The Physiology of the Cuticle and of Ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera); with special reference to the function of the oenocytes and of the dermal glands.

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With 15 Text-figures.

CONTENTS.

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Structure and Chemistry of the Cuticle and its Bristles</td>
<td>270</td>
</tr>
<tr>
<td>Cuticle of the Adult</td>
<td>270</td>
</tr>
<tr>
<td>Cuticle of the Nymphs</td>
<td>275</td>
</tr>
<tr>
<td>Mechanism of Stretching</td>
<td>276</td>
</tr>
<tr>
<td>Ducts of Dermal Glands</td>
<td>278</td>
</tr>
<tr>
<td>2. The Structure of the Epidermis (Hypodermis)</td>
<td>279</td>
</tr>
<tr>
<td>3. General Account of the Process of Moultling</td>
<td>281</td>
</tr>
<tr>
<td>Methods</td>
<td>281</td>
</tr>
<tr>
<td>Duration of Moult</td>
<td>281</td>
</tr>
<tr>
<td>4. The Epidermal Cells, Uric Acid Cells, and the Red Pigment</td>
<td>282</td>
</tr>
<tr>
<td>5. Formation of the New Cuticle and Bristles</td>
<td>285</td>
</tr>
<tr>
<td>In the Fourth Nymph</td>
<td>285</td>
</tr>
<tr>
<td>In the Fifth Nymph</td>
<td>288</td>
</tr>
<tr>
<td>Formation of the Bristles</td>
<td>289</td>
</tr>
<tr>
<td>Pigmentation of the Cuticle</td>
<td>291</td>
</tr>
<tr>
<td>6. The Dermal Glands and the Moultting Fluid</td>
<td>291</td>
</tr>
<tr>
<td>Function of the Dermal Glands</td>
<td>294</td>
</tr>
<tr>
<td>7. The Oenocytes</td>
<td>299</td>
</tr>
<tr>
<td>Changes in the Oenocytes during Moultling</td>
<td>300</td>
</tr>
<tr>
<td>Behaviour of the Oenocytes in the Adult</td>
<td>303</td>
</tr>
<tr>
<td>Function of the Oenocytes</td>
<td>304</td>
</tr>
<tr>
<td>8. The Basement Membrane and Haematocytes</td>
<td>308</td>
</tr>
<tr>
<td>9. Summary and Conclusions</td>
<td>312</td>
</tr>
<tr>
<td>10. References</td>
<td>315</td>
</tr>
</tbody>
</table>

Ecdysis in insects is the homologue of metamorphosis, and there can be small prospect of understanding the complex process of metamorphosis until the physiology of ecdysis has been
adequately described. It is somewhat surprising, therefore, that so little attention should have been paid to the histological changes which take place during the moulting of non-metabolic insects; and the present work is an attempt to describe certain of these changes in the blood-sucking bug, *Rhodnius prolixus*.

Most authors dealing with ecdysis, usually in caterpillars, date the commencement of the process from the time when the insect ceases to eat and to move about. But it is probable that the sequence of events which terminates in the casting of the old skin has been proceeding far longer than this, perhaps from the time of the previous moult, and that many of the essential stages have been overlooked. It so happens that in *Rhodnius* a single meal of blood suffices for each instar, and that ecdysis occurs at a definite interval after this meal, the whole process following a more or less constant time-table. This circumstance makes *Rhodnius* an admirable subject for such an investigation.

But the ingestion of a meal is followed by three processes: (i) the digestion, assimilation, and excretion of the ingested food (this occurs at all stages of life); (ii) growth, leading up to ecdysis (in the nymphal stages); and (iii) the maturation of the reproductive elements (in the adult and to a lesser degree in the fifth or last nymphal stage). In order to separate these processes, and to establish clearly which of the observed phenomena are truly connected with ecdysis, it has been necessary to study the changes which take place after a meal in the fourth nymphs, fifth nymphs, and adults of both sexes; and to observe, also, the changes which follow moulting when no meal is given.

For the present, the observations have been limited to the tergites and sternites of the abdomen; for these alone have proved sufficiently complex, and it is probable that the principles established apply equally to the remainder of the body.


*Cuticle of the Adult.*—Text-fig 1A shows a longitudinal section of the sternites of the adult cut with the freezing micro-
tome and mounted without staining. The cuticle consists of two primary layers: (i) a very thin epicuticle, and (ii) a relatively thick endocuticle.

Text-fig. 1.

A. Longitudinal section of abdominal sternites of adult *Rhodnius*.  
B. Detail of same just in front of intersegmental membrane.  
C. Section of abdominal tergites of fifth nymph.  
D. Detail of same.

All unstained.  

*d*, ducts of dermal glands;  
*end*$_1$, outer part of endocuticle, formed before moulting;  
*end*$_2$, inner part of endocuticle, formed after moulting;  
*ep*, epicuticle;  
*ism*, intersegmental membrane;  
*pl*, thickened plaque bearing bristle;  
*sec*, dried secretion from dermal glands.

The epicuticle is a uniform, refractile membrane less than 1 $\mu$ in thickness, either amber coloured or more or less
impregnated with melanin. It corresponds with the 'Grenz-lamelle' of German authors.

The endocuticle is divided by a more or less distinct horizontal line into an outer half (laid down before moulting) and an inner half (laid down after moulting). Its essential structure is most clearly seen at the intersegmental membranes (Text-fig. 1 a), where it is a thick colourless layer traversed by a great number of undulating vertical lines extending from the hypodermis to the epicuticle. These lines are the 'pore canals' of authors, the nature of which is a problem that has provoked much controversy. In the case of Rhodnius, however, there can be no doubt that they are fine canals containing protoplasmic filaments or fluid; for if the sections are allowed to dry on the slide and then mounted in Canada balsam, many of the pore canals contain air, appearing black by transmitted light and silvery-white by reflected light. (Similar observations were made by Hass (1916) on the cuticle of Crustacea.)

In sections fixed with Carnoy's or Bouin's fixatives and stained with haematoxylin and eosin, the pore canals are very indistinct, and they are apt to stain pink like the rest of the endocuticle. This pink staining has often been regarded (quite gratuitously) as evidence of chitinization; it is on these grounds that Holmgren (1902 a) and Plotnikow (1904) describe the 'pore canals' as solid filaments that have been transformed into chitin, and Berlese (1909) also adopts this view. On the other hand, if the whole cuticle after fixation is deeply stained with haematoxylin, the pore canals usually stain blue at least in the deepest parts of the cuticle.

The endocuticle shows also a horizontal striation, the laminae being most conspicuous in the inner half.

Such is the essential structure of the endocuticle as seen at the more flexible regions. But over the greater part of the abdomen the outer half of the endocuticle is impregnated with amber coloured material, more or less mixed with melanin, like the epicuticle. This rigid 'cuticulinized' layer (or 'sclerotized' layer, to use the term proposed by Ferris and Chamberlin (1928) ) (Text-fig. 1 b, end.) corresponds with the 'Emailschicht' of Biedermann (1903), the 'Lackschicht' of P. Schulze (1918), the
'Pigmentschicht' of Kremer (1920), and the 'exocuticula' of Campbell (1929); but since, at the intersegmental membranes and elsewhere, it is continuous with an entirely colourless endocuticle, and since it has the same basic structure as this, it seems preferable to regard it as an integral part of this layer. In these rigid areas the vertical striation (due to the pore canals) is very distinct, but the horizontal lamination seems wanting; in this it agrees with the structure of the dorsal plates of queen termites, as described by Ahrens (1930).

Chemically, also, the cuticle consists of two layers. Considering first the flexible type of cuticle seen at the intersegmental membranes: if the sections are heated in saturated caustic potash at 140° C. for five minutes, both epicuticle and endocuticle usually remain intact; and if the sections are now freed from alkali and treated with iodine in 1 per cent. sulphuric acid, the endocuticle shows an intense violet colour (chitosan test) while the epicuticle turns yellow. Chitin is therefore confined to the endocuticle. Similarly the biuret, xanthoproteic, and Millon's reactions are all intensely positive in the endocuticle but negative in the epicuticle. Thus protein also is limited to the endocuticle. Where the outer part of the endocuticle is impregnated with the amber coloured material, it still shows both the chitosan and protein reactions, although these may not be quite so intense; and in the core of the bristles likewise both these reactions are positive. More severe treatment with alkali (heating at 140-150° C. for an hour) removes entirely the amber coloured outer parts, leaving only the chitin in the endocuticle and in the small and limp remains of the bristles.

The converse experiment can be performed and the outer layers obtained free from chitin and protein by cautiously heating the sections in concentrated nitric acid. The colourless endocuticle swells up and becomes bright yellow, there is a vigorous evolution of gas,1 the melanin is destroyed, and the protein and chitin of the endocuticle dissolve. At the intersegmental membranes nothing remains but the very thin epicuticle; elsewhere the amber coloured outer layer of the

1 This gas is, doubtless, the product of oxidation by the nitric acid; it does not occur with sulphuric or hydrochloric acid.
endocuticle also remains with the pore canals intact, and the
bristles still retain their normal structure.

When the inner part of the cuticle has been eliminated in
this way, it is possible to study the properties of the outer part.
This, as has been seen, is exceedingly resistant to strong nitric
acid, although on prolonged heating it ultimately dissolves;
and it is equally resistant to concentrated sulphuric acid in the
cold and to hydrochloric acid. It gives an intense yellow colour
with iodine, but does not stain with fat stains. It is insoluble
in carbon disulphide and other fat solvents. The furfurol test
is negative. On heating with a saturated solution of potassium
chlorate in nitric acid, it melts into oily droplets (cerinic acid
test). On warming in 40 per cent. caustic soda it fuses into an
amorphous mass and dissolves. The biuret reaction is negative.
On heating the dried membrane it partially fuses, and oily
droplets appear which stain with Sudan III.

It is evident from these tests that the epicuticular substance
is not a protein, carbohydrate, or a simple fat; its properties
suggest that it might be a complex fatty or waxy substance or
mixture. It forms the main constituent of the epicuticle or
‘Grenzlamelle’, which Kühnelt (1928) has shown to have the
properties described above; but in addition it impregnates the
protein and chitin of the outer part of the endocuticle. As
Kühnelt points out, this material has the same function and the
same chemical properties as the cuticle of plants; it is possibly
allied chemically to ‘cutin’ or ‘suberin’. These substances are
themselves mixtures,¹ but it is convenient to have a name for
them; similarly, it will be convenient to refer to the correspond-
ing product of the insect cuticle as ‘cuticulin’.

But just as the ‘cutin’ does not make up the whole cuticle
of plants (van Wisselingh, 1895), so there are other substances
besides ‘cuticulin’ in the outer parts of the cuticle of insects.
For the nitric acid not only eliminates the melanin, but it

¹ Lee (1925) concludes that ‘cutin is a complex mixture of fatty acids,
both free and combined with alcohols, that have undergone condensation
and oxidation; soaps of fatty acids and unsaponifiable material which
probably contains some higher alcohols; resinous substances, and a com-
 pound giving tannin reactions’.
removes the constituents which are responsible for the rigidity of the thicker parts. These are the constituents referred to by Schulze (1923) and Kühnelt (1928) as 'Inkrusten', and these authors give some evidence, derived from a study of the cuticle of beetles, for their being carbohydrates.

Thus the composition of the cuticle in Rhodnius may be summed up as follows:

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<tbody>
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<td>Epicuticle</td>
<td>Cuticulin+ melanin, &amp;c.</td>
<td>Grenzlamelle</td>
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<tr>
<td>Endocuticle</td>
<td>Outer pigmented layer present only in rigid portions</td>
<td>Emailschicht</td>
</tr>
<tr>
<td></td>
<td>Cuticulin+ melanin, &amp;c.+ protein+chitin</td>
<td>Lacksschicht</td>
</tr>
<tr>
<td></td>
<td>Inner colourless layer</td>
<td>Pigmentschicht</td>
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<td></td>
<td>Protein+chitin</td>
<td>Exocuticle</td>
</tr>
</tbody>
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This agrees with the conception of Kühnelt (1928) except that he describes what is here called ‘cuticulin’ as being limited to the ‘Grenzlamelle’ (epicuticle), whereas in Rhodnius it is evident that the same material permeates also the outer layers of the endocuticle (‘Pigmentschicht’).

Finally, there is often mentioned in insects an outermost layer or ‘Sekretschicht’ supposedly produced by the dermal glands pouring out their secretion over the surface of the cuticle (Schulze, 1913; Stegemann, 1930). In Rhodnius this layer is discontinuous, and occurs only in little patches round the orifices of the dermal glands. As will be shown later, its presence is adventitious; it has no true relation to the structure of the cuticle and need not be referred to further here.

Cuticle of the Nymphs.—Text-fig. 1 c shows a longitudinal section of the tergites of the abdomen of the fifth nymph. The epicuticle again forms a very thin amber coloured membrane more or less impregnated with melanin. It is thrown into complicated folds except immediately around the bristles; here it is smoothed out to cover the prominent little plaques from which the bristles arise. The endocuticle is everywhere colourless and flexible, except around the bristles where the outer part is permeated with ‘cuticulin’ so as to form the rigid little plaques. It is again divided into an outer part (usually
about two-thirds) laid down before moulting, and an inner part (one-third) laid down after; and it is traversed by 'pore canals' like those described in the adult. This vertebral striation is very much more conspicuous (in sections from the freezing microtome) than the horizontal striation. In fresh material the latter is very difficult to make out, but it can generally be seen if

![Text-fig. 2.](image)

A. Surface view of abdomen of unfed fifth nymph showing plaques bearing bristles, and stellate folds in epicuticle. B. Ditto, in fully fed fifth nymph.

the cut surface of the section is brought into focus. It is readily seen in sections cut with the freezing microtome, fixed in Carnoy, and stained with haematoxylin; and it is then apparent that the laminae are more widely spaced in the inner third. The chemistry of the two layers is the same as in the adult.

**Mechanism of Stretching.**—The nymphal stages of *Rhodnius* ingest from six to twelve times their own weight of blood at a single meal (Buxton, 1930) and the adult may take three times its own weight. The abdomen must therefore be capable of enormous distension, which is accomplished in nymphs and adults in a totally different way.

In the nymphs, as we have seen, the endocuticle is flexible and free from 'cuticulin', except immediately around the bristles, and in the fasting insect the overlying epicuticle is thrown into deep stellate folds (Text-figs. 2 A and 3 A). When
the abdomen is distended with blood, the endocuticle is stretched and attenuated (Text-fig. 3 A), while the stellate folds of the epicuticle become smoothed out (Text-fig. 2 B); it is this mechanism which provides for the enormous changes in volume. At the sides of the segments the tergites and sternites unite directly without the intervention of any elastic pleural membrane; but the unfolding of the intersegmental membranes contributes somewhat to the stretching process.

In the adult, on the other hand, the outer part of the endocuticle is permeated with 'cuticulin' and is so rigid that it cannot be stretched. The chief provision for distension now lies at the sides of the abdomen, where there is a deep longitudinal fold or pleat (Text-fig. 3 B and C). The upper wall of this fold has a rigid cuticle like the rest of the abdomen; but in the lower
wall the endocuticle is entirely soft and elastic and the epicuticle is excessively folded (Text-fig. 3 c and d). Consequently, when the adult is gorged with blood, not only are the intersegmental membranes and the lateral folds expanded, but the lower wall of each lateral fold becomes enormously stretched in the same manner as, but to an even greater degree than, the general cuticle of the nymphs (Text-fig. 3 b'). In addition to this, there is a line of weakness along either side of the tergites where the cutinization of the endocuticle is wanting (Text-fig. 3 c and e). Folding of the tergites occurs also along this line (Text-fig. 3 b').

Ducts of Dermal Glands.—Besides the fine ‘pore canals’ which do not pierce the surface of the epicuticle, the cuticle is traversed by a great number of relatively large ducts connected with the dermal glands. In the nymphs these are fairly evenly distributed over the tergites and sternites of the abdomen, though they are more numerous in the immediate vicinity of the bristles. In the adult, they are evenly distributed over the sternites, but in the tergites they are very scanty in the central region (except over the last two segments) and are concentrated at the sides of the abdomen.

Their distribution is most readily demonstrated by placing the tergites or sternites (removed from a freshly killed insect) without fixation, in 0.05 per cent. methylene blue in Ringer's solution, and then fixing with ammonium picrate and acidified ammonium molybdate (Dogiel's method). This stains selectively the dried secretion around the orifices of the ducts, and renders them very conspicuous. Text-fig. 4 A shows the distribution of the ducts as demonstrated in this way; and Text-fig. 4 B the detailed appearance of the stained secretion. It is this secretion which constitutes the 'Sekretschicht' already mentioned (Text-fig. 1, sec).

The ducts of dermal glands are themselves sometimes referred to as ‘pore canals’ (the term is used in this sense, for example, by Henke (1924) in the case of Pyrrhocoris apterus) and it has sometimes been stated that they occur only in the pigmented areas. In unstained preparations this appears to be the case in Rhodnius, but only because the orifices of the ducts are difficult to make out in unpigmented places.
Text-fig. 4 A shows the lack of any true relation with the pigment, and this confirms the observations of Henke (1924) on *Pyrrhocoris*.

2. **The Structure of the Epidermis.**

Text-fig. 5 is a slightly schematized longitudinal section of the cuticle to show the various elements which compose the ectoderm or epidermis (hypodermis).

The epidermal cells themselves (Text-fig. 5, *kd*) are usually conical with their pointed ends attached to the basement membrane, and their broad ends giving off very delicate processes which extend into the 'pore canals' of the cuticle. They contain numerous spheres of a brick-red pigment.

The epidermal cells beneath the plaques that bear the bristles
differ from the others in being stuffed with spheres of uric acid and containing very little of the red pigment. These will be referred to as urate cells (uc). In the centre of these are the large trichogen or hair-forming cell (hc) and the 'tormogen' or socket-forming cell (sc).

Between the bases of the epidermal cells are the following structures: (i) the vestiges of dermal glands (gl) with their ducts opening through the cuticle; these are developed and functional only during moulting and will be described later; (ii) the oenocytes (oe), large cells with uniform eosinophil

1 The chemical form of the uric acid, whether free or combined, is unknown. It gives a murexide test and Folin's colour reaction, and forms a precipitate with silver nitrate which quickly blackens in the light.

2 Greek τόρμος: 'any hole or socket, in which a pin or peg is stuck' (Liddell and Scott).
cytoplasm; and (iii) undifferentiated or embryonic cells (ec), some of which, in due course, will give rise to the new dermal glands at the time of moulting, while others will give rise to the next generation of oenocytes.

The basement membrane (bm) is well developed and spreads as an unbroken sheet all over the body-wall, save where it is pierced at intervals by the muscles and by small tracheae, which break up into numerous tracheoles that ramify in all directions beneath the epidermis, adding their matrix cells to the elements mentioned above. Adhering to the lower surface of the basement membrane are haematocytes (h) of several kinds.

The fat body forms a rather slight fenestrated sheet below and often adherent to the basement membrane. It contains only a single type of cell, urate cells and oenocytes being restricted to the other side of the basement membrane.


Methods.—The morphological changes in the various elements mentioned in the last section have been studied throughout the fourth and fifth nymphaal instars, and in adults of both sexes at all stages of digestion and reproduction.

Many of these changes are most readily observed in ‘whole mounts’ of the abdominal tergites and sternites. The dorsal and ventral walls of the abdomen have been fixed in Carnoy, freed from fat body and other deep structures, and stained with haematoxylin and eosin; or, for studying the uric acid cells and the pigment of the epidermis, they have been mounted unstained. A corresponding series of preparations has been embedded in paraffin and cut into serial longitudinal sections. Certain other experimental procedures for the elucidation of particular points will be described in the course of the argument.

Duration of Moulting.—The fourth nymphs moult for the most part on the fifteenth and sixteenth days after feeding, though some have moulted as early as the thirteenth day and

1 The insects have been kept throughout at 24° C. in a damp incubator; all the times quoted refer to these conditions.
one as late as the twenty-first. The cause of these differences is obscure; they are certainly not due to differences in the interval since the previous moult, for batches of ten insects fed 1 week, 2 weeks, 3 weeks, 6 weeks, and 9 weeks after moulting all showed the same degree of variation, more than half the insects in each batch always moulting on the fifteenth and sixteenth days.

In the fifth nymphs, moulting has occurred from the twenty-fifth to the thirty-first day after feeding, but most individuals moulting on the twenty-eighth day.

In the descriptions which follow, it will be convenient to allot a standard time of fifteen days to the fourth moult and twenty-eight days to the fifth, and to describe the sequence of events as though all the insects kept rigidly to the same timetable.

4. **THE EPIDERMAL CELLS, URIC ACID CELLS, AND THE RED PIGMENT.**

In the fourth nymph, within a day or so after feeding, the epidermal cells swell up and the granules of red pigment they contain begin to increase. By the fourth day, many of the cells along the intersegmental membranes have the nuclear chromatin shrunken away from the wall of the nucleus, and by the fifth day mitosis has begun. Multiplication is most intense along the intersegmental membranes, but by the sixth day mitotic figures can be seen all over the tergites and sternites (Text-fig. 6 A).

Now it is obvious that, in order to divide, the cell must detach itself from the cuticle, and it is highly probable that once it has become detached it must remain so. Hence, if division occurs in all the epidermal cells (and it is so widespread that this appears to be the case), then once it has taken place over the whole surface of the body, the insect will have freed itself from the old cuticle; it only remains for the new cuticle to be laid down and the old cuticle to be absorbed for moulting to be complete.

Conversely, it is clear that multiplication of the epidermal cells cannot take place once the new cuticle is laid down, and
thus it is that the first appearance of the cuticle coincides with the cessation of mitosis at about the ninth day.

During this multiplication process, the red pigment continues to increase, reaching a maximum at the eighth or ninth day. The increase is most marked along the intersegmental membranes, where the cells become so densely crowded and later folded to form a triple layer. Exactly parallel with the pigment the uric acid also increases. The urate cells, as they become stuffed with the uric acid spheres, form opaque white spots beneath the bristles; and besides these cells there are others, scattered at intervals along the intersegmental membranes and along the margin of the abdomen where the tergites and sternites join, which also become charged with uric acid. Like the red pigment, the uric acid reaches a maximum at about the eighth day. Thereafter it begins to disappear, and by the time moulting takes place it is greatly reduced. The pigment follows the same course, but it diminishes more slowly.

**Text-fig. 6.**

A. Epidermal cells on abdominal tergites of fourth nymph six days after feeding. B. Epidermal cells on abdominal tergites of fifth nymph nine days after feeding, showing uric acid spheres many of which have formed around granules of red pigment, shown black (unstained).
(Spheres of uric acid (or urate) were observed by Poisson (1925) in the epidermal cells of Notonecta in the nymphal stages, but no explanation of the phenomenon was suggested.)

What is the significance of this accumulation? The uric acid is certainly a waste product, but since it is the chief excretory substance removed from the blood by the Malpighian tubes (Wigglesworth, 1931), it seems more probable that the uric acid in the urate cells is produced locally, than that it is taken up from the blood. Now one of the chief constituents of the cuticle is chitin, a substance very poor in nitrogen, and this is produced by the insect from the highly nitrogenous blood protein on which it feeds. Consequently in the formation of chitin there must be a large production of nitrogenous waste, and it seems likely that this is the explanation of the appearance of uric acid in the epidermis during ecdysis.

It remains to be explained why the uric acid is confined to certain cells. Now it is very striking that it is deposited chiefly in the cells below the plaques bearing the bristles. These cells are crowded into deep pits, and are therefore much more protected from the circulating blood than the epidermal cells elsewhere; and it is possibly this circumstance, that the uric acid is produced more rapidly than it can be eliminated, which is responsible for its crystallization.¹ There are two other facts which lend colour to this hypothesis: (i) uric acid spheres appear also in other places sheltered from the blood, for example, along the intersegmental membranes, at the margin of the abdomen where the tergites and sternites unite, and in the mid-dorsal line where the heart is closely applied to the body-wall; (ii) as soon as the cells retract from the pits below the bristles, the uric acid begins to disappear; but soon the new pits are formed and uric acid persists in the depths of these until the next moult. On the other hand, in the formation of the adult tergites, in which these pits do not occur, the disappearance of the uric acid takes place very strikingly as soon as the new cuticle begins to form. By the time moulting occurs very little remains; most

¹ On the other hand, uric acid spheres do not occur in the hair-forming and socket-forming cells which lie at the centre of the urate cells.
of this dissolves away during the next ten days, and none reappears after feeding in the adult stage.

If the view put forward here is correct, the uric acid that appears in the epidermal cells is simply the product of their own metabolic activity and there is no reason to regard these cells as organs of excretion.

The red pigment of the epidermal cells increases in amount exactly parallel with the uric acid, and this suggests that it also is an excretory by-product of the synthetic activity of these cells. It is much more widespread than the uric acid, occurring in all the epidermal cells, but this may be due to its much greater insolubility. There is no sharp distinction between the cells that contain uric acid and those that contain pigment; many of the cells contain both, the red granules of pigment serving as nuclei upon which the uric acid crystallizes out (Text-fig. 6 b).

The fate of the pigment is probably the same as that of the uric acid—slow diffusion into the blood and excretion by the Malpighian tubes. It is an exceedingly insoluble substance, and this perhaps explains the slowness of its disappearance; but after passing a maximum it gradually decreases, and in the adult insect that has moulted some weeks before very little remains. Perhaps this is the source of the yellow pigment that occurs in the urine in Rhodnius (Wigglesworth, 1931), for the brick-red pigment and the yellow pigments of Hemiptera are closely related (Henke, 1924).

In the fifth nymph the sequence of events is precisely the same; mitoses occur from the sixth to the fifteenth day, and cease just before the new cuticle is laid down. The uric acid and red pigment reach a maximum round about the twelfth day. None of these changes take place after a meal in the adult; they are all clearly associated with moulting.

5. Formation of the New Cuticle and Bristles.

In the Fourth Nymph.—At the ninth day, when mitoses are almost at an end, the epidermal cells can readily be detached as a continuous layer from the old cuticle; and if this layer is examined in surface view, it is seen to be covered by an exceedingly delicate smooth membrane which in stained.
preparations shows a finely fibrillar structure. By the tenth day (Text-fig. 7 A) this membrane shows signs of crinkling, and by the eleventh day it is thrown into conspicuous stellate folds (Text-fig. 7 B). It therefore corresponds clearly with the epicuticle, but at this stage it stains with haematoxylin.

![Text-fig. 7.](image)

Longitudinal sections showing formation of new cuticle during moulting of fourth nymph. A. Ten days after feeding. B. Eleven days. C. Twelve days. D. Just before moulting (fifteen days). E. Twenty-four hours after moulting (now fifth nymph). F. Four days after moulting.

It is interesting to consider the mechanism by which the folding of this membrane may be effected. The folding must be a spontaneous change occurring in the membrane itself, for the folds bear no relation to the arrangement of the underlying cells. The secretion of the cells evidently coagulates at first to form a smooth membrane, which then expands (perhaps by the imbibition of water from the moulting fluid outside it) and
CUTICLE OF RHODNIUS

becomes folded, falling naturally into stellate folds (see Text-fig. 2A) like those in the skin which appears at the surface of hot milk.

At this stage the cuticle contains no chitin, and it quickly dissolves on heating in saturated caustic potash. It gives a very weakly positive Millon's reaction, but on warming in the Millon's reagent, it persists for a time as a colourless membrane free from protein, and then dissolves. Although evidently the epicuticle, it has not yet acquired its resistant qualities.

The membrane that is going to form the plaques around the bristles does not become folded but remains smoothly adherent to the 'urate cells' beneath it. Then these cells develop large basal vacuoles which cause the cuticular surface to bulge outwards; in this way the raised plaques are moulded (Text-fig. 7B and C).

At the same time the formative cell of the bristle, which lies at the centre of the group of urate cells, sends out a fibrillar prolongation which forms the basis of the new bristle (see p. 289).

As soon as the epicuticle is laid down, the formation of the endocuticle begins, and the chitosan test applied at the twelfth day reveals a delicate chitinous sheet moulded to the lower surface of the epicuticle. The endocuticle shows its vertically striated appearance almost from the outset. The exact mechanism of its formation is not known, but it must evidently be separated from the cells in fluid form around the protoplasmic filaments that occupy the 'pore canals', because there is no sign of any partitioning ('Felderung') of the endocuticle to correspond with the underlying epithelium, such as occurs in those cases where the cuticle arises by a transformation of the superficial layers of the epidermal cells. It can often be noticed that the granules of pigment in the epidermal cells arrange themselves in vertical lines (Text-fig. 9C and D). This suggests that there are invisible filaments running through the cells, perhaps continuous with those in the 'pore canals'.

As Berlese (1909) points out, the formation of the chitinous parts of Arthropods is certainly accomplished in many different ways. A fluid secretion may condense into a solid sheet, as in the formation of the peritrophic membrane in many insects.
(Vignon, 1901; Wigglesworth, 1930), or the chitin may be separated within the body of the cell itself, as in the formation of the cuticle of certain Crustacea (Vitzou, 1882) and in the development of cuticular hairs and bristles (see p. 289). The mechanism in Rhodnius seems to agree most closely with that described by Leydig (1864) and by Braun (1875) in Astacus, by Holmgren (1902 a) in the oviduct of Calliphora, and by Poisson (1924) in aquatic Hemiptera, which consists in the deposition of chitin in fluid or semi-fluid form around filiform outgrowths from the epidermal cells, which outgrowths later constitute the pore canals. As has often been pointed out before, this mechanism recalls the formation of dentine by the odontoblasts of vertebrates (Hass, 1916).

By the thirteenth day, when the endocuticle is about one-third its final thickness, the epicuticle and the bristles are becoming amber coloured; they are more resistant to solution in concentrated nitric acid than the endocuticle, but dissolve fairly easily on heating. The endocuticle contains its normal constituents: protein and chitin. At this stage it is very difficult to detect any horizontal striation of the endocuticle.

Text-fig. 7 D shows the appearance of the cuticle at the time of moulting, before the epicuticle has darkened. Twenty-four hours later (Text-fig. 7 E) the epicuticle has darkened and the inner layer of the endocuticle is appearing. By the end of four days (Text-fig. 7 F) the endocuticle is fully developed. It will be seen that as the pore canals cross the boundary between the inner and outer layers, they often change their direction slightly. This indicates that at the time of moulting the cuticle has been displaced somewhat in relation to the epidermal cells.

In the fifth nymph the sequence of events is the same. The non-chitinous epicuticle first appears at about the sixteenth day after feeding. It becomes slightly folded during the seventeenth and eighteenth days, and then the chitinous endocuticle is formed. At the time of moulting, the bristles are strongly 'cuticulinized' and amber coloured, but the impregnation of the outer part of the endocuticle with 'cuticulin' is incomplete, and this layer is still almost colourless. It darkens and hardens
during the next day or so, while the inner half of the endocuticle is being deposited.

The most important conclusion from these observations is that the outermost layer, the epicuticle, is laid down first, and the endocuticle then formed below it; and that the impregnation of the cuticle with 'cuticulin' takes place progressively while the endocuticle is being laid down. This impregnation may possibly continue after moulting, or it may be that the 'cuticulin' already present merely undergoes oxidation when exposed to the air, becoming more resistant to reagents, like a 'drying oil' or varnish.

This mode of formation of the epicuticle agrees with that described in Leptinotarsa by Tower (1906), who calls the epicuticle 'primary cuticula' and the endocuticle 'secondary cuticula'. It is contrary to the belief of Schulze (1913) deduced from a study of the elytra of beetles, that the 'Grenzlamelle' (epicuticle) is formed by the outpouring of the secretion of the dermal glands over the surface of the endocuticle; nor does it agree with the recent observations of Yonge (1932), who has shown that the outer layer of the cuticle of Decapod Crustacea is almost certainly formed by the dermal glands in this way.\(^1\)

**Formation of the Bristles.**—The method of formation of the bristles agrees exactly with that described in Saturniid larvae by Haffer (1921) and in Hyponometa (Lep.) by Hufnagel (1918). Two cells are concerned in the formation of each bristle, a hair-forming cell ('Haarbildungszelle'), and a socket-forming cell ('Haarpfannenzelle'). Haffer describes how the hair-forming cell sends out a process which passes through the annular socket-forming cell 'as the finger is passed through a signet ring'. He describes and figures this process from sections, and it is similarly figured from the fourth nymph of Rhodnius in Text-fig. 7. But the aptness of the signet ring analogy is best appreciated when the process is studied in surface view. Text-fig. 8 shows several stages in the formation of the bristles.

\(^1\) It may be pointed out here that the outer layer of the cuticle in Decapod Crustacea as described by Yonge has very different properties from the epicuticle of insects; it stains with basic dyes, and with lipoid stains, and it readily imbibes water.
on the sternites during the moulting of the fifth nymph, as seen in whole mounts.

The outgrowth stains rather deeply at first and is covered by a delicate basophil membrane continuous with the newly formed epicuticle over the epidermal cells. When fully grown, the bristle gradually becomes eosinophil; finally, it becomes amber coloured and impregnated with 'cuticulin' and will not stain at all. A central canal always remains. This process demonstrates very clearly (i) the deposition of chitin within a cellular outgrowth, and (ii) the progressive impregnation of this chitin with 'cuticulin'.

Text-fig. 8 A shows a basophil filament (f) which lies immediately in front of the bristle. This represents the other end of the filament (f) shown in Text-fig. 10 A and comes from the corresponding bristle of the old cuticle. Judging from Hafler's account, this is perhaps the distal filament of the neurone which innervates the bristle, but the nerve supply of these structures
has not been studied in *Rhodnius*. Apparently the same hair-forming and socket-forming cells persist from one moult to the next.

**Pigmentation of the Cuticle.**—The late progressive changes in the cuticle, described above, suggest that the epidermal cells, presumably by way of the pore-canals, can still exert an influence upon the epicuticle after the endocuticle is formed; and that this is so is proved by certain observations on the pigmentation of the cuticle. In one experiment, which has been several times repeated and confirmed, the old cuticle was stripped from the region of the fourth and fifth abdominal tergites in a fifth nymph forty-eight hours before moulting. The epicuticle was already fully formed; but instead of darkening within an hour or so after exposure to the air, as occurs in the insect moulting normally, the exposed area remained pink and soft for two days. The insect then moulted, and pigmentation at once began in all parts of the body.

In many parts of the cuticle, particularly in the nymphs, melanin is restricted to the epicuticle. We have already seen (p. 278) that the formation of melanin cannot be connected with the secretion of the dermal glands. The influence which induces pigmentation must therefore be exerted from within the body, through the substance of the epicuticle. Perhaps this is the function of the pore-canals—to enable the epidermal cells to effect enzymic changes in the epicuticle after moulting is complete.

6. **The Dermal Glands and the Moulting Fluid.**

The dermal glands are formed anew at each moult from undifferentiated or embryonic cells in the epidermis. They are functional only during moulting and then degenerate. They are absent in the adult.

In the fourth nymph they first become recognizable at about the eighth day after feeding as clusters of four cells, each of which has a nucleus of a distinct type which it is not necessary to describe in detail. Clearly each of these cells is destined to contribute a different element to the final gland. One becomes the secretory cell proper, one forms the intracellular duct of the
secretory cell, one appears to be responsible for the part of the
duct which passes through the epidermis and will later be
included in the new cuticle, and perhaps the fourth supplies a
delicate capsule for the whole structure. In the late stages of
development the fourth cell often cannot be found.

Text-fig. 9 A shows the rudiments of a gland at the eighth
day; the duct is being laid down, and it is already evident which
cell is going to form the gland proper. By the eleventh day
(Text-fig. 9 B) the gland-cell is becoming vacuolated and the
duct is well developed. Up to this stage it is impossible to detect
any certain differences between one gland and another, but in
the later stages of moulting there are two very distinct types.
Text-fig. 9 c and d shows these at the time of maximum activity
shortly before moulting. In type 'A' (Text-fig. 9 c) the gland-
cell is excessively vacuolated, the vacuoles being larger peri-
pherally; the intracellular duct ends in a small round vesicle,
which in some sections contains material staining blue with
haematoxylin, and the whole structure lies close beneath the
cuticle. In type 'B' (Text-fig. 9 d) the vacuolation of the gland-
cell is not so extreme, there is a very large intracellular saccule
distended with blue staining secretion, and the gland lies below
the general level of the epidermal cells. The duct of type 'B'
is narrower. Both types are evenly distributed all over the
tergites and sternites, but there is always a group of three or
four immediately round each bristle. Type 'B' is by far the
more numerous.

In the fifth nymph the two types are distinguishable at
a relatively much earlier stage. By the sixteenth day type 'A'
is already recognizable (Text-fig. 10, a), but type 'B' is still at
the stage of four scarcely differentiated cells (Text-fig. 10, b).
The subsequent development of type 'A' is just like that in the
fourth nymph, the vacuolation becoming more and more
extreme so that the adjacent epidermal cells are forced aside;
Text-fig. 10, g shows their appearance at the twenty-seventh
day, shortly before moulting. Text-fig. 10, b to f, shows the
subsequent development of type 'B', as seen in whole mounts.
When fully formed (Text-fig. 10, f) the gland-cell is less vacuo-
lated than in type 'A', but the intracellular duct is very similar,
Formation of dermal glands during moulting of fourth nymph; longitudinal sections.

A. Eight days after feeding; cells separated from cuticle, new dermal gland forming.
B. Eleven days after feeding; dermal gland becoming active; new epicuticle formed; digestion of old cuticle just beginning.
C. Fourteen days after feeding; dermal gland type 'A' at height of activity; old cuticle almost entirely digested, the part in process of digestion appears as blue staining layer.
D. Thirteen days after feeding; dermal gland type 'B' fully active, with its saccule containing basophil material. oc, old cuticle; dc, layer of old cuticle in process of digestion; nc, new cuticle.
ending in a small round vesicle and not a large saccule as it does in the fourth nymphs.

In the sternites of the fifth nymph (that is to say the sternites of the developing adult) both types are evenly distributed; but in the tergites, type 'A' occur chiefly over the more central regions, while type 'B' are concentrated in enormous numbers along the margins of the segments (Text-fig. 4 A).

During moulting the cells composing the old moulting glands break down; their nuclei undergo chromatolysis, and chromatin spheres derived from them are set free among the epidermal cells. These will be mentioned again later (p. 308).

Function of the Dermal Glands.—Dermal glands associated with moulting occur in many groups of insects (Plotnikow, 1904). They were first recognized by Verson (1902) in the larva of the silkworm, and he ascribed to them two functions: (i) the production of an exuvial fluid which serves
as a lubricant and facilitates the casting of the old skin, and
(ii) the excretion of urates and oxalates vicariously on behalf
of the Malpighian tubes, which are supposed to be temporarily
out of action through a sort of physiological stoppage.

The excretory function of these organs can probably be
dismissed, for it was shown by Plotnikow (1904) that the
crystals which appear in the exuvial fluid are really derived
from the Malpighian tubes, and have spread forwards from the
anus beneath the old cuticle; an observation which has recently
been confirmed by Shimizu (1931). There is no sign of any
excretory substances in the moulting fluid of *Rhodnius*.

The origin of the moulting fluid from the dermal glands has
been generally accepted by Plotnikow (1904), Tower (1906),
Schulze (1912), &c.; on the other hand, v. Buddenbrock (1930,
1931), in the case of Lepidoptera, regards this fluid as a product
of the epidermal cells (as also do some other authors, notably
Blunck (1923) in *Dytiscus*), and gives some rather indirect
evidence for the view that the glands of Verson are endocrine
organs discharging into the blood of the larva a hormone which
induces moulting.1 In the case of *Rhodnius* this hypothesis
is untenable, because moulting (as indicated by the mitosis and
separation of the epidermal cells) has already begun by the
fifth day after feeding in the fourth nymph and by the seventh
day after feeding in the fifth nymph—long before the dermal
glands are differentiated, far less fully active. Moreover, the
ducts of the glands are so distinct that there is no reason to
doubt that they are responsible for the secretion of the moulting
fluid.

The chief question is the function of this fluid. The majority
of authors regard it primarily as a lubricant to facilitate
moulting. Yet it is a matter of observation that most insects
are almost dry at the time the skin is cast, whereas if they are
dissected a day or two before moulting, there is abundant fluid
present. It is probable, therefore, that the main function of
the moulting fluid is accomplished before ecdysis actually occurs.

Various authors (Plotnikow, 1904; Tower, 1906) have noted
that the old cuticle is largely softened and absorbed during

1 See also Hoop (1933).
moult ing, and Tower suggests that perhaps an enzyme is concerned in this process; but the extent to which such absorption occurs does not seem to have been generally appreciated. It is illustrated by the following observations.

The abdominal tergites and sternites were dissected off ten fifth nymphs of *Rhodnius* twenty-four hours after feeding, freed from cellular tissue by rubbing the inner surfaces with cotton wool, dried in the air and weighed. The average weight was 3.7 mg. The corresponding parts were then dissected from the cast skins of ten fifth nymphs. They showed an average weight of 0.5 mg.; in other words, 86.5 per cent. of the abdominal cuticle has been digested and reabsorbed by the insect.

The course of this absorption is shown clearly in Text-fig. 9. It begins when the dermal glands become functional (at about the eleventh day in the fourth nymph and the twenty-first day in the fifth nymph) and it is most rapid in the last few days of moulting when these glands are at their zenith. Thus it is almost certain that they are responsible for the digestion of the cuticle, and their secretion must therefore contain both a chitinase and a proteinase.¹ (Perhaps each type of gland is responsible for one of these enzymes, but this possibility has not been proved experimentally.)

The properties of the moulting fluid have been further studied by stripping off the old cuticle from the fifth nymph a few days before moulting and collecting the fluid in a capillary tube. It is a neutral fluid (pH about 7 as tested with phenol red and B.D.H. universal indicator; litmus being turned slightly bluish); it appears to be free from chloride, giving no precipitate with silver nitrate and nitric acid, but it shows the protein colour reactions.

Only the endocuticle is digested by the moulting fluid; the epicuticle is quite untouched, a fact which can be very clearly demonstrated in the following way. If the old cuticle is stripped off in the last few days of moulting, a very delicate moist

¹ A minute fragment of congo red fibrin prepared by the method of Roaf (1908) was inserted beneath the old cuticle of a fifth nymph three days before moulting. When the skin was cast three days later, there was a small pink stained area on the cast skin at the site of the fibrin particle.
pellicle usually separates from its inner surface. This is the innermost layer in process of digestion. If it is mounted in water and examined microscopically, it is found to bear a great number of little sacs connected to fine ducts (Text-fig. 10 A, s). These are the linings of the old dermal glands that have been cast; they belong to the epicuticle and have so escaped digestion.¹ Their epicuticular nature is readily shown by heating this membrane for a minute or so in strong potash and then treating with iodine. The membrane gives a violet colour; the saccules and ducts stain bright yellow. On further heating in potash they dissolve entirely.

Were the moulting fluid capable of digesting the epicuticle, there is no apparent reason why the new cuticle also should not be digested. It is protected by the epicuticle being laid down first (see p. 286).

Now the quantity of fluid present at a given moment is very small; it amounts to little more than a moist film (see Text-fig. 9 s and p). It is obvious, therefore, that if the relatively massive endocuticle is to be digested and absorbed, there must be a continuous circulation of the moulting fluid carrying the digestion products into the body of the insect; and this raises the question of the route by which this absorption takes place.

The only author who appears to have considered the question of absorption of the moulting fluid is Wachter (1930), who gives a very good description of moulting in the silk-worm; but she was concerned not with a circulation of the fluid but only with the absorption that takes place just before ecdysis, and she found that in the silk-worm much of the fluid is then swallowed. It seemed very unlikely that it should be ingested by the mouth in Rhodnius, and this possibility was readily excluded by tying a tight ligature round the front of the head at an early stage of moulting. The absorption of the old cuticle and the casting of the skin occurred normally, although the front part of the head was missing and the mouth occluded.²

¹ That the ducts of the moulting glands are not dissolved by the moulting fluid was observed also by Plotnikow (1904).
² The insect did not extricate itself completely, and the wings were not fully expanded; but this experiment shows incidentally that in
The question was then studied by taking a number of fifth nymphs at the twenty-fifth day after feeding (that is about three days before moulting), making a small cut with scissors along the margin of the abdomen without injury to the new cuticle, and injecting beneath the old cuticle a saturated solution of neutral red in distilled water. This stained the old endocuticle around the site of injection deep red. Insects dissected next day showed neutral red in the lower segments of the Malpighian tubes (Wigglesworth, 1931) and a diffuse staining of the new cuticle with the dye round the site of injection.

On succeeding days, as the old cuticle was digested and absorbed, the colour at the site of injection became less, and when insects moulted the old cuticle showed only a very faint colour and there was a slight diffuse staining of the new cuticle.

The same results were obtained with indigo-carmine, which appeared in crystalline form in the Malpighian tubes although this dye seemed to inhibit to some extent the digestion of the cuticle around the site of injection.

These experiments demonstrate clearly the continuous absorption of the fluid, and they suggest very strongly that the absorption takes place through the general surface of the new cuticle.

If this is so, the new cuticle cannot attain its waterproof properties until the very end of the moulting period, and that this is the case can be shown by stripping the old cuticle away from the abdomen a day or two before moulting. Within twenty-four hours the abdomen is greatly shrunk and desiccated, although the insect remains alive for several days. It is true that at this stage the endocuticle is not fully formed, but it is almost certain that in the normal insect the impermeability of the cuticle to water is due entirely to the epicuticle or 'Grenzlamelle' (Kühnelt, 1928).

It is thus pretty certain that the function of the dermal glands in Rhodnius is to digest the old cuticle and make it available to the insect for the formation of the new cuticle; a process Rhodnius the swallowing of air is not an essential process, at least in the early stages of casting the old skin.
which forms an interesting commentary on the view that chitin in insects is a waste product (Bounoure, 1919) and ecdysis an excretory phenomenon.

But one of the objects of the present investigation was to find out whether the function of the dermal glands in *Rhodnius* was the same as that recently demonstrated by Yonge (1932) in Decapod Crustacea, namely, to form the outermost layer of the cuticle (epicuticle) by pouring their secretion over the surface of the endocuticle; or perhaps to contribute to the epicuticle those constituents which render it waterproof. It will therefore be desirable to give the reasons for abandoning this hypothesis.

We have seen that the secretion in the ducts of the dermal glands is strongly basophil; it stains just like the moulting fluid and the innermost layer of the old cuticle as it is being dissolved (Text-fig. 9, d), whereas the new epicuticle is unstained during the later part of moulting when the dermal glands are most active. Furthermore, there is no reason to doubt that the basophil secretion which is present around the orifices of the ducts in the adult, and which collects within an hour or two after moulting, represents the normal product of the glands; and this secretion remains soft and basophil throughout the life of the insect. It never hardens into a waterproof layer like the epicuticle.

7. THE OENO CYTES.

The oenocytes in *Rhodnius* are all confined between the basement membrane and the epidermis; so that by stripping off the tergites and sternites of the abdomen and mounting them whole, it is possible to expose the entire oenocyte system in a single preparation. Their distribution is the same at all stages of the life-history. On the tergites they are concentrated at the sides, along the intersegmental membranes, and all over the first segment (Text-fig. 11 A). On the sternites their distribution is similar, but the concentration in the first segment is not so marked.

They are very variable in size and shape, sometimes reaching a diameter of 100 μ. Their eosinophil cytoplasm is usually homogenous, or very finely vacuolated; occasionally it is faintly granular (Text-fig. 11, c and d); often, in fixed material, it
shows spindle-shaped clefts or canals (which may be artefacts), as described in *Thrixion* by Pantel (1898) (Text-fig. 11, e, f), and sometimes it contains elongated, apparently crystalline bodies, best seen in fresh preparations, like those described in many insects by Hollande (1914) (Text-fig. 11, h, i). The cytoplasm is not naturally yellow as it is in many insects, nor does it contain granules of pigment.

**TEXT-FIG. 11.**

A. Distribution of oenocytes on abdominal tergites of fifth nymph (schematic). B. Forms of oenocytes as seen in fifth nymph. a–d, at time of feeding; e–g, six days after feeding; h, fourteen days; i–k, twenty-six days after feeding.

**Changes in the Oenocytes during Moulting.**—During moulting the oenocytes go through a well-defined cycle, which is illustrated in the case of the fourth nymph in Text-fig. 12. At the time of feeding (Text-fig. 12 a) they are of two kinds: (a) large solitary more or less lobulated forms with the chromatin massed chiefly near the centre of the nucleus, and (b) small rounded forms, evidently a new generation, which are nearly always in pairs and are sometimes united by an unbroken strand of cytoplasm; in these the chromatin granules are more evenly distributed over the nuclear membrane.
TEXT-FIG. 12.

Changes in oenocytes during moulting of fourth nymph. A. Before feeding. B. Five days after feeding. C. Nine days after feeding; several cells showing chromatolysis; chromatin droplets free among epidermal cells. D. Twelve days after feeding; new generation present in pairs. E. At time of moulting. F. Six days after moulting.
After feeding, the oenocytes grow rapidly and the nuclei of the small forms acquire the characters of the large forms (Text-fig. 12 a). By the ninth day (Text-fig. 12 c) they have attained their maximum size, and most have lobes like pseudopodia projecting from their periphery. At this stage many of the older generation break down completely; the cytoplasm disintegrates, and the chromatin of the nuclei collects in deeply staining spheres; the nuclear membrane then dissolves, and these spheres are set free among the bases of the epidermal cells. It is difficult to judge how many of the oenocytes disintegrate in this way, but I am of the opinion that none of the young generation are concerned and only a part of the older generation.

(Chromatolysis and dissolution of a certain number of the oenocytes with the formation of 'chromatin droplets' was described by Weissenberg (1907) during pupation of the Chalcid Torymus and by Stendell (1912) in Ephesia. Poyarkoff (1910), Pérez (1910), Hufnagel (1918), Poisson (1924), and others have observed spheres or droplets of this type among the epidermal cells of various insects at the time of moulting, and have usually regarded them as waste products discharged from the nuclei during a process of rejuvenation. We shall return to the matter of these spheres in dealing with the basement membrane and its cells.)

After the 9th day the oenocytes gradually become smaller. Many large lobulated forms persist until the twelfth day or later (Text-fig. 12 d), but by the time of moulting, at the fifteenth day (Text-fig. 12 e) they are markedly reduced. Reduction continues for a few days after moulting (Text-fig. 12 f), and then they show no further change until another meal initiates a new moulting cycle.

It can now be seen (Text-fig. 12 r) that a new generation of oenocytes has arisen; young forms lie in pairs between the older forms, just as these lay among their predecessors before the last moult began. If the series of preparations is traced backwards in the reverse order, it is found that these young oenocytes have not arisen from the older generation of oenocytes, but from undifferentiated cells in the epidermis. These paired cells can be recognized as early as the tenth day, but before this they
cannot be distinguished with certainty from the cells which are going to form the new dermal glands.

Thus a new generation of oenocytes arises from the epidermis at each moult, although some of the old oenocytes also persist. This recalls the conditions described by Weissenberg (1907) in the Chalcid Torymus at the time of pupation, when the imaginal oenocytes arise from the epidermis, and a part only of the larval oenocytes disintegrates. It appears to be a general rule in metabolic insects (see Karawajew (1898) in Lasius (Hym.), Verson (1900) in Bombyx mori, Kreuscher (1922) in Dytiscus, &c.) for a second generation of oenocytes to arise from the ectoderm at the time of pupation; but the appearance of a new generation at each moult, such as occurs in Rhodnius, does not seem to have been described before, although Poisson (1924) notes that, in Notonecta, oenocytes continue to arise from very small sub-epidermal cells throughout post-embryonic life.

During the moulting of the fifth nymph the oenocytes go through the same cycle. The young cells grow rapidly and reach a maximum at about the fourteenth day, when many of the old cells break down; they remain very conspicuous until about the twenty-fourth day, and during the last few days they become reduced in size. During the moulting of the fifth nymph, however, no new forms appear, so that the oenocytes present in the adult are mainly those which arose during the moulting of the fourth nymph. This is analogous to what happens in metabolic insects, where the so-called 'imaginal oenocytes' always arise at the time of pupation, that is, during the penultimate moult.

Cyclical changes of a more or less similar nature and associated with the moulting rhythm were described by Verson and Bisson (1891) in the oenocytes of the silk-worm, and more recently by Albro (1930) in the Chrysomelid beetle Galeruella. No other authors seem to have found any strict relation between the secretory changes in the oenocytes and the moulting cycle, although changes in volume during metamorphosis have been very commonly observed.

Behaviour of the Oenocytes in the Adult.—The
oenocytes persist throughout the life of the adult *Rhodnius*; but this does not necessarily imply that they are still functional at this stage, for the epidermal cells and the matrix cells of the tracheae also persist, although their function of laying down the cuticle is completed a few days after the final moult. The most important question is whether the oenocytes show signs of renewed activity at any stage, and to decide this they have been examined in both sexes at all periods of fasting, digestion, and reproduction.

After the final moult, the oenocytes in both sexes tend to lose their regular distribution and collect together into clumps, in which the cell boundaries are very difficult to detect. Their cytoplasm is considerably reduced as compared with their state during moulting, but this reduction is not so extreme in the female. Text-fig 13 A and B shows a typical group of oenocytes in both sexes three weeks after moulting.

At this stage the fasting adults were fed and allowed to mate, and the oenocytes examined on succeeding days. In the males they show only a very slight increase in size, whereas in the females they swell up markedly and develop large 'pseudopodia'. Text-fig. 13 A' and B' shows this difference between the sexes ten days after the feed; the same difference persists until the digestion of the blood is complete, that is throughout the period of oviposition.

Function of the Oenocytes.—At the present day there is general agreement that the oenocytes are secretory organs which discharge their products into the blood (Koller, 1929), and their behaviour in *Rhodnius* during moulting and oviposition leaves no doubt that this view is correct.

The chief question is the nature or function of their secretion. Koller (1929) has suggested that it may be a hormone which induces moulting; but in the case of *Rhodnius*, moulting, as indicated by the mitoses of the epidermal cells, has begun within five or six days after feeding, whereas the secretory activity of the oenocytes does not reach its maximum until about the ninth day in the fourth nymph and the fourteenth day in the fifth nymph. Moreover, the bulk of the oenocytes in relation to the size of the insect is so great that it seems more
likely that they are contributing some material substance to
the insect body, than that they are producing only a physiologi-
cal stimulant or hormone.

Now the cycle of secretory activity in the oenocytes of

**TEXT-FIG. 13.**

A. Oenocytes in adult male, three weeks after moulting, unfed. B. Ditto, in female. A'. Oenocytes in adult male, ten days after feeding (thirty-one days after moulting). B'. Ditto, in female.

*Rhodnius* is clearly associated with moulting, and not merely with digestion and assimilation. For in the first place, the oenocytes do not reach their maximum until ten days or so after the meal; and in the second place, when the insect moults,
the stomach still contains a large amount of undigested blood which is assimilated during the ensuing weeks. Yet the oenocytes decline markedly as soon as moulting has occurred; indeed, they are becoming reduced before the old skin is cast. They cannot, therefore, be concerned in any of the ordinary processes of intermediary metabolism; they must contribute something specific to ecdysis.

Now the most important changes in the moulting of a non-metabolic insect like Rhodnius are the growth of the epidermis, the digestion of the old cuticle, and the formation of the new. We have seen that the oenocytes show their greatest activity after the growth of the epidermis is complete; they cannot, therefore, be concerned in the process of growth as such. We have seen that the dermal glands are probably responsible for the digestion of the old cuticle, and that there is certainly no 'genetic relation' between the oenocytes and the dermal glands, such as Kremer (1925) claims to have shown in Melasoma (Col.). Moreover, if the oenocytes were connected with the digestion of the cuticle, it would be difficult to understand their renewed activity in the adult female. It is therefore reasonable to suppose that the oenocytes are concerned in the formation of the new cuticle; and that they synthesize, and secrete into the blood, materials which go to form a part of this cuticle.2

There are certain facts which favour this hypothesis. (i) The oenocytes are specialized epidermal cells, and this suggests that they have some functional relation with the cuticle. (ii) The oenocytes reach their maximum size just before the new cuticle is laid down (at the ninth day after feeding in the fourth nymph, and the fourteenth day in the fifth nymph). (iii) They become reduced as the cuticle is formed; and this reduction continues, as does the formation of the cuticle, for some days after moulting.

1 The mid-gut of Rhodnius consists of a capacious 'stomach' and a narrow convoluted 'intestine'. The blood is stored in the stomach but is not digested until passed on into the intestine. It may take five or six weeks before this store of undigested blood in the stomach is exhausted.

2 A similar suggestion was put forward in the posthumous paper by Willers (1916).
(Stendell (1912) showed that in *Ephestia* (Lep.), the oenocytes are large and active during intra-ovum development, when the cuticle of the larva is being formed, but they are greatly reduced in the newly hatched larva.)

The next question is the behaviour of the oenocytes in the adult. In both sexes, during reproduction, there is abundant formation of new tissue (eggs and spermatozoa), and in both there is digestion, excretion, and the synthesis of fat &c., in the fat body; but the oenocytes become conspicuously active again only in the female. Now the most obvious difference between the sexes, from a chemical point of view, is the formation of the egg-shells by the follicular cells of the female, and the egg-shells have much in common with the cuticle of the insect itself. They do not contain chitin, but they are composed of an insoluble protein and are covered by a very delicate outer membrane\(^1\) which corresponds with the "cuticulin" of the insect's epicuticle.

If, therefore, it is supposed that the oenocytes synthesize the protein or the "cuticulin" elements of the cuticle, then both the cycle of their activity during moulting and their renewed activity in the adult female are understandable. It is reasonable to suppose that by relieving the epidermal cells themselves of this synthetic function, the oenocytes will accelerate the actual deposition of the cuticle. The extraordinary rapidity with which the solid substance of the cuticle is laid down may be judged from Text-figs. 7 and 9.

Whether this hypothesis will obtain support from other groups of insects only further observations can show. There is little information available on the behaviour of the oenocytes in the adult insect, and still less on sexual differences. Wielowiejski (1886) describes differences between the oenocytes of male and female *Tipula*, but does not clearly state that they

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\(^1\) If the empty shell is immersed in strong nitric acid, it becomes bright yellow and bubbles of gas are evolved. But these bubbles come only from the inside of the egg, and when they have ceased to come off there remains this delicate outer membrane. It is insoluble in strong hydrochloric and sulphuric acids in the cold, dissolves readily in alkali, the biuret reaction is negative, and it gives a yellow colour with iodine.
are larger in the female. Hollande (1914) records that in many insects the oenocytes diminish in volume after mating in the male and oviposition in the female. Kremer (1925) observed that in young adults of *Melasoma* (Col., Chrysomelidae), in the autumn, the oenocytes show a progressive reduction in size in spite of the insect taking in a large supply of food, and the fat body becoming enlarged ready for hibernation. But during sexual activity in the following summer they become enlarged again, and this enlargement is greater in the males. The conditions here, however, are not quite comparable with those in *Rhodnius* because Kremer describes as oenocytes the enlarged fat body cells charged with albuminoid materials. He regards the oenocytes of the adult beetle as metabolic intermediaries ('Vermittler') between the fat body and sexual organs; at the time of mating they are said to have practically disappeared.

8. The Basement Membrane and Haematocytes.

Text-fig. 14A shows the appearance of the basement membrane in the fourth nymph at the time of feeding. Two chief kinds of cell occur on its surface: stellate cells with radiating processes and granular inclusions, and rounded cells with clear eosinophil cytoplasm devoid of granules. Here and there, especially around the tracheae where they pierce the basement membrane and along the sides of the dorsal vessel, there are clumps of haematocytes of varied form and size (Text-fig. 15A).

A few days after feeding, all these cells begin to multiply exceedingly and numerous mitotic figures are seen; by the fifth day many parts of the basement membrane are covered with densely packed cells, which present a most varied appearance. Many of them are of the stellate type described above; most are spindle-shaped or pyriform, and scattered through them are always some of the rounded eosinophil type.

The time of greatest development of these cells is reached about the eighth or ninth day (Text-fig. 14B), when the oenocytes are breaking down and the 'chromatin droplets' are most abundant in the epidermis. At this time many of the cells below the basement membrane become charged with similar blue staining droplets or granules.
After the tenth day, when the ‘chromatin droplets’ are no longer present in the epidermis, many of the cells on the base-

ment membrane begin to degenerate and they are then ingested by their neighbours (Text-fig. 14 c). In this way their numbers become greatly reduced, and by the time of moulting they are
relatively sparse (although occasional mitoses can still be seen), save in the occasional clumps around the tracheae and at the sides of the dorsal vessel.

If Indian ink is injected into a nymph at, say, twenty-four hours after feeding, most of the stellate cells forsake the basement membrane, take up particles of the Indian ink and collect in clumps, like the haematocytes, along the dorsal heart and elsewhere.\(^1\) But the round eosinophil cells do not engage in this phagocytic activity (Text-fig. 15 B).

These observations suggest that all these cells are simply haematocytes, which multiply and collect over the surface of the basement membrane during moulting because that is where their phagocytic activities are in greatest demand.

On the other hand, the appearance shown in Text-figs. 14 A and B recalls that described by Mayer (1896), and suggests that the stellate cells at least are matrix cells of the basement

\(^1\) Muttkowski (1924) has shown how amoebocytes in insect blood apply themselves to smooth surfaces and assume the stellate form described above.
membrane, which might be supposed to arise from their modified cytoplasm.

If the views of Lazarenko (1925) are correct, there is nothing incompatible about these two alternatives. For, according to this author, the connective tissue membranes of insects (of which the epidermal basement membrane is a typical example) are formed in just this way—by the transformation of the cytoplasm of phagocytic blood-cells.

All the forms present among these cells can be readily homologized with the leucocytes described by Hollande (1911) in Pyrrhocoris apterus. (i) The small forms with little cytoplasm and large nucleus, frequently seen dividing, are the 'proleucocytes'; (ii) the larger forms showing endless variation in shape and size are the 'phagocytes'; and (iii) the round forms with eosinophil cytoplasm are the 'oenocytoids'. Finally (iv) there appear, especially towards the end of the moulting period (Text-fig. 14 D), large forms with abundant fat droplets in the cytoplasm. These are the 'adipoleucocytes' of Hollande, the ultimate fate of which in Rhodnius has not been traced.

The function of the rounded 'oenocytoids' is unknown; they certainly have no connexion with the oenocytes as suggested by Poisson (1924) for similar cells in the aquatic Hemiptera. But all the other types appear to take up and digest the cellular débris resulting from the breakdown of the oenocytes and the old dermal glands and possibly some of the epidermal cells themselves. Poyarkoff (1910), Pérez (1910), and Hufnagel (1918) have described how the basophil spheres and granules in the epidermis of Galerucella (Col.), Calliphora (Dipt.), and Hyponomeuta (Lep.) are taken up by the phagocytes; and the role of phagocytes in the histolysis of the muscles of Diptera and Hymenoptera is well known (Pérez, 1910, 1911). The only difficulty about this view is that in Rhodnius the basement membrane appears always to be a continuous unbroken sheet.

During moulting in the fifth nymph, the haematocytes go through the same cycle of changes. They multiply to reach a maximum about the fourteenth day, becoming filled with granular débris at this time, when the oenocytes, &c., are breaking down; they then become reduced by ingesting one another.
The same wide range of forms is present as in the fourth nymph. In the adult they forsake the basement membrane, for the most part, and persist chiefly in clumps along the tracheae and at the sides of the dorsal heart. They do not show any marked increase after feeding or during oviposition. Their activity is clearly associated with moulting and not merely with digestion and assimilation.

9. SUMMARY AND CONCLUSIONS.

1. Structure and Composition of the Cuticle.

The cuticle of *Rhodnius* consists of two primary layers: a very thin epicuticle ('Grenzlamelle') and a relatively thick endocuticle traversed by fine pore canals containing protoplasmic filaments or fluid.

The endocuticle is composed of protein and chitin. The epicuticle is composed of material for which the name 'cuticulin' is proposed, which has chemical properties like those of the cutin or suberin of plants and is perhaps a complex mixture of fatty or waxy substances. Melanin is often present in the epicuticle.

The endocuticle is made up of two parts separated by a distinct horizontal line; of these, only the outer is laid down before moulting. The endocuticle shows also rather faint horizontal laminæ.

In the adult, over the greater part of the abdominal tergites and sternites, the outer part of the endocuticle is impregnated with 'cuticulin' more or less mixed with melanin. This layer corresponds with the 'Emailschicht', 'Pigmentschicht', or 'exocuticle' of various authors. In the nymphs, the endocuticle of the abdomen is impregnated with cuticulin only below the little plaques which bear the bristles. Consequently, the stretching of the abdomen to receive the large meals of blood is accomplished by a different mechanism in nymphs and adult (p. 276).

The cuticle is traversed by numerous ducts opening to the exterior. In the nymphs and in the abdominal sternites of the adult, these are evenly distributed; in the tergites of the adult
they occur chiefly at the sides of the abdomen. Their distribution is not related to the pigmentation of the cuticle.

Around the orifice of each duct is a little patch of dried secretion which stains readily with methylene blue. This corresponds, presumably, with the 'Sekretschicht'; but in *Rhodnius* it is not an integral part of the cuticle.

2. Structure of the Epidermis (hypodermis).

This consists mainly of conical cells containing granules of a brick-red pigment; but beneath the bristles, and in some other places, the cells are stuffed with spheres of uric acid. Among the epidermal cells are the dermal glands, the oenocytes, and embryonic cells which give rise to new dermal glands and oenocytes at the time of moulting. The basement membrane is well developed, and many haematocytes adhere to its lower surface.


After a full meal, the fourth nymph moults, as a rule, in fifteen days at 24° C. and the fifth nymph in twenty-eight days. These times do not seem to be affected by the duration of the fast preceding the meal.

4. The Epidermis, Red Pigment, and Uric Acid Cells.

The epidermal cells swell up within a day or two after feeding; then they separate from the cuticle, and multiply by mitosis from the fifth to the ninth day in the fourth nymph and from the sixth to the fifteenth day in the fifth nymph. The red pigment and the uric acid both increase until about the eighth day in the fourth nymph and the twelfth day in the fifth nymph. The uric acid then decreases rapidly; the red pigment more slowly.

It is suggested that both the uric acid and the red pigment may be waste products of the synthesis of various constituents of the cuticle. They reach a maximum shortly before the cuticle is laid down.
5. The Formation of the New Cuticle.

The non-chitinous epicuticle is laid down first. It appears as a smooth membrane which later becomes folded, particularly in the nymphs. The chitinous endocuticle is laid down below the epicuticle. It appears to be secreted in fluid form around filamentous processes from the epidermal cells which become the pore canals.

The active intervention of the underlying cells, effected through the endocuticle, presumably by way of the pore canals, is necessary for the pigmentation of the epicuticle at the time of moulting. The impregnation of the outer part of the endocuticle with 'cuticulin' also appears to take place gradually after the endocuticle is formed. It is suggested that the function of the pore canals may be to permit this action at a distance.

The bristles are formed by trichogen cells; these give out processes which pass through annular 'tormogen' or socket-forming cells and later become chitinized and impregnated with cuticulin.

6. The Dermal Glands.

These are associated with moulting, and afterwards degenerate. They are formed anew at each moult from embryonic cells in the epidermis. Their development and structure is described. They are of two distinct types, one of which is much more numerous than the other.

These glands secrete the moulting fluid, the chief function of which is to digest the old cuticle. Their secretion must contain a proteinase and a chitinase. All but about 14 per cent. of the old cuticle of the abdomen is digested and absorbed; but the epicuticular structures, such as the linings of the ducts of the old dermal glands, are not affected. Experimental evidence is given that there is a continuous circulation of the moulting fluid, and that the products of digestion are absorbed through the general surface of the new cuticle, which is shown not to develop its waterproof properties until the time of moulting. When the skin is cast, practically no moulting fluid is present.

The dermal glands appear to play no part in the formation of the new cuticle.
7. The Oenocytes.

The oenocytes are all confined between the basement membrane and the epidermis. A new generation arises at each moult (except the last) from the embryonic cells in the epidermis.

During moulting they go through a definite cycle, growing rapidly and reaching a maximum just before the new cuticle is laid down; that is, at the ninth day in the fourth nymph and the fourteenth day in the fifth nymph. At this stage many of them undergo chromatolysis with the liberation of chromatin droplets. From this point until the time of moulting the survivors gradually become reduced in size.

The oenocytes persist in the adult; after feeding, they become conspicuously active again in the female, but show very little change in the male.

It is suggested that they synthesize some of the (non-chitinous) constituents of the cuticle during moulting, and of the egg-shells during maturation of the ova.

8. The Basement Membrane and Haematocytes.

The cells of the basement membrane appear to be haematocytes. The forms of those present are described.

During moulting they increase greatly by mitosis, and the phagocytic forms become charged with blue staining granules and débris possibly derived from the chromatolysis of the oenocytes, old dermal glands, and perhaps some of the epidermal cells themselves. In the later stages of moulting their numbers are reduced. Many of them degenerate and are ingested by the survivors.

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CUTICLE OF RHODNIUS

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