OBSERVATIONS ON THE FINE STRUCTURE OF THE DEVELOPING CUTICLE OF A SOIL MITE *OPPIA COLORADENSIS* (ACARINA : CRYPTOSTIGMATA) [1]

BY

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Introduction.

The typical life cycle of an oribatid mite includes the egg, larva, protonymph, deutonymph, tritonymph, and adult stages. The gross morphology and duration of these stages, including quiescent periods, has been studied in detail for the soil mite, *Oppia coloradensis* (Dolan, 1969). The integumental fine structure of *O. coloradensis* has also been investigated (Brody, 1969 *a*). Through an understanding of the life cycle and the cuticular morphology of *O. coloradensis*, it is now possible to report on several developmental features which may be significant in understanding the integumental structure and its formation in a comparative manner.

There is a large amount of information available on insect cuticle. A review by Richards (1951), portions of a text by Wigglesworth (1965), and articles by Hackman (1964), Locke (1959, 1960, 1961, 1965, 1966), Richards (1967), and Beament (1955, 1959, 1961, 1964, 1968) cover studies of many types including investigations of the fine structure.

The information available on the fine structure of mite cuticle has been published by Gibbs and Morrison (1959), Henneberry et al. (1965), Nathonson (1967), Wharton et al. (1968), and Brody (1969 *b*). Among other kinds of studies of acarine integument that have been published are those of Hughes (1959) and Lees (1946, 1947).

Several good descriptions of arthropod molt cycles are available (Richards, 1951 ; Jones, 1954 ; Hinton, 1958 ; Locke, 1964 ; Wigglesworth, 1965 ; and Johnston, 1967). The application of the term " molt " — a separation of the hypodermis from the old cuticle with subsequent formation of a new cuticle, and the term " ecdysis " — the splitting and shedding of the old cuticle, is intended to coincide with modern usage (Hinton, 1958 ; Snodgrass, 1960). The term " pharate " (Hinton, 1958) is used to describe a stage in the period during molting, but preceding ecdysis.

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Methods and materials.

O. coloradensis were maintained in laboratory culture on a diet of yeast and dextrose. All life stages were kept in two ounce jars at a constant 95 % relative humidity (Dolan, 1969).

Living mites were placed directly into a fixative of 6.2 % glutaraldehyde and then a post-fixative of 1 % osmium tetroxide at pH 7.4. Primary aldehyde fixation was from 3-4 hours, followed by post-fixation in buffered osmium for 30-40 minutes. After fixation and before embedding, the mites were dehydrated in a graded series of ethyl alcohols (50 %, 70 %, 95 %, and 100 %). The tissue was allowed at least 10 minutes in the 50 % to 95 % range, and 20-30 minutes in the absolute alcohol.

Propylene oxide was the most satisfactory transitional solvent. Geisy's resin (Geisy, 1968) was used in the following proportions (propylene oxide to resin) ; 3 : 1 for one hour, 2 : 2 for one hour, 1 : 3 overnight and then 24 hours in 100 % fresh resin before final embedment in new plastic in an oven at 60° C for two to three days.

Glass knives were used to cut sections on an LKB ' Ultratome ' or Porter-Blum MT-2 ultramicrotome. Sections were cut 2-5 microns thick for light microscopy and 350-900Å thick for electron microscopy.

Sections were picked up on 250-mesh, formvar coated copper grids and stained with uranyl acetate and lead citrate. Thin sections were examined primarily on a Zeiss transmission electron microscope model EM9-A. Initial magnification of micrographs ranged from 1,750 to 39,500 × and subsequently was enlarged photographically.

Results.

In the pharate adult of *Oppia coloradensis*, the new integument is separated from the old, sclerotized, outer, tritonymphal cuticle (Fig. 1). The tritonymphal cuticle (Fig. 1) is approximately 1 micron thick at a time when the developing adult integument is only about .20 microns thick. No sections of the pharate adult were cut in which the adult integument was less than .20 microns thick. At this earliest of observed stages, a well defined, although apparently distended ecdysial space, is clearly evident. Locke (1964) has described a molting gel in the ecdysial space of several insects. Secretion products overlying the proteinaceous epicuticle at this stage may represent a molting gel (Fig. 2).

Two stages following the initial separation of the new epicuticle from the old cuticle have been recognized. The first of these stages (Figs. 3 & 4) is characterized by a completed inner epicuticle and a thicker procuticle (about .65 microns) in which granules are deployed in a parabolic array. In the stage following this development (Fig. 5), the granules have disappeared and microfibers can be recognized. It is assumed that the granules have coalesced to produce the microfibers. At this stage only a single layer or lamina is complete, but a new lamination has started to form as the cuticle thickens during deposition. Cavities seen in the developing procuticle at these stages are interpreted as developing pore canals (Figs. 4 & 5).

In fully formed adult cuticle (several days after ecdysis), microfibers can be seen extending from the laminations into the more electron dense interlaminar spaces (Fig. 6) (see Discussion). The cuticle of a mature adult mite is approximately 4-7 microns thick. In the case of figure 6 however, the section was cut in a rather tangential plane, thus causing the laminations to appear wider than they actually are.

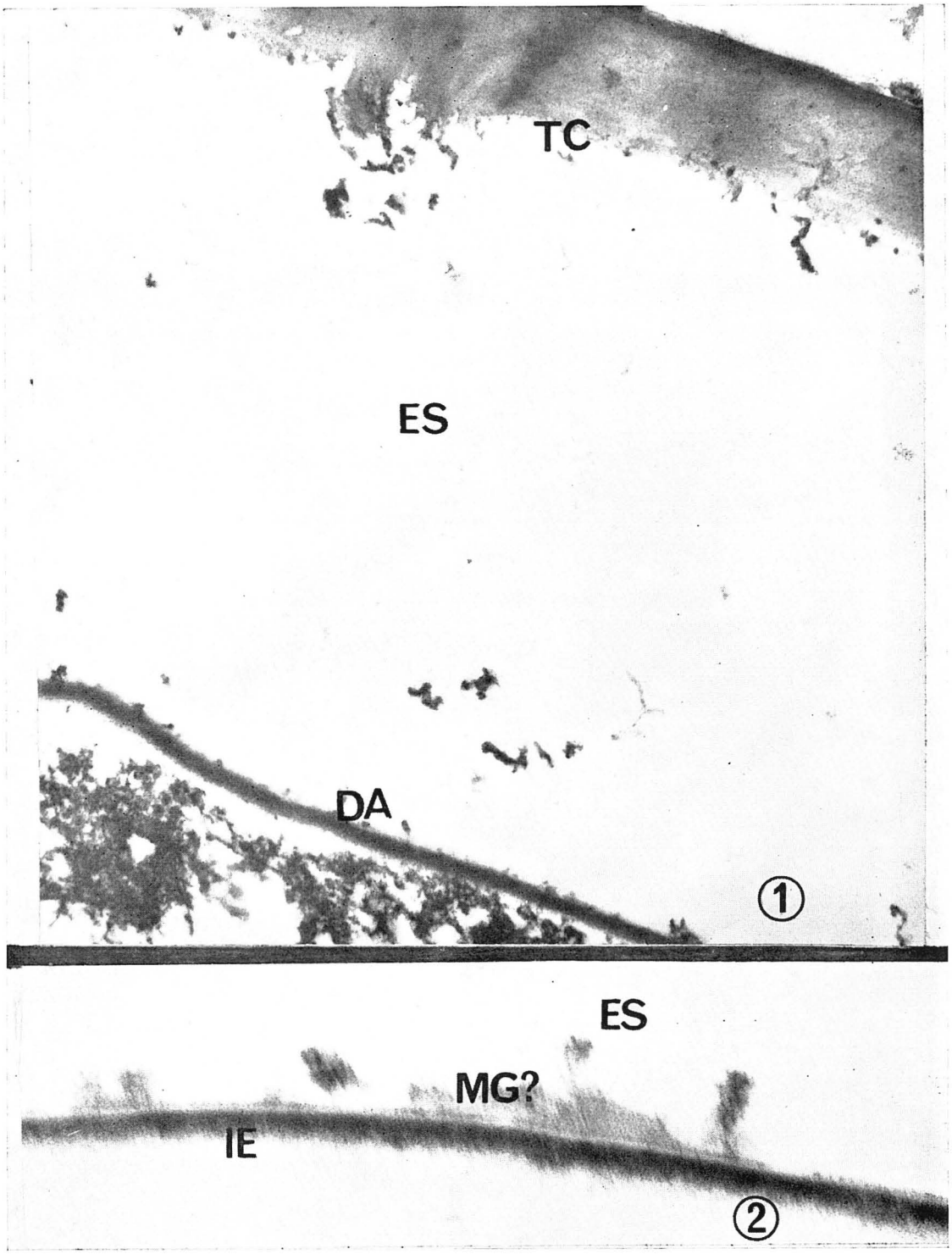


FIG. 1 : Section through a quiescent tritonymph showing developing adult integument (DA) beneath old tritonymphal cuticle (TC) separated by an ecdysial space (ES). × 18,000.

FIG. 2 : An electron micrograph of developing adult integument before deposition of the endocuticle. Inner proteinaceous epiculticle (IE), ecdysial space (ES), molting gel ? (MG). × 35,000.

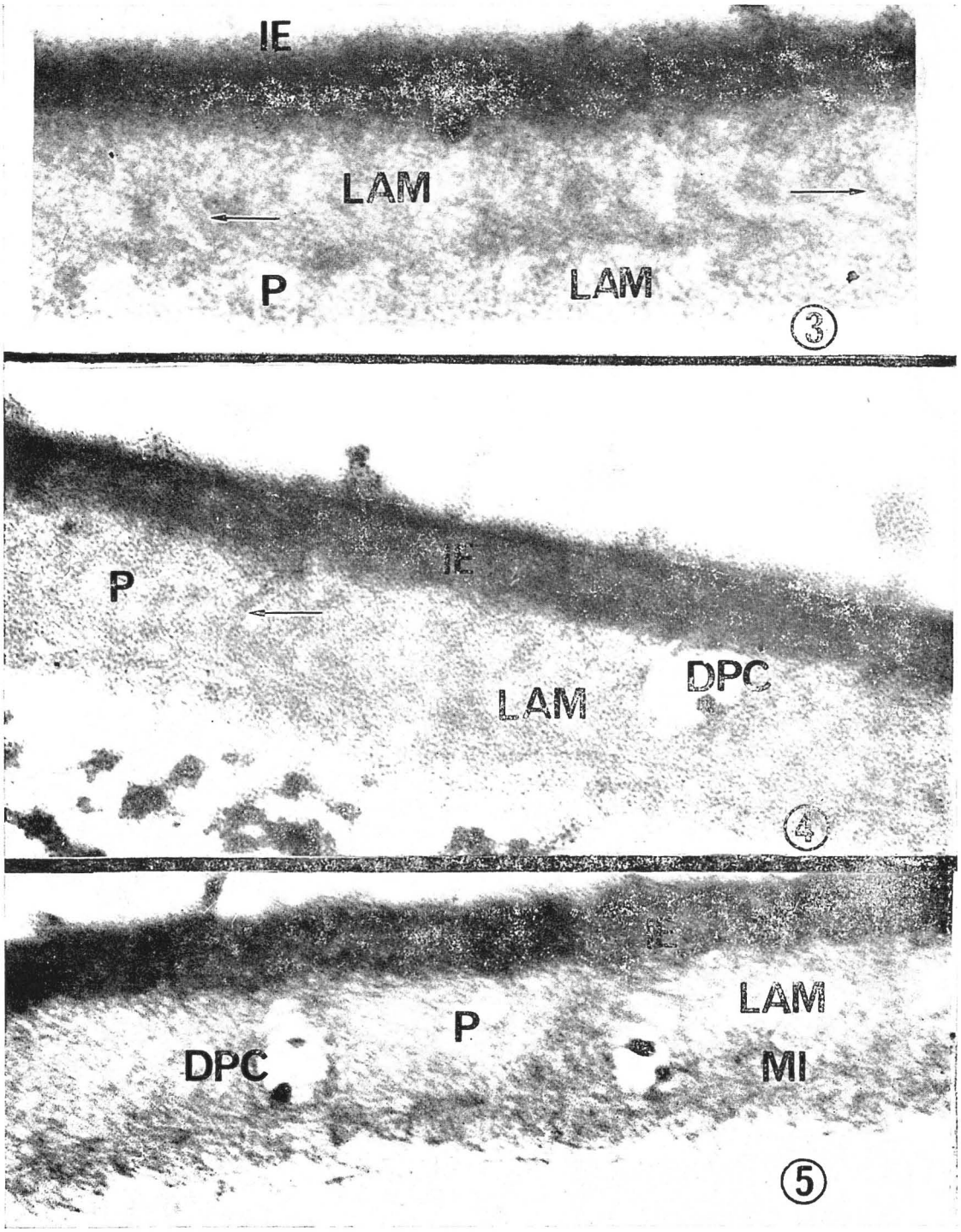


FIG. 3 : The parabolic array of granules (arrows) has formed laminations (LAM) below the electron dense inner epicuticle (IE). Procuticle (P). × 76,000.

FIG. 4 : Section through the developing adult integument of the quiescent tritonymph. Deposition of the procuticle (P) has commenced beneath the inner epicuticle (IE). The arrow indicates the parabolic array of granules which form a lamination (LAM). Developing pore canal (DPC). × 70,000.

FIG. 5 : Section of developing adult integument in which the granules have coalesced to form microfibers (MI). The fibers form a lamination (LAM) in the procuticle (P). Developing pore canal (DPC) beneath the inner epicuticle (IE). × 66,500.

The developing pore canals are approximately .25 microns in diameter, within the range of pore canals found in some other mites (WHARTON et al, 1968 ; and BRODY, 1969 *b*). The origin of the single, or sometimes double, electrondense granule that is observed within the developing canals is difficult to positively identify (Figs. 4 & 5). However, the dark spheres are most likely cytoplasmic extensions of the underlying hypodermal cells. These extensions may prevent

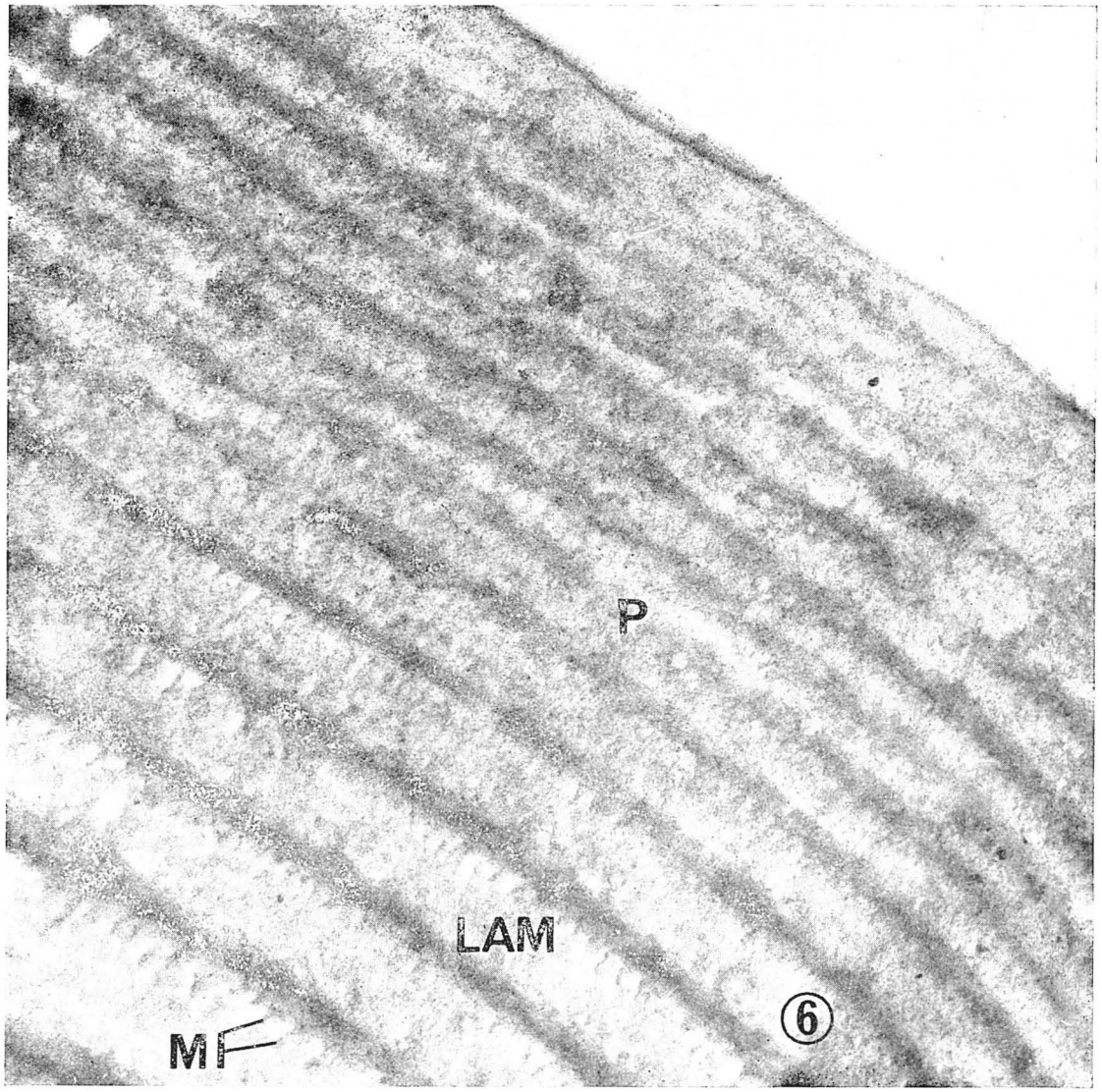


FIG. 6 : A rather tangential section through the mature cuticle (at least four days after molting) of an adult mite. The procuticle (P) is a series of laminations (LAM) formed from microfibers (MI). × 57,000.

the cuticular microfibers from forming in the immediate vicinity, and the spaces are thus maintained as canals when the cuticle becomes sclerotized (a theory proposed for insect cuticle by LOCKE, 1964 ; and WIGGLESWORTH, 1965). Several pore canals which may have been formed as a result of the process can be seen in the newly molted adult cuticle of *O. coloradensis* (Fig. 7).

The superficial epicuticular layers that are known to be exuded from the pore canals and ducts

of dermal glands in other arthropods were best observed in sections of the immature stages of this mite. These exudations are identified as a lipid layer (about .03 microns thick), a cement layer (about .03 microns thick), and wax blooms of varying size and shape (some rising .80 microns above the cuticle) (Figs. 8 & 9). The blooms are only about 1 micron apart and are often much closer together. The wax blooms in immature stages are invariably a portion of the epicuticular lipid layer which forms a universal covering over the mite (Figs. 8 & 9). Coating the blooms as well as the lipid layer, and also ubiquitous on the cuticle of the larval and nymphal stages of *O. coloradensis*, is a well defined cement layer that is occasionally seen to be folded back and forth upon itself (fig. 8).

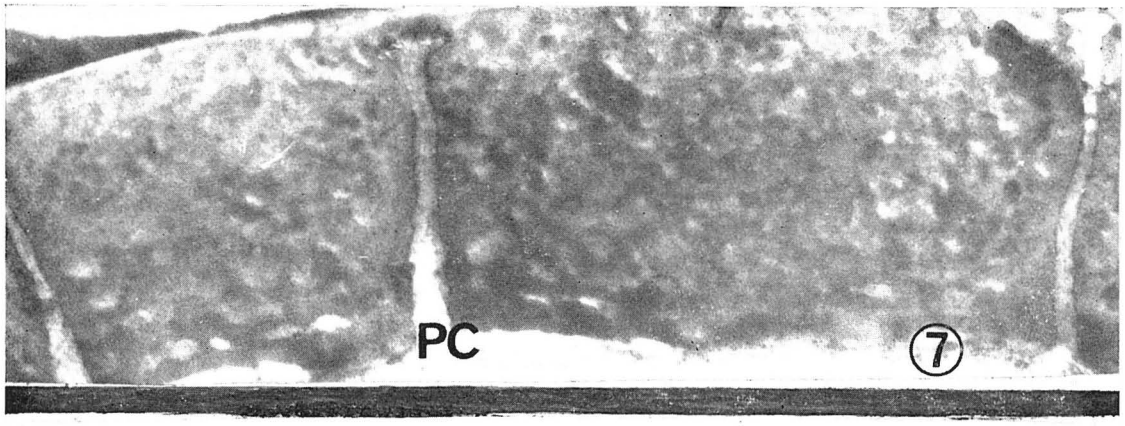
Discussion and conclusions.

It has been shown in the description of the pharate adult cuticle of *O. coloradensis* that each lamina can be interpreted as a series of layers of parabolic microfibers, with each fiber formed from a number of coalesced granules (Figs. 3, 4, 5, & 6). These granules appear to be deposited as procuticle at the surface of the hypodermis. They later are pushed up as more granules are deposited beneath them. This idea is in agreement with work reported by Condoulis and Locke (1966). They used labeled glucose as a chitin tracer and labelled amino acids as protein tracers. With these tracers, they followed the deposition of endocuticle autoradiographically in the insect *Calpodes*. Incorporation of glucose-labelled chitin in the endocuticle occurred in a sharply delineated band.

Without exception, electron-dense layers are seen between the cuticular laminations of this mite (Figs. 3, 4, 5, 6, & 8). The density of these areas is apparently caused by a merging of laminar constituents (granules or microfibers) between each pair of laminations (Figs. 3 & 6). This situation may add a measure of stability to the entire cuticle and would provide a reasonable explanation for the ever-present pattern of alternating light and dark bands observed in cross-sections of mite and insect cuticle.

The cuticle of *O. coloradensis* is covered by a thin (about .10 micron) epicuticle. Four layers (cement, lipid, cuticulin, inner epicuticle) have been identified in the epicuticle of both an insect (Locke, 1966) and this mite (Figs. 8 & 9). The epicuticle has been considered by several investigators to be of primary significance in the waterproofing mechanism of most arthropods (Lees, 1946 and 1947 ; Locke, 1959 and 1960 ; Beament, 1959 and 1961 ; Wharton & Devine, 1968).

It is interesting to note that the epicuticular wax blooms and the cement and lipid layers are most highly developed in the larval, protonymphal, and deutonymphal stages of *O. coloradensis*. These structures are not as distinct in the tritonymph and are cryptic or lacking in the adult. The complexity of development of the epicuticle appears to be in an inverse proportion to the amount of sclerotization in the procuticle. The lightly sclerotized cuticle of the protonymph and the deutonymph appears to have an epicuticle which is clearly subdivided into various layers (Figs. 8 & 9). The extensive, well developed wax blooms and the folding of the cement layer upon itself are examples of an unusual organization of this cuticular element. Conversely, the cuticular surface of the heavily sclerotized adult is relatively undifferentiated (Fig. 6). The presence or absence of a substantial epicuticle may be indicative of the amount of protection needed by the procuticle. Simply stated, the lightly sclerotized cuticle of the larvae and nymphs probably requires a more effective barrier to environmental moisture than the heavily sclerotized cuticle does. This assumption appears to be in agreement with work by Neville (1965). He indicated that swelling agents such as water reduce the cohesion of micellae in lightly sclerotized or unsclerotized cuticle of insects.



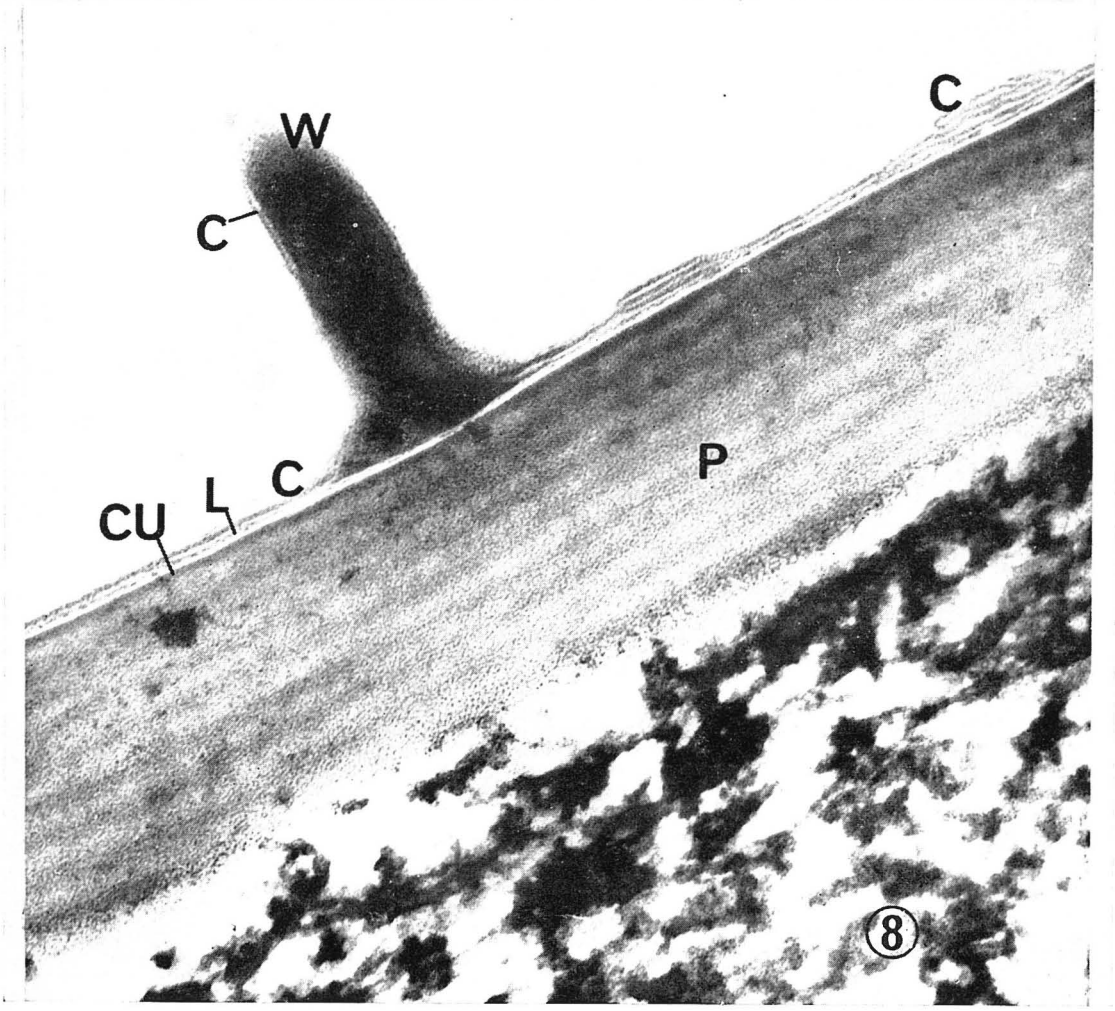


FIG. 7 : Three pore canals (PC) in the cuticle of a newly molted adult. × 38,000.

FIG. 8 : An electron micrograph of a section through the cuticle of the protonymphal stage. Covering the laminated procuticle (P) is a cuticulin layer (CU), a lipid layer (L), and a cement layer (C) which is folded back and forth on itself. A wax bloom (W), also covered by cement, is continuous with the lipid layer. × 38,000.

As part of the epicuticle, lipid secretions known as wax blooms have been identified on the cuticle of two mites. WHARTON et AL. (1968) demonstrated wax blooms opposite the wax secreting pore canals on the cuticle of *Laelaps echidnina*. BRODY (1969 *a*) found that the wax blooms are continuous with the lipid layer of the epicuticle and are ubiquitous on the cuticular surface of all the stages of *Oppia coloradensis* except on the dorsum of the adult. Some of these wax blooms

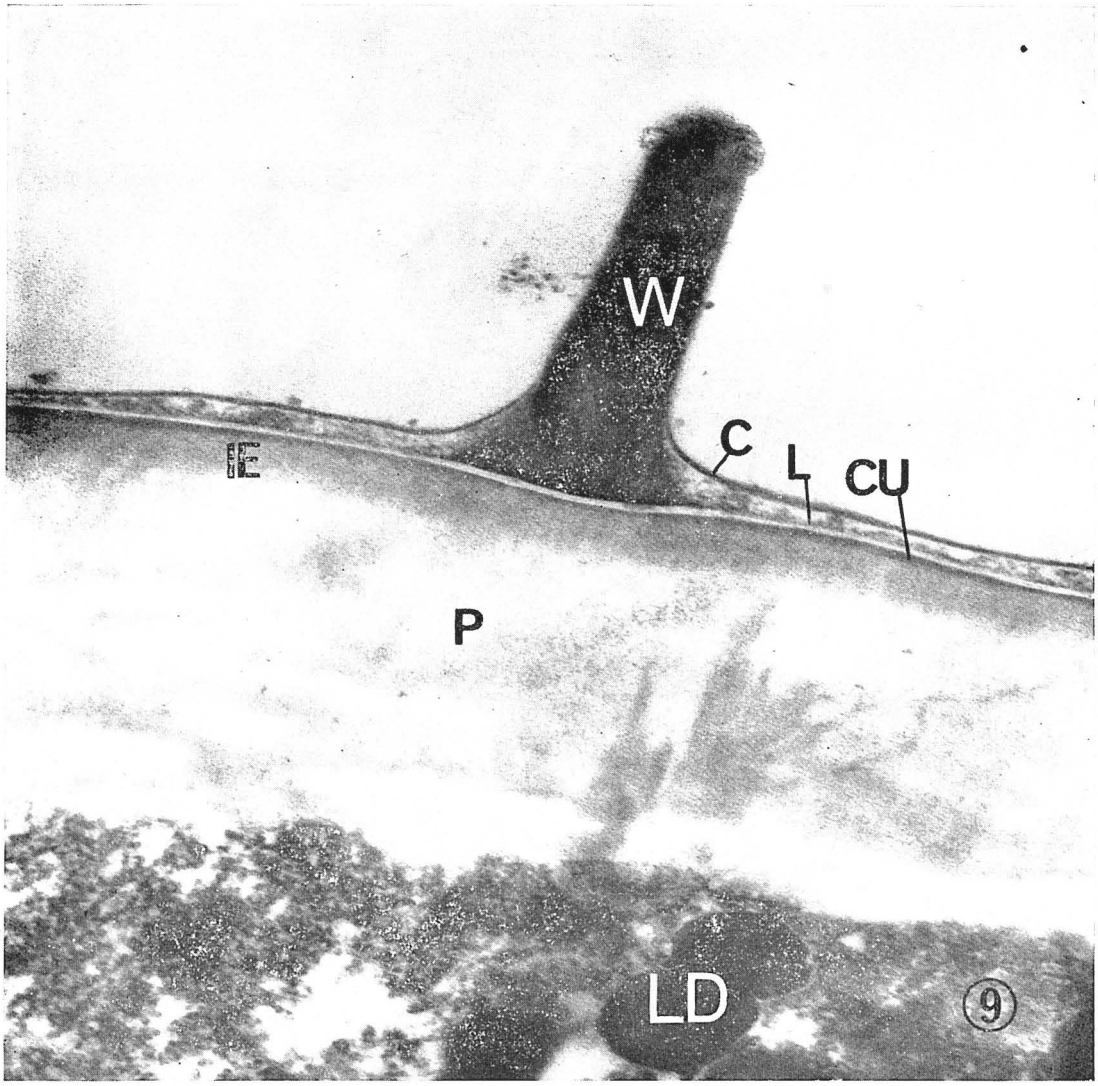


FIG. 9 : A section through deutonymphal cuticle demonstrating the inner epicuticle (IE), cuticulin (CU), lipid layer (L), and cement layer (C) above the procuticle (P). A wax bloom (W), apparently covered by cement, is continuous with the lipid layer. Lipid droplets (LD) are seen in the hypodermis. × 38,000.

rise to a height of about .80 microns and are porous (Figs. 10 & 11). A cement layer (Figs. 8 & 9) covers the lipid layer and wax blooms. Furthermore, these wax blooms, as a regular feature of the epicuticle of *O. coloradensis*, may have several important implications in the formation of a waterproofing system. With a hydrophilic, protective cement cover, the blooms may conserve water among them. This situation may result in subsequent evaporation and cooling of the

mite. On the other hand, if the cement layer is hydrophobic, the cuticular surface would have decreased wettability and air may be trapped between a layer of water and the cuticle as moisture spreads over the surface of the mite.

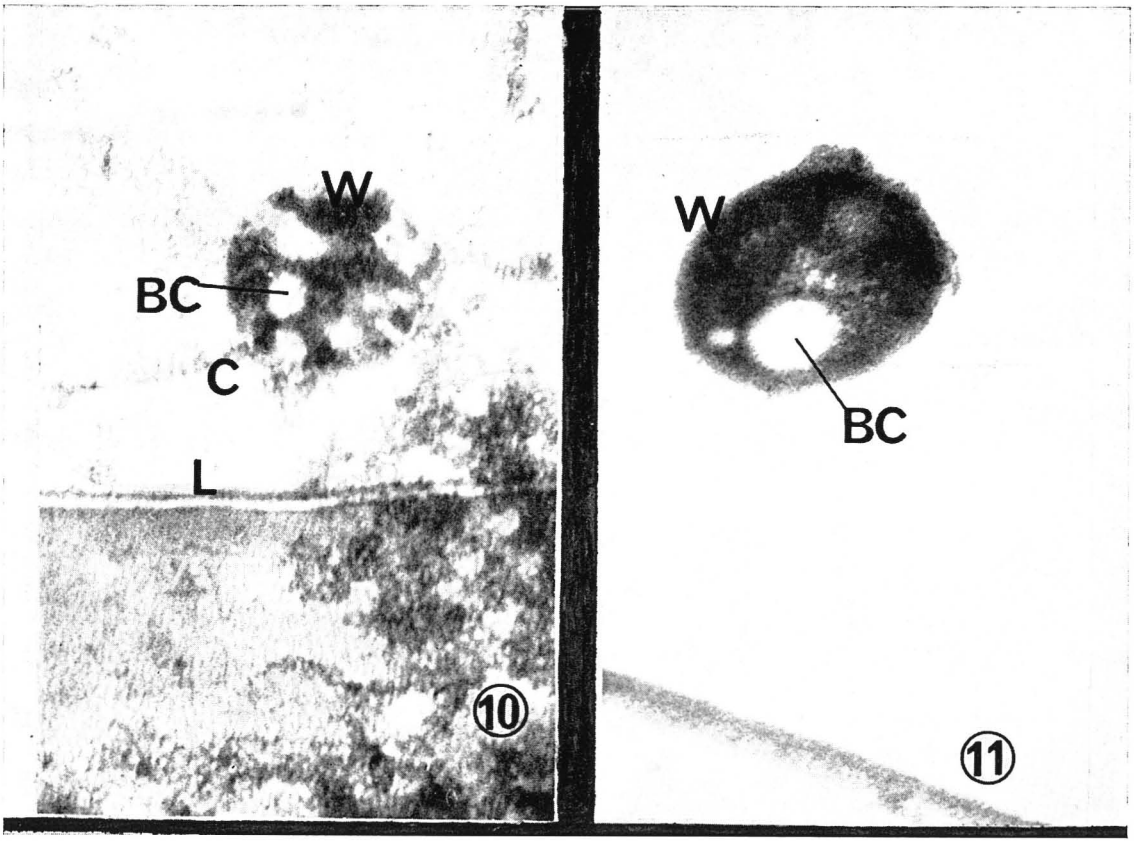


FIG. 10 : Section of larval cuticle showing a cross section through a wax bloom (W) with bloom canals (BC). Cement (C) and lipid layer (L). × 57,000.

FIG. 11 : Section of deutonymphal cuticle showing a bloom canal (BC) in a cross section of a wax bloom (W). × 57,000.

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ABSTRACT.

Whole mites were fixed with glutaraldehyde and osmium and embedded in a mixed resin of Epon and Araldite. Sections were stained with uranyl acetate and lead citrate and viewed on a Zeiss transmission electron miscroscope.

As new adult cuticle is produced beneath sclerotized, tritonymphal cuticle in the pre-ecdysial condition, numerous granules are secreted in layers by the hypodermis. These granules apparently coalesce into microfibers which are arranged in a parabolic pattern. The fibers are then consolidated to form

laminations as the cuticle matures. An ecdysial space is present between the pharate adult and the tritonymphal cuticle.

An epicuticle is present which consists of several layers. The fine structure of the epicuticle reveals a cement layer, a lipid layer with wax blooms, a cuticulin layer, and a proteinaceous layer below. The cement layer appears to cover the wax blooms and probably is a distinct factor in the water balance mechanism of this mite.

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