THE INTEGUMENT OF THE LARVA OF THE ALDER FLEA BEETLE.

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Summary.

As in insects generally, the body wall of the larva of the alder flea beetle (*Altica bimarginata* Say: Coleoptera, Chrysomelidae) is composed of three layers: (1) a *cuticula,* secreted by (2) an underlying *hypodermis,* which rests upon (3) a structureless *basement membrane.* Three sorts of glands are differentiated in the hypodermal layer: molting fluid glands, glands connected with the trichogens, and "prothoracic glands."

The Cuticula.

The cuticula of the body wall consists of three layers: a very thin faintly staining *epicuticula* (the "*Grenzhäutchen*" of German authors), a deeply pigmented *primary cuticula* which does not stain with an eosin-haematoxylin combination, and a non-pigmented *secondary cuticula* which does stain. This staining reaction has probably little significance: i.e., in another chrysomelid larva (*Deloyala clavata* Fabr.) under similar conditions the primary cuticula stains violet, and the secondary pinkish.

The cuticula is covered with conspicuous nodules which lie thickly scattered between the setiferous tubercles; their appearance in a caustic potash preparation is shown in figure 1 and in cross section in figure 2. Together with the tubercles these nodules are by far the most deeply pigmented portions of the cuticula, although pigment granules occur in other parts. The nodule, most of which is primary cuticula, is covered by a comparatively thick cap of epicuticula which is very deeply pigmented.

The writer has made no chemical tests and does not know the extent of chitinization in the cuticula. The primary layer is probably the only sclerotized portion: (1) the cuticula of a newly molted larva is devoid of pigment; sclerotization after ecdysis is correlated with pigmentation, and with the exception noted above, pigment is restricted to the primary cuticula; (2) the secondary cuticula increases in thickness during the instar, whereas sclerotization is complete two or three hours subsequent to the molt.

Even the primary cuticula must be somewhat elastic since the integument stretches considerably during the instar; the cuticula nodules lie much closer together just after the molt than they do a few days later. This is a generalization true of this type of chrysomelid larvae. In all those studied, the internodular cuticula is sufficiently transparent to allow the fat body to show through so as to determine the general body color of the larva; in most species, it is yellow or orange as in *bimarginata,* but in *A. corni* Woods, for example, it is white. In each species the color of the eggs and of the pupae is correlated with that of the fat body, which is constant in color throughout all the stages.

The body wall is of course continuous with the lining of the fore-intestine, the hind-intestine, the spiracular invaginations and those of the salivary glands. Primary and secondary intima may be traced in the fore- and hind-intestines, and at the very beginning of the spiracular invaginations. The writer cannot detect any such differentiation in the tracheae or in the salivary glands (even at the point of invagination), and there is apparently no "epi-intima" developed anywhere, the epicuticula ceasing at the point of invagination.

The Hypodermis.

The hypodermis consists of cuboidal or rectangular cells, somewhat variable in size and shape, which present as such no special modifications in the larva, Late in the prepupal period, the cells become greatly attenuated, a condition quite different from the typical columnar epithelium of the larva and one which persists through most of the pupal instar. Except in the region of the imaginal discs, the hypodermis is but a single layer of cells in thickness.

The Basement Membrane.

The basement membrane is a delicate structureless limiting membrane developed at the base of the hypodermal cells. In well fixed material, its presence is always clear, but the writer has never detected any trace of nuclei in it.

The Molting Fluid Glands

The molting fluid glands are the most conspicuous of the hypodermal glands. They are large unicellular glands, occurring in all parts of the body apparently without regular arrangement, not infrequent in sections, but not developed in great numbers. The writer prefers to treat them as unicellular glands, although strictly each gland consists of three cells, two small cells which serve as neck or guard cells, and a much larger secreting cell.

In larvae sectioned immediately after ecdysis, the glands are not charged with secretion vacuoles, and the canaliculi through which the gland empties may be seen plainly. (The writer has no data as to whether or not these canaliculi are chitinized.) The course of this canal is quite variable. It is always somewhat convoluted, and apparently is always two branched, the forking occurring usually close to the cuticula as in figure 4, but sometimes farther back as in figure 3. (As the portions of the canal lie at different levels, these figures are necessarily reconstructions from several successive serial sections.)

Both figure 3 and figure 4 are drawn from a larva fixed during ecdysis. The molting fluid is elaborated during the instar and the gland gradually becomes charged with secretion vacuoles. During this process the glands become somewhat but not very greatly enlarged. An enlarged and vacuolar gland, drawn from a two day prepupa, is shown in figure 5. Typically the discharge of the molting fluid takes place on this day, for sections of three day prepupae usually show the glands somewhat reduced in size and without secretion vacuoles, a very faint trace of the forming pupal cuticula, and a coagulum between the hypodermis and the now separated larval cuticula.

Since well formed molting fluid glands are to be found in sections of larvae fixed at eclosion or just after ecdysis, and since there is no appearance of "incipient" glands in a larva just ready to molt, the writer believes that each gland is persistent from embryonic life up to the pupal stadium, and functions several times. (The cells do increase in actual size, but the increase is proportional with those of the hypodermis and epithelium.) These glands persist through the prepupal period (after their functioning on the second day) in a sort of resting condition, and the writer has found them in sections of pupae up to the fifth day of pupal life. He is unable to state definitely when and how they disappear.

That the molting fluid complex is entirely surrounded by the basement membrane is always clear in well fixed material. The writer has already suggested his interpretation of the three cells of this complex. The two upper cells, much smaller than the lower, are protecting neck cells. Frequently if not typically the nucleus of one cells is decidedly smaller than that of the other.

The cell divisions, always difficult to make out, were in no case examined so sharply defined as in the figures. In some series only one of the neck cell nuclei is apparent (as in figure 4), but the writer does not consider this a normal condition even though careful search has failed to reveal the missing nucleus. The lowermost cell is the secreting cell, but the canaliculi go without break through the neck cells.

The writer does not believe that the canaliculi ever open by a cuticular pore to the outside, as has been figured for certain species. He has found no evidence of such a pore, and apparently the molting fluid is poured out when the new cuticula is just forming, before it has covered over the hypodermis at the base of the gland. Were the canaliculi to penetrate through special pores, it is evident that new glands would have to be formed at each ecdysis, which is almost certainly not the case.

In caustic potash preparations, what seems to be cuticular pores appear rarely in the setiferous tubercles. These pores are really trichopores, and usually a tiny seta may be found connected with them. In figure 1, such a trichopore with a tiny seta is shown on the left of the tubercle, and on the right is a trichopore where the seta has entirely failed to develop. These pores have nothing to do with the molting fluid; they are vestiges which indicate (as comparative studies make abundantly clear) that the evolution of the setal pattern in *Altica* has been a process of reduction.

As one would naturally expect, the molting fluid glands of *Altica* are almost exactly similar to those of *Galerucella,* which Poyarkoff (1910, p. 31–40, fig. 12) has described as the "glandes à trois noyaux" of the larva, save that in the elm leaf beetle the neck is somewhat more drawn out, and the cell divisions are even less apparent than in *Altica.*

The Trichogen Cells.

The setae of the larvae are hollow and each one is connected with two cells, the seta forming, or trichogen cell proper, and an associated gland cell. The hair is slightly enlarged at the tip, where there is a pore to permit the exit of the secretion of the gland (see figure 1). The secretion is odorless and is not put forth in droplets when the larva is disturbed. It certainly is not of a repugnatorial nature. Since the integument sheds water easily, and since minute particles easily adhere to the more or less sticky skin, the secretion is perhaps of a waxy nature. The writer interprets the setae as primarily sense hairs, with which a glandular secretion (perhaps, as the larvae feed exposed on the upper surface of the leaf, concerned with making the integument waterproof, so as to shed rain and to check evaporation) is secondarily and incidentally associated.

In a larva fixed and sectioned immediately after a molt, the gland cell extends some little distance up into the seta (see figure 6) but glandular activity is indicated by the appearance of a lumen which becomes progressively larger during the instar. This condition is shown in figure 7, from a full fed larva. A very thin protoplasmic film which encloses the secretion droplet, running about half way up the seta, is not shown in the figure.

Trichogen cells may be found in larvae sectioned immediately after their hatching, and the same trichogen cells function throughout larval life, merely showing, as do the molting fluid glands also, an increase in size proportional to that of the other cells. The trichogen cells of the pupa are of a very different type, and are similar to those figured by Poyarkoff (1910, fig. 10) for *Galerucella*. What the writer interprets as the trichogen cell proper is a large elongate cell extending up into the seta, and the gland cell beside it is relatively small (the "cellule compagne" of Poyarkoff). The writer believes that during the prepupal period most of the larval trichogens are destroyed, and that the others are converted into trichogens of the pupal type. Not only the pupal setae fewer in number but they are of a very different type. They are solid, not hollow, and have no glandular function; they support the pupa from contact with the pupal cell and are therefore practically confined to the back, as the pupa always lies with the ventral aspect uppermost. They are probably not even sense hairs; at least, the writer has never detected any innervation.

In some sections of larvae the nervous connection is apparent, and it is shown in both trichogens figured. Two or three of the nerve nuclei of the complex are always larger than the others, and these large cells are confined to the region just below the trichogen. As indicated in the figures, the trichogen and gland cells are always sharply distinct from the nerve tissue. The writer has no material properly stained to study the detailed innervation. In nearly all cases, as in figure 6, one can detect only the nuclei, but one section shows clearly the joining of several of these nuclei as illustrated in figure 7. The nerve nuclei resemble the "Sinneszellgruppe" of Korschelt (1924, p. 218) and are likewise divisible into larger nuclei (Sinneszellkerne) near the seta and smaller nuclei (Neurilemmkerne) which may occur anywhere along the nerve. The writer does however hesitate to call the smaller nuclei in *Altica* "neurilemma nuclei" since in all his sections the neurilemma seems to be a very thin structureless membrane devoid of nuclei, and, as shown in figures 6 and 7, is in all cases plainly continuous with the basement membrane of the hypodermis which is certainly non-nucleated. (The basement membrane is also apparently continuous with the sarcolemmata of the muscles as they attach to the cuticula through the hypodermal tendons.) As shown by figure 7, the trichogen nerve arises from the subhypodermal plexus, but the writer has not been able to trace the nerve in his sections much farther than is designated in the figure.

Aside from the innervation which is not discussed in the papers referred to below, conditions are very similar to the poison setae of *Hemerocampa, Apatela* and *Euchaetias* figured by Gilmer (1925, p. 210); there is however no tendency for the trichogen cell proper to degenerate as in *Euchaetias,* and the hairs are not crowded together in tufts as in lepidopterous larvae. The setae also resemble those figured by Matheson (1923, p. 55) as type IV of *Pseudococcus,* except that in *Altica* the trichogen complex is made up of two cells instead of three. Most of all naturally they resemble the trichogen cells of *Galerucella* (Poyarkoff 1910, p. 27–31, fig. 8). Poyarkoff figures two nuclei in his drawing of the trichogen complex, but he does not distinguish between the gland cell and the trichogen proper in his text.

The Prothoracic Glands.

The head of the *Altica* larva is somewhat retracted under the prothoracic shield, with which it is connected by a non-sclerotized fold of the cuticula. The hypodermis of this membranous fold is very glandular, and probably secretes a lubricating fluid. These glandular cells are several times as large as the ordinary cells, and show secretion vacuoles.

The cells were noted by Poyarkoff (1910, p. 33) in *Galerucella* larvae, and called by him "les glandes prothoraciques." In that species, each gland is remarkable in possessing from five to twelve separate canaliculi. One would expect to find a similar condition in *Altica,* but the writer has not been able to detect such canaliculi in his sections.

The Rectal Glands.

The writer has already described (Woods, 1918, p. 305) unicellular glands in the rectal epithelium of this larva, which are interpreted as the molting fluid glands of the hind intestine. In structure and staining reactions they are entirely unlike the molting fluid glands of the hypodermis. These glands may occur in the otherwise unmodified hypodermis at some little distance from the point of the rectal invagination.

The glands are of course totally unlike the so-called rectal glands of certain insects, which are more properly termed "pygidial glands."

The Eversible Glands.

Segmentally arranged eversible glands such as were described in detail by Garb (1915) for *Melasoma lapponica* are totally wanting in *Altica,* and so far as the writer knows in all alticine and galerucine larvae. They are probably confined to and characteristic of the tribes Phaedonini and Phyllodectini of the subfamily Chrysomelini. The writer has found these eversible glands in all larvae of these two tribes which he has been able to examine: *Plagiodera versicolor* Laich., *Gastroidea polygoni* L., *Lina interrupta* Fab., *Lina scripta* Fab. and *Phyllodecta americana* Schaef. He has sectioned larvae of only the last named species but in it conditions are exactly similar to those described so clearly and carefully by Garb.

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Explanation of Figures. Plate XIX.

Figure 1. Caustic potash preparation of integument, showing seta with pore, setiferous tubercle with very small trichopore and seta (left) and very small trichopore without seta (right); a few of the cuticular nodules are shown above the tubercle.

Figure 2. Section of the integument, showing the nodules in cross section. E is epicuticula; P is primary cuticula; S is secondary cuticula; H is hypodermis; BM is basement membrane.

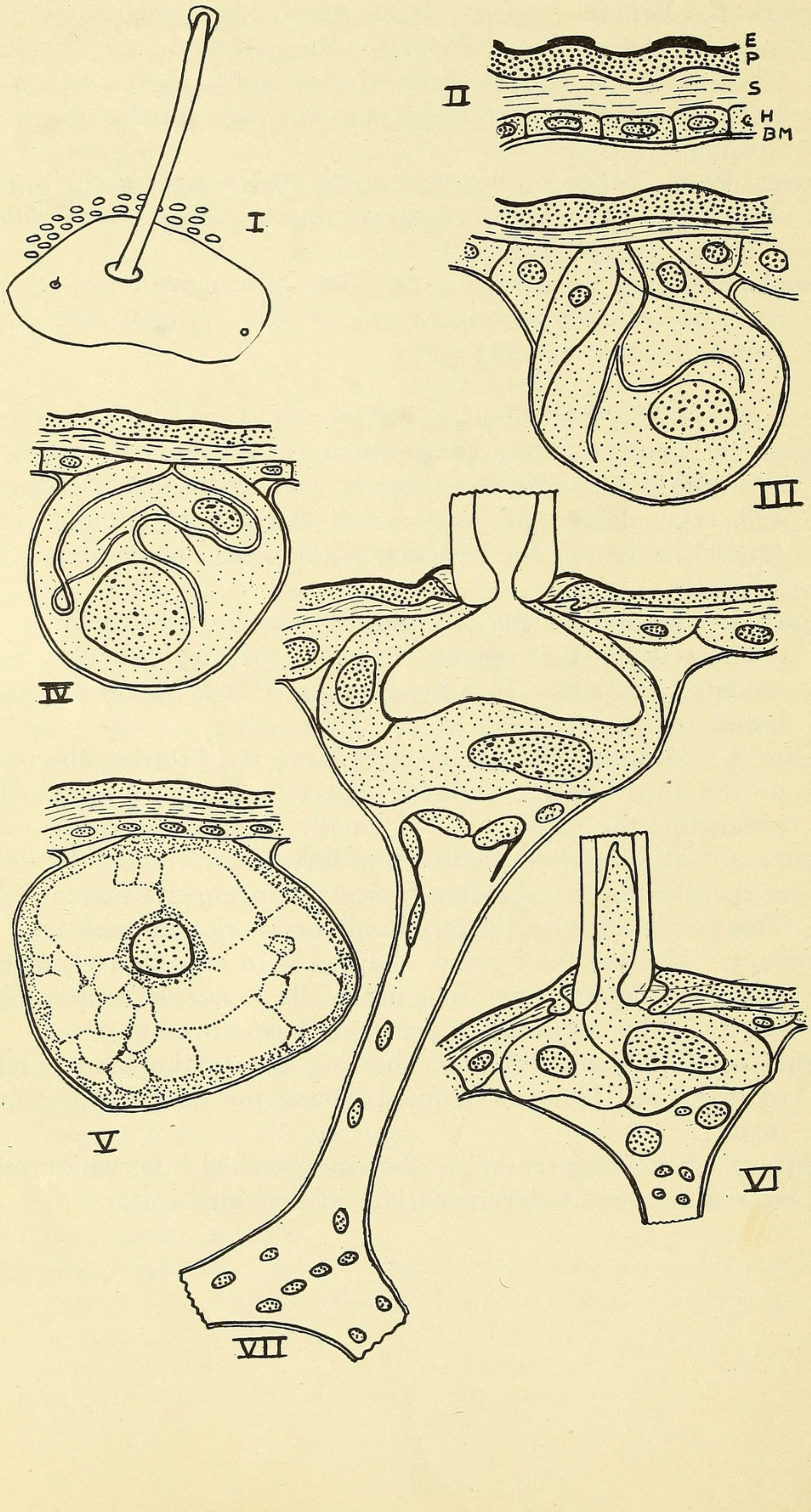
Figure 3. Molting fluid gland, from larva fixed during the molt to the third instar, showing the two neck cells and the canaliculi; the forking is not usually so far back in the cell.

Figure 4. Molting fluid gland, from larva fixed during the molt to the third instar, showing typical arrangement of canaliculi. This particular gland showed only one neck cell nucleus.

Figure 5. Molting fluid gland, charged with secretion vacuoles, from two day prepupa. The neck cells do not show up as distinctly in this section, but are nevertheless present.

Figure 6. Trichogen complex showing innervation and extent of gland cell from larva fixed during the molt to the third instar.

Figure 7. Trichogen complex showing detailed innervation and secretion vacuole developed late in the third instar.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