ADAPTIVE FEATURES OF THE EXOSKELETON AND "PIGMENT" DEPOSITS IN *DEMODEX* SPP. (DEMODICIDAE) *

BY

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ABSTRACT.

The demodicid exoskeleton shows the three (epicuticle, exocuticle, endocuticle) layers typical of the arthropod exoskeleton. It is remarkably thin (under 0.6 μ) in all stages of the life cycle, relatively non-porous, and highly chitinous. A keratin-like protein present in the mid-layer (exocuticle) is apparently of adaptive significance in lending flexibility, especially in the egg and in mature stages, to the exoskeleton. The epicuticle is very thin and, in part, lipid in nature. The endocuticle was most reactive to all tests for chitin.

The pigment granules found in all stages (even ova) of the demodicid life cycle are not guanine. They seem to play a dual role as (1) inert repositories of waste material and (2) elements used in "excretory coding" — since they are incorporated in the egg and probably form a substrate for waste immobilization during development.

Members of the three genera, *Demodex*, *Harpyrhynchus*, and *Psorergates*, show birefringence on polariscopic examination and so fall within GRANDJEAN'S phylogenetic grouping of Actinochitinosi.

INTRODUCTION.

The exoskeleton and "pigment" granules of the parasitic mites of the Grade Eleutherengona have received little attention despite their obvious adaptive values. These inert structures, in the Demodicidae are concerned in, for example, oviposition of the huge egg, unusual adaptive egg shapes (NUTTING *et al*, 1968). A proneness of all stages to dessication, a lack of conduits for waste removal, and the matching of body shape and dimension to habitat (see NUTTING, 1965). Such factors are all related to mite survival. Closely allied cheyletids, psorergatids and harpyrhynchids also share some of these adaptive features.

This report presents some data on the physical characteristics and chemistry of the exoskeleton and of the pigment granules of *Demodex* spp. (esp. *D. caprae*) with brief note of their adaptive significance. Sections and whole specimens of *Demodex* sp. from the marsupial mouse, *Antechinus stuartii* (NUTTING and WOLLEY, 1965) were used for comparison. Some information is given, also, for the related genera *Harpyrhynchus* and *Psorergates*.

* Work supported in part by a grant from the National Science Foundation (GB-3516).

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Materials and Methods.

The physical characteristics of both the exoskeleton and pigment granules in four stages of the life cycle (egg, larva, nymph, adult) of *D. caprae* were examined using light, phase, polariscopic and electron microscopy (EM). A few specimens of *Harpyrhynchus* sp. and *Psorergates* sp. were also examined by light miscroscope and polariscope.

The exoskeleton of all stages of *D. caprae* was examined for the presence of chitin, sclerotin, acid mucopolysaccharides and keratin. For these studies the mites were first freed from the paste-like material in which they are found in the papule (skin lesion of the goat) by rapid agitation in physiological saline using a VirTIS micro-blendor. They were then washed and treated as follows.

The chitosan-iodine method for the detection of chitin (Campbell, 1929) was used initially. Mites were rewashed in chloroform : methanol (2 : 1) to remove lipids and then reacted with hot (160°C) KOH. The sample of exoskeletons was divided in half ; one-half was treated with 3 % acetic acid, the other was stained with 0.02 % iodine and 1 % sulfuric acid (Richards, 1951).

Secondly the chitinase digestion method using the procedures of Hackman (1953 a), Reynolds (1954) and Okafor (1965) was employed. Mite exoskeletons prepared in a manner similar to above were tested with a 0.01 % chitinase (Nutritional Biochemical Company) against known chitin (NBC), the degradation being carried out at 30°C for 48 hours. Breakdown products were identified with paper and thin-layer chromatography (TLC). Paper chromatography followed the method of Hackman (1953 b) with 1 ml samples developed in a descending system using n-butanol : acetic acid : water (77 : 6 : 17) as solvent system for 12 hours. For TLC the samples were spotted on a 0.25 mm layer of silica gel G and developed by an ascending method in a solvent system of n-butanol : ethanol : water (30 : 60 : 10). Standards of glucosamine and n-acetyl glucosamine were run with each separation. Separations were visualized with the Morgan-Elson reaction for hexosamine (Partridge, 1948) and by charring with 50 % sulfuric acid saturated with potassium dichromate.

Thirdly chitin was detected in mites *in situ* by treating paraffin sections of papules with chitinase. A 0.01 % chitinase was added to the section at 30°C for 24 hours. Control and experimental slides were stained in haematoxylin and eosin for comparison.

Polyphenoloxidase, a precursor of sclerotin was decteted by the catechol method (Johri and Smyth, 1961) and the protein precursors were searched for with the bromphenol blue method (in Pearse, 1968).

In addition the following histochemical tests (see Humason, 1962 ; Pearse 1968) were performed on paraffin sections of mites (*D. caprae* and *D. sp.*) *in situ* : Mallory triple stain for chitin, Periodic acid Schiff reaction for mucopolysaccharides, Bromphenol blue for protein and Masson trichrome stain for keratin.

Prior to staining, the mites and paraffin sections of papules were treated with benzene or ether since this increased the intensity of the staining of the mites especially in acid fuchsin.

Both mite exoskeletons and their pigment granules were incubated with saturated KOH and 1 % trypsin for 12 hours to note the denaturation of their protein components.

Results.

Physical characteristics of the egg shell, larval, nymphal and adult exoskeletons of D. caprae.

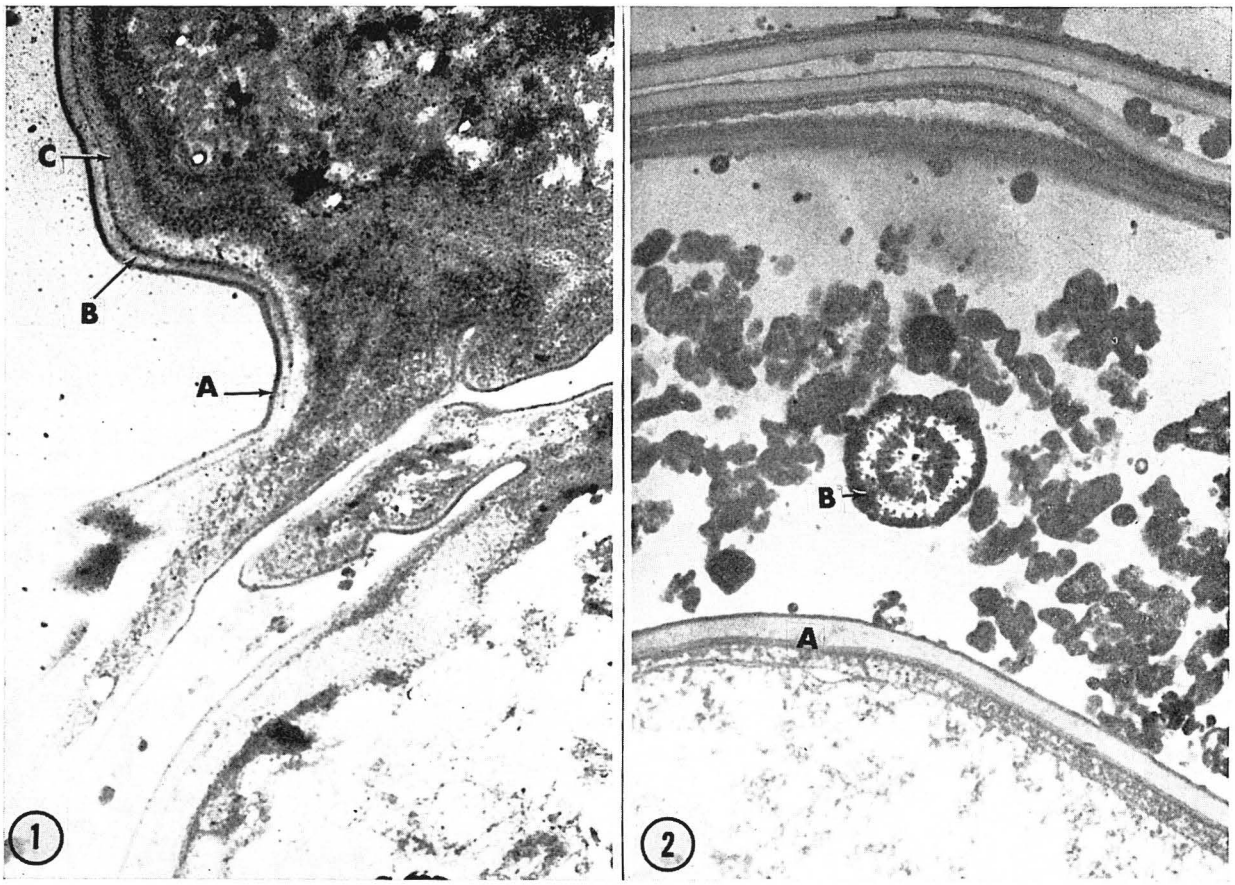

Fig. 1. — EM photomicrograph of a section through the podosoma of *Demodex caprae* : A — epicuticle ; B — exocuticle ; C — endocuticle. (Photo by Dr. Oscar Bradfute).
Fig. 2. — EM photomicrograph of a section of the immature (nymphal ?) opisthosoma of *Demodex caprae* : A — exoskeleton ; B — cross section of pigment granule. (Photo by Dr. Herbert Postswald).

The thickness of the structures varied from 0.11 μ (egg shell) to 0.54 μ (adult exoskeleton in podosomal region). The fine structural studies (Figures 1,2) are preliminary observations since difficulties with fixation and infiltration may have introduced artifacts. Nevertheless an outer epicuticle area is evident and below this there are two distinct layers, an exocuticle and an endocuticle. In all sections the endocuticle appeared to be non-porous and relatively homogeneious.

Nymphal and larval (Figure 2) exoskeletons are thinner (0.4 μ) with both the exo- and endocuticles less well developed than in the adult. The adult exoskeleton shows a thickened saddle-shaped area in the anterior-dorsal podosomal region. The posterior portion and sides of this saddle represent the areas of origin of the major muscles of the podosoma and capitulum.

The pigment granules are irregular, bean- or rod-shaped objects measuring 0.5 to 13.2 μ

in length and 0.5 to 2.5 μ in diameter. In fine structure (Figure 2) the large granules seem concretionary in nature. In contrast to the granules of *D. caprae*, those in *Harpyrhynchus* sp. are larger (c. 6.6 to 16.5 μ × 3.3 to 6.6 μ) and more rod-like, and in *Psorergates* sp. smaller (under 1.0 μ), rounded and more dispersed.

In all three genera certain exoskeletal setae, the chelicerae, and the pigment granules showed birefringence in the polariscope.

Chemical Composition.

All tests employed showed that chitin was present in the egg shell and in larval, nymphal and adult exoskeletons. Chitinase degradation yielded glucosamine and n-acetyl glucosamine (Figure 3). The rapidity of degradation in KOH and trypsin followed the sequence of egg shell, larval exoskeleton, nymphal exoskeleton, adult opisthosomae and adult podosomae. This is in keeping, not only with their relative dimensions as assessed by electron microscopy but also, and more significant, with the amount of protein material which was detected by histochemical staining.

The polyphenol oxidase could not be identified in any stage of development, and this indicates that sclerotin is most likely absent from the exoskeleton.

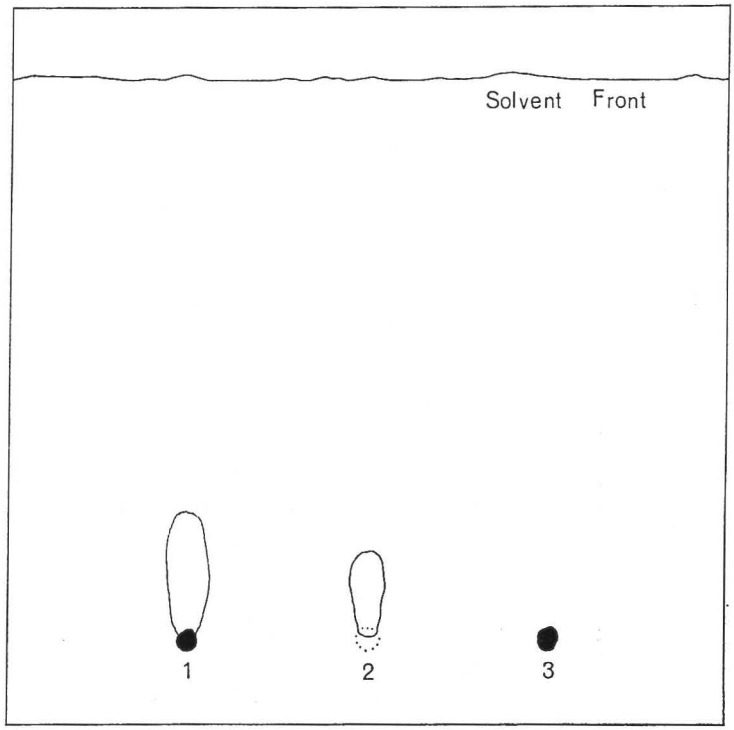

FIG. 3. — Separation of degradation products of *Demodex caprae* exoskeletons after chitinase digestion (1) and controls n-acetyl glucosamine (2) and glucosamine (3). (Solvent front 22.4 cm).

Histochemical Studies.

Since stains and fixatives penetrate more readily after the mites are rinsed in benzene or ether it is assumed that the epicuticle is in part lipid in nature. This lipid component is represented by the dense outer cuticular layer upon electron microscope examination. Two layers

in the exoskeleton were visible with trichrome staining, the outer layer (exocuticle) was positive for keratin and the inner layer (endocuticle) was positive for mucopolysaccharides (most likely chitin). The exoskeleton of the female and immature developmental stages of the demodicid from *Antechinus* appeared very bright (birefringent) when examined with phase and polarized light microscopy. This may have been due to the keratin components. Moulted exoskeletons in *D. caprae* were usually thin, the endocuticle being absent, perhaps due to the premoult degradation of chitin.

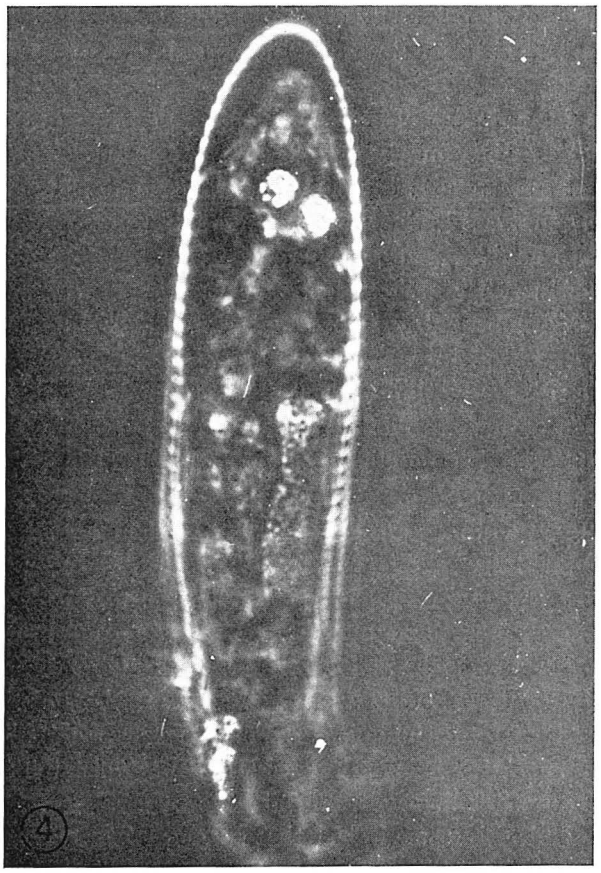

FIG. 4. — Phase contrast photomicrograph of the opisthosoma of a demodicid of *Antechinus stuartii*. Shows segregation of pigment granules just prior to incorporation into developing ovum.

Pigment Granules.

Observation of the life cycles of *D. caprae* and *D.* sp. of *Antechinus* show that the pigment granules are segregated into two nearly equal groups just prior to egg development (Figure 4). One group is incorporated into the egg early in development, while the other remains in the female. In the egg the crystals are aggregated in the (future) posterior of the mite and they were found in the posterior opisthosomal region in all ensuing stages. Separation of the granule aggregate does not occur in the male mite and for example no granules were found in the testes.

Preliminary tests with KOH show that the crystals were not soluble in this and so probably not guanine.

Discussion.

Histochemical tests and electron microscopy preparation were hampered by the physical characteristics of the mite exoskeleton. For the former the thinness of the exoskeleton made it difficult to distinguish the stains accurately. In the electron microscope studies we found that fixation and infiltration by conventional methods were inadequate especially for soft structures. This would suggest that the exoskeleton is relatively impermeable to large molecules, although it obviously permits water and gas exchange.

The endocuticle is composed primarily of chitin and is well developed. This is especially true of the saddle-shaped plate in the podosoma which provides greater strength to the thin exoskeleton for muscle attachment.

As mentioned above, for a demodicid in *Clethrionomys* (Nutting *et al*, 1968) the shell of the relatively large demodicid egg is remarkably flexible. This can in part be explained by the thinness of the shell but it seems more intimately related to the high levels of protein.

In all other stages our analyses indicate that demodicid mites rely heavily on the production of chitin for adaptive fit to environmental demands rather than on high levels of keratin or of tanned sclerotin as occurs in other arthropods.

The pigment deposits are probably waste material. It is interesting that approximately one-half of these are incorporated into the egg. It seems likely that two purposes are served by this unique mechanism : the reduction of waste in the female and incorporation of a substrate for waste deposition in the embryo. The first may in part explain the relative longevity (see Spickett, 1961) of the adult female demodicid as compared with the male. The latter apparently has no such waste removal mechanism. If the parental granules are used as a substrate for ensuig stages this « excretory coding » would represent a biochemical short-cut in evolution for inactivating deleterious metabolic waste products.

The pigment granules (" guanine spheroids ") found by Wright and Newell (1964) in *Anystis* sp. were assumed, by them, to be guanine. The pigment granules found in *Demodex* spp. appear to be more refractile and to have fewer concentric layers than those in *Anystis*. The guanine spheroids found by Wright and Newell were in the epidermis, while demodicid pigment spots are free in the posterior end of the opisthosoma closely associated with the mid-gut and/or gonads. Wright and Newell also believe that epidermal crystals are involved in waste removal : apparently, however, they are not incorporated into the ova.

The demonstration of birefringence in the three genera (*Demodex, Harpyrhynchus, Psorergates*) from three closely related families confirms Grandjean's (1935) view that these trombidiform mites can be grouped phylogenetically as Actinochitinosi.

Acknowledgments.

The authors would like to thank Dr. O. Bradfuten for the electron photomicrographs, Dr. H. Postswald for help with interpreting the photomicrographs and Dr. M. Kaulenas for the initial chemical analysis of the pigment granules.

REFERENCES

Campbell (F. L.), 1929. — The detection and estimation of insect chitin, and the correlation of " chitinisation " to hardness and pigmentation of the cuticle of the American cockroach, *Periplaneta americana*. — Ann. Ent. Soc. Amer., 22 : 401-426.

GRANDJEAN (F.), 1935. — Observations sur les Acariens. — Bull. Mus. Hist. nat., **7** (2) : 119-126.

HACKMAN (R. H.), 1953 *a*. — Chemistry of the insect cuticle and darkening of the cuticle. — Biochem. J., **54** : 371-377.

HACKMAN (R. H.), 1953 *b*. — Studies on Chitin. I. Enzymatic degradation of chitin and chitin esters. — Aust. J. Biol. Sci., **7** : 168-178

HUMASON (G. L.), 1962. — Animal tissue techniques. — W. H. Freeman and Co. San Francisco.

JOHRI (L. N.) and SMYTH (J. D.), 1965. — A histochemical approach to the study of helminth morphology. — Parasitology, **46** : 107-116.

NUTTING (W. B.), 1965. — Host Parasite Relations : Demodicidae. — Acarologia, **7** : 301-317.

NUTTING (W. B.) ,TISDEL (M. O.) and EMEJUAIWE (S. O.), 1968. — Y-shaped ovum of a demodicid from *Clethrionomys gapperi*. — J. Parasitol., **54** : (2) 393-394.

NUTTING (W. B.) and WOOLLEY (P.), 1965. — Pathology in *Antechinus stuartii* (Marsupialia) due to *Demodex* sp. — Parasitology, **55** : 383-389.

OKAFAR (N.), 1965. — Isolation of chitin from the shell of the cuttlefish *Sepia officinalis* L. — Biochim. Biophys. Acta., **101** : 193-200.

PARTRIDGE (S. M.), 1948. — Filter-paper partition chromatography of sugar. — Biochem. J., **42** : 238-248.

PEARSE (A. G. E.), 1968. — Histochemistry : Theoretical and applied. — Little, Brown and Co., Boston.

REYNOLDS (D. M.), 1954. — Exocellular chitinase from *Streptomyces* sp. — J. Gen. Microbiol., **11** : 150-159.

RICHARDS (A. G.), 1951. — The integument of arthropods. — Univ. Minnesota Press., Minneapolis.

SPICKETT (S. G.), 1961. — Studies on *Demodex folliculorum* Simon (1842) I. Life History. — Parasitology, **51** : 181-192.

WRIGHT (K. A.) and NEWELL (I. M.), 1964. — Some observations on the fine structure of the midgut of the mite *Anystis* sp. — Ann. Ent. Soc. Amer., **57** : 684-693.