å thick (figs. 1, B-E; 2, B, C). Its inner surface is less regular and may extend in small islands.

Wax canals and convoluted tubules. Emerging round the periphery of each crater there are 3 or 4 rows of tubes about 60 Å in diameter (fig. 1, B, D). The tubes do not always appear hollow (fig. 1, C) and their contrast with the rest of the cuticle is probably due to staining of the material which they contain. Without lead staining they are barely distinguishable. The arrangement of the rows is strikingly regular, each tube being equidistant (about 160 Å) from 6 neighbours (fig. 2, B, C). On the inside the tubes ramify in an irregular way after they emerge from the dense layer, and in this region the cuticle round them appears less dense. The less-dense areas are particularly obvious at the base of each crater, where they extend into the dense layer as the tubes emerge (fig. 1, E). There is a matrix of microfibres without any preferred orientation separating the lamellae of the endocuticle from the region where the tubes ramify.

The hollow wax cylinders. The wax filaments seen with the light microscope have a melting-point of 81 or 82° C and they can be viewed in the electron microscope without melting. Under this higher resolution they are seen to be hollow cylinders with the same diameter as the apices of the craters which secrete them (fig. 1, F). The wax follows exactly the distribution

FIG. 3 (plate). A, Transverse section of the cuticle where the wax secretion takes place. Note the absence of pore canals and the fragmented cement layer. (Electron micrograph.)
B, section at right angles to the plane of the lamellae in the endocuticle. Three lamellae are visible crossing the field obliquely. (Electron micrograph.)
C, vertical section through a tubercle on a tergite. Note the gaps in the epicuticle which accompany the wax canals. (Electron micrograph.)
D, the epicuticle and wax canals from a tergite at higher magnification than C. (Electron micrograph.)
Locke—Wax Secretion in Calpodes

of tubes in the walls of the crater, and this provides strong circumstantial evidence that it is secreted through them. The tubes have therefore been termed ‘wax canals’. For the same reason it seems probable that the wax canals traverse not only the dense layer but also the cuticulin layer and they have been drawn thus in fig. 4, although they cannot with certainty be seen to do so. Fig. 4 presents in diagrammatic form the chief findings concerning the structure responsible for the secretion of a single hollow wax cylinder.

Fig. 4. Diagram of the most probable structure of the cuticle which secretes the hollow wax filaments.

**Cuticle from Other Parts of the Caterpillar**

Over most of the sternites and tergites the cuticle is thrown into rounded tubercles similar to those described by Way (1950) and Takahashi (1956). With the electron microscope these are seen to have basically the same components as the wax-secreting cuticle (figs. 3, c, d). The cement layer is irregularly preserved. The cuticulin layer is of approximately the same thickness as in the wax-secreting cuticle, but the dense layer is everywhere much thicker. There are a few tubes penetrating the dense layer, having the same dimensions as the wax canals, but they are irregularly and sparsely scattered. For about two-thirds of their journey through the dense layer they are accompanied by a finger-like projection of less dense material from the inside. The tubes themselves ramify little when they emerge, but pursue a much straighter course for a short distance into the non-laminated endocuticle. The endocuticle does not differ from that described already.

The epicuticle has also been studied on the tracheae. It has no wax canals
and is similar to that described in *Rhodnius* and other insects, consisting of two layers, cuticulin and the dense layer (Locke, 1957).

The chief features of the tergal cuticle are summarized in fig. 5.

**DISCUSSION**

Although the epicuticle is known to contain waxes (Wigglesworth, 1957), it is perhaps not surprising from its dense appearance under the electron microscope that pores through it should be necessary for the movement of wax. But if the wax canals were only necessary to provide unimpeded movement through the epicuticle, they might be expected to terminate or even funnel out on the inner surface. However, the wax canals are more than this, for the straight course of the canal through the epicuticle is only a half or a third of the total length of the tube. The convoluted course is improbable if the tube is only a vehicle for wax-transport from the inside to the outside of the epicuticle. If, as has been suggested, the wax is synthesized in this region, it would seem plausible to suppose that the site of synthesis should be as close to the site of deposition as possible. This being so, the walls of the convoluted tube have an obvious claim as a seat for this synthesis. Two other details support this hypothesis. The cuticle round the convoluted tubes is less dense and looks as though it might provide free access to them, and in the tergal cuticle there is an apparently empty sleeve for the tube for over half its passage through the epicuticle.

If wax canals should prove a common feature in the wax secretion of insects, they may play a significant part in the permeability of the cuticle, particularly to non-polar substances, including many insecticides. There is some physiological evidence favouring this. Holdgate (1956), for example, suggests that water loss/temperature curves may be explained by diffusion through pinholes.

According to Watson (1958) lead hydroxide stains many cellular constituents. A possible rationale for its staining action in the epicuticle would be...
the formation of lead soaps from fatty acids, if the fatty acids were not entirely leached out during embedding.

The double nature of the epicuticle (excluding the cement) is now well documented for a number of insects (Sarcophaga, Dennell, 1946; Calliphora, Wolfe, 1954; Periplaneta, Dennell and Malek, 1955; Apis, Richards, 1952; Bombyx, Ito, 1954; Rhodnius, Locke, 1957, 1958). For convenience in this paper the outer layer has been referred to as cuticulin, although the term as introduced applied to both layers. Its characteristic feature in Calpodes as in Rhodnius is its extreme thinness and uniformity. The thicker layer within has been referred to with a non-committal term, the dense layer. No more specific name is useful until its chemical nature has been established. In tracheae it contains chitin but this may not be true in general.

According to Richards (1958, 1959) microfibres have not been found in cuticle, nor from his cross-grid model of chitin/protein structure, based on his work on the optical properties of isolated chitin and anthropodin, does he expect to find them. Fig. 3, B shows that such fibres exist and their orientation may go some way to explain his results. The effect of the orientation on the integument may be to allow the stretching which occurs during an instar and it might also impart some elasticity.

I greatly appreciated the hospitality of Professor V. B. Wigglesworth during my 6 months' stay in his laboratory. I am very grateful also to Dr. V. E. Cossett for the electron-microscope facilities provided in the electron-microscope department of the Cavendish Laboratory. I particularly thank Mrs. A. Cossett for her helpful advice and the use of her equipment for the preparation of ultra-thin sections. My thanks are also due to Mr. T. Morley and Mr. R. Horne for taking the electron micrographs. Lastly, I am happy to acknowledge my debt to Dr. J. W. L. Beament's encouragement.

REFERENCES