The Structure and Deposition of the Cuticle in the Adult Mealworm, *Tenebrio molitor* L. (Coleoptera)

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With two Plates and eight Text-figures

In three earlier papers (Wigglesworth, 1933, 1945, 1947a) the structure and deposition of the cuticle of *Rhodnius prolixus* have been described. The main conclusions reached were as follows. The epicuticle is a composite structure made up of four layers: (i) a 'cuticulin' layer composed of a condensed lipoprotein subsequently tanned by quinones; (ii) a layer of material rich in dihydroxyphenols; (iii) a thin layer of crystalline, orientated wax molecules responsible for the waterproofing of the cuticle (cf. Beament, 1945); and (iv) an outermost 'cement' layer protecting the wax.

The lipoproteins which form the foundation of the epicuticle are apparently synthesized by the oenocytes before being transferred to the epidermal cells. The oenocytes reach their maximum development immediately before the cuticulin layer is deposited and then diminish rapidly in size. The polyphenol layer is secreted from the epidermal cells by way of the pore canals which appear to penetrate the cuticulin layer. The wax is secreted in the same way immediately before moulting. The cement layer is poured out from the dermal glands over the surface of the wax immediately after moulting.

The hardening which takes place shortly after moulting is due to the tanning of the lipoprotein of the epicuticle and the cuticular protein of the exocuticle by quinones produced by the oxidation of the dihydroxyphenols (Pryor, 1940b; Pryor, Russell and Todd, 1947). The endocuticle is laid down by the epidermal cells during the next few days.

In the deposition of the cuticle the epidermal cells first separate from the old cuticle and undergo active mitosis. Many more cells are produced than are required. Consequently, great numbers of them suffer autolysis with the formation of the 'chromatic droplets', until, finally, a regular epithelium with the nuclei evenly spaced is produced, which then proceeds to lay down the new cuticle (Wigglesworth, 1943a).

If the cuticle of *Rhodnius* (and many other insects) is gently rubbed with alumina dust, the cement layer and the wax layer are abraded; the layer containing the polyphenols is then exposed and will stain a deep chestnut-brown if the insect is immersed in ammoniacal silver hydroxide. The polyphenol layer is likewise exposed more or less readily by extraction of the cuticle surface with chloroform and other wax solvents.
The ease with which the polyphenols are exposed by these two procedures varies greatly in different insects and in different parts of the same insect. In adult beetles it was found that there is no detectable abrasion (or, at least, no exposure of silver-reducing materials) over the hard regions of the cuticle in insects left in contact with alumina dust (Wigglesworth, 1947b). Silver staining after this treatment is confined to the soft dorsal cuticle of the abdomen and to the various intersegmental membranes.

The object of the present work was to compare the structure and formation of the cuticle in these different regions of the body in the adult beetle *Tenebrio*, and to see how far the conclusions arrived at from the study of the *Rhodnius* cuticle were applicable to this unrelated insect.

**STRUCTURE OF THE ADULT CUTICLE AND EPIDERMIS**

The dorsal cuticle of the abdomen, where it is covered by the elytra, is excessively thin (total thickness 4 μ). The tergites are distinguishable only by their shining surface and faintly amber tint which contrasts with the colourless matt surface of the lateral and intersegmental regions. The varied sculpturing and spicules of the different parts will not be described.

The ventral cuticle is very hard and a deep amber-brown in colour (total thickness in an old insect 36 μ). Evenly dispersed over the sternites are shallow oval depressions each with a tiny slender bristle curving backwards from its anterior end (Text-fig. 1). In the floor of these pits are minute pores—1–3 in the female, a closely packed group of about 25–30 forming a diminutive ‘pore plate’ in the male. In both dorsal and ventral cuticle the limits of the epidermal cells are clearly indicated in the sculpturing of the cuticular surface. There is a shallow groove between each cell area.

Both tergites and sternites are pierced by the ducts of dermal moulting glands; in the sternites these occur as a circle of some 8–10 glands around
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each pit, but they are seen with difficulty in ordinary preparations. They occur also in the pleural regions.

Text-fig. 2 shows histological sections of the dorsal and ventral integument as seen in a recently moulted adult about 6 days old. The cuticle of the tergites (Text-fig. 2A) consists of an excessively thin 'epicuticle', an unstained exocuticle about 1.5 μ thick, and an endocuticle of about 2.5 μ, but no further details can be seen. In the sternites (Text-fig. 2B) the cuticle is made up of a fairly thick amber-coloured 'epicuticle', an amber exocuticle about 12 μ in thickness in which the vertical striation of the pore canals is just visible, and a laminated acidophil endocuticle which, in an insect several weeks old, may be double the thickness of the exocuticle. The exocuticle is differentiated faintly into an outer and inner half. The outer half has a slightly greyer shade in the unstained state, and in the recently moulted adult it stains bluish with haematoxylin. At intervals the cuticle is pierced by the ducts of dermal glands, and in the male the groups of pores in the pits form conspicuous interruptions in the sections.

There is the usual complex of cellular elements in the epidermis, best seen in the recently moulted adult (Text-fig. 3). On the sternites, besides the epidermal cells, there are scattered oenocytes, the dermal moulting glands (unicellular glands with some three other cells forming the duct, &c. (Hundertmark, 1935)), and the pit glands. Each pore of the pit glands is connected with a single large cell. In the mature male these cells become enormously swollen and the glands project like closely packed buttons far beyond the
The Structure and Deposition of the surface of the shrunken epidermis. In the female, with usually not more than two pores, the associated gland cells are comparatively inconspicuous.

The structure of the cuticle, particularly the ventral cuticle, has been studied by the methods used on *Rhodnius*.

(i) *Fresh Sections*. In sections cut with the freezing microtome and examined fresh in water the pore canals appear much as in stained sections.

(ii) *Fresh Sections treated with Ammoniacal Silver*. Similar sections, 10 µ thick, were immersed for 1 hour in 5 per cent. ammoniacal silver hydroxide and mounted in Canada balsam. All the pore canals in the exocuticle run a parallel vertical course. In the outer half of the exocuticle their contents stain brown, but only where they come very close to the exposed surface of the sections or where the canals have been actually cut open (Text-fig. 4). These filaments are almost certainly in the form of a close spiral (as described by Richards and Anderson, 1942, in the cockroach), but only here and there can this be resolved with certainty with the light microscope. In the endocuticle the pore canals run a somewhat uneven spiral course, often changing direction as they cross from one lamina to the next and converging gradually towards the base. Throughout the endocuticle they contain a black deposit.

These glands resemble those described by Hoffbauer (1892) in the elytra of the Cerambycid *Tetropium*. Hoffbauer noted great differences in closely related species but makes no mention of sexual dimorphism. The glands in *Tenebrio* must presumably have some sexual function. Perhaps in the male they produce an aphrodisiac secretion. It is pointed out to me by Dr. H. E. Hinton of the British Museum, in a personal communication, that hair tufts, presumably the outlets of glands, occur in many male Coleoptera. In other parts of the body (legs, prothorax, &c.) the male *Tenebrio* has only two or three pores in the pits, like the female.
of silver along their course; and this is not limited to the canals exposed on the surface of the sections.

The staining of the pore canal contents is seen even more clearly if the sections after treatment with the silver are bleached for 24 hours in perhydrol; and then it is seen that the exposed surface of the exocuticular matrix also stains weakly with the silver. Beyond the limit of the brown-staining pore canals in the exocuticle there is an 'epicuticle' apparently devoid of pore canals; but in many places the brown filaments come so close to the surface that it is not possible to ascribe a measurable thickness to this layer.

(iii) Fresh Sections dried in Air. Fresh sections were dried thoroughly in warm air and then mounted direct in Canada balsam. The pore canals of the endocuticle contain air in many places and appear as black threads. In the exocuticle they contain no air.

(iv) Silver Staining after Abrasion with Alumina. If the intact insect is immersed in ammoniacal silver hydroxide no staining of the cuticle occurs. If it is rubbed gently on filter paper dusted with alumina and then immersed in the silver solution, the dorsal cuticle shows a uniform pinkish-brown staining over all the raised points, with the tips of the pore canals, extending apparently into the substance of the brown-staining membrane, seen in surface view as minute black points. The ventral cuticle shows no staining with silver after abrasion with alumina except at the intersegmental membranes which stain in the same manner as the dorsal cuticle.

(v) Silver Staining after Scratching with Glass. If the hard parts of the ventral cuticle are treated more brutally by scratching, with varying degrees
of severity, with a fragment of glass and then immersed in ammoniacal silver, it is again found that where the injury is very superficial the scratches are scarcely visible and there is no silver reduction. Where the scratches go deeper the distal ends of the pore canals stain dark brown in a pale brown background. (There are occasional areas where there is a uniform pale brown staining and the pore canals show up as white points. The explanation of this, which does not appear to be an optical effect, is uncertain.)

Text-fig. 5. Longitudinal sections of cuticle of adult male Tenebrio, 1 week after moulting, boiled 5 minutes in chloroform and then treated with ammoniacal silver hydroxide. A, dorsal cuticle and intersegmental joint; A', detail of same; B, ventral cuticle and intersegmental joint; B', detail of same. x, silver-stained epicuticle and outer exocuticle; y, silver-stained cuticle lining the openings of the male pit glands.

(vi) Silver Staining after Chloroform Extraction. Extraction with chloroform likewise leads to the exposure of the silver-staining material much more readily in the same regions which are affected by abrasion with alumina. This is most clearly demonstrated by boiling the insect for 5 minutes in chloroform before immersion in the ammoniacal silver. Text-fig. 5 shows longitudinal sections of the dorsal and ventral abdominal cuticle in a Tenebrio adult so treated. The dorsal epicuticle, including the spicules, stains everywhere an intense brown. The same staining extends into the outer part of the exocuticle—sometimes as a uniform brown, sometimes limited to pore canals. The inner half of the exocuticle (stained red with Mann's methyl blue eosin) and the blue-staining endocuticle show no silver reduction.
In the ventral integument, on the other hand, silver staining of the epicuticle is limited to the outer folds of the intersegmental membranes. Elsewhere (apart from the 'cement layer' which will be discussed later) there is no silver staining of epicuticle or exocuticle except in the lining of the orifices of the pit glands or 'pore plates'. The residual contents of the dermal moulting glands also stain deeply and are very conspicuous after this treatment.

(vii) Chitosan Test. The ventral cuticle of the fully hardened adult some weeks old was treated with saturated potassium hydroxide at 150°C. until just colourless, then washed in alcohol and examined under a coverslip in surface view. In the deeper layers (endocuticle) the criss-cross fibrils of chitin running in different directions in successive layers are clearly seen; and the areas corresponding with each epidermal cell are evident. In the superficial layers (exocuticle) the pore canals are readily visible; they appear evenly distributed and the cell limits cannot be seen. Now that the amber material has been dissolved away, the pore canals, as seen in optical section, are open to the surface.

If acid iodine is allowed to diffuse slowly in below the coverslip, the matrix of the cuticle in the superficial layers takes on a violet colour, but as this colour spreads it is quite evident that the pore canals remain colourless as minute white points. There is certainly no chitinization of the contents of the pore canals of the exocuticle.

(viii) Disintegration in Nitric Acid and Potassium Chlorate. The abdominal cuticle was boiled in chloroform for 10 minutes and then immersed in concentrated nitric acid saturated with potassium chlorate. The dorsal cuticle shows little ebullition as the thin endocuticle dissolves. A very thin epicuticle is left which on warming breaks up with the liberation of small oily droplets.

In the ventral cuticle there is much evolution of gas as the inner layers are dissolved. On warming fairly strongly the exocuticle then fuses and disperses in the form of feebly refractile spheres. If at this stage the preparation is washed with 50 per cent. alcohol and flooded with Sudan B in 70 per cent. alcohol, very few of the droplets stain. They consist presumably of broken-down protein and chitin. There are, however, a few fat-staining droplets, particularly where the epicuticle is beginning to disintegrate. If the heating of the preparations is continued the epicuticle remains last; and as it breaks up, undoubted oil droplets, highly refractile and staining with Sudan B, appear in great quantity. They are far more copious than in the dorsal cuticle, a fact which agrees with the much greater thickness of the epicuticle on the ventral surface as seen in sections.

(ix) Demonstration of Wax and Cement Layers. It has not been possible to demonstrate a wax layer in the cuticle of the mature Tenebrio adult; the evidence for the presence of this layer will be given when the deposition of the cuticle is described. The existence of a cement layer is readily demonstrated.

If the legs or elytra are immersed fresh in xylene, droplets of water slowly exude from the epicuticle (Wigglesworth, 1942). In many places during this
process a thin layer flakes away in fragments; in other places a delicate continuous membrane, the 'cement layer' is lifted from the surface.

This layer can also be demonstrated by silver staining. If the mature adult beetle is boiled in chloroform for 5 minutes and then treated with ammoniacal silver, a delicate membrane can be seen over the surface of the sternites, extending into the bristle-bearing pits. It consists of very fine evenly dispersed brown granules in a colourless substance. Where the cuticle has been rubbed in mounting it is torn and partially removed; and in sections of such insects it is detached and very easily seen.

Around the openings of the dermal glands, in some preparations, there is a non-staining disk devoid of granules (Pl. I, fig. 1). And extending from the orifice of a certain number of glands (always the glands at the centre of the spaces between the pits, which are a little larger than the others) there is a brown-staining convoluted filament lying on the surface of the cuticle (Pl. I, fig. 2). This appears to be the extruded residue of the secretion from the glands.

A series of adult beetles, 2 weeks after moulting, were subjected to extraction with boiling chloroform for periods of 1, 5, 15, 30, and 60 minutes before treatment with the silver. Insects untreated with chloroform showed no silver staining. All the others showed the granular cement layer over the sternites. The staining was often most intense in the furrows between the cell boundaries on the cuticle surface, giving a net-like appearance (cf. Pl. II, fig. 10). In the insects extracted for longer periods disintegration of the membrane was more advanced; the granules had fused in many places to give larger silver-staining spheres and the convoluted filaments from the dermal glands showed the same change (Pl. I, fig. 3).

It thus appears that the cement layer, as was suggested in *Rhodnius*, also consists of tanned material; but that the silver-reducing groups of the polyphenol concerned are not accessible until the material has been subjected to boiling chloroform. Presumably it is compounded in some way with lipides.

(x) Summary of the Conclusions on the Structure of the Cuticle. From these varied observations we may conclude that the dorsal cuticle is not only excessively thin compared with the ventral cuticle, but the substance of the outer layers (the epicuticle and the very thin exocuticle of the delicate sclerites), like the same parts in *Rhodnius*, still contain polyphenol material in a state accessible to the silver solution if the cuticle is cut or the protective coverings on the surface are removed by abrasion or by extraction with lipid solvents.

The thick and brittle cuticle of the sternites has very different properties. It has a relatively thick and hard epicuticle which will not reduce ammoniacal silver (when immersed for 1 hour) even when exposed by abrasion, section, or lipid extraction. The exocuticle is likewise hard and impermeable; polyphenol material easily accessible to the silver solution is confined to the contents of the outer parts of the pore canals, and the limitation of silver staining to the surface of the fresh sections shows how impermeable is the
exocuticle to the diffusion of silver through its substance. (The black deposits
in the pore canals of the endocuticle may well be simply a precipitate of the
silver by salt or protein.)

The state of the material in the lumen of the pore canals is difficult to
determine. In the endocuticle many of the canals contain air on drying; but
as Dennell (1947b) points out, this does not disprove the existence of solid
filaments within them. In the exocuticle they appear to be solid; perhaps
their contents consist of protein which has been tanned along with the
substance of the exocuticle during the hardening process. It is certain that
they do not contain filaments of chitin.

As will be shown later a wax layer covers the cuticulin of the epicuticle,
and over the surface of this is a cement layer which likewise consists of tanned
materials in intimate association with lipides. In ordinary sections neither
cement nor wax layer are optically distinguishable from the cuticulin layer.
It has not, therefore, proved possible to homologize the structures here
described with the inner and outer epicuticle as defined by Dennell (1946) in
Sarcophaga larvae and in Tenebrio and other adult beetles.

**Deposition of the New Cuticle in the Pupa and Young Adult**

**Outward Changes in the Pupa and Young Adult**

Newly moulted pupae of *Tenebrio* were kept at 25°C. Formation of the
adult beetle then takes place according to the following time-table. During
the first 24 hours the pupa has a glassy appearance and the eyes show only
brown central points in the facets; no separation of the adult is visible in the
living pupa. By the second day the adult is just beginning to separate from
the pupal cuticle, for example, at the tips of the appendages. At 3 days the
separation is obvious in the claws, palps, &c., but the new cuticle has not
begun to appear. The eyes are just beginning to darken. By 5 days the eyes
are fairly dark and the new cuticle is well defined. By 6 days the eyes are
quite dark; the claws, tarsal segments, the femero-tibial articulations, and the
distal third of the mandibles are darkening. By 7 days the head and legs are
becoming dark generally. Moulting to the adult occurs on the eighth day.

The last stages of moulting can be roughly timed by observing the sheaths
of the appendages. By the end of the seventh day the limbs are chestnut-
brown in colour but the pupal sheaths enclosing them are fully distended
with moulting fluid. About 6–10 hours before moulting to the adult, as the
inner layers of the pupal cuticle are digested and the moulting fluid absorbed,
the sheaths of the last pair of legs begin to collapse, followed by the middle
and anterior pairs. Finally, the sheaths around the palps, labrum, and
mandibles crumple and collapse, and moulting usually takes place from 2 to
4 hours later. By this time the moulting fluid has disappeared, the pupal
cuticle is excessively thin and fragile, and the surface of the insect is quite dry.

The newly emerged adult has the head and prothorax amber; the legs,
particularly the joints and claws, are likewise amber, and so are the margins
and posterior extremity of the abdomen; but the tergites and sternites of the
abdomen, and the elytra, are alike colourless. Within a few hours the beetle becomes amber all over. After 1 day the general colour is pale chestnut; after 2 days, dark brown; and after 3 days it is fully darkened and almost black.

**Histological Changes in the Epidermis and Cuticle**

The histological changes have been observed in serial transverse sections of the abdomen fixed with Carnoy’s and Bouin’s fixatives at all stages, stained with haematoxylin, Mann’s methyl blue and eosin, and Mallory’s triple stain. Likewise at all stages the dorsal and ventral integument of the abdomen have been removed, freed from underlying tissues, and mounted flat after (i) fixation in Carnoy and staining with haematoxylin; (ii) fixation in Bouin and staining with Sudan black B; (iii) fixation in Altmann and counterstaining with carmine.

There is no interruption in the process of growth when *Tenebrio* pupates. Within less than 24 hours after moulting mitosis in the epidermis of the sternites has already begun. At this stage there is no obvious difference between the cuticle and epidermis of the dorsal and ventral walls of the abdomen (Text-fig. 6A, a and b), apart from the fact that oenocytes are confined to the sternites. The epidermis is thin and the nuclei lie all in one plane, not nearly contiguous. The oenocytes of the ventral wall still occur in pairs, embedded among the epidermal cells (Text-fig. 6A, c).

By one day after pupation a difference is apparent between the epidermis of the dorsal and ventral integument. In the sternites the epidermal cells are now much more numerous, the nuclei are so crowded that they lie in several planes, and there are abundant mitoses. Certain of the nuclei are in chromatolysis (Pl. I, fig. 5). The whole epidermis is in such a violent state of flux that the centres of formation of dermal glands, bristles, &c., cannot be recognized. The oenocytes are still mostly in pairs; they are enlarging slightly and now lie wholly on the inner surface of the epidermis (Pl. I, fig. 6). In the dorsal integument there are no mitoses, no oenocytes, and, as yet, little chromatolysis.

By two days (Text-fig. 6B) the same differences between the dorsal and ventral epidermis are becoming exaggerated. That on the dorsal integument is still extremely thin and many of the nuclei are breaking down so that chromatic droplets in all stages of formation are abundant (Pl. I, fig. 4).

By three days (Text-fig. 6C) mitosis and chromatolysis in the ventral epidermis is still in active progress; the dermal glands are becoming visibly differentiated and the oenocytes are becoming large and acidophil. In the dorsal epidermis chromatolysis is well advanced and, since no mitosis has occurred, the cells are becoming very sparse as well as very attenuated.

By four days (Text-fig. 6D) the epidermis has been detached from the old cuticle, chromatic droplets have almost disappeared, the nuclei are evenly distributed, and the oenocytes are large, lobulated, and strongly acidophil (Pl. I, fig. 7). The innermost layer of the old cuticle stains with haematoxylin
TEXT-FIG. 6. The formation of the adult cuticle in the pupa of Tenebrio. a, section of dorsal integument; b, section of ventral integument; c, surface view of oenocytes below the ventral epidermis. A, newly moulted pupa; B, pupa at 2 days; C, 3 days; D, 4 days; E, 5 days; F, 6 days; G, shortly before moulting to adult. The details are given in the text.
and the secretion of the new cuticle is imminent. Particularly in the ventral epidermis the cells now show distinct intercellular membranes and in many places these have a remarkably exact hexagonal arrangement (Pl. I, fig. 8). In preparations fixed with Altmann the great lobulated oenocytes stain dark grey; and they stain conspicuously in Sudan black B.

By five days (Text-fig. 6E) (occasionally by 4 days) the new epicuticle is formed. It is refractile and eosinophil, excessively thin on the dorsal surface as compared with the ventral. In ordinary histological sections it appears to be homogeneous although the outer extremity of the cell body lying immediately below is seen to be vertically striated. In the Altmann preparations, in which it can be studied in surface view or in optical section, the epicuticle stains grey and the pore canals are clearly visible as colourless threads in a dark matrix; their outer ends are closed only by an excessively thin refractile membrane. The oenocytes are still large but do not stain so deeply in Altmann.

By six days (Text-fig. 6F) the formation of the exocuticle is in progress and the oenocytes are becoming reduced in size; many of them are clearly dying with chromatolysis of their nuclei. The new cuticle, particularly the epicuticle, still stains grey with osmic acid. The arrangement of the epidermal cells, as seen in surface view, is not so regular, but they still lie in rows, this being associated no doubt with the deposition of the parallel strands ('Balken') of chitin extending from cell to cell. Highly vacuolated dermal glands occur in both dorsal and ventral integument.

By seven days the formation of the ventral integument is becoming much thicker and its inner and outer halves can be distinguished. For example, with Mallory's stain the inner half stains blue and the outer half stains red, being traversed by blue-staining pore-canal fibrillae which can be traced almost if not quite to the surface of the epicuticle. The dermal moulting glands are even more distended and vacuolated and the pit glands in the male are beginning to enlarge. The oenocytes are reduced in size and stain only a faint grey in osmium tetroxide. The osmic staining of the epicuticle has almost disappeared. Digestion of the inner layers of the pupal cuticle is beginning and is completed by the eighth day (Text-fig. 6G).

In the young adult there are no very striking histological changes. The regular arrangement of the epidermal cells is largely lost, but in many places the cells, as seen in surface view, still lie in parallel rows with filaments extending from one to the next as the criss-crossing chitin strands of the endocuticle are formed. As the endocuticle is being secreted the outer parts of the cells stain deeply with haematoxylin, and blue-staining filaments extend into the inner ends of the pore canals. The endocuticle is just about complete in 4 days after moulting. By that time the epidermal cells are small and shrunken and the pit glands of the male are greatly swollen and project far below the epidermis (see Text-fig. 2).

In addition to the sub-epidermal oenocytes described above there are clusters of large oenocytes segmentally arranged in the neighbourhood of the
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spiracles (Koch, 1940). These appear to go through the same cycle as the oenocytes below the epidermis. They reach their maximum size at about the fourth day of pupal development and appear then to be discharging their secretion into the blood.

Formation of the Epicuticular Layers

(i) Polyphenol Layer. The formation of the cuticulin layer of the epicuticle takes place as we have seen between the fourth and fifth days after pupation by the secretion of lipoprotein from the epidermal cells. Upon this is deposited a layer of material rich in silver-reducing substances, the 'polyphenol' layer.

If the epidermis with its new epicuticle, 5–6 days after pupation, is immersed directly in the ammoniacal silver solution there is a clear-cut silver precipitate marking out the membrane between the cell bodies. At the outer limit of each cell there is some finely punctate silver staining, but the epicuticle does not stain. Here and there are single cells in which the outer region stains intensely with the silver (Pl. I, fig. 9) and from the strongly staining cells black filaments run through the pore canals of the newly formed epicuticle to give discrete and evenly distributed black points on the surface.

Soon the black points become general, though always confined to the limits of the cells. It is often possible when examining these preparations in surface view to note the grouping of the pore canals in optical section, to follow them upwards, and to observe that the black droplets on the surface of the cuticle have the same grouping. There can be no doubt that the droplets are formed, as was claimed in Rhodnius, by the extrusion of material from the pore canals (Pl. I, figs. 10, 11).

Gradually the exuded droplets enlarge and fuse, so that in pupae in which the new cuticle has been exposed and treated with the silver during the seventh day all intermediate stages can be seen leading up to a condition where the epicuticular cap of each cell is covered by a deep-chestnut-staining layer, more or less continuous, with the punctate black staining of the tips of the pore canals below (Pl. I, fig. 12). Finally this layer joins up with that covering adjacent cells until the cell boundaries almost disappear. At no stage does this superficial polyphenol layer extend over the floor of the pits. Consequently, these show up as oval white spots in the preparations (Pl. I, fig. 12; Pl. II, figs. 4, 5, &c.).

If, during this process, the exposed cuticle, before treatment with the silver, is wiped with a piece of filter-paper cut to a point, the silver-staining material will run together into rounded droplets. If it is more strongly rubbed with filter-paper, the material may be removed completely, leaving only the black-staining tips of the pore canals.

These observations, made upon whole preparations seen in surface view, can be confirmed in sections. Text-fig. 7 shows sections of the new cuticle which had been exposed in the 6-day pupa and treated with silver. In Text-fig. 7A the cuticulin layer is visible as a clear zone beyond the silver-staining
filaments in the exocuticle. But here and there these filaments cross the clear zone, becoming continuous apparently with the polyphenol droplets on the surface. At the stage represented in Text-fig. 7B the polyphenol is being actively secreted; it appears chiefly in the form of tapering filaments passing outwards through the cuticle, with their pointed end leading, to fuse upon the surface. The form of these filaments bears a striking resemblance to that assumed by droplets of biliverdin as they pass through the striated border of the Malpighian tubes in *Rhodnius* (Wigglesworth, 1943b).

(ii) *Wax Layer.* During the last few hours before moulting the polyphenol layer is largely covered over. Soon after the silver staining has reached its greatest intensity and become practically continuous, it begins to break up into rounded patches with non-staining areas between; and gradually the staining spots become reduced in number and in size (Pl. II, fig. 1). Around each spot can be seen a pale-brown halo. This is due to the diffuse staining of the exocuticle and the dark-brown staining of the filaments in the pore canals, the distal limits of which now lie on a distinctly lower plane than the dark-brown spots (Pl. II, fig. 2)—though sections through the dark spots often show brown filaments running from them into the pore canals.

These observations show that at this stage silver can get into the deeper layers of the cuticle only through the silver-staining patches; the intervening areas are impermeable. Having got in it will diffuse laterally through the exocuticle to reach adjacent pore canals. The slightest abrasion of the surface with alumina, or brief immersion in chloroform, will expose the polyphenol layer everywhere.

It would appear that, as in *Rhodnius*, the polyphenol layer is being covered by a layer of lipoid or wax immediately before moulting. At the time of moulti...
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silver staining in the form of scattered rounded spots, particularly along the side of the abdomen and on the intersegmental membranes. Here and there the stained spots lie in rows, mostly running in an antero-posterior direction (Pl. II, fig. 3). These have evidently resulted from abrasion during moulting and show how fragile is the protective layer at this time. Within 2 hours the silver staining is much reduced, though what remains has the same disti-
tion as before. By 6 hours after moulting there is a further marked dimen-

(iii) Cement Layer. Meanwhile the cement layer is being poured out over the surface of the wax. At the time of moulting, droplets of water, at the tip of a very fine waxed pipette, will not adhere to the surface of the sternites. After 1 hour they usually begin to adhere slightly, particularly in the lateral regions. When brought into contact with the cuticle the droplet on the pipette breaks and a very tiny drop remains on the insect. After 6 hours the droplets will adhere all over the cuticle with an angle of contact ranging from 90° upwards, but they never spread over the surface. This slightly increased affinity for water takes place in spite of the fact that the hardening process that is going on in the substance of the cuticle will be tending to make it hydrophobe; it is certainly the result of the outpouring of the cement layer.

The cement comes from the dermal glands. We have seen that after extraction with boiling chloroform it will stain with ammoniacal silver, and this property may be utilized to demonstrate its secretion. Adult Tenebrio in the act of moulting and at periods of 30 minutes, 1 hour, 2 hours, &c., afterwards have been fixed briefly in Carnoy, boiled in chloroform for 5 minutes, and then immersed in the ammoniacal silver.

At the time of moulting the sternites stain deeply after this treatment; excepting the floor of the pits, the dark-brown colour is continuous and extends for the most part right across the abdomen. The contents of the dermal glands stain black (Pl. II, figs. 4, 5, and 6). The gland vesicle has a convoluted form tapering to a point, and in many of the glands the secretion appears to be pressed into a distended mass near the orifice. Towards the sides of the abdomen in some places the dense polyphenol staining of the exocuticle is becoming discontinuous (Pl. II, fig. 7); and where that happens the dermal glands are partially emptied and there is some superficial dark-

At 30 minutes after moulting there is hardly any increased wetting of the cuticle by water. Staining with ammoniacal silver now shows, particularly in the lateral regions, that the cement is being discharged, with the result that the polyphenol layer is no longer exposed by chloroform extraction. Where this process is most advanced the glands are empty; where the polyphenol staining is still continuous, as in the middle of the segments, the glands are...
distended as at moulting; and all intermediate stages occur (Pl. II, figs. 8 and 9). The new cement layer shows regular fine brown granules in a colourless matrix.

One hour after moulting only the middle regions of the sternites show intense silver staining. The lateral parts stain a diffuse brown. In the central area the dermal glands still show intense black contents. In the lateral part they are completely empty, the polyphenol layer is entirely covered, and the only silver staining is in the granular cement. This extends over the whole surface, including the floor of the pits and the bristles, but it is most intense in the furrows between the cellular impressions. Here it may form more or less continuous brown lines, giving a net-like appearance (Pl. II, fig. 10). In the intermediate zone occasional dermal glands can be seen in which the black-staining contents are in process of discharge.

After 6–8 hours the dermal glands are mostly emptied and the cement layer is evident everywhere. There is little change at 1 day after moulting. At 2 days after moulting the black-staining filaments discharged from the largest of the dermal glands have made their appearance (Pl. I, fig. 2) and practically all the glands are emptied.

It was interesting to apply this technique of staining with silver after extraction with boiling chloroform to *Rhodnius* at the time of moulting. In the *Rhodnius* fifth-stage nymph there are two sorts of dermal glands (Wigglesworth, 1933): type 'B' present in great numbers, particularly around the bristle-bearing plaques, with a large distended oval vesicle; and type 'A' much less plentiful, with an elongated intracellular vesicle. In the nymph at the time of moulting it is only type 'A' whose contents stain black with silver after chloroform extraction; the vesicle contents of type 'B' are unstained. If the preparation is allowed to dry on the slide after dehydrating in alcohol the vesicles of type 'B' fill with air and on mounting in Canada balsam they show up very conspicuously.

In order to see whether glands of this type had been overlooked in *Tenebrio* a preparation of a newly moulted *Tenebrio* adult was likewise allowed to dry in the air, but no glands were revealed. It appears that in *Tenebrio* there is one type only, and its contents reduce silver after chloroform extraction.

It appears from these observations that the cement layer in *Rhodnius* resembles the substance of the ootheca in the cockroach, as described by Pryor (1940a), in being the product of two glands, one of which secretes a protein solution and the other a polyphenol. When the cement in *Rhodnius* is first discharged it is strongly hydrophil and droplets of water spread actively on the surface (Wigglesworth, 1947a). In *Tenebrio* there is only a slight increase in this adhesion of water when the cement is secreted. This is probably because the cement in *Tenebrio* contains more lipoid material; for it is the product of a single type of gland, the contents of which reduce silver only after extraction with boiling chloroform.1

It is unlikely that the glands opening into the floor of the pits in *Tenebrio* contribute to the cement. There is a very great difference in their form and development in the two sexes and they do not appear to reach full activity until some days after moulting.
Hardening of the Cuticle

No attempt has been made to follow the movements and distribution of the enzymes concerned in the hardening and darkening of the cuticle (cf. Dennell, 1947a).

The most striking feature of the hard ventral abdominal cuticle of *Tenebrio* as compared with the cuticle of *Rhodnius* is the failure of the epicuticle and of the substance of the exocuticle to reduce silver once hardening is complete.¹

Text-fig. 8 shows a fresh section of the cuticle in the young adult one day after moulting, when hardening is still far from complete, cut with the freezing microtome and treated with ammoniacal silver. The outer half of the exocuticle is stained a uniform intense blackish-brown in which the pore canals can scarcely be differentiated. The inner half also stains brown, but not so deeply, and the pore canals are more distinct. The endocuticle is about equal in thickness to the exocuticle. The lamination is faintly visible and the slightly converging pore canals are brown-stained throughout their course. The much stronger silver reduction in the outer half of the exocuticle agrees with the limitation of polyphenol to the outer half of the pore canals in the fully hardened cuticle (p. 200). Perhaps this outer half represents the amount of exocuticle that has been laid down at the stage when the most active secretion of polyphenol is taking place.

The process of hardening in the sternites is most readily studied by rubbing the cuticle surface gently with alumina before immersion for 1 hour in the silver (Wigglesworth, 1945). If this is done at the moment of moulting the wax layer is removed and everywhere there is a strong diffuse staining of

¹ This difference is one of degree. Immersion in ammoniacal silver for 1 hour has been used for the test, and this gives only a very faint staining of sections of the exocuticle. On prolonged immersion more intense reduction would doubtless be obtained.
the exocuticular matrix and an intense black staining of the pore canals (Pl. II, fig. 11). At 6 hours after moulting, when the cuticle is just beginning to darken, the abraded areas stain dark brown as before, but in the form of small spots instead of extensive rounded patches (Pl. II, fig. 12). The exocuticle still stains readily, but now the cement has been laid down the covering is less fragile. At 1 day after moulting the silver staining after abrasion consists of faint pink areas with the punctate brown staining of the tips of the pore canals distributed through them, while in the fully hardened beetle at 4 days, as we have seen, there is no silver staining of the sternites after abrasion with alumina.

These results indicate that although silver-reducing material is probably still present in the epicuticle and exocuticle, the substance of these layers in the fully hardened insect has become so impermeable that the silver does not have access to it.

Along with the hardening, the exposed parts are blackened. If fragments of the fresh cuticle of the newly moulted insect are immersed in dilute ferric chloride, the exocuticle develops a diffuse violet coloration. This is most evident in the parts which will become darkened. Perhaps it indicates the concentration of tyrosine in the cuticle of these regions. The cuticle removed at an earlier stage (in pupae at 6 or 7 days) and immersed in dilute ferric chloride gives a diffuse greenish coloration which changes to red in the presence of alkalis. This colour is said to be given by ortho-dihydroxyphenols only when they are in solution (Lison, 1936). No definite coloration has been obtained with the 'polyphenol layer' on the surface of the cuticulin. Perhaps the phenol responsible for the silver reduction is bound to protein and is insoluble.

**Waterproofing of the Cuticle at the Time of Moulting**

Pupae of *Tenebrio* were kept at 25° C. in dry air and weighed daily. Among 12 pupae with an average weight of 97.7 mg. (85-119) the loss of weight per diem was about 1.3 mg. The loss in the young beetles after moulting ranged from 1.2 to 1.8 mg. per diem with a mean of about 1.6 mg. During the 24 hours which included the moult the loss of weight (excluding the dry weight of the cast skin) varied widely from 4.1 to 12.6 mg., with an average of 7.7 mg.

In three of these insects weighings were made during the act of moulting in order to estimate the relative importance of the water lost with the skin and that lost by transpiration afterwards. The results were as follows:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total loss of water during 24 hours from moulting, in mg.</td>
<td>11.8</td>
<td>6.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Water lost with the skin</td>
<td>4.2</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>&quot; &quot; by transpiration</td>
<td>7.5</td>
<td>3.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Weight of the dry cast skin</td>
<td>1.2</td>
<td>1.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Weighings of insects at intervals after moulting showed that the increased rate of transpiration occurs chiefly in the early hours after shedding the skin; but here again there is great individual variation. For example, the insect B
above, which lost 3.4 mg. by transpiration in 24 hours, lost 1.2 mg. in the
first 6 hours, 2.2 mg. in the next 18 hours; while the insect C, which lost
5.5 mg. in 24 hours, lost 3.2 mg. in the first 3 hours and 2.3 mg. in the next
21 hours.

These results agree with the observations on the newly moulted Tenebrio
which show a variable amount of silver staining that disappears during the
24 hours after moultina. They are entirely consonant with the view that
waterproofing results from the deposition of a layer of wax over the polyphenol.

In Rhodnius, during the day on which moultina occurs, the water loss is
rather more than doubled (Wigglesworth and Gillett, 1936). Smallman (1942),
in the case of Dixippos, found that the rate of loss was increased about four
times but returned to normal within one day. The values in Tenebrio likewise
show an increased loss on the day of moultina about four to six times that of
the beetle during the succeeding days.

I am indebted to Mrs. A. Whittingham for a large amount of careful
technical assistance and to Mr. F. J. Bloy for taking the photomicrographs.

SUMMARY

The conclusions on the structure of the cuticle in Tenebrio have been
summarized on p. 204.

Observations on the deposition of the cuticle are in general agreement with
those made on Rhodnius.

Mitosis and chromatolysis precede the formation of the definitive epidermis.
The basic layer of the epicuticle, 'cuticulin', is then laid down. It consists of
condensed lipoproteins (subsequently tanned, it is supposed, by quinones)
and its deposition is immediately preceded by the peak in the secretory cycle
of the subepidermal oenocytes.

Pore canals from the epidermal cells penetrate the cuticulin layer and pour
out silver-reducing material (believed to be dihydroxyphenols in insoluble
form) upon its surface. This material is confined to the areas overlying the
cell bodies during all but the last stages in its formation, when it fuses to give
a more or less continuous layer.

During the last few hours before moultina a wax layer appears to be laid
down over this polyphenol layer. By the time moultina occurs the polyphenol
layer is almost covered and the insect is nearly waterproof. During the first
day after moultina, while the secretion of the wax is being completed, the loss
of water by transpiration is about four to six times the normal.

Very soon after moultina the dermal glands discharge the cement layer
over the surface of the wax. The substance of this layer and the contents of
the dermal glands reduce ammoniacal silver after extraction with boiling
chloroform. It is suggested that it consists of polyphenol-containing material
associated with protein and lipides.

(It is shown that in Rhodnius the cement layer is formed by the admixture
of secretion from the two types of dermal gland previously described. The
one produces a solution of protein, the secretion of the other agrees in proper-
ties with that here described in Tenebrio. The similarity of this arrangement
to that discovered by Pryor in the colleterial glands of the cockroach is
pointed out.)

In addition to these cement glands there are glands of unknown function
opening into the floor of the pits in the cuticle. These are highly developed
in the sternites of the male, small and inconspicuous in the female.

REFERENCES


—— 1947a. Ibid., 134, 79.

—— 1947b. Ibid., 348.


Koch, A., 1940. Ibid., 37, 38.


—— 1940b. Ibid., 393.


—— and Gillett, J. D., 1936. Ibid., 11, 104.

EXPLANATION OF PLATES

PLATE I

All figures at the same magnification with 4-mm. objective except figs. 9, 10, and 11 taken
with 2-mm. objective.

Fig. 1. Surface view of ventral abdominal cuticle. Boiling chloroform 5 minutes, followed
by ammonical silver hydroxide. The black spots are the ducts of the male pit glands (cf.
Text-fig. 5j). The cement layer is visible as a granular film; it shows colourless round areas
and at the centre of each of these is a black point which is the opening of a dermal gland.

Fig. 2. The same. Towards the left the silver-stained cement layer has been partially
rubbed off, leaving unstained areas. Silver-staining convoluted filaments protrude from the
mouths of some of the dermal glands; others appear as small black spots.

Fig. 3. The same, but treated in boiling chloroform for 30 minutes. The cement layer is
fusing into rounded silver-staining droplets. Above and below are convoluted filaments
which have partially fused.

Fig. 4. Epidermis of dorsal abdominal integument in the 2-day-old pupa seen in surface
view. Ehrlich’s haematoxylin. Many of the nuclei are breaking down with the formation of
chromatic droplets.

Fig. 5. Epidermis of ventral abdominal integument in the 1-day-old pupa seen in surface
view. Ehrlich’s haematoxylin. Numerous mitoses; abundant and crowded epidermal cells;
some nuclei breaking down to form chromatic droplets.

Fig. 6. The same. More superficial view (from inside) showing the oenocytes, many of
them still in pairs. Numerous chromatic droplets.

Fig. 7. The same in 4-day-old pupa showing the oenocytes very large and lobulated