a. *Megoperculata* m.[42].

4. Ordnung: *Pedipalpi* Latr.

5. - *Araneae* Sund.

b. *Cryptoperculata* m.[42].

6. Ordnung: *Meridogastra* Thor. (*Cryptostemma* Guér.).

B. *Holotracheata* m.

a. *Holosomata* Pocock.

7. Ordnung: *Chelonethi* Thor.

8. - *Opiliones* Sund.

9. - *Acarina* Nitzsch.

b. *Mycetophora* Pocock.

10. Ordnung: *Solifugae* Sund.

Marburg, den 20. Februar 1902.

---

2. Observations on the Structure of the Exuvial Glands and the Formation of the Exuvial Fluid in Insects.

By W. L. Tower.

(With 8 figs.)

eingeg. 22. Februar 1902.

In Insects, the end of one developmental stage and the beginning of another are marked by the casting of the cuticular covering, and of the intima of all ducts and tracheae and of the alimentary canal. These are then replaced by a fresh development of the cuticula over all the hypodermal tissues thus laid bare. This process has been repeatedly observed from the outside, and we know in detail the manner of the casting and the time required to complete it in many insects. Of the internal changes, however, which take place during ecdysis, we have almost no mention, excepting a few scattered and incomplete accounts.

The observations which are recorded in this paper were made upon larvae of the Chrysomelid beetle, *Leptinotarsa decem-lineata* Say.

My material was killed in:

Saturated Cor. Sub. ($HgCl_2$) in 35 % alcohol . . .	70 ccm.
Glacial Acetic Acid	30 ccm.

This was used for one minute or less at a temperature of 60°—65° C. and was followed by a solution of:

Saturated Cor. Sub. ($HgCl_2$) in 35 % alcohol . . .	99 ccm.
Glacial Acetic Acid	1 ccm.

---

[42] Μέγας = groß, operculum = Deckel, wegen der großen Bauchplatte (Genitalopercula) des 2. Mesosomalsegmentes, die die Geschlechtsausführungsgänge nach außen bedeckt; κρυπτός = verborgen, operculum, wegen des Fehlens der großen Bauchplatte vor der Geschlechtsöffnung.

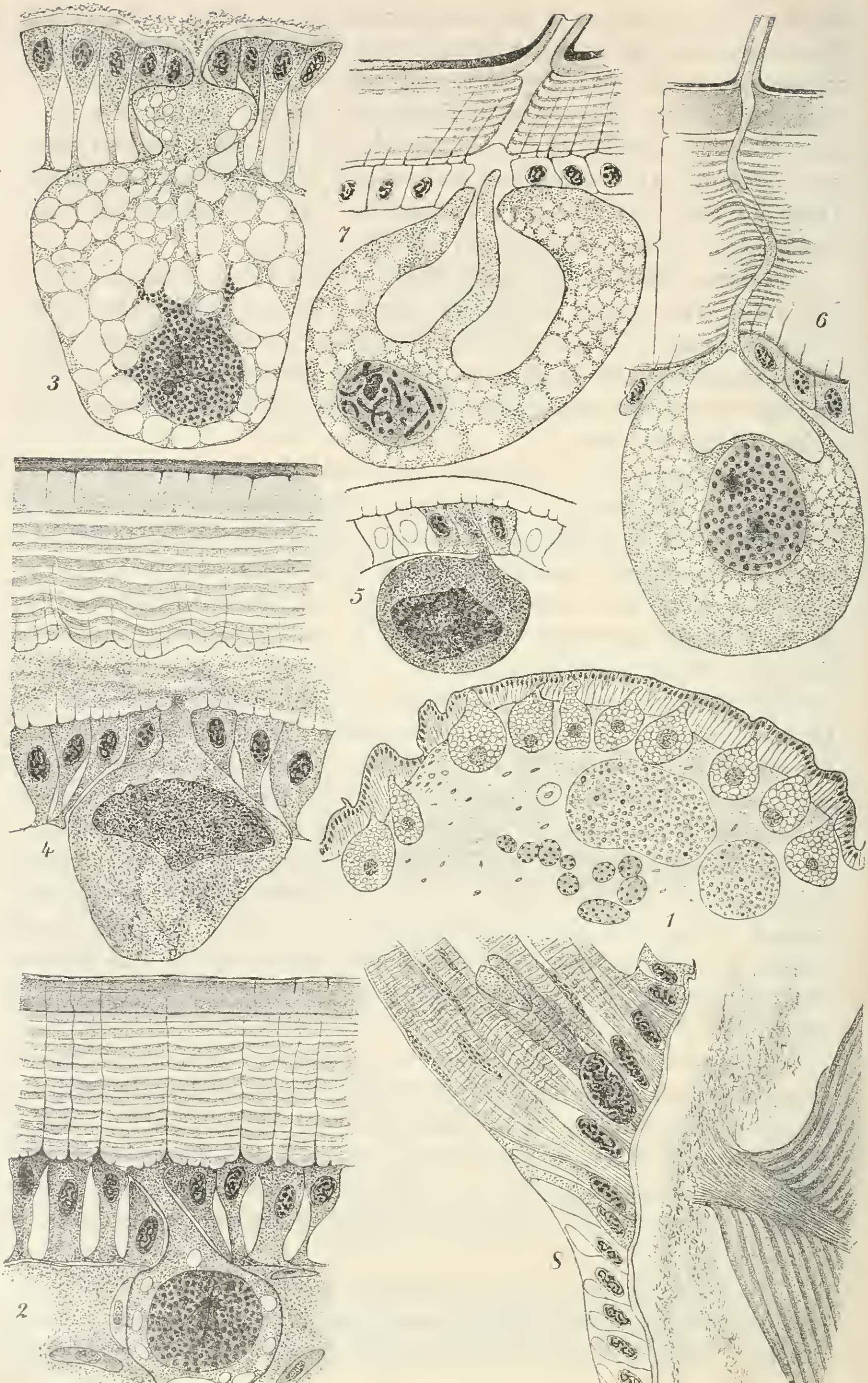
for from thirty minutes to six hours according to the size of the larvae. Specimens were put into the first solution alive and were then quickly withdrawn, cut in several places, and put into the weaker solution. The material was dehydrated in the usual manner, cleared in a mixture of Cedar oil and Xylol, and imbedded in paraffin. Sections were cut $6^2/_3 \mu$ and 10μ in thickness, and stained with Heidenhain's Iron Haematoxylin, and Mayer's Haemalum, and double stained with Orange G., or Zwaardemaker's Safranin.

Material killed in Perenyi's fluid, several Picric Acid mixtures, hot water, Hermann, Fleming (strong formula) and vom Rath, was also studied. The three latter gave results which were good, but not as uniform as the sublimate-acetic acid mixtures. I found Perenyi and the Picric compounds entirely unreliable, whereas hot water gave only a fair preservation.

The existence of a fluid between the old cuticula and the new at ecdysis was first observed by Newport. This discovery has been confirmed many times by others, but Gonin (1894) was the first to demonstrate its presence by modern histological methods. According to the observations of this author and of Professor Bugnion (Packard, 1898), a liquid was found which nearly or quite covered the surface of the larva (*Pieris brassicae*) at ecdysis. At the same time, the authors noticed certain large cells, lying as Bugnion states, in the region between the head and the prothoracic segments. These cells were seen to be extruding between the two layers of cuticula a substance which was coagulated by the reagents, but they did not seem to the authors to be true glands like the unicellular glands of other forms. The authors further state that if these cells do function as organs secreting this exuvial fluid, they ought to be found scattered all over the body instead of restricted to a small area, and this seemed to them a good reason for thinking that these cells do not secrete all of this fluid or perhaps any of it, but rather that it is secreted by the hypodermis in every part of the body.

In the form which I have studied, there is a distinct, and often a large coagulum in sections made of larvae that are undergoing ecdysis. Not only at pupation, as has been observed by Newport, Gonin, Bugnion and others, but at every moult this fluid is formed in greater or less abundance, separating the old and new cuticulas, but it is most copious at the moult which the animal undergoes when the larva becomes a pupa.

In *L. decem-lineata* there are found at ecdysis in, or just below the hypodermis, large, highly vacuolated cells with deeply staining nuclei. These cells often send blunt processes through the hypodermis


3
7
6
5
4
1
2
8

All figures are from sections of *L. decem-lineata.* and were drawn with a Abbe Camera. from material killed in Acetic Sublimate and stained with Mayer's Haemalum.

Fig. 1. Transverse section of the pronotum of a pro-pupa just before the final transformation, showing the closely packed, and highly vacuolated exuvial cells. Bausch & Lomb $^2/_3$ in. obj., 2 in. oc., 160 mm.

Fig. 2. Exuvial cell in process of degeneration. Bausch & Lomb $^1/_8$ in. obj., 1 in. oc., 160 mm.

Fig. 3. Exuvial cell in the most extended condition pouring out its contents between the old and freshly forming cuticula. Bausch & Lomb $^1/_6$ in. obj., 1 in. oc., 160 mm.

Fig. 4. Exuvial cell in stage of degeneration. Bausch & Lomb $^1/_6$ in. obj., 1 in. oc., 170 mm.

Fig. 5. Exuvial gland in a late stage of degeneration, just before being attacked by phagocytes. Bausch & Lomb $^1/_6$ in. obj., 1 in. oc., 170 mm.

Fig. 6. Exuvial or setigerous cell in larvae of third instar. Bausch & Lomb $^1/_8$ in. obj , 1 in. oc., 160 mm.

Fig. 7. Setigerous cell in the third larval stage just before ecdysis, showing a stage in the formation of a new hair. Bausch & Lomb $^1/_8$ in. obj., 1 in. oc., 165 mm.

Fig. 8. Section of the body wall showing separation of the old cuticula from the hypodermis by the exuvial fluid in the pro-pupal stage. Bausch & Lomb $^1/_6$ in. obj., 2 in. oc., 160 mm.

by which they are seen to be pouring out their contents (Fig. 8) between the old and the rapidly forming new layer of cuticula. They are most abundant along the anterior edge of the pronotum, where they are often thickly crowded together (Fig. 1), but they are also found scattered irregularly over the body surface, being quite abundant about the base of the legs. In sections made through a larva about to pupate these cells (Fig. 1) are found to be well developed and distended with a secretion (Figs. 1 and 3). Soon, however, their contents are extruded between the old cuticula and the hypodermis, forming a thin layer between them. The hypodermal cells are now perfectly naked and no trace of the new cuticula can be found (Fig. 4).

At this time these cells are large with a deeply staining nucleus and two dense nucleoli. They connect with the exterior by a more or less blunt process which may open in a small pore (Fig. 3) or in a large rupture in one side of the cell. The cell wall is relatively thin, but perfectly distinct, and shows no modifications of any kind. The Cytoplasm is composed of a watery albuminous material in the form of globules surrounded by the denser plasma. The vacuoles found in preparations are probably in life filled with a fluid which is extracted in preparation, for they remain unstained by anything I have used. Only in rare cases have I found a faint staining of the contents of these vacuoles. The plasma is finely granular and shows no characters of interest. The nucleus of these cells is large, round or oval in shape, and often branching more or less (Fig. 3) in fine dendritic processes between the chylema globules of the cytoplasm. The nuclear membrane is at this stage sharply defined over the body of the nucleus, but less so on the ramifications. The densely granular nucleoplasm is filled with a large number of rounded, deeply staining bodies which react like chromatin towards all stains and alone are stained by thionin. A delicate linin reticulum connects these bodies with one another. Two dense masses — nucleoli (?) —are rather characteristic of this stage. These stain intensely and from them radiate larger fibres through the nuclear reticulum, often in the form of an aster, but there is no regularity in this character or in their position.

It is these cells which have been seen by Gonin and Bugnion, at pupation, when they are large and extremely vacuolated. Bugnion, however, doubts the glandular character of these cells, but offers no explanation of their origin, use, or fate. In the embryo and younger larval stages these cells are not found in the same condition in which Gonin found them or as described above, but they exist as different structures as far as appearance and function are concerned. The changes which these cells undergo during larval life are interesting. In a late embryonic stage certain cells of the hypodermis are seen to be enlarging, the nucleus becomes large and round and develops a dense nucleolus. This growth continues until near the time for hatching, when the cell develops a single hollow hair. After hatching and during the early larval stages these short hollow hairs are scattered over the entire surface of the body. They are most numerous in the first larval stage, becoming less and less so until all trace of them is lost in the fourth larval instar.

The hair is a hollow cuticular tube open at the distal end and continuous with the primary cuticula at the proximal end (Fig. 6, 1). Its interior is filled with protoplasm which is continuous witht hat of the cell beneath the hypodermis, connection between the hair and cell being maintained by a delicate canal which pierces the cuticula. The cell body is composed of rather dense protoplasm with numerous small, clear vacuoles. The nucleus is large, its diameter being equal to the radius of the cell. The nuclear membrane is well developed and numerous small chromatin bodies are scattered through the nucleoplasm and connected by a linin network. One or two densely staining bodies are found in each nucleus and may be the nucleoli or aggregations of chromatin. I have been unable to detect any nerve fibres connected with these cells.

This condition is found in the middle of one of the early instars, while the animal is actively feeding. When the time draws near for ecdysis, the protoplasmic process is withdrawn from the hair and canal, and the cell itself draws away from the cuticula, leaving a small cavity in which the hair lies. At first the hair is without any chitin and has only a thin external layer of homogeneous substance. The hair may be much curved as it lies in the cavity (Fig. 7), which is filled with a fluid secretion of the cell. After the hair is completely formed the secretion of this fluid is very rapid, the cavity becomes large and the nucleus and protoplasm of the cell are crowded down and to one side. The fluid, however, is not stored in the cell, but in the cup-shaped invagination in which the hair lies. Small globules of this material can be seen in the cytoplasm, but they are evidently extruded before any great amount is collected in the cell. This secretion is largely water, but a small precipitate forms when the animal is killed. This precipitate is finely granular, occasionally stringy, and stains precisely like the exuvial fluid which is found a few hours later, separating the hypodermis and cuticula. It does not resemble the haemolymph: it stains differently with all analins; and it is more finely granular and often shows a peculiar stellate form of coagulation which the haemolymph never does. This latter stellate form of coagulation appears after killing with sublimate acetic, Hermann, Fleming (strong), or vom Rath, and is probably not due to the peculiar action of any one killing fluid.

The withdrawal of the hair, and the secretion of the fluid require from thirty to eighty hours for their completion. When the time for ecdysis arrives, the hair and the liquid contents of the cavity about it, are, by the contraction of the cell, extruded between the old cuticula and the hypodermis. The liquid serves as a cushion, protecting the delicate hypodermis from injury by being brought into too violent contact with the hard cuticula, and allowing room for the new cuticula to form. This now develops as a thin, continuous layer covering the whole body surface as well as the hair. In the later stages of the larva the hairs are lost and these cells remain as glandular looking cells lying beneath the cuticula. It was in this stage that I first noticed them. In the younger larval stages the cells do not change much in character during ecdysis, excepting that there is a decrease of size due to the extrusion of material to form the exuvial fluid. In the last two larval stages almost all the hairs have been lost and these cells exist as large unicellular glands, which now take on the peculiar and characteristic structure shown in Fig. 3.

At pupation these cells are turgescent with the exuvial fluid (Fig. 1), which is extruded between the hypodermis and cuticula, separating them. During the expansion of the wings, mouth parts and legs, and the formation of the pupa, this fluid surrounds them, and its solid part is gradually precipitated upon the surface of the animal, leaving the more fluid portion in contact with the old cuticula. When the rupture of the old cuticula comes and the fluid is exposed to air it hardens, and cements the appendages to the body. However, if the pupa be put into water or weak alcohol the cementing material is dissolved, and the appendages are set free, a fact also observed by Gonin and Bugnion. I am ignorant of the nature of this fluid.

In the pupa most of these cells degenerate, and during the life of the pupa many entirely disappear. A considerable number remain upon the pronotum to secrete enough fluid to start the animal in the final ecdysis. In the breaking down of one of these cells the body of the cell becomes densely granular (Fig. 2), the nucleus exceedingly granular, and the nucleoli(?) are lost (Fig. 4). This continues until the cell body is small, the nuclear membrane lost, and the nucleus a mass of irregular, dense granules (Fig. 5). Soon the cell is detached from the hypodermis, and floats free in the haemolymph, where it is destroyed.

In *Clisiocampa americana*, I have observed the cells which form the hair and secrete the exuvial fluid degenerate to a condition like that shown in Fig. 5, then develop into one of the large scales which cover the body of the imago.

Summary: The exuvial glands are not true glands, but the setigerous cells which, in early life, are chiefly concerned with the formation of the hairs upon the body; but upon the loss of these, the cell takes on the function of secreting the exuvial fluid, which is most copious at pupation. These cells degenerate in the pupa, and take no part in the formation of the imaginal ornamentation.

Hull Zoological Laboratory. University of Chicago, Chicago, Ill., U. S. A.
1 Feb. 1902.

Bibliography.

1894. Gonin, J., Recherches sur la métamorphose des lépidoptères. De la formation des appendices imaginaux dans la chenille du *Pieris brassicae*. Bull. Soc. Vaud. sci. Nat. Vol. XXX. pls. 11—15. p. 89—138.
1898. Packard, A. S., A Text-Book of Entomology. New York. (XVII, 729 p.
1898. Bugnion, E., Letter to Packard (1898). p. 613—615.

3. Verzeichnis der in der näheren Umgebung von Göttingen gesammelten Milben.

Von Hans Voigts, Oslebshausen (Bremen).

eingeg. 5. März 1902.

Ende April bis Anfang Juni vor. Jahres habe ich in der näheren Umgebung von Göttingen (i. Hannover) auf meinen coleopterologischen Excursionen auch auf die Milben und Collembolen meine Sammelthätigkeit ausgedehnt, die erfreulicher Weise recht gute Resultate ergeben hat. In liebenswürdiger Weise hat die Bestimmung der Milben