The Subimaginal Cuticle of the Mayfly Callibaetis sp. (Ephemeroptera)1,2

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ABSTRACT

Mayflies are the only group of insects in which there is usually another molt after a functionally winged stage is attained. Despite being a well-studied area in the literature, whether the cuticle of the subimago is a true cuticle, and hence whether the subimaginal represents a true instar, has not been confirmed. Examination of stage by stage during subimaginal to imaginal of a species of Callibaetis shows that the subimago has a normal cuticle and that its exuvia is also normal. During the subimaginal stage there is a normal molt cycle producing the adult cuticle. Therefore, the subimaginal stage represents a true instar. The fact that the exuvia remains present at the time of the molt is suggested from the series of hypothetical instars employed. Other details of the subimaginal cuticle are given, including that the shading structures of intersegmental membranes are distinct, that both the aquatic and the exuvial membranes are distinctly different, and that the subimaginal is composed of at least two different materials.

Mayflies are the only insects which normally molt after acquiring functional wings. This is one of the reasons why they have been the subject of much interest and speculation among biologists. It should be noted, however, that this peculiar molt does not occur in all the Ephemeridae. In our genus it does not occur at all, it is present in some, and it occurs only in the male in others (Noodham et al. 1935). The aquatic (immature) stages of the Ephemeroptera are called nymphs. The term “subimaginal” is applied to the winged stage between the last aquatic instar and the final form of the insect, the adult or imago. There is some doubt expressed in the literature as to the true nature of the subimaginal “cuticle.” After it has been cast, various authors describe it as a “delicate pellicle,” “shroud,” or “skin,” but seldom as a cuticle. Lamere (1926) stated that the cuticle of the subimaginal divides to form an outer layer which is shed and an inner layer which is retained as the imaginal cuticle. Noodham et al. also do not refer to the shedding of the subimaginal “cuticle” as a true molt but as a

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partial shedding of skin after the adult form has been assumed;" it is only a casting of the delimited hairy outer cuticle. Neither Lawrence nor Needham et al. presented any direct evidence to substantiate their statements.

The subimaginal period is extremely short, lasting from a few minutes in some species to 24 hours or more in others. Judging from the number of rings in the Pulexim body of an adult mite, which Needham et al. state as representing successive molts, the mummy Callicorbius appears to undergo approximately 15 molts. Since the interval between batch- ing from the egg and attainment of the adult stage in Callicorbius is 5 to 6 weeks (Needham et al.), each natal instar, therefore, averages 2 to 3 days. The subimaginal period of Callibrobus was observed to last approximately 12 hours under laboratory con- ditions. Either the subimaginal cuticle is not a com- plete cuticle, or the adult cuticle is secreted in a few hours, or the adult cuticle is already present when the subimagmo emerges from the last natal instar. In order to ascertain which of these possibilities was true and in order to learn more about the nature of the subimaginal cuticle, this research was under- taken.

MATERIALS AND METHODS

Callibrobus, probably ferrugineus (Wash.), was chosen for this study because it is a pond-water mite, therefore comparatively easy to rear in the laboratory, and it has a comparatively short life span with sometimes two generations per summer.

Naisis of various ages were collected during the summer of 1950 from the shallow water of a small lake a few miles north of St. Paul, Minnesota. They were brought to the laboratory and kept at room temperature in pans of aerated water.

For histological study, specimens were severed at the thoraco-abdominal intersegmental membrane and fixed in Bouin's fluid (picro-formol) for 5 to 18 hours; those tissues which were to be treated with enzymes were fixed in Carnoy's fluid. It was not possible to determine precisely the developmental age within a natal instar; they could only be desig- nated as "young" and "old." Specimens of the subimaginal stage were fixed at approximately hourly intervals from emergence until the ecdisis to the imago. Imago, like the subimagmo, were fixed at known intervals after the last ecdisis. Tissues were dehydrated by causes of the butanone series, and im- bedded in 30 parts paraffin (m.p. 54°-56° C.) to one part bayberry wax. Head and thoraces were handled separately from the abdomen and were set aside for possible later studies. Each abdomen was sectioned longitudinally at 5μ and the ribbon was divided and placed on five slides. One of the five slides of each specimen was stained with Mallory's triple stain (consisting of orange G). This histological stain was used for observing the various layers of the cuticle. A second slide was stained by the PAS (periodic acid-Schiff) test. A third was stained with Gomori's aldehyde-fuchsin stain following, in general, the procedure outlined by Halini (1952), with the precautions listed by Eifman (1959), but omitting the use of benzoin (fig. 35). A fourth slide was stained with alcin blue following the method de- scribed by Balbin and Saltmanse (1959). The fifth slide was reserved for confirmative tests.

The methods used for the tryptic, pepsi, and hyaluronidase extractions are those given by Pusser (1960). Hyaluronidase was used at a concentra- tion of 1 mg/ml. in 0.85 percent saline solution, trypsin at a concentration of 0.2 mg/ml. in 0.025 m boro buffer at pH 8.9, and pepsi at 2 mg/ml. in 0.2 m HCl at pH 1.6.

In general, the terms used in this paper are those used by Richards (1958a).

RESULTS

Changes in Old Cuticle

Thickness.—The fully formed cuticle of Callicorbus in all three stages examined is very thin, averaging around 2.5μ. It is perhaps slightly thicker than this in the last natal instar. Endo- and mesocuticle are present, the former being twice the thickness of the latter. An epicuticle is only slightly developed in Callibrobus. It is seen only in the latter stages of an instar and then it is usually confined to the spine on the surface of the cuticle. The epicuticle is extreme- ly thin; it was ignored in this study since its develop- ment could not be readily followed. Just prior to ecdisis, as a result of the action of the melting fluid, the endocuticle is reduced to about half its previous thickness. Here and in the exuvia the cuticle is approximately 1.5μ thick (fig. 5).

The cuticle of the imago presents an interesting case. It continues to increase slightly in thickness until shortly after the molt (fig. 3), but then it de- creases in thickness until it is one-half to one-third as thick at 24 hours after the ecdisis (fig. 4) (the endocuticle is still thicker than the mesocuticle). A possible explanation for this phenomenon will be presented below in the discussion of the epidermis.

Separation of Cuticle from Epidermis.—In sec- tioning the specimens, the cuticle was often unac- tually separated from the underlying epidermal cells. As a result it was difficult to determine at what time the subimaginal cuticle separated from the epidermis. Since the new imaginal cuticle appears at 3 to 4 hours after emergence of the subimagmo, one would assume that the separation had probably oc- curred shortly prior to this. However, at about 7 hours after emergence the subimaginal cuticle is still joined to the new cuticle in the regions im- mediately posterior to the intersegmental membranes,

1 Identification by Dr. Lewis Harvey, University of Florida.
and is essentially the same thickness as in the newly emerged subimagos. At 8 hours, the exuvial cuticle of the subimaginal cuticle begins to thin, and at 10 hours it is completely free from all underlying structures. It should be noted that the cuticles are two to three times thicker in the regions that separate last, and that in these regions instead of the endocuticle being twice the thickness of the exocuticle as elsewhere, the mesocuticle is approximately three times thicker than the endocuticle; this is the region marking the separation of primary segments.

Subcuticle.—The subcuticle is seen as a distinct layer between the cuticle and the epidermis after certain staining methods such as aldehyde-fuchsin and PAS (Schmidt 1936); it was found to be readily stained by alcian blue in all stages except the imagos (figs. 1, 2). The subcuticle is present at all stages in Callobia. When the cuticle separates from the epidermis in molting, the subcuticle remains attached to the inner surface, and seems to give rise to the exocuticle membrane. In regions where the cuticle has separated from the epidermis as a result of sectioning, the subcuticle can sometimes be seen to adhere to the epidermis, sometimes to the cuticle, and sometimes split and adhering to both. When the subcuticle is thus split, fibrous material can often be seen filling the space between the old cuticle and epidermis. This may represent the subcuticle in a torn condition.

Endocutal Membrane.—An endocutal membrane is present between the new and old cuticles of a last naiadial instar. It varies in thickness at all stages and at all instars from less than 0.5 μ to 1 μ. Its normal position appears to be appressed to the old cuticle, though it is often seen free as in figures 5 and 6. It does not appear to separate from the intersegmental membrane as is shown in figure 7. The endocutal membrane is part of the last naiadial exuvia. It is sometimes separated from the endocuticle of the exuvia, and it is sometimes contiguous with it.

In the subimagos, the endocutal membrane is first seen 8 hours after emergence, i.e., three-fourths of the way through the instar. It seems to arise from what had been the subcuticle—both having identified histochemical reactions; these are described in a later section. No significant differences in thickness or staining reactions were noted at any time between the subcuticle and endocutal membrane. It should be noted here, however, that even after the endocutal membrane is formed, a histochemically identical layer still remains attached to the inner surface of the old cuticle.

The mesocuticle membrane is more difficult to find in the subimaginal exuvia than it was earlier. It is possible that during drying it becomes adherent to the inner surface of the exuvia.

Pore Canals.—Pore canals are seen clearly only in the aldehyde-fuchsin preparations. They are extremely numerous with much too small a diameter to be measured with the light microscope. They are seen passing from the epidermal cells part way through the cuticle.

Surface Spines.—The cuticle of the last naiadial instar appears serrated in longitudinal section (fig. 6). From the tip of each caudally directed "tooth" projects a short spine approximately 1 μ long. These spines appear about 5 μ to 8 μ apart. Similarly directed and distributed spines are present on the subimaginal cuticle. Here, however, the spines are considerably longer (9 μ to 12 μ) apart. In surface view, the subimaginal cuticle appears to be surface-sculptured into somewhat hexagonal-shaped areas; one or two of the 9 μ- to 12 μ-long spines arise from each of the "hexagonal" regions. There are no spines present on the surface of the imaginal cuticle.

In the last naiadial instar only, there is a ring of long amber-colored spines (10 μ to 40 μ long) encircling the body and projecting caudally at the posterior margin of each abdominal segment.

Intersegmental Membrane.—The endocuticle of the intersegmental membranes is always thicker than the mesocuticle, even after action by the molting fluid. In fact, they do not appear to be acted on by the molting fluid at all, being the same thickness in the old last naiadial instar and in the exuvia as in the young last naiadial instar. Because of this, the intersegmental membranes of the last naiadial exuvia are thicker than the rest of the exuvia (fig. 7). The intersegmental membranes of the subimaginal exuvia, however, are as thin as or thinner.
then the rest of the exuvia. The explanation for this point possibly has to do with the fact that the last naiadial exuvia is shed in water whereas the subimaginal exuvia is shed in air. The intersegmental zeugmata of the latter could therefore come to be of lesser thickness than those of the last naiadial exuvia by dehydration.

**Formation of New Cuticle**

As mentioned earlier, the last naiadial instar could not be aged with any precision. As a result, the development of the subimaginal cuticle in that instar could not be readily followed. In the subimagino, how- ever, the marginal cuticle first becomes visible at 3 to 4 hours after emergence, i.e., one-third to one-fourth of the way through the instar. It is then a very thin membrane less than 0.5μ thick (fig. 10). By 8 hours after emergence a definite endo- and mesocuticle can be seen. The new cuticle continues to increase in thickness through ecdysis and into the imaginal stage.

**Epidermis**

The nuclei of the epidermal cells are more or less round and granular. They appear the same in all stages (except the old imagos) demonstrating no obvious process of breakdown and build-up. No mitotic figures were seen at any stage. Granules are seen in the cytoplasm of the epidermal cells to many of the preparations. These granules appear to fol- low no developmental process and possibly represent a fixation artifact.

The epidermis of a last naia first appears to remain apparently unchanged over the surface of the naia. In the young subimagino, the epidermis increases gradually from this thickness until 7 to 10 hours after emergence when it is at its greatest—approximately 5μ. It then decreases slightly until ecdysis. By 24 hours after the molt to the imagino, the epidermis has sharply de- creased in thickness to 2.5μ to 1μ (fig. 4). The nuclei of the epidermis are now darker-staining, smaller, more elongate, and more granular than previously. They are against the basement membrane while the remainder of the epidermis appears trans- parent over the surface of the naia (as was observed in the tritiated earlier) and the epidermis is possibly due to (1) the loss of cuticular water or of other cuticular or epidermal constituent to the internal environment of the naia. (Lower 1959) demonstrated that certain cuticle constituents can be utilized by a starving naia. Male imagos, under laboratory conditions, were observed to live approximately 24 hours; the last specimen described above, a male imago 24 hours old, was very near death and possibly undergoing starvation since adult mayflies are thought not to feed. Females live 3 weeks or more under laboratory conditions.

**Dermal Glands**—Dermal glands, when present, are round or ovoid shaped with ducts extending out through the cuticle as seen in figure 9. No dermal glands were seen in the epidermis of the young last naiadial instar; some were seen in the old last naiadial instar, but they were very few in number and appeared to be empty. In the subimagino, they become gradually more and more prevalent until 8 hours after emergence when they appear to stop in- creasing in number. They continue to increase in size, however, as the epidermis increases in thick- ness. They also have some material in them at this time. They appear to be more numerous in the anterior abdominal segments than elsewhere. Twelve hours after emergence of the subimagino they are at their largest and contain more material than previ- ously. There were no dermal glands seen in the six specimens that was fixed halfway through the shedding of the subimaginal cuticle. Also, these were no dermal glands seen in the imagino.

**Ecdysis**

The wing pads of the last naiadial instar appear darker in color than those of earlier instars. One- half to one hour prior to moltning the naia takes on a silvery, silken-like appearance and becomes very active, darting rapidly here and there just below the surface of the water. If the naia darts toward the bottom of the rearing pan and ceases all muscular movement it immediately floats to the surface, ap- parently buoyed up by a gaseous layer between the old and new integuments. At all other stages of naiadial life the mayfly remains on the bottom of the rearing pan amongst aquatic plants. Edmon (1882), Cassid (1898), and Daggy (1935) note the pres- ence of this gas between the new and old cuticles of emerging mayflies as they are separating. In the passage of gas is derived from trachea. Daggy theories that it may be a layer of carbon dioxide being given off from the body wall and trapped between the two integument layers as they are separating, and that it apparently functions in holding the naia at the surface while transformation is taking place. An analysis of this gas would be a profitable area of investigation.

At emergence, the naia suddenly darts across the surface of the water, withdraws from the exuvia and flies to the center top of the rearing pan. Some naia do this in less than 15 seconds, others take somewhat longer.

The subimaginal ecdysis is no less dramatic than that of the last naia. Again for 30 minutes to an hour prior to emergence, the subimagino becomes quite active. The abdomen begins twitching from side to side, a couple of legs begin flexing, then one or two of the others—there being no particular sequence to any of these movements. While these events occur, the wings separate slightly and are held more or less vertical, whereas at all other stages the wings are held horizontally over the body. Suddenly the mayfly lowers its wings to slightly less than a horizontal position, the cuticle bursts suddenly along the ecdysial suture, and the mayfly quickly withdraws from the cuticle and walks away.
from it. From the time the salvinago begins to lower its wings until it walks away from the exuvis takes approximately 15 to 30 seconds.

**Histochemistry**

**Subcuticle and Ecdyson Membrane.**—The subcuticle and ecdysial membrane will be discussed jointly since they seem to represent different stages in the development of the same structure, and also since their histochimical reactions are identical. The subcuticle and ecdysial membrane at all stages of Calliheres are positive to the PAS test and are also stained by aldehyde-fuchsin; the latter is always a much stronger reaction. The PAS test is negative if the oxidation with periodic acid is omitted. Identical reactions have been previously noted by Schmidt (1936) for the subcuticle of all six insects of the five orders he examined. The ability of materials to stain with aldehyde-fuchsin closely parallels their giving a positive reaction to the PAS test (Scott and Clayton 1935; Halimi and Davies 1953). As the PAS test is widely considered as specific for certain carbohydrate constituents of tissues, similarity with aldehyde-fuchsin must suggest the probable presence of carbohydrate. In parafin sections, the only PAS-positive materials are glycogen, or various mucoid substances such as the neutral muco polysaccharides, muco- or glycoproteins or perhaps a complex of these; acid muco polysaccharides are PAS negative (Pettebre 1969). It has been shown by Schmidt (1936) that muco- and glycoproteins are probably present in the subcuticle.

The subcuticle and ecdysial membrane are also alcin blue-positive. Alcin blue staining, however, is extremely weak in the imaged subcuticle and in the area under the thick part of the cuticle immediately posterior to each intersegmental membrane (recall that this is the area which separates last in molting). When alcin blue is used under carefully controlled conditions, positively stained areas suggest the presence of acidic carbohydrates, specifically, the acid muco polysaccharides (Lison 1933; Mowry 1936; Wagner and Shapiro 1957). Further evidence is provided by the fact that the metachromasia produced to toluidine blue 0 and the positive reaction with alcin blue have been shown to parallel one another closely (Wagner and Shapiro 1957). The subcuticle and ecdysial membrane, however, demonstrated only very weak metachromasia. No explanation can be offered for this. On digestion by hyaluronidase, the alacin blue-positive material in the subcuticle and ecdysial membrane remained intact. The only acid muco polysaccharides known to be fast to hyaluronidase are epibranchial mucin and chondroitin sulphate acid Type B (Pettebre 1960). Of these two substances, it would seem more probable that the former would be present in the subcuticle.

As acid muco polysaccharides are PAS-negative, approximately there are at least two different materials in the subcuticle and ecdysial membrane. One of the materials, which is PAS-positive, may be either glycogen, a neutral polysaccharide, muco- or glycoprotein, or a complex of these; and the other, which is PAS-negative but alcin blue-positive, is an acid muco polysaccharide. This latter material is present in the imaginal subcuticle in extremely small amounts.

The rhinos material that is sometimes observed between the old cuticle and epidermis when they have separated in sectioning is positive to aldehyde-fuchsin and alcin blue. It probably also stains with PAS but so weakly that it cannot be readily ob-

**Cuticle.**—The cuticle proper is negative to alcin blue and only very weakly positive to the PAS test, especially in those areas stained and as shown in metachromasia with alcin blue. However, it does not seem likely that it is involved in the mechanism being studied here.

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**Table 1—Summary of the histochimical reactions of the cuticular materials.**

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**Summary**

- The subcuticle and ecdysial membrane are PAS-positive and also stained by aldehyde-fuchsin; however, the latter is always a much stronger reaction. The PAS test is negative if the oxidation with periodic acid is omitted. Identical reactions have been previously noted by Schmidt (1936) for the subcuticle of all six insects of the five orders he examined.

- The ability of materials to stain with aldehyde-fuchsin closely parallels their giving a positive reaction to the PAS test (Scott and Clayton 1935; Halimi and Davies 1953).

- The subcuticle and ecdysial membrane are also alcin blue-positive. Alcin blue staining, however, is extremely weak in the imaged subcuticle and in the area under the thick part of the cuticle immediately posterior to each intersegmental membrane.

- Further evidence is provided by the fact that the metachromasia produced to toluidine blue 0 and the positive reaction with alcin blue have been shown to parallel one another closely.

- The subcuticle and ecdysial membrane, however, demonstrated only very weak metachromasia. No explanation can be offered for this. On digestion by hyaluronidase, the alacin blue-positive material in the subcuticle and ecdysial membrane remained intact.

- As acid muco polysaccharides are PAS-negative, approximately there are at least two different materials in the subcuticle and ecdysial membrane. One of the materials, which is PAS-positive, may be either glycogen, a neutral polysaccharide, muco- or glycoprotein, or a complex of these; and the other, which is PAS-negative but alcin blue-positive, is an acid muco polysaccharide.

- This latter material is present in the imaginal subcuticle in extremely small amounts.

- The rhinos material that is sometimes observed between the old cuticle and epidermis when they have separated in sectioning is positive to aldehyde-fuchsin and alcin blue. It probably also stains with PAS but so weakly that it cannot be readily observed.

- It should be noted that not all structures are stained identically with PAS, aldehyde-fuchsin, and alcin blue. The treated fining, gut fining, fining of the ovatocut, cuticle, connective tissue of fat body, basement membrane, and muscle sarcoplasma all stain positively by the PAS test and aldehyde-fuchsin; of these structures, only the first three are stained by alcin blue. It may be significant that of the structures listed, the first three are those in which one would most expect to find an epithelial muscle.
and to aldehyde-fuchsin. This weak PAS positivity could possibly be due to the presence of some tyro-
sine, which would give a reaction.

**Interssegmental Membranes**—The staining reac-
tions of the different stages of the integument and of the cuticle are sharply contrasted with the rest of the cuticle. They are strongly positive to the PAS test and to aldehyde-
fuchsin. The thicker inner portions of the mem-
branes are alcin blue-positive; the thin outer layer is alcin blue-negative. This condition exists in all Stages examined except for the imaginal imaginal i
segments studied. The individual membranes are almost completely alcin blue-negative; such a condition is comparable to the almost complete lack of any alcin blue stain-
ing in the imaginal subcuticle.

**Epdhema**—The cuticle is stained weakly posi-
tive to the PAS test and to aldehyde-fuchsin. This result may be due to the presence of some glycogen, but, since no diastase digestion were performed, no definite conclusions can be reached. The basal membrane is positive to PAS and aldehyde-fuchsin. It is negative to alcin blue, apparently lacking any acid mucopolysaccharides.

**Dermal Glands**—The dermal glands, like the base-
nent membrane, are positive to PAS and aldehyde-
fuchsin. A small percent of them are very weakly positive to alcin blue. The positive function of these glands will be briefly discussed later.

**DISCUSSION**

The subimaginal imaginal of Calliphora shows obviously each represent a true instar (defining instar as a stage in the life cycle of an insect separated by another stage by a molt). Shortly after emergence to the subimaginal imaginal cuticle begins to separate from the epidermis, and the imaginal cuticle begins to form. After spending approximately 12 hours as a subimaginal imaginal, the imaginal cuticle begins to one of the length of each aquatic instar, the maggot sheds the subimaginal cuticle and emerges as an imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal imaginal 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turdness or nothirous when viewed under the light microscope (Richards 1955; Lower 1957). They may, however, show a fibrous structure in electron micrographs (Richards 1953). The ecdyial membrane of Callithrix, as already noted, is fibrous under the light microscope (fig. 8). Therefore, in view of this and other recorded differences (Richards 1955; Malek 1958), it would appear that the ecdyial membrane could best be defined as any layer which is apparent between the old and new cuticles at some time in the molting cycle. It re- mains to be determined whether or not such membranes have the same origin, morphology, and composition in different species of insects (to date ecdyial membranes have been recorded only for several moths, the honey bee, a locust, and now this mayfly; no such membrane is to be seen in cockroaches, Rhodnius, or Tenebrio).

Schmidt (1956) suggested that (1) the subcuticle functions in fastening the cuticle to the epidermis, and that (2) it possibly functions in the loosening of the cuticle at the time of molting. Possibly, the PAS-positive material of the subcuticle is responsible for the first of Schmidt's suggestions, and the alcin blue-positive material is responsible for the second. This latter statement is derived from the knowledge that the cuticular region that separates last from the epidermis during molting is a region of very little alcin blue-positive material compared to the rest of the cuticle. After the cuticle has completely separated from the epidermis then more alcin blue-positive material is seen in this region. A third possible function of the subcuticle (at least in Callithrix) is that after it changes into an ecdyial membrane, it might causally function as a lubricating layer aiding the process of molting. The almost complete lack of an acid mucopolysaccharide in the imaginal subcuticle could possibly be correlated with the fact that the imago has no need of a lubricating layer, only an adhesive layer provided by the aldehyde-fuchsin- and PAS-positive material.

Bailey and Sallhouse (1959) showed with alcin blue staining that the majority of dermal glands in Rhodnius prolixus secrete a mucin at the time of molting which they suggest serves as a lubricant in the molting process. The morphological development of the dermal glands in Callithrix, as reported earlier, would tend to support this suggestion; the histological data, however, do not confirm. The majority of the dermal glands are negative to alcin blue; the others are only very weakly positive. They are, however, strongly positive to aldehyde-fuchsin and PAS. If they did secrete a lubricating material at molting, it would appear not to be an acid mucopolysaccharide. Since the dermal glands increase in size and stabidensibility at the time when the new cuticle begins to increase in size and when the old cuticle begins to be digested, it is possible that they function somehow in one of these processes.

From the evidence presented in this paper for California, it cannot be assumed that the same developmental process occurs in all the Ephemerinae species. As was mentioned in the introduction, not all mayflies undergo a complete subimaginal molt. Also, some mayflies, such as Cercida and Ephemeretum, spend only a few minutes as subimagos. Though these few minutes probably do represent a true molt, it would seem that the imaginal cuticle must be formed (or at least partially formed) during the last naiadal instar. Otherwise, it would be difficult to examine one or both of these genera to see if this were true.

ADDITION

Since submitting this manuscript we have learned of experimental work by Dr. C. M. Williams which has certain intriguing aspects relative to our findings. Williams' new communication (to be published in a few months) has found that when adult Cercopidae moths are induced to molt again by being joined in parabiosis with pupae sup- plying ecdysone, the abdomen of the adult molt alone in a manner normal but the cuticle of the second adult is nearly or completely devoid of scales. This data parallel closely what we found in the subimaginal-imaginal stages of Callithrix. That is, the cuticle of the subimagos is setose whereas that of the imagos is smooth and shaly; this change is re- corded as general for mayflies (Needham et al. 1953).

It therefore appears that when an adult molts again—regardless of whether the molt is normal or induced—it can form a normal cuticle but cannot form setae or scales. This gives another point suggest- ing that the subimagos represent a true instar, and, further, that it is a true first adult instar (as opposed to the possibility of being a last nymphal instar or "hyper-nymphal instar" which it might otherwise be considered since subimagos have not been shown to mate and lay eggs). On the basis of cuticle development, then, the subimagos of mayflies compared with the normal adult of Cercopidae and other insects whereas the true imagos of the mayfly copareses with the induced second adult stage of Williams' Cercopidae moths.

REFERENCES CITED


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