

OBSERVATIONS ON THE FINE STRUCTURE OF THE CUTICLE
OF THE SPINY RAT MITE, *LAELAPS ECHIDNINA*
(ACARI — MESOSTIGMATA)¹

BY

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The classification of Acari is almost entirely a summary of statements derived from study of the cuticle and cuticular structures. Despite this reliance on the cuticle as the primary source of taxonomic information there have been few attempts to extend the study of cuticles beyond the level of light microscopy and to relate observations made by light microscopy to the fine structure of the cuticle. The only such papers with which we are familiar are those on spider mites (Tetranychidae) by ANWARULLAH (1963) and HENNEBERRY, *et al.* (1965); and those on ticks by DANIEL and LUDVIK (1954) and NATHANSON (1967). It is the purpose of the present paper to describe some features of the cuticle as observed by light microscopy and to analyze these features in terms of the fine structure as observed with the techniques of electron microscopy. These observations were made on the spiny rat mite, *Laelaps echidnina* Berlese, 1887, a species used in physiological studies in our laboratories over the past ten years.

MATERIALS AND METHODS.

Females of the spiny rat mite from cultures maintained in the Institute of Acarology were used in all of the work. Entire mites and dissected portions were mounted in Berlese's fluid. Serial sections were prepared for study with conventional histological techniques and with EM techniques using an ultramicrotome. Electron micrographs were made of thin sections prepared in a number of ways. Sections fixed in osmium tetroxide and imbedded in methacrylate resin proved unsatisfactory. Fixation with osmium-tetroxide and imbedding in epoxy resin was more successful. The best method used is as follows :

1. A suitable specimen was immobilized by attaching its dorsal shield to a piece of masking tape.

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2. The tarsi of two or more legs were cut off with a micro-knife constructed of a small fragment of a razor blade.
3. A micro-needle was inserted into the haemocoel through a coxal acetabulum or through the soft cuticle just posterior to the anal plate.
4. The body cavity was perfused with 5 % gluteraldehyde at pH 7.2 (preceded by phosphate buffer for a few seconds to clear the micro-needle of cell debris).
5. Perfused specimens were allowed to fix in the gluteraldehyde solution for periods ranging from 3 1/2 hours to overnight.
6. Specimens were washed one hour in pH 7.35 phosphate buffer and postfixed in osmium tetroxide for 1 hour.
7. Dehydration and imbedding in epoxy resin was accomplished according to the method of LUFT (1961).
8. Ultra thin sections were cut with a Porter-Blum microtome using either a glass knife or a 42-43° angle du Pont diamond knife.
9. Sections between 0.050 and 0.100 microns in thickness were placed on carbon-coated grids and were stained in uranyl acetate, followed by lead citrate.
10. Observations and photographs were made with either an RCA EMU2B or an RCA EMU3G electron microscope.

A few electronmicrographs of the cuticle of *Caloglyphus berlesei* (Michael, 1903) were prepared using osmium tetroxide fixation and epoxy imbedding. These have been included here for comparative purposes.

RESULTS AND INTERPRETATIONS.

With the use of light microscopy on entire specimens, the following discontinuities of the general surface of the idiosoma can be seen : (1) sclerotized cuticle in the form of shields ; (2) lines dividing the sclerotized cuticle into polygonal areas ; (3) puncta within the polygonal areas of the hard cuticle ; (4) soft cuticle between the shields and striae on the soft cuticle ; (5) setal bases and setae on both the soft and hard cuticle ; and (6) pores on both the hard and soft cuticle. In the following paragraphs we attempt an interpretation of these features in terms of the fine structure of the cuticle.

Excellent accounts of the fine structure of insect cuticle have been given by LOCKE (1961, 1965) and it is on the basis of these accounts that our identifications of the components of the integument of the mites studied here have been made. While the necessary chemical tests to identify the various layers unequivocally have not been made, it would seem desirable to emphasize the visual similarities between these observations and LOCKE's until such time as any chemical incongruities are in fact discovered.

Electronmicrographs of sections of the sclerotized cuticle, and especially that of the dorsal shield, made possible an analysis of the fine structure (Figure 1). The

inner-most layer of the cuticle is a granular region at the interface with the epidermal cell (Figure 1, 2; S1). This granular layer varies in thickness from 0.025 micron to 0.050 micron and is here identified as SCHMIDT's layer. Above the granular layer the cuticle lacks lamellae for a distance of some 0.4 micron; this portion is regarded as endocuticle (Figures 1, 2; endo). Above this non-laminate layer the structure of the cuticle remains essentially constant for a distance of about 10 microns. Within this region of the cuticle lamellae about 0.1 micron in thickness are separated by electron dense material which is some 0.01 to 0.02 micron in thickness. Thus the main portion of the sclerotized cuticle consists of about 100 lamellae separated by thin electron dense plates. This main body of sclerotized cuticle is the exocuticle (Figure 1; exo). A thin outermost region, characterized by closely spaced thin lamellae, is the so-called dense layer (Figure 1; dl). External to the dense layer are the other layers of the epicuticle (Figures 3, 4; epi); here three thin layers can be differentiated. Innermost is a (relatively) thick, very dense layer. This is followed by a clear layer which is overlaid by a thin dense layer. These three layers are identified (Figures 3, 4) as, respectively, the cuticulin (cut), the wax layer (wl), and the cement layer (cem). The four layers of the epicuticle together comprise a thickness of 0.15 microns. The entire sclerotized cuticle varies in thickness between 10 and 11 microns.

Each of the shields or plates of the idiosoma appears to be divided into polygonal areas by a series of lines on the surface (Figure 5). Electron microscopy reveals that the lines are actually ridges that are deflected posteriorly (Figure 6). While the ridges probably represent the posterior margins of the epidermal cells that underlie the cuticle, no indication of cell boundaries was visible through the main body of the cuticle underlying the ridges. The puncta that are so characteristic of sclerotized acarine cuticle can be clearly seen within the polygonal areas of the dorsal shield (Figure 5). In electron micrographs of thin sections made approximately parallel to the surface of the sclerotized cuticle they can be seen in cross section (Figure 7). In cross sections of the sclerotized cuticle they can be seen to be canals originating at the epidermal cell and extending to but not through the cuticulin layer (Figure 1). These are the pore canals. Most of the canals bifurcate about 2 microns from the surface of the cuticle and on one occasion a triple branch was observed. Extending from the distal tips of the canals are electron dense wax filaments (Figures 3, 4). In some electron micrographs, clumps of secretion could be seen on the surface of the cuticle opposite the tips of the canals (Figure 8; bl); these are interpreted as wax blooms (comp. LOCKE, 1961). The similarity of the pore canals and polygonal areas to those of insects can be seen from the following description of insect cuticle by WIGGLESWORTH (1965, p. 28): "The endo- and exocuticle are traversed by numerous vertical lines. When seen in surface view these appear as minute dots, often arranged in polygonal fields separated by clear boundaries, corresponding to the limits of the epidermal cells."

Electron micrographs of *Caloglyphus berlesei* (Michael, 1903) revealed that the

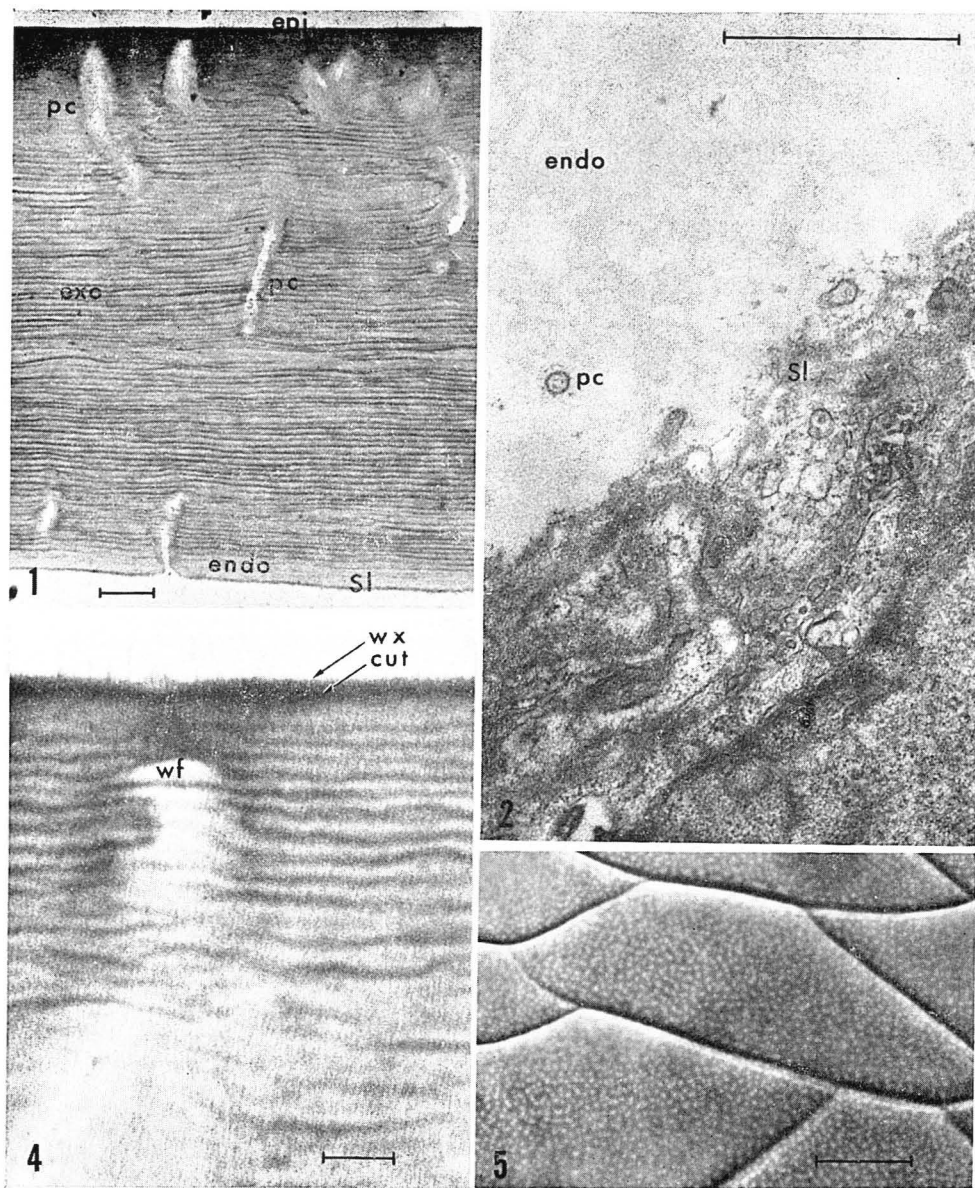


FIG. 1. — Transverse section of sclerotized cuticle of spiny rat mite.

Sl = Schmidt layer; endo = endocuticle; exo = exocuticle; epi = epicuticle;
pc = pore canal. Scale = 1 μ .

FIG. 2. — Section of the interface between the epidermal cell and the cuticle cut obliquely to a plane normal to the surface so that pore canals (pc) are shown in approximate cross section. The boundary (the Schmidt layer, Sl) between the cell and the endocuticle can be seen to be poorly defined. Note the extensive endoplasmic reticulum. Scale = 1 μ .

FIG. 4. — Electronmicrograph of structures shown schematically in Figure 3.

wf = wax filaments; cut = cuticulin; wx = wax layer. Scale = 0.1 μ .

Fig. 5. — Surface of dorsal shield as seen with oil immersion phase contrast microscopy.
Scale = 10 μ .

puncta visible in the sclerotized portion of the cuticle were pore canals (Figures 9, 10). Furthermore the same fine filaments associated with the canals in the spiny rat mite were visible in *Caloglyphus*.

Not preserved in cleared whole mounts but clearly visible in ultra thin sections with either light or electron microscopy are the epithelial cells that are responsible for the secretion of the cuticle. These cells are flattened in the adult mite and are usually less than 1 micron in thickness. Despite their extreme thinness they are apparently quite active judging from the well developed ER system adjacent to the SCHMIDT layer and the numerous mitochondria and ribosomes (Figures 2, 11).

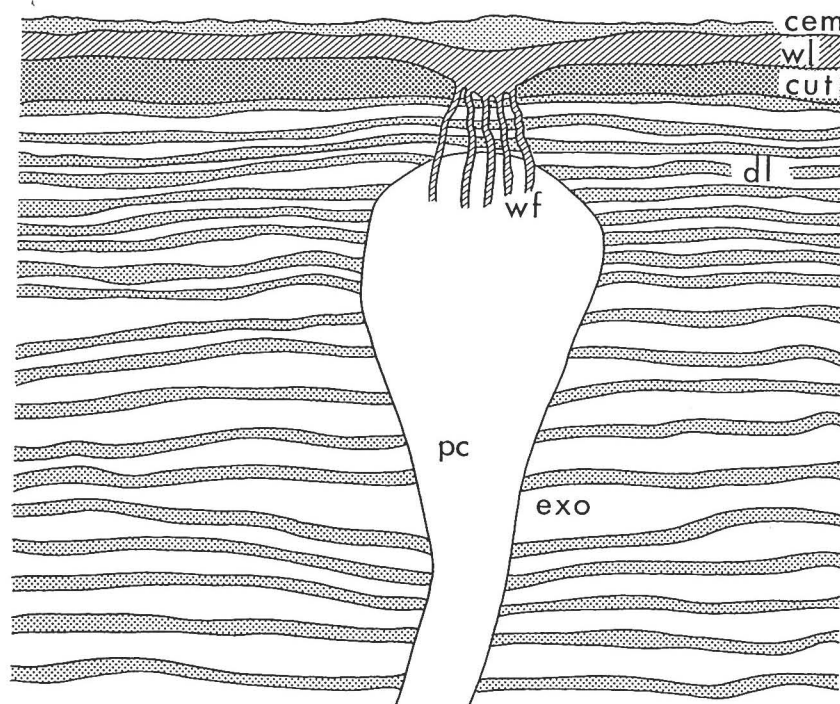


FIG. 3. — Diagram of the epicuticle and the terminus of a pore canal in the spiny rat mite.
cem = cement ; wl = wax layer ; cut = cuticulin ; dl = dense layer ; pc = pore canal ;
wf = wax filament ; exo = exocuticle.

Two seemingly unrelated functions must involve these cells. The first, that of production of wax, follows from the presence of "blooms" seen on the surface of the cuticle opposite the tips of the pore canals. The second that of absorbing water follows from the fact that the spiny rat mite can extract water through the cuticle from unsaturated air (WHARTON and KANUNGO, 1962 ; KNÜLLE, 1967).

The soft or unsclerotized cuticle is about twice as thick as the sclerotized cuticle (Figure 12). Its inner and outermost layers are similar to those of the sclerotized cuticle but its main body is quite different. About nine or ten electron dense lamellae

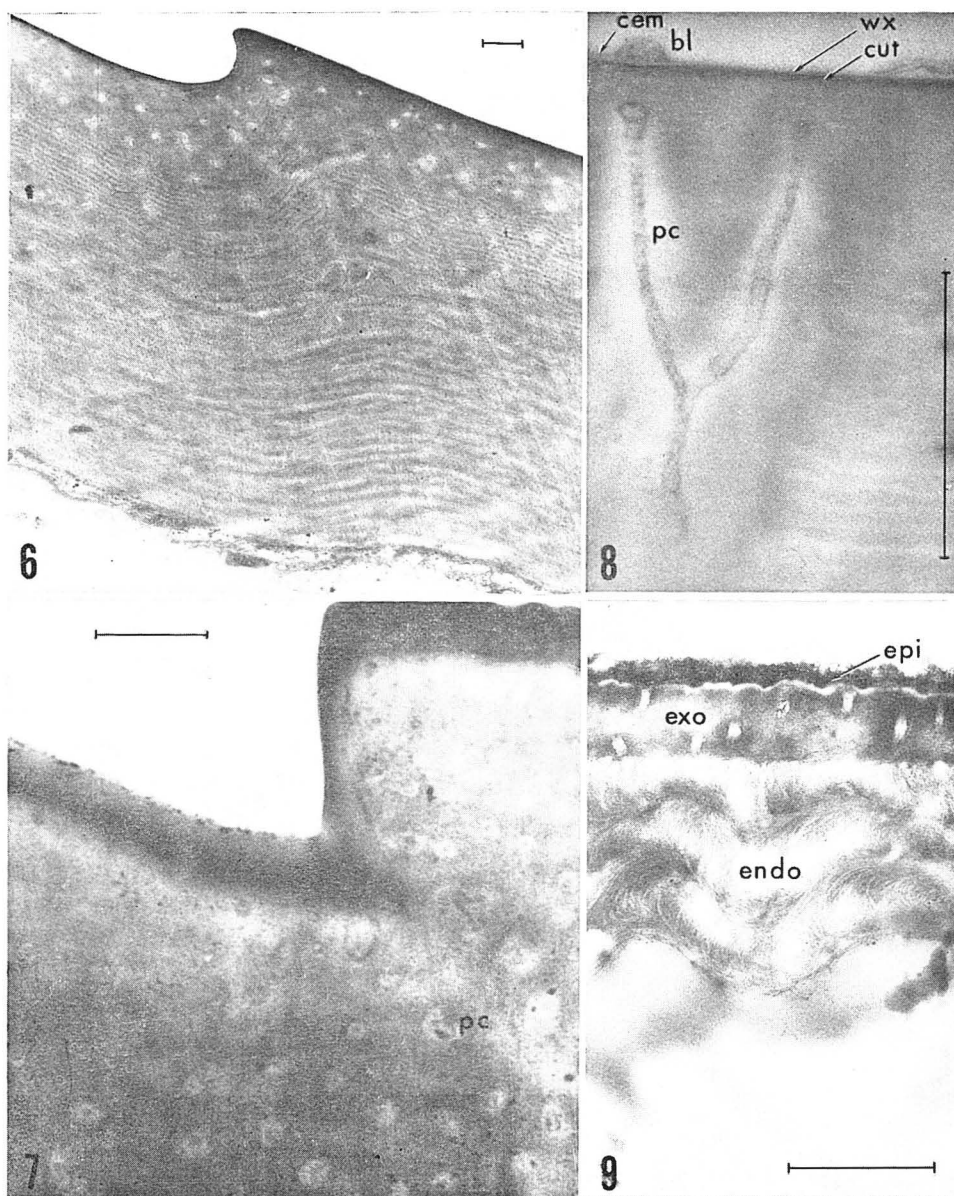


FIG. 6. — Oblique section of cuticle through one of the posteriorly projecting surface ridges responsible for the polygonal pattern observed at the surface of the sclerotized plates. Scale = 1 μ .

FIG. 7. — Section of cuticle showing the pore canals in transverse section.
pc = pore canal. Scale = 1 μ .

FIG. 8. — Transverse section through sclerotized cuticle showing a "bloom" opposite the termination of a pore canal. bl = bloom; pc = pore canal; cut = cuticulin; wl = wax layer; cem = cement. Scale = 1 μ .

FIG. 9. — Cuticle of *Caloglyphus berlesei*. endo = endocuticle; exo = exocuticle; epi = epicuticle. Note pore canals in exocuticle. Scale = 1 μ .

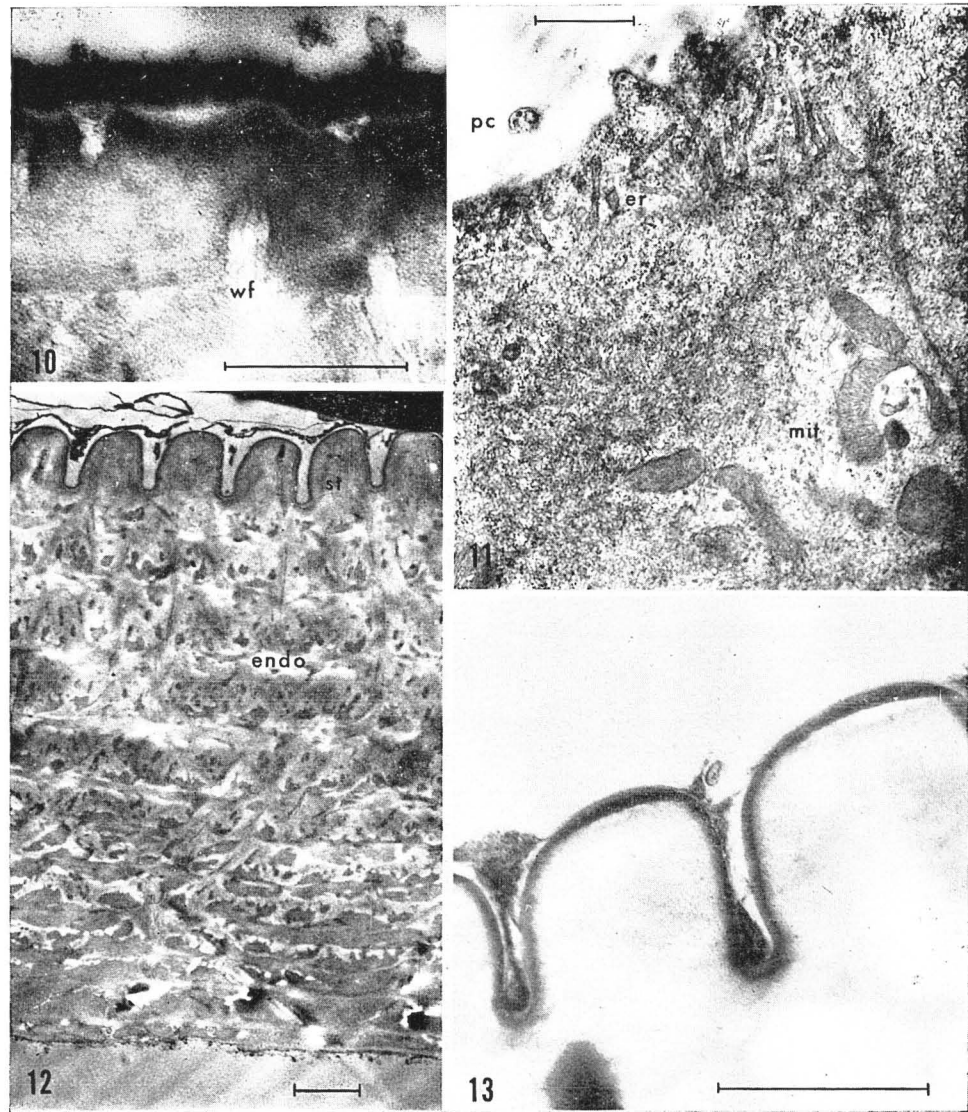


FIG. 10. — Cuticle of *Caloglyphus berlesei*. Note wax filaments (wf).

Scale = 0.5μ

FIG. 11. — Section of epidermal cell of spiny rat mite showing : pc = pore canal ;
er = endoplasmic reticulum ; mit = mitochondrion. Scale = 1μ .

FIG. 12. — Section through the unsclerotized cuticle of spiny rat mite.
endo = endocuticle ; st = stria. Scale = 1μ .

FIG. 13. — Section of striae from the unsclerotized cuticle of spiny rat mite.
Scale = 1μ .

separating less dense areas can be seen. Each lamella is about 2 microns in thickness. The outer surface of the smooth cuticle is thrown into folds or striae; these striae are entirely superficial (Figures 12, 13).

The setal bases and setae were found to have the same basic fine structure as the dense layer of the sclerotized cuticle. Phase contrast oil immersion microscopy of ultra thin sections revealed that the setal bases extended through the whole thickness of the cuticle and thus in effect formed a discontinuous portion of the body wall.

In insects an outer covering of material secreted by dermal glands is considered to be the typical upper most layer of the cuticle. Such an upper most layer is visible in the spiny rat mite. If such a layer in mites is comparable to that in insects then dermal glands should be present in the mites. Among acarines there have been described several types of pores that open on the surface of the cuticle (VAN DER HAMMEN, 1964; EVANS and TILL, 1965; FAIN, 1966; and WILKINSON, 1965). In the spiny rat mite 22 pairs of pores occur on the dorsal shield; their distribution is the same as that shown in the generalized diagram of *Laelaps* given by EVANS and TILL (1965, Figure 9 C). Our studies of the pores are quite incomplete but it is clear that several types are present. Three pairs of the pores are associated with an internal cuticular sac. Excretion or secretion from these pores could be responsible for a cement layer. On the other hand there are four pairs of pores on the dorsal shield that provide an opening for a large glandular type cell to the surface. These also could contribute to or be solely responsible for the cement layer. In some insects more than one type of gland is thought to be responsible for the cement, e. g. *Rhodnius* (WIGGLESWORTH, 1965, p. 39).

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