

INTEGUMENTARY SYSTEM

The evolutionary and physiological impacts of having a cuticle + not skin.

Huge diversity



It must be emphasized that our knowledge of insect physiology is limited and is usually based on a few model systems. We know that insects are enormously diverse and numerous. Physiologists have not tapped into this rich source of diversity. Because of this, our knowledge is not complete. We make generalizations and make suggestions based on these limited models; when in fact, there are probably insects out there that defy our hypotheses and generalizations. In fact, there are insects out there that have physiological processes that we haven't even discovered. A good example of this is Ron Hoy's work at Cornell. Ron discovered a new hearing mechanism in a parasitic fly of crickets that the army is interested in. Another is the recent find that some insects have a 'breathing-like' apparatus.

INTEGUMENTARY SYSTEM

1. Function(s) of the integument + evolutionary significance to arthropods
2. Structure of the cuticle
3. Chemical composition
4. Sclerotization
5. Physical properties of cuticle
6. Coloration and melanization
7. Techniques and tools used to study the integument
8. Basic and applied research

Evolutionary significance of arthropod integument or cuticle

POSITIVE EFFECTS

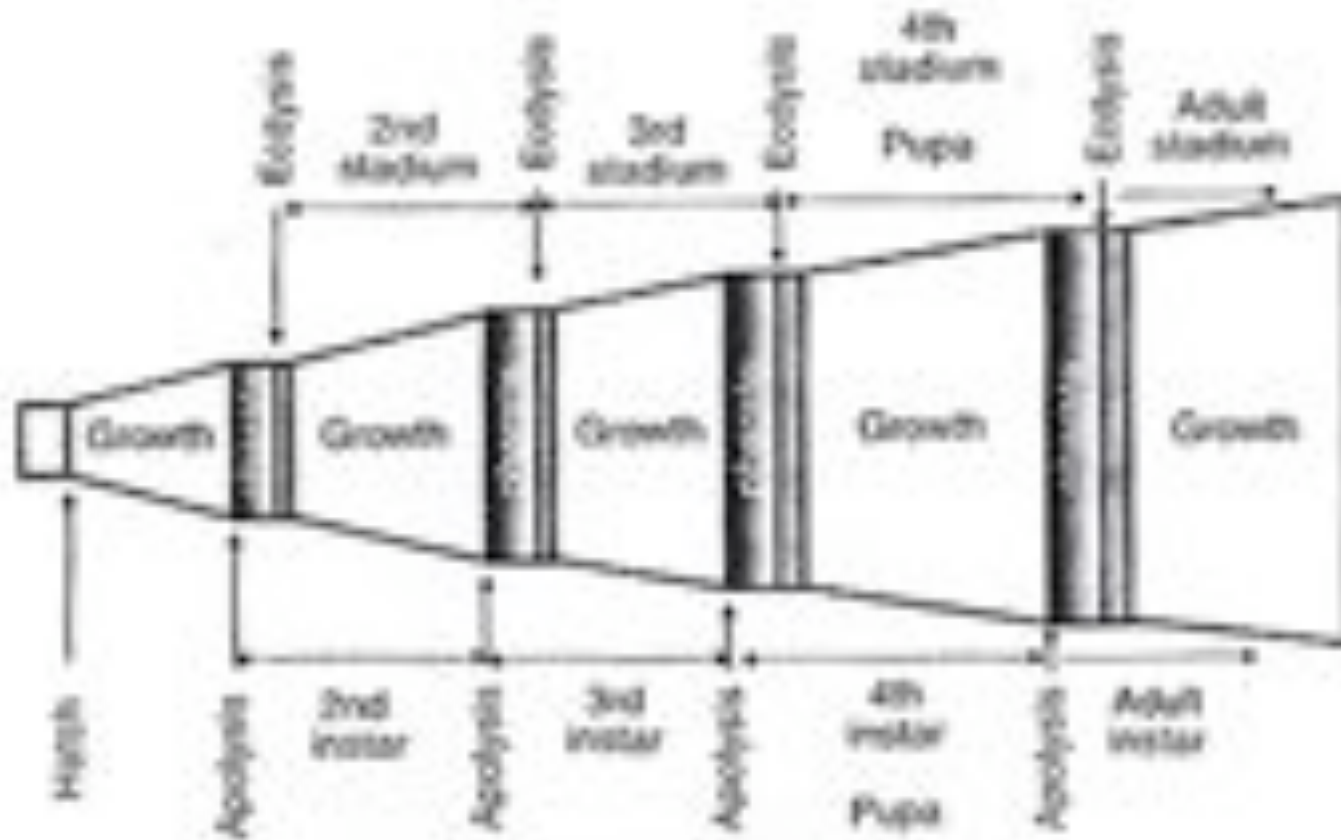
- Prevention of water loss because of wax layer and cuticular composition. Smaller the organism the greater the amount of surface area per unit volume-greater tendency to lose water.
- Preventative barrier for pathogens, parasites and predators.

NEGATIVE EFFECTS

- As size increases, problems with cuticle being too heavy and also gaseous exchange (oxygen uptake). Evolutionary experiment with *Meganeuron*.

1. FUNCTIONS OF THE INTEGUMENTARY SYSTEM

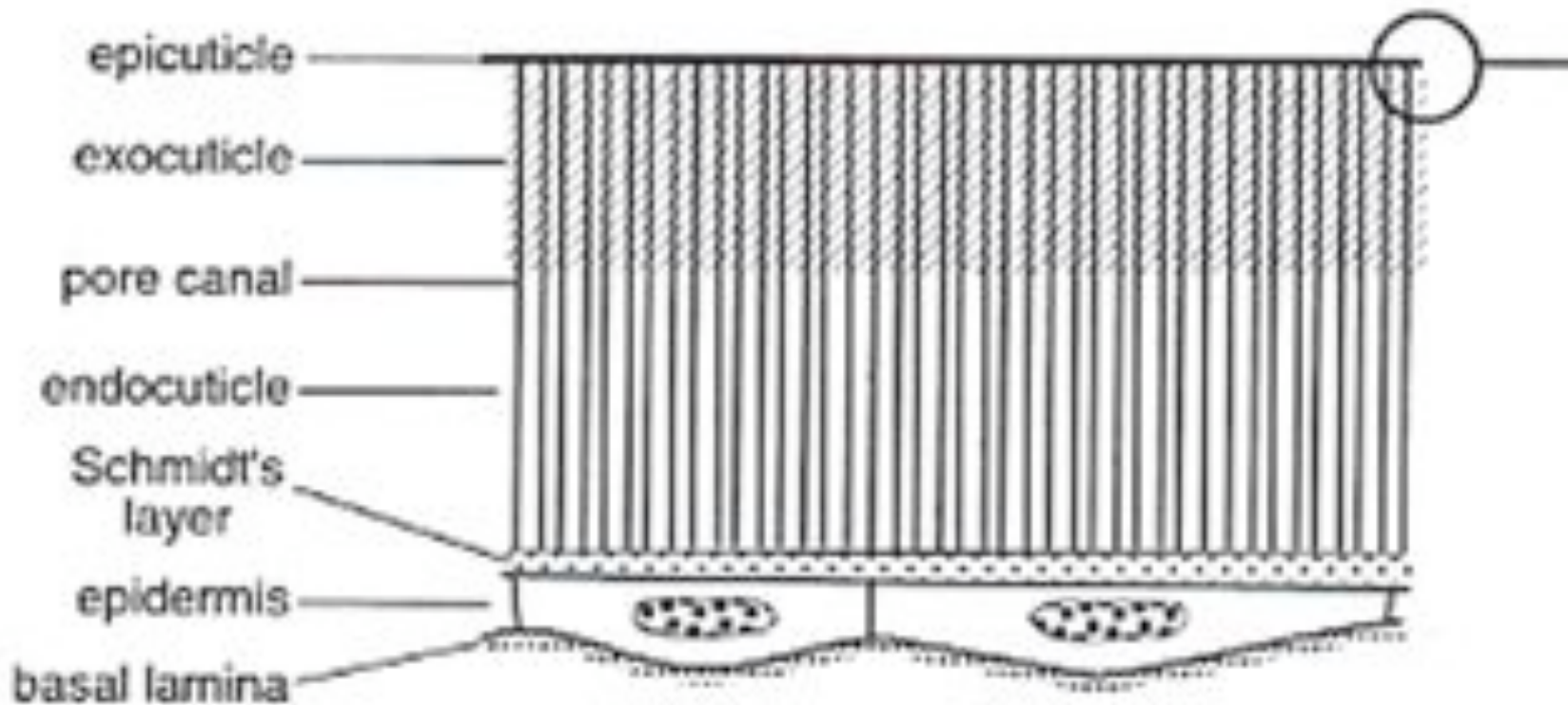
1. Protection of internal organs and tissues
2. Protective barrier against entry of pathogens, parasites, and predators. Now also pesticides.
3. Preventive barrier against water loss
4. Provides for the insect the sensory “windows to the outside world”
- 5. Also lines the tracheae, tracheoles, salivary glands and portions of reproductive tract. At the molt, all of this is shed.**
- 6. Protective barrier for foregut and hindgut.** What kind of questions do I want you to learn to ask about things in this course? **What could you ask here?**



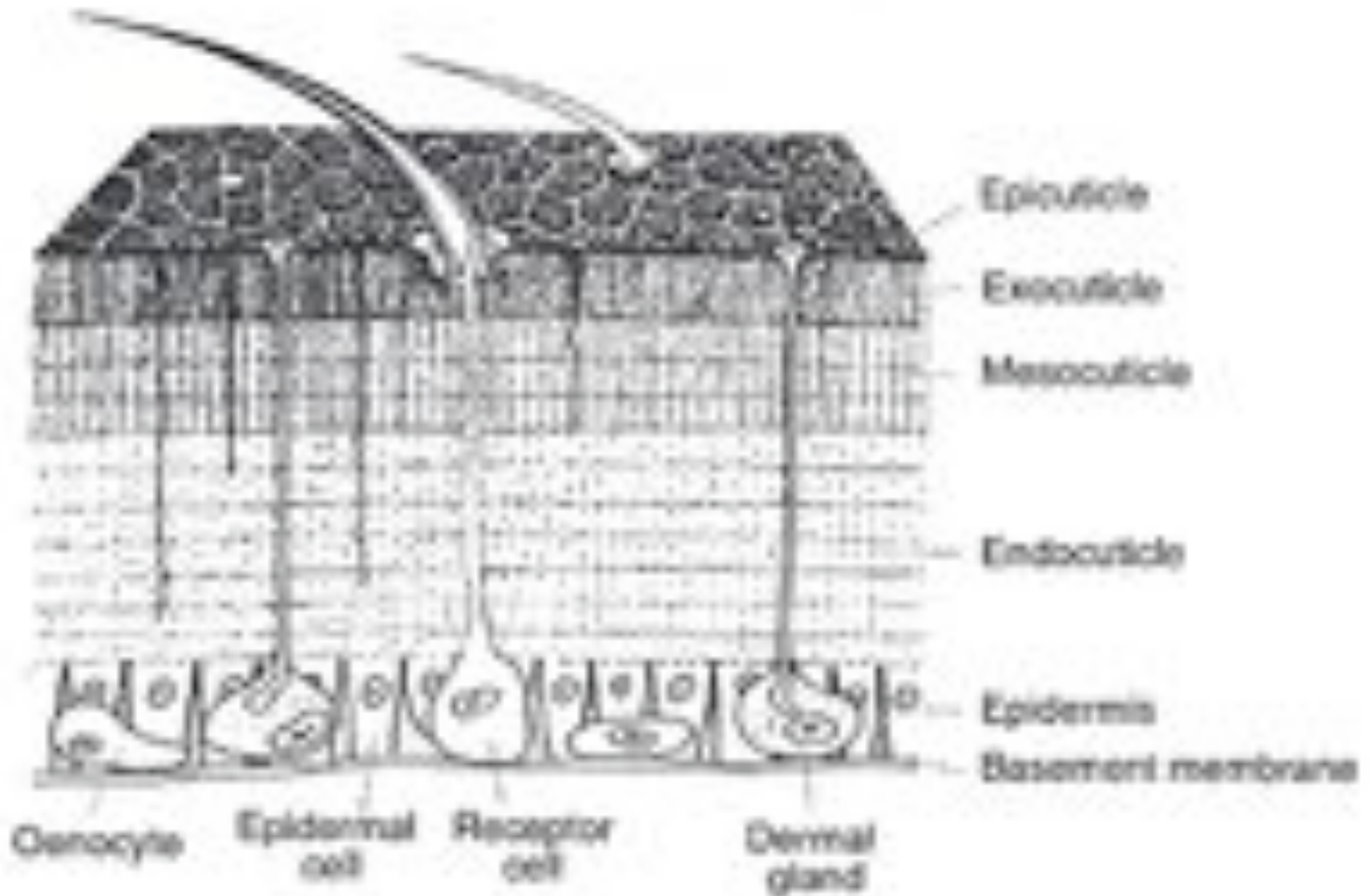
INSECT GROWTH AND THE TERMS APPLIED

Etymology: 19c in sense 1; 17c, meaning ‘a race course’; originally one *stadium* in length, from Greek *stadion* ‘an ancient Greek measure of length of about 1/8th of a mile.’

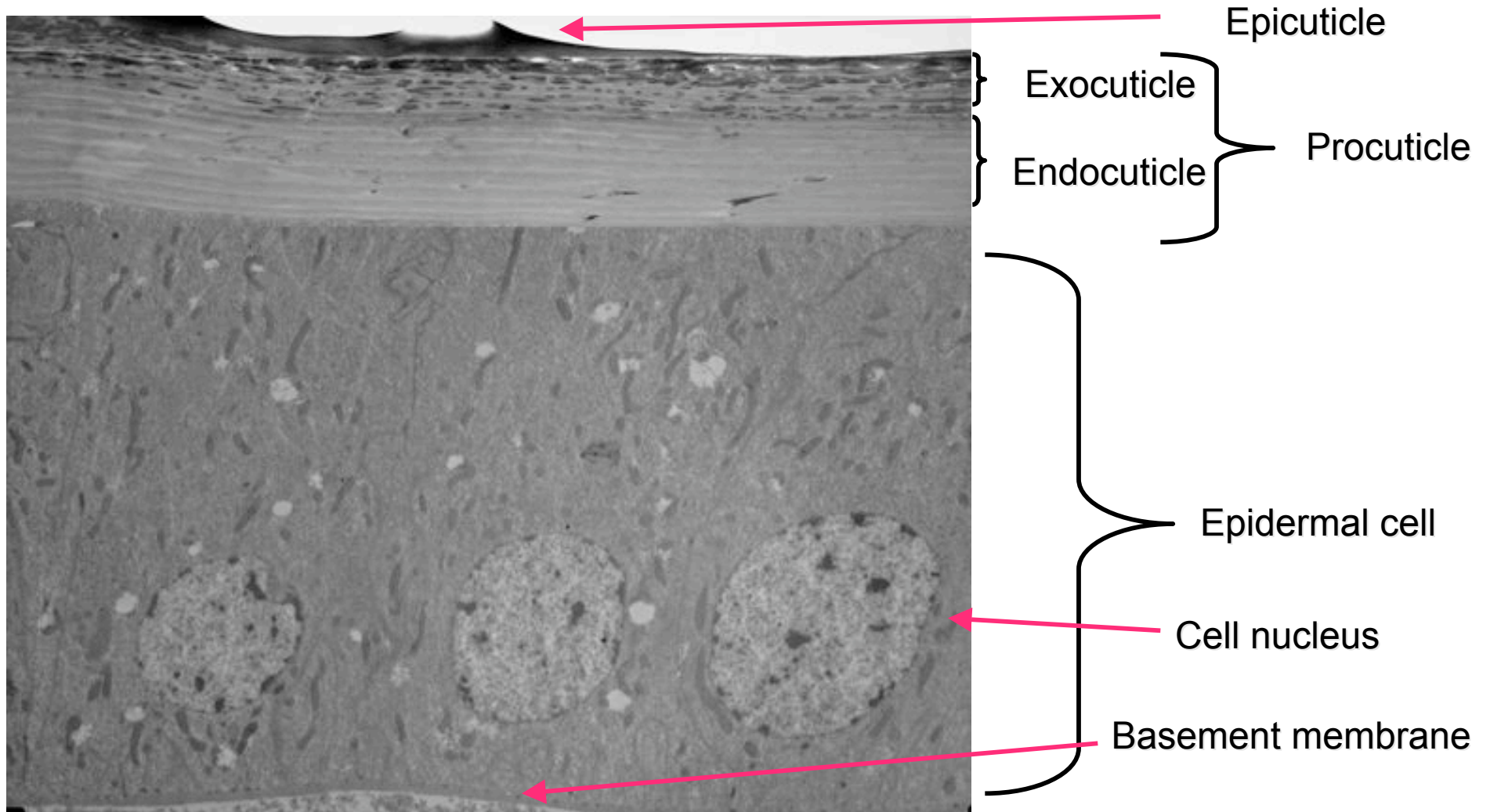
2. STRUCTURE AND FUNCTION OF THE INSECT CUTICLE & HYPODERMAL OR EPIDERMAL CELLS

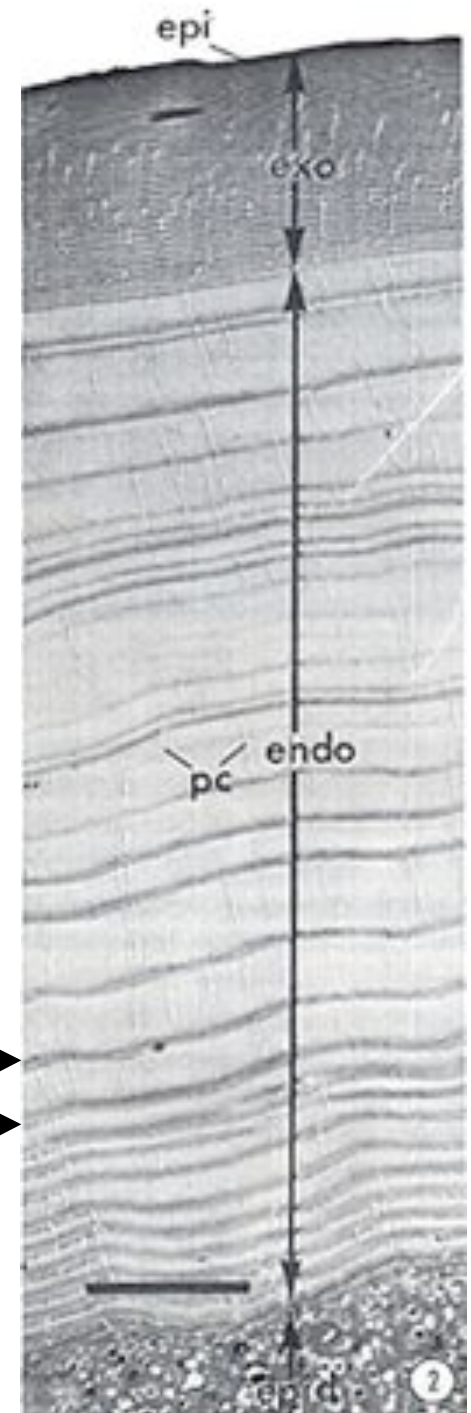
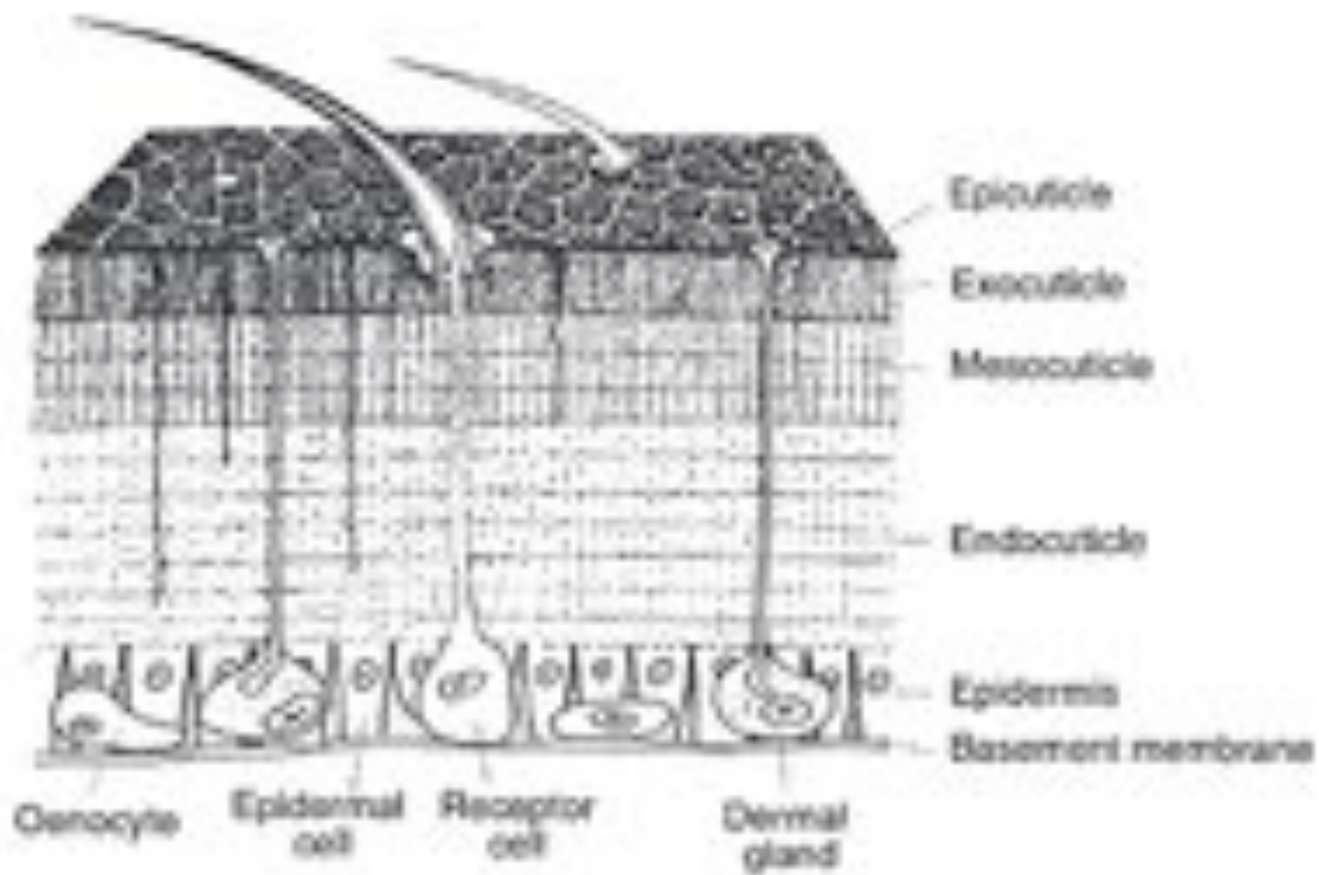


2. STRUCTURE AND FUNCTION OF THE INSECT CUTICLE & HYPODERMAL OR EPIDERMAL CELLS



Transmission Electron Micrograph of Insect Cuticle – Adult female of *Heliothis zea*





**Arrows point to 'growth' layers: day/night
Thus, exact aging not physiological aging**

**Cross-section of *Oncopeltus*
cuticle to right-**

Function(s) of each major layer of cuticle and epidermal layer

- **Epicuticle-Complex layer.** The outermost layer and probably the most important layer. Water proofing and general impermeability of cuticle. Produced by hypodermis/epidermis and dermal glands.
- **Exocuticle-Region** in which cross-linking of proteins occurs to give cuticle hardness. Region not broken down by proteases at molt and is what usually remains in form of exuviae. Melanin and other pigments found in this area. Sclerotization mainly occurs here. Also arthropodin and sclerotin.

Function(s) of each major layer of cuticle and hypodermal layer

- **Endocuticle**-That region directly above the hypodermal cells. Is continually being synthesized (in a dark/light way-24 hrs) and often is laid down in layers, thus can often be used to age-grade some insects. Contains most of the chitin, which is broken-down at the molt by chitinase. Little cross-linking of proteins, thus most is broken-down at molting and reabsorbed. In soft-bodied insects and regions of flexibility (eg. Arthrodistal membranes or intersegmental membranes), this layer is well developed and not the exocuticle.

Function(s) of each major layer of cuticle and hypodermal layer

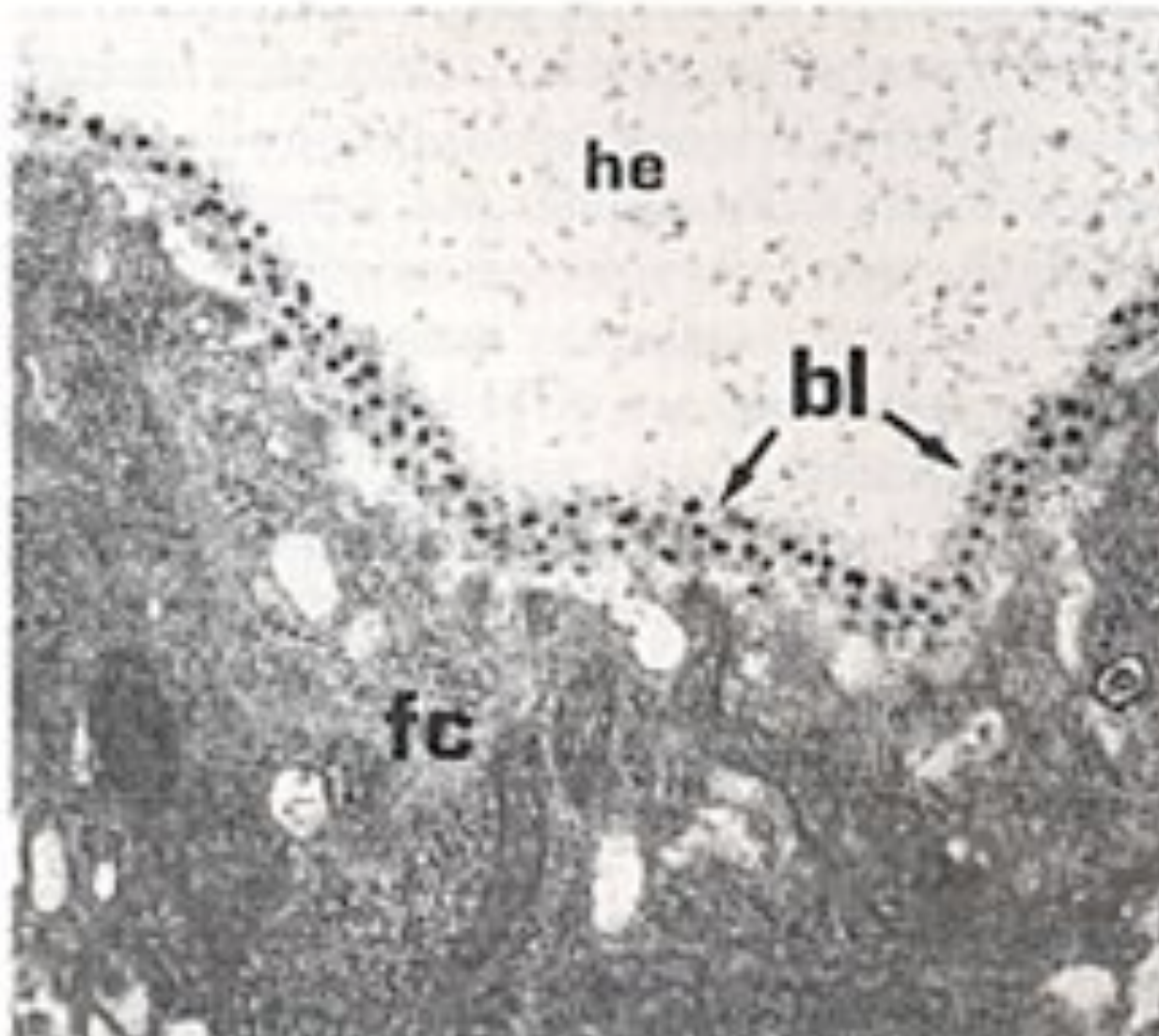
- **Pore canals**-Narrow pores that extend from the hypodermal cells to the inner most part of the epicuticle. Believed to be involved in transport of lipids from hypodermal cells to the epicuticle or outer layer.
- **Schmidt's layer**-The zone of deposition of new cuticle or procuticle.
- **Hypodermis or epidermis**-A single layer of cells that underlies the cuticle and is responsible for its production. It is the only living portion of the integument that is ectodermal in origin. These cells can be modified to form dermal glands, sensory receptors and oenocytes. **What every epidermal cell must know?**

Function(s) of each major layer of cuticle and hypodermal layer

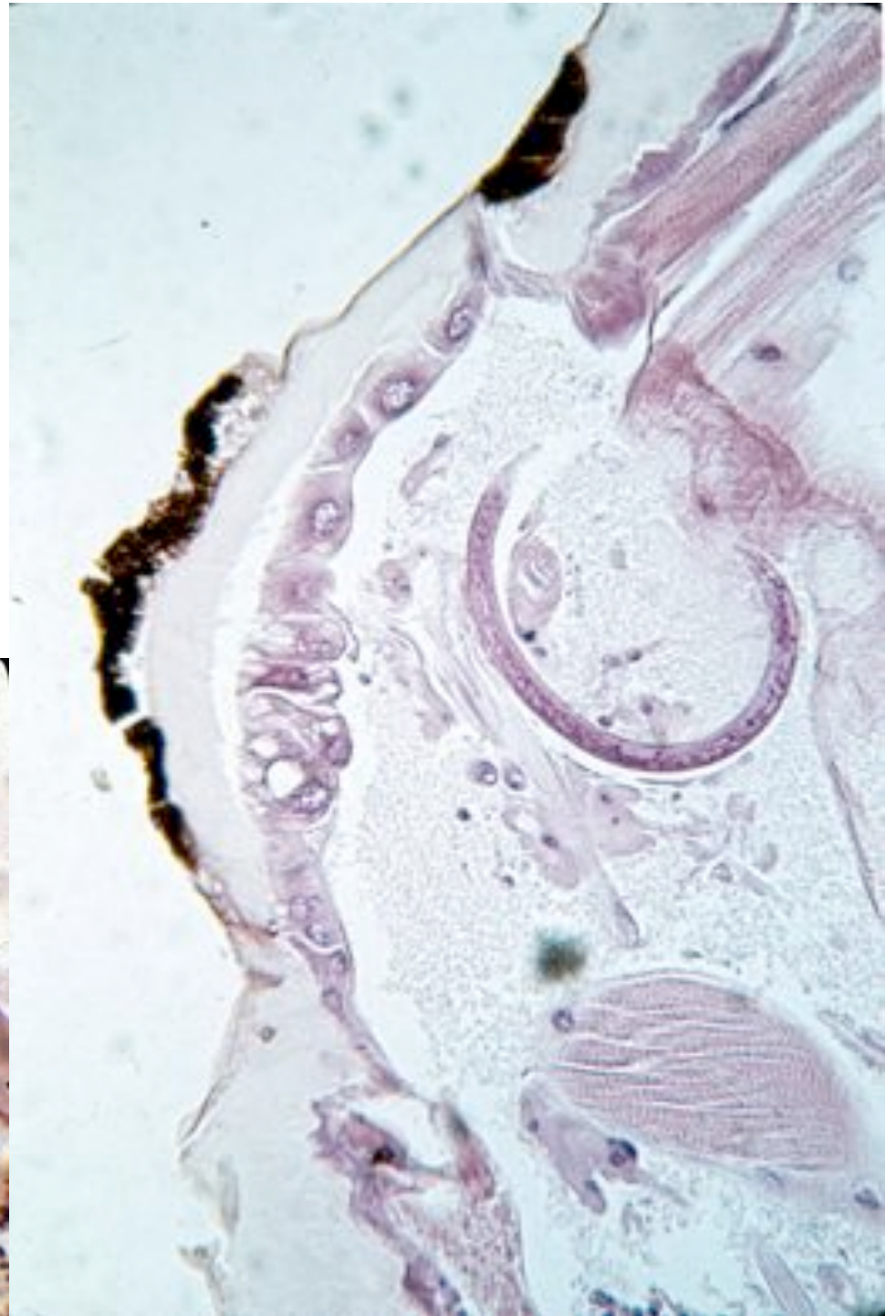
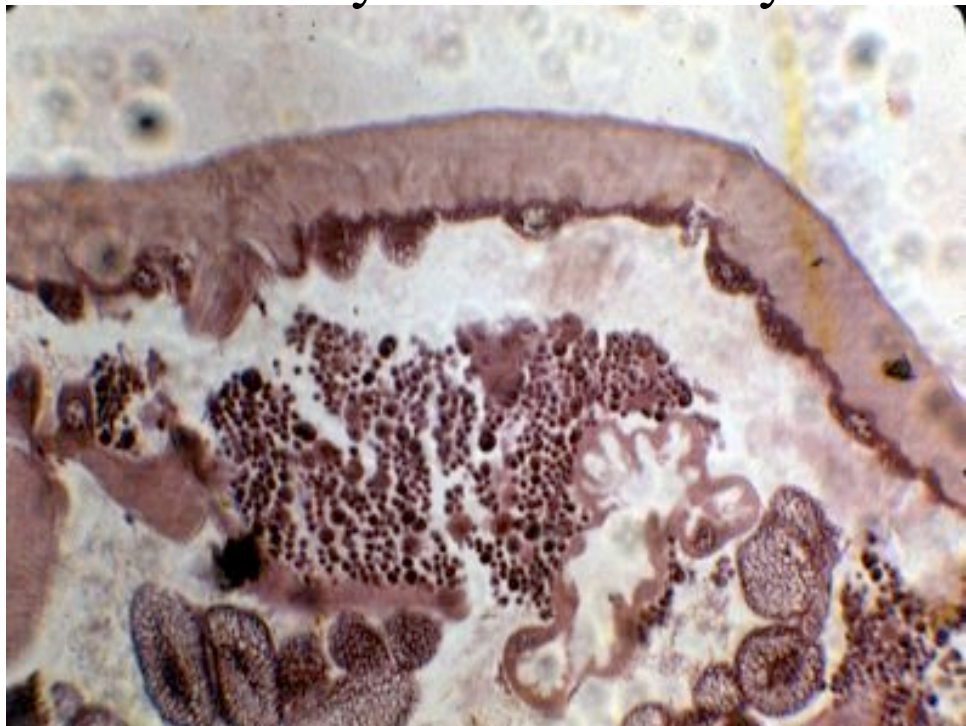
- **Basement membrane or basement envelope (Basal lamina)** -Mucopolysaccharide layer that is secreted by the hemocytes, is penetrated by nerves and tracheae going to hypodermis, and is a selective barrier between hemolymph and epidermal cells. Hormones and other nutrients can pass through this selectively permeable layer to reach the hypodermal cells. Is important in the recognition of ‘self’; thus, the insect’s blood cells do not recognize it as ‘foreign.’ Molecules in this layer are charged and probably act like a molecular sieve. **POORLY UNDERSTOOD, YET EXTREMELY IMPORTANT IN SELF RECOGNITION AND CUTICULAR INTEGRITY.**

In this micrograph we added cationized ferritin molecules and one can see that they are attracted to the anionized sites in the basal lamina. This was in *Phormia regina* follicle cells (fc).

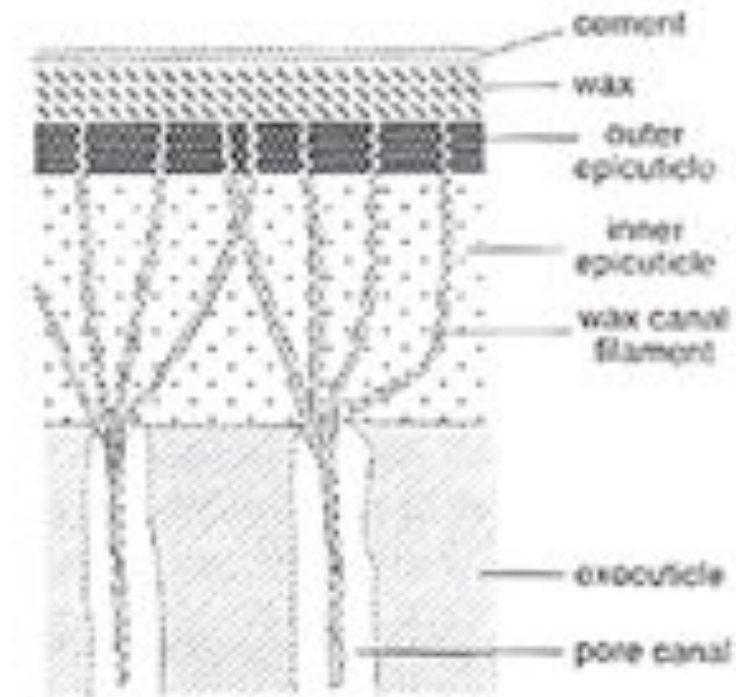
He=hemolymph



Below and to the right are cross-sections through the cuticle of a house fly larva. Note hypodermal cells, epicuticle and rest of the cuticle, which is mainly endocuticle-thus flexibility. Note wound healing and tonofibrillar attachment for the muscle. Also note hemocytes and fat body cells.



Significance of each layer of the epicuticle



One major problem with any insect, especially those that live in soil is:

Erosion of the cuticle by abrasion and adhesion

Significance of each layer of the epicuticle

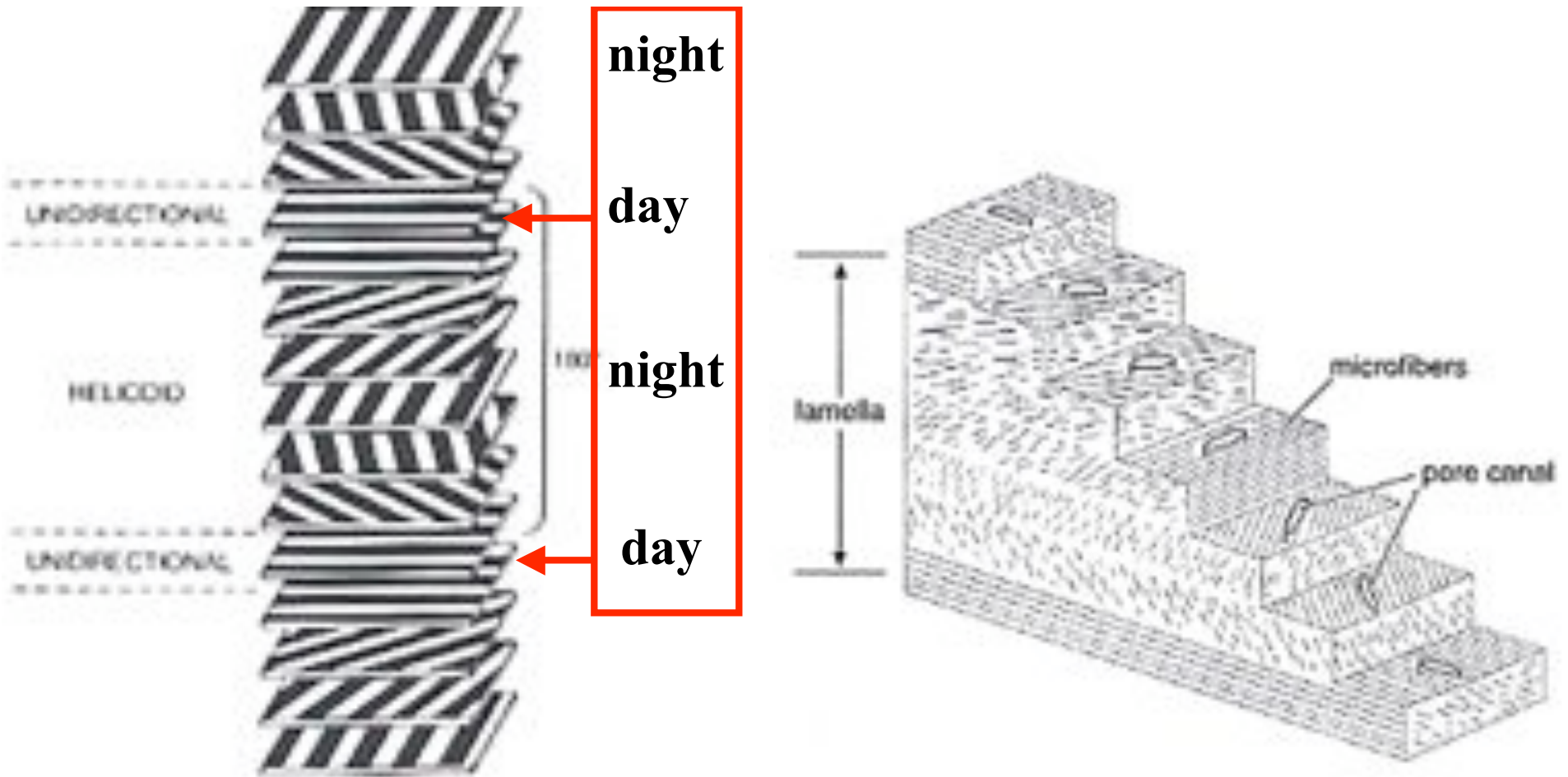
1. **Cement layer**-Thin outside layer. Closely associated with wax layer and may serve to protect it. Not found in all insects.
2. **Wax layer**-Hydrocarbons constitute 90% of this layer. Important to insects for water loss, thus **waterproofing** of cuticle. In some insects (e.g., Fulgoridae and scales), the insects produce a large bloom of wax on outside. Bees have special glands, wax glands, on ventral abdominal segments 4-7 that produce wax, which is then formed into flakes used by the bees to make their cells. Hydrocarbons in this layer that are used by insects for both inter- and intraspecific communication signals. **SYSTEMATISTS USE THESE CUTICULAR HYDROCARBONS**. They are also important communication molecules for social insects especially.
3. **Outer epicuticle**-First layer formed following the molt and is the layer that protects the new procuticle from digestion by molting enzymes (i.e., chitinases and proteinases). Also called **cuticulin layer**

Significance of each layer of the epicuticle

- 4. Inner epicuticle-**Function not that clear but it is a much thicker layer than the outer epicuticle.
- 5. Wax canal filament-**A filament of wax that is produced by the hypodermal cells and extends to the inner part of the epicuticle. Probably is a filament that continually is moved towards the surface of the cuticle.
- 6. Pore canals-**Tiny pores that run from the hypodermal cells to the inner part of the inner epicuticular layer. Inside the canals are wax filaments that extend up to the epicuticular layer. Probably serve as a transport passage for wax from hypodermal cells up to wax layer.

Insects needing a renewable epicuticular layer

- Soil or sand dwelling insects



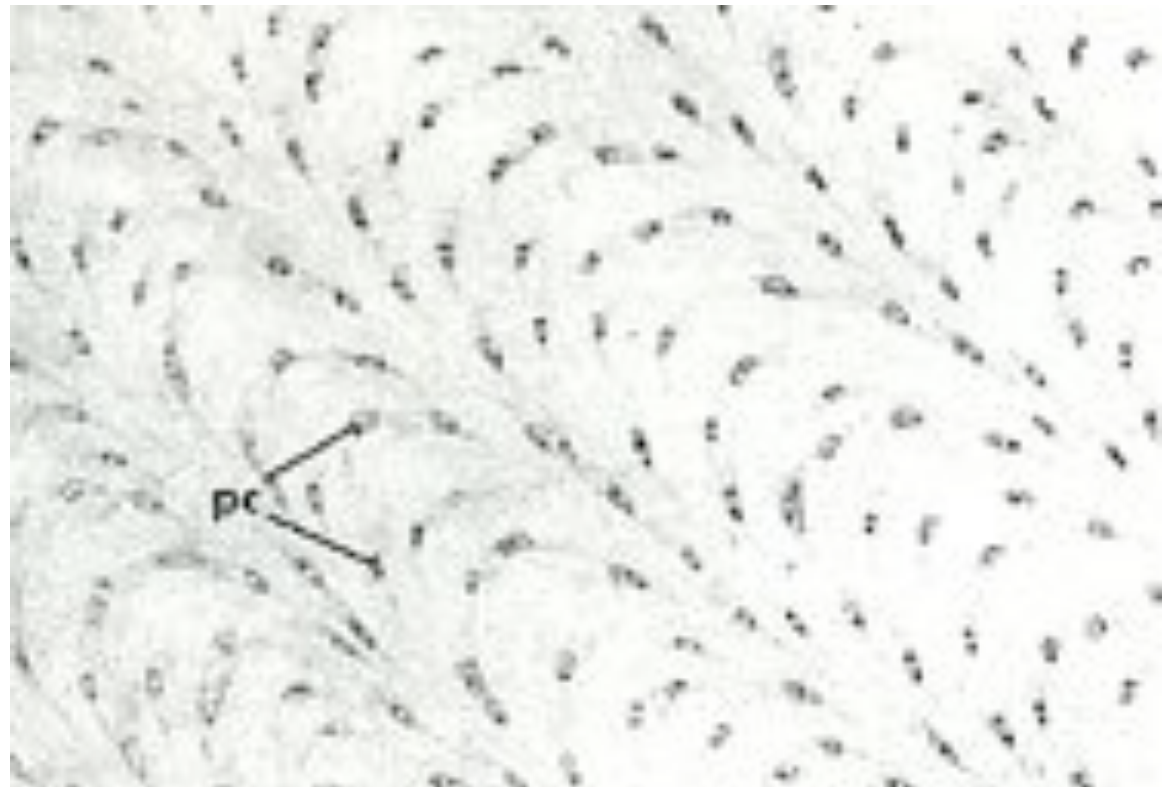
Helicoidal arrangement of pore canals suggests the way in which the arrangement of the cuticle is produced or laid down in sheets or lamellae. Neville, using *Locusta*, showed that single sheets were deposited during daylight in a unidirectional way and helicoidal at night. Reared at constant light or dark deposited only that cuticle for the respective light/dark cycle.

Openings or specializations of areas in the cuticle

1. Openings in the cuticle
 - a. Pore canals
 - b. ‘Sweat pores’ of Sonoran desert cicada
2. Specialized areas of cuticle
 - a. Anal organ of dipterous larvae
 - b. Sense organs
 - c. Pheromone or deterrent chemical site release

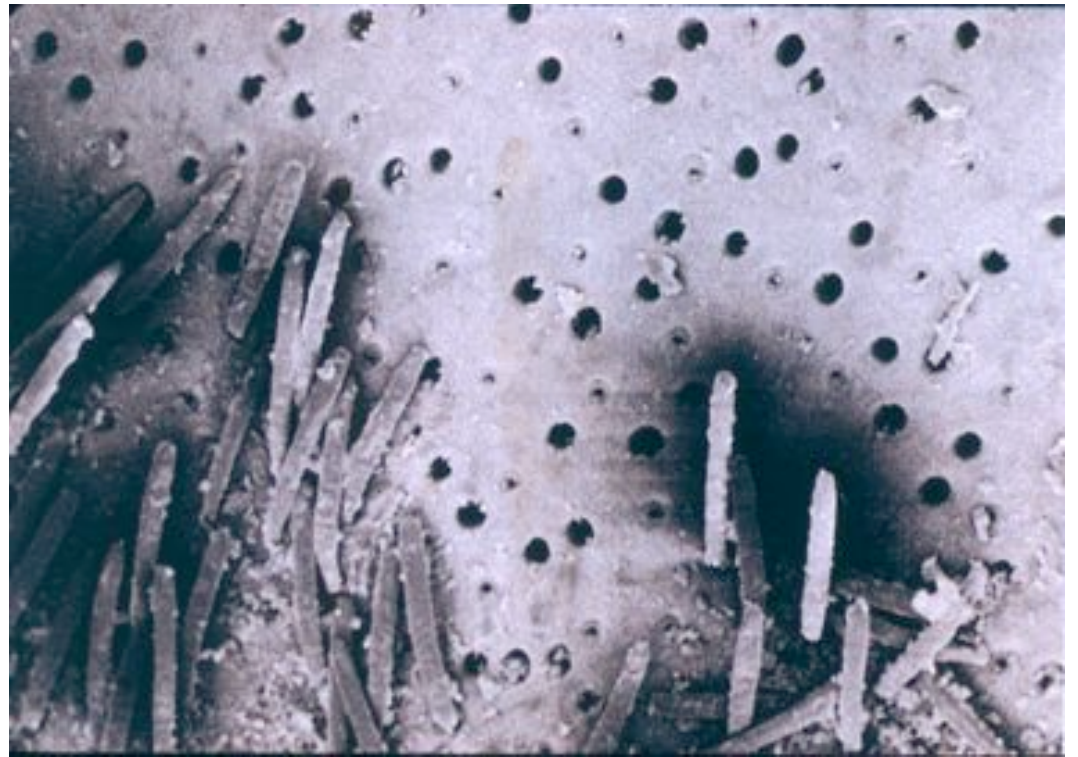
Pore canals-Tiny pores that extend from the top of the hypodermal cells to the inner most part of the epicuticle. Believed to be involved in the transport of lipids from the hypodermal cells to the epicuticle.

Photo taken from pg. 8 of
Insect Cells-their
structure
and Function by David
Smith, 1968.

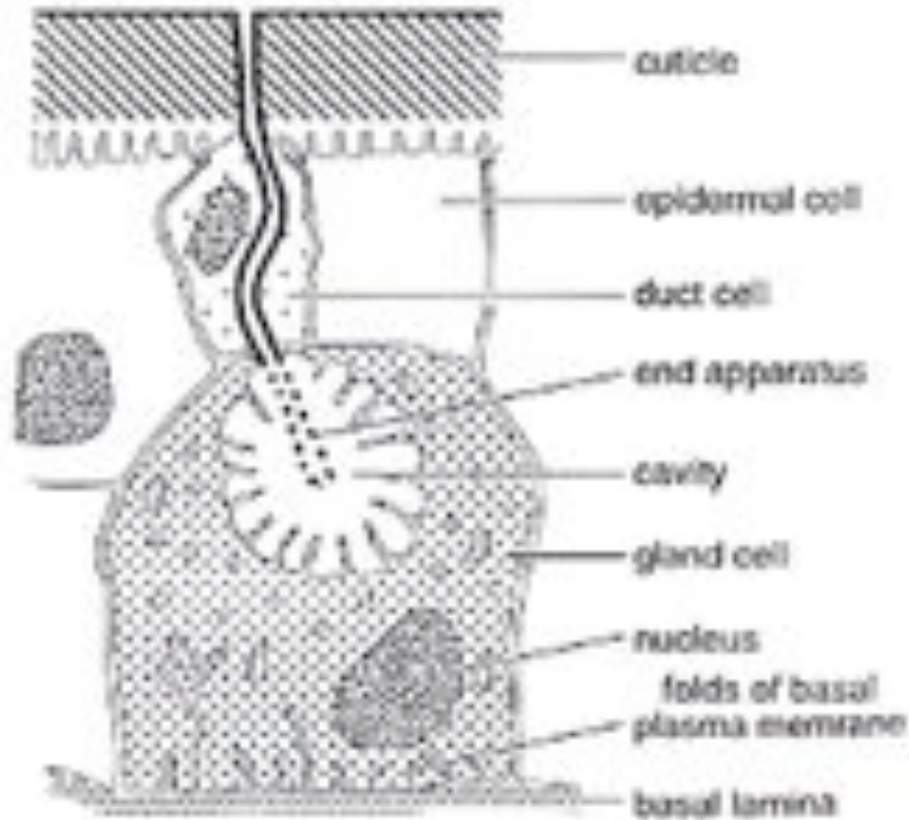




Sonoran desert cicada.
Pores 7X size of pore
canals located on dorsal
mesonotum + connected
to special dermal glands
via cuticular ducts are
involved in water transport
to the surface. Cooling of
2-5°C below ambient of
42-45°C.

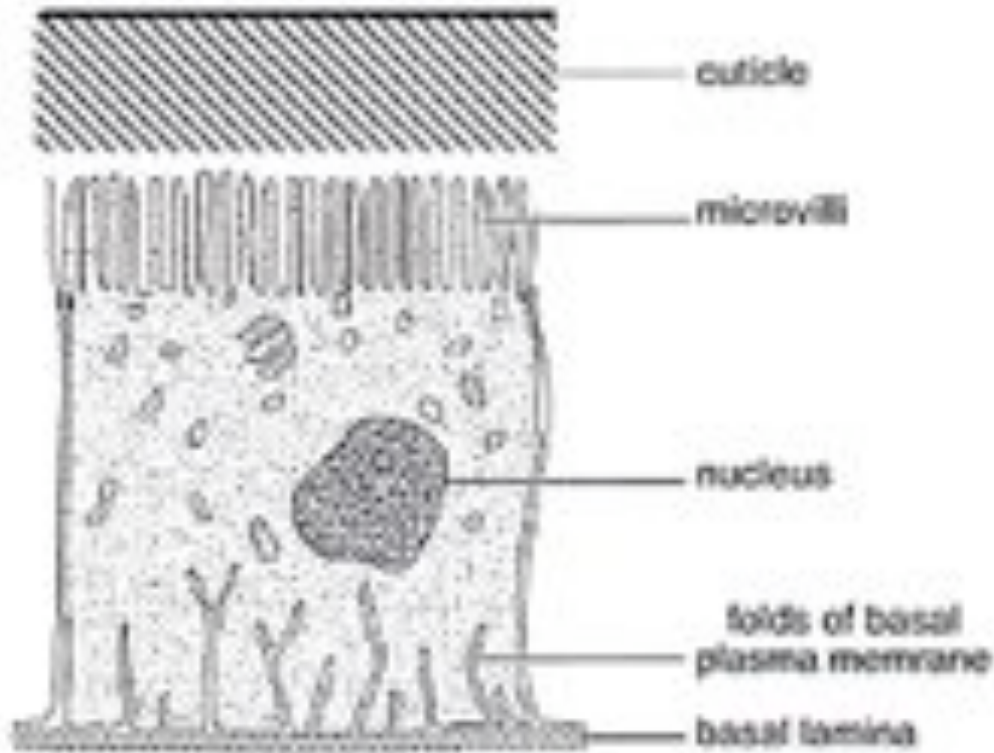


c) class 3 gland cell



Dermal gland of *Tenebrio* has a ductule gland cell that produces a duct through which the secretion passes directly to the outside. In the desert cicada the special dermal glands are able to extract water from the hemolymph and pass it up to the cuticle via the duct and out the pore.

b) class 1 gland cell



Class 1 gland cell: Gilson's gland of *Phryganea* larva. Here the glandular secretion passes directly through the cuticle.

Specializations of areas

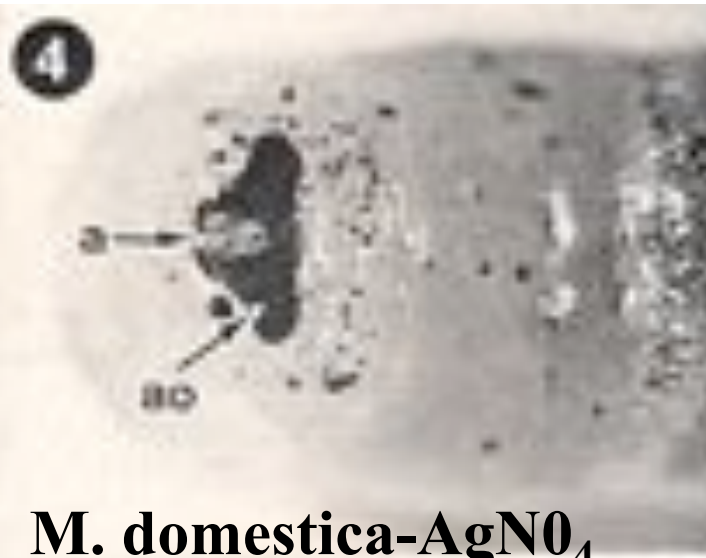
Anal organ of dipterous larvae



Organ is involved in osmoregulation in the dipterous larva.

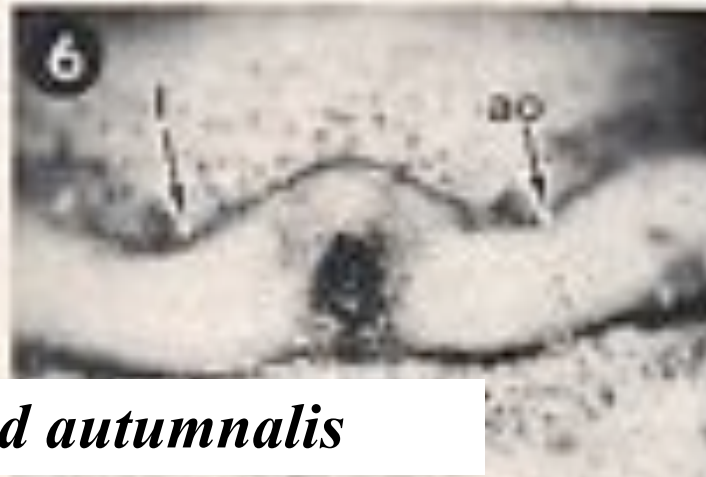
Structure tells you something about its function-thinner cuticle, larger epidermal cells, and separation from rest of cuticle. TEM needed

Anal organ of different dipterous species. Surrounds the anus of the larva. Cuticle is much thinner than elsewhere, it has larger epidermal cells, and an infolding separating it from the rest of the cuticle. Not usually visible but stained with acetocarmine or silver nitrate it stands out. **A good key for larval taxonomy. Why the carry over of the anal organ into the pupa? See fig. 5 below.**



***M. autumnalis*-silver nitrate**

***M. domestica*-AgNO₄**



M. domestica and autumnalis

Specializations of areas

What sorts of problems does the apple maggot female face when she attempts to lay an egg in a fruit?

- 1. Hardness of the fruit**
- 2. Quality of the fruit**

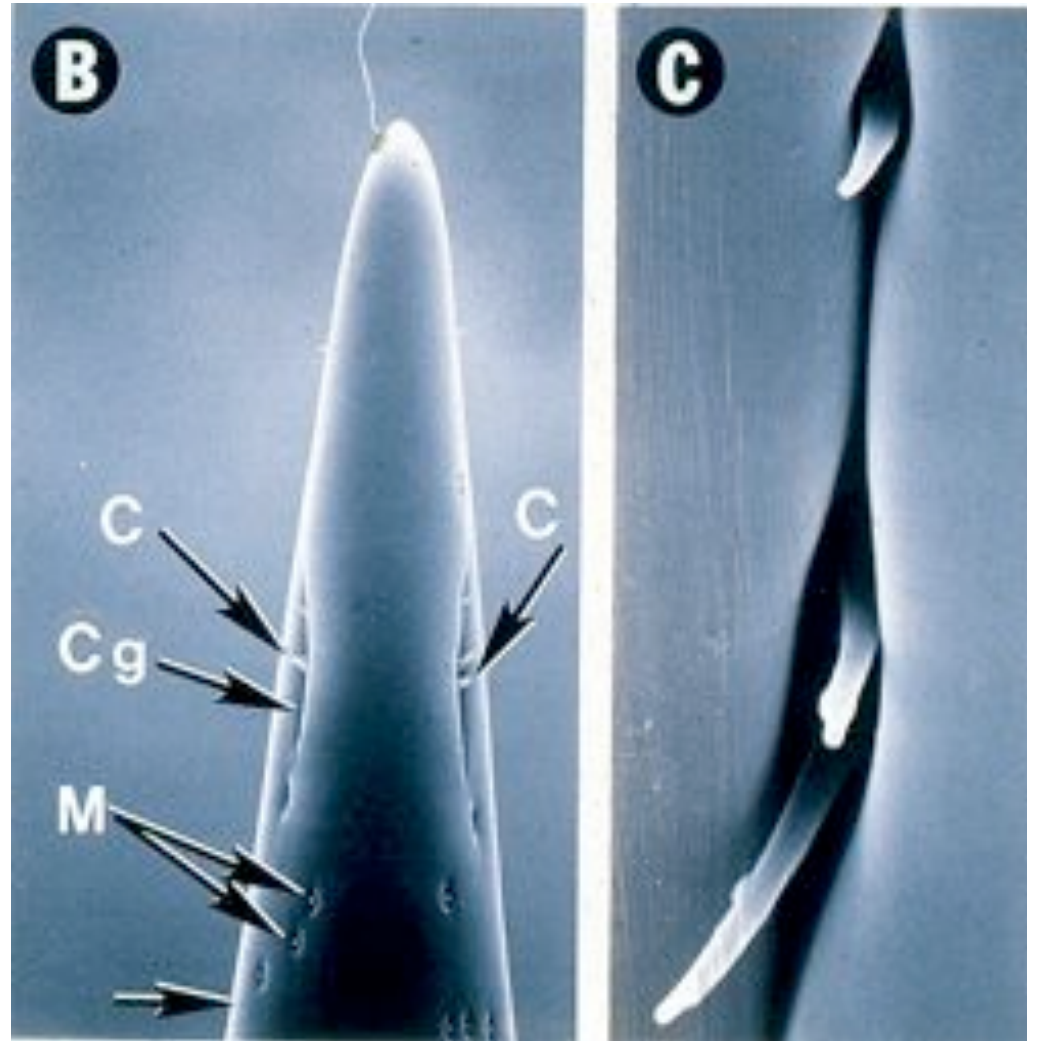
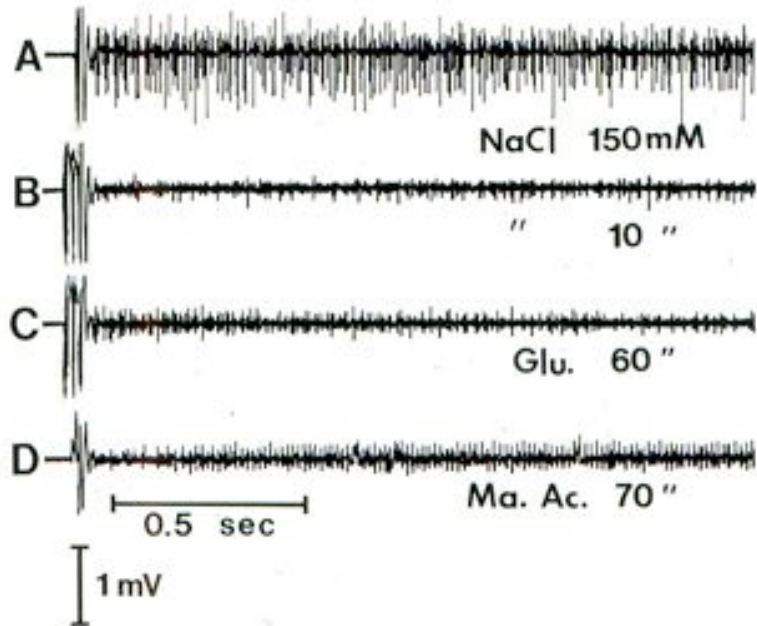
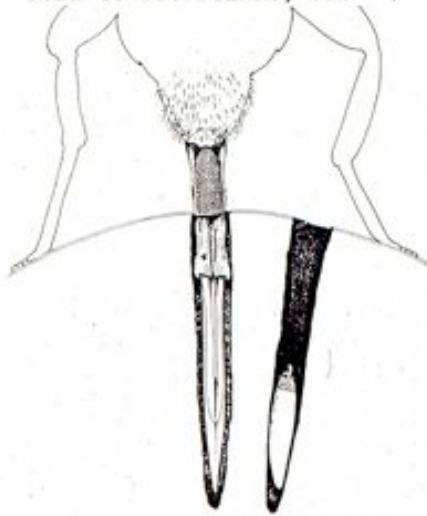
How does the cuticle come into play in this situation?



OVIPOSITION BEHAVIOR IN THE TEPHRITIDS

BY

JOHN G. STOFFOLANO, JR.



Formation of sensilla

Trichogen cell-

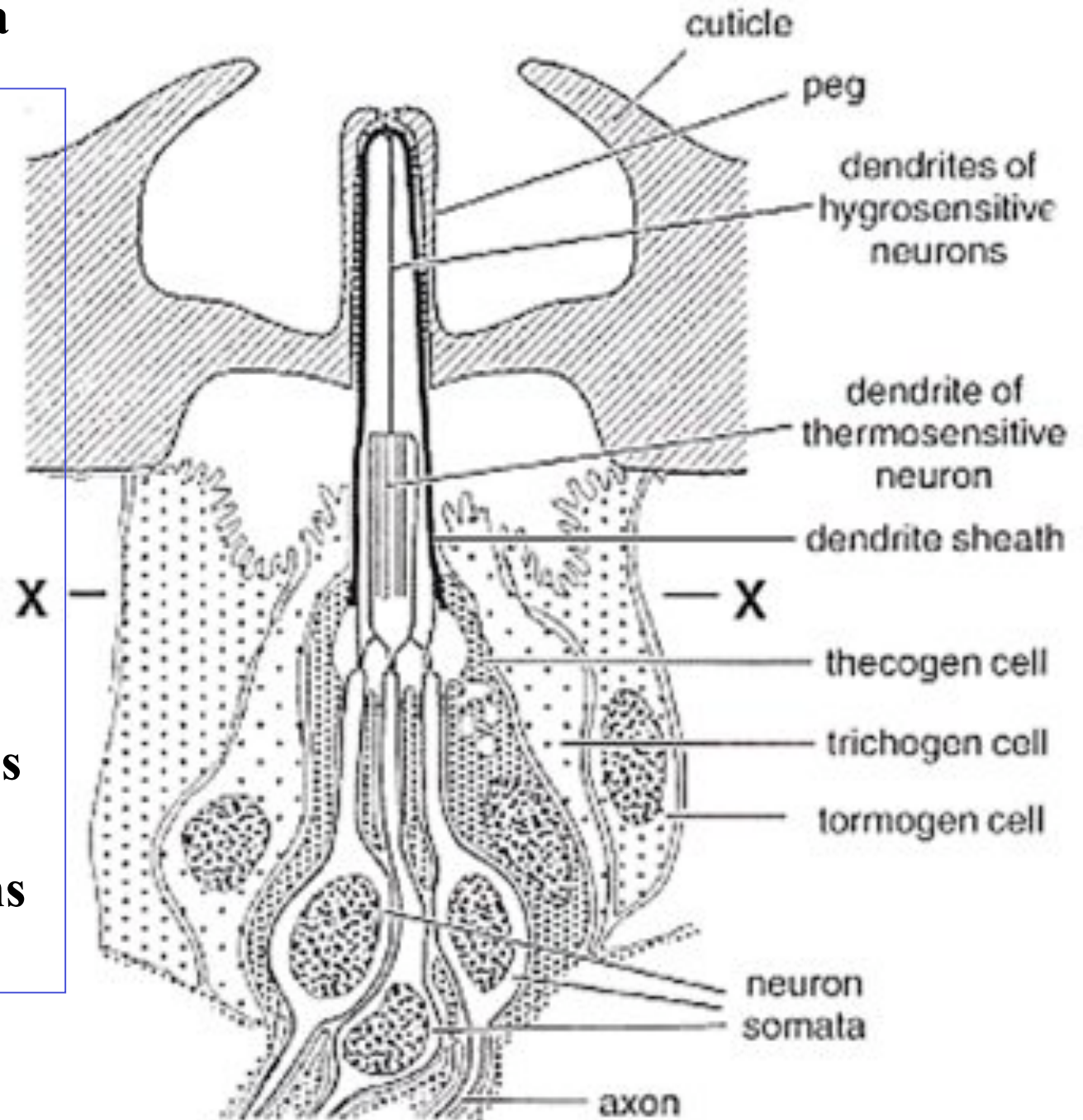
Creates the shaft of hair

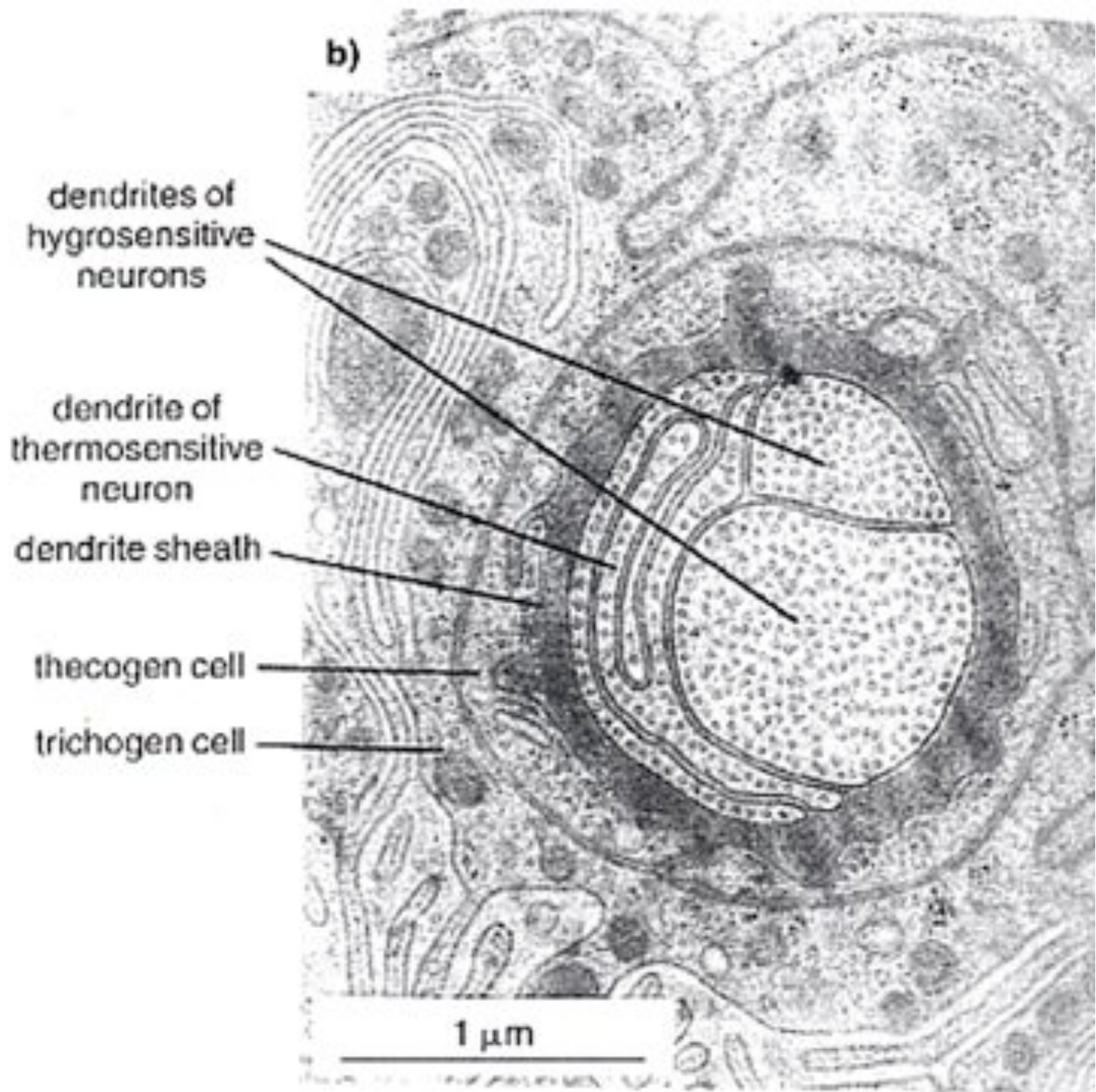
Tormogen cell-

Creates the socket of the hair or sensillum

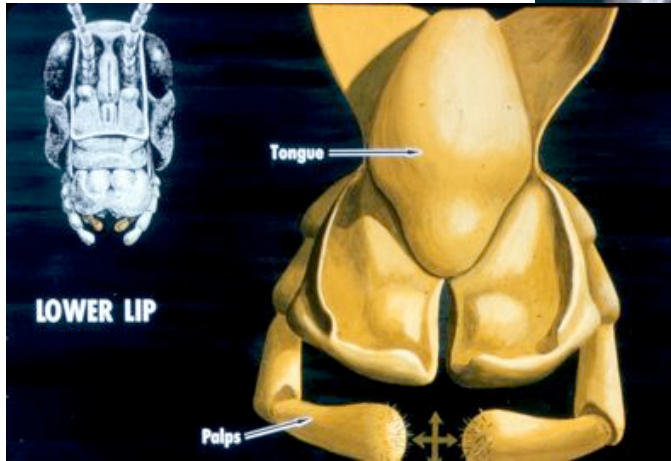
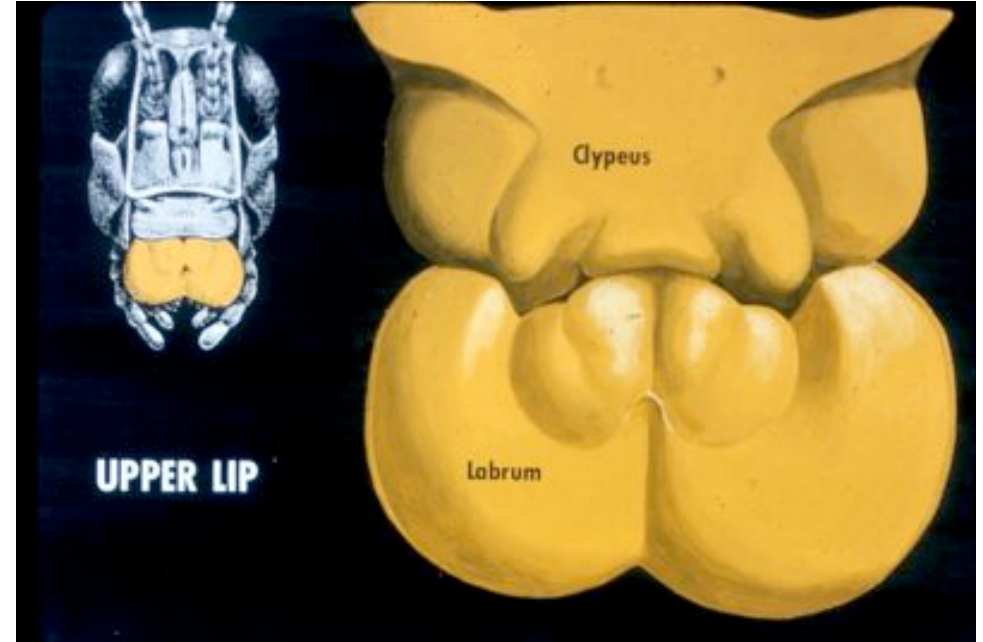
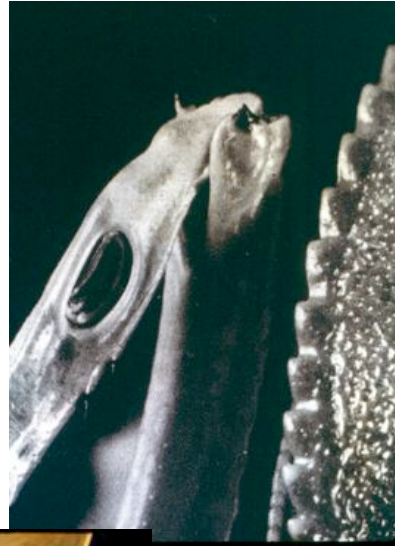
Thecogen cell-

Creates the sheath that surrounds the neurons and isolates them and provides the neuron with ions and nutrients.

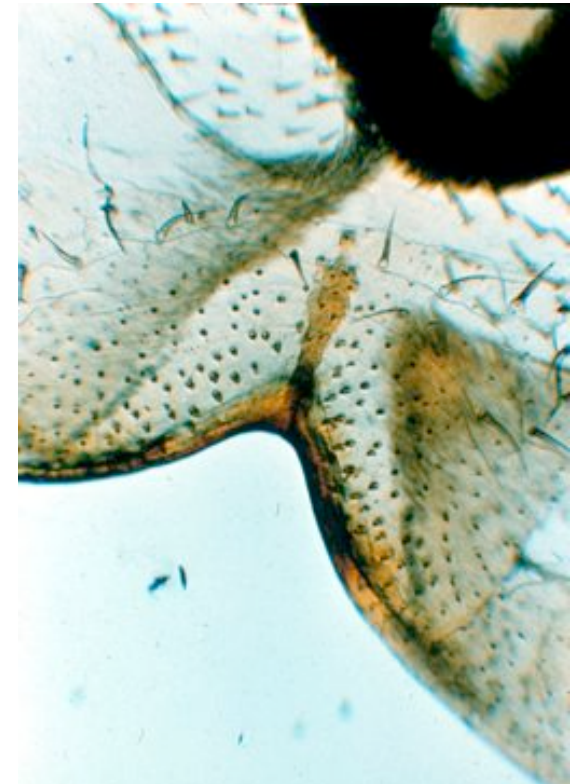




Openings and production of sense organs



Different types of sensilla:
Tympanum
Chemosensilla on labial palps
Chemosensilla on undersurface of the labrum



3. Chemical composition of the cuticle

- Lipids-
 - a. **Waxes**-Wax blooms produced by several groups of insects
 - b. **Cuticular hydrocarbons-also used by systematists**
- Carbohydrates
 - a. **Chitin**-polysaccharide with repeating units of *N*-acetylglucosamine residues. Chitin gives the cuticle its strength, not its hardness.
- Proteins
 - a. **Arthropodin**-Untanned protein that during sclerotization is cross-linked (thus tanning) to produce sclerotin and give the cuticle its hardness
 - b. **Sclerotin**-Tanned protein that gives cuticle hardness. This is brought about by a process called sclerotization and involves eclosion hormone and bursicon, another hormone.
 - c. **Chitinase**-Enzyme involved in digestion of chitin at the molt. Is released and produced by the epidermal cells.

3. Chemical composition of the cuticle

- d. **Proteinases**-Enzymes other than chitinase that aid in the digestion of the endocuticle at the molts.
- e. **Resilin**-Colorless rubber-like protein. Can be stretched and stores energy due to tension. Places found: Wing hinges; food pump of reduviid bugs; hind legs of jumpers; aids in inspiration in beetles, which lack inspiration muscles. Pads between dorsal and ventral sclerites. Thus, during expiration, pads are compressed and then release energy for inspiration.

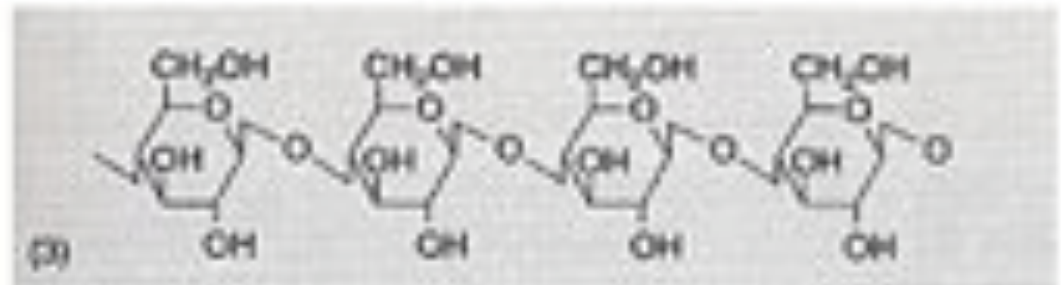
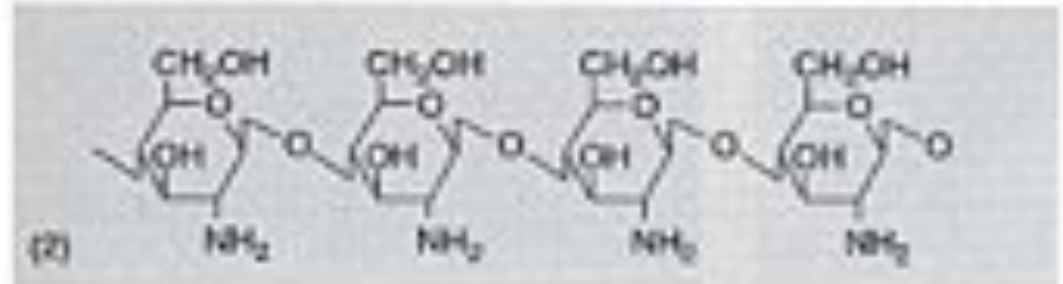
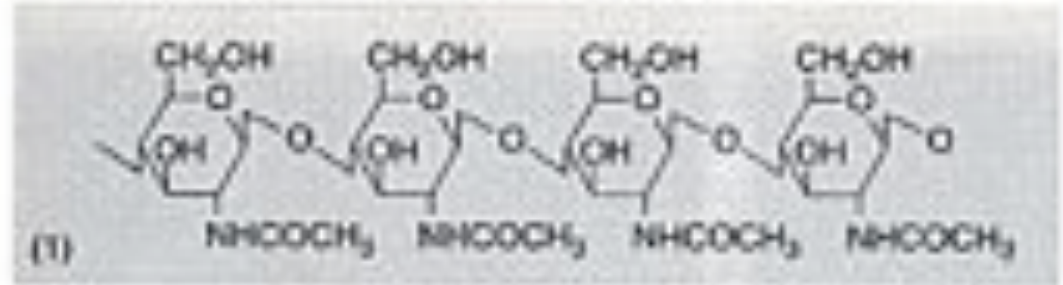
CHITIN-2nd most common molecule

N-acetylglucosamine polymer

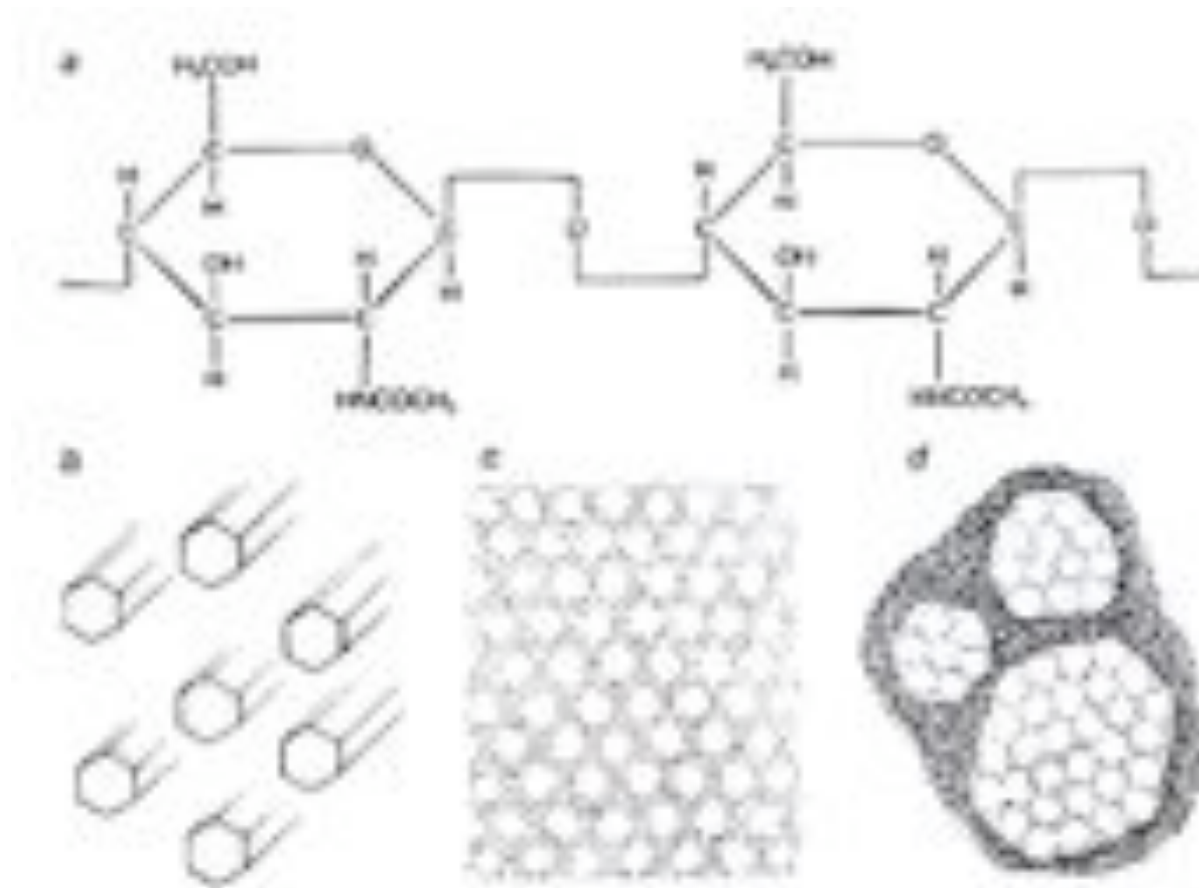
CHITOSAN

CELLULOSE-most common molecule in nature

Figure 2.2 Chemical course: Chitin, chitosan, and cellulose. Adapted from E. Pevsot, 1993, in Science News.



Surface view of the cuticle using TEM and shown in c below. Here the chitinous rods (b) are regularly spaced above each epidermal cell that produces them. In crustaceans they are not as regularly spaced, as shown in fig. d. The darker matrix material is sclerotized protein for the insect and calcium for the Crustacean and protein for the crab.



INVESTIGATION OF CUTICULAR HYDROCARBONS FOR DETERMINING THE AGE AND SURVIVORSHIP OF AUSTRALASIAN MOSQUITOES. Am. J. Trop. Med. Hyg., 74(3), 2006, pp. 462-474

LEON E. HUGO^{*}, BRIAN H. KAY, GEOFF K. EAGLESHAM, NEIL HOLLING, AND PETER A. RYAN

Australian Centre for International and Tropical Health and Nutrition, and Mosquito Control Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; Pathology and Scientific Services, Queensland Health, Brisbane, Queensland, Australia

4. Sclerotization of cuticle

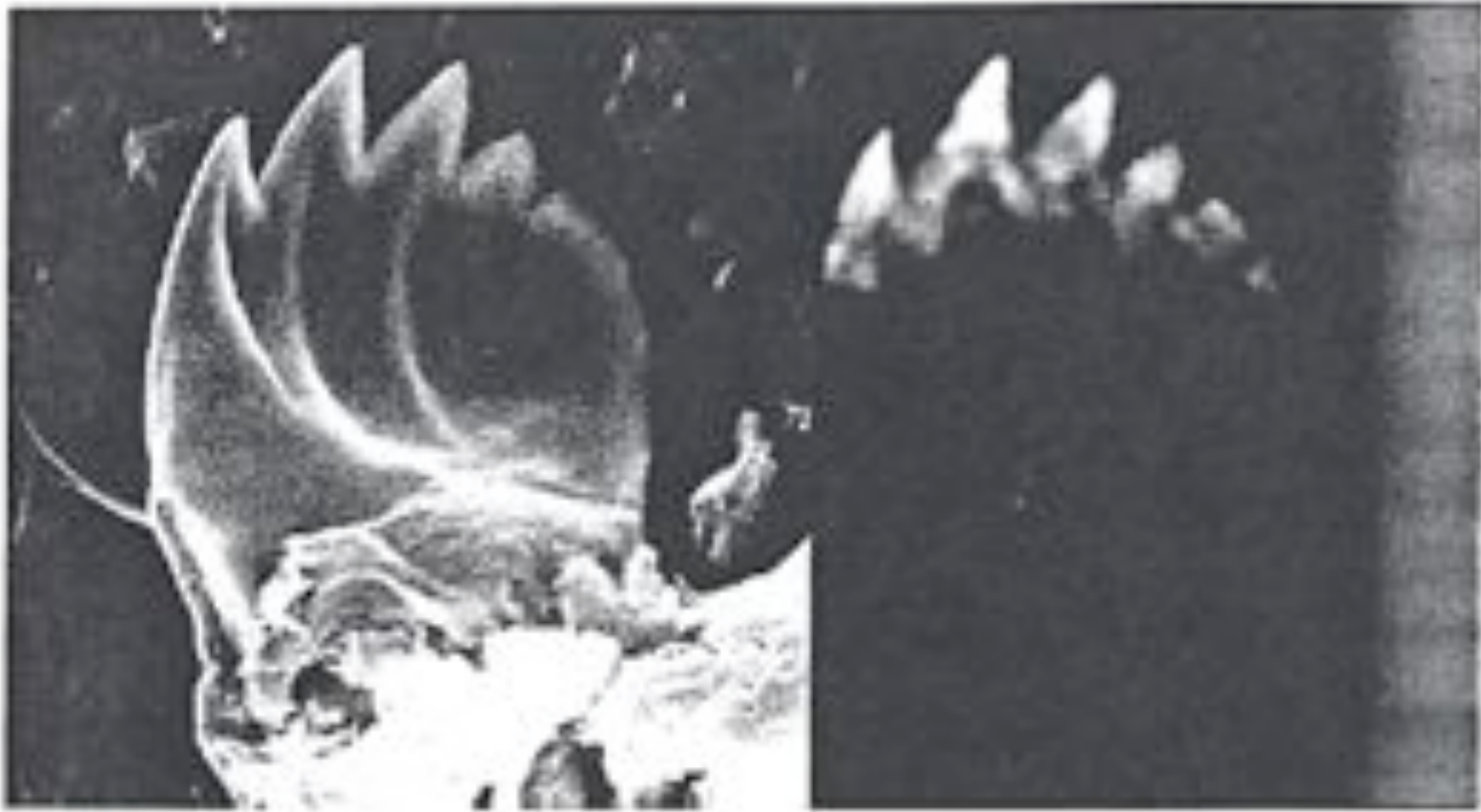
- The process whereby untanned or unlinked proteins become cross-linked to form strong linkages that give the cuticle its hardness. This process occurs mainly in the exocuticle area.

5. Physical properties of the cuticle

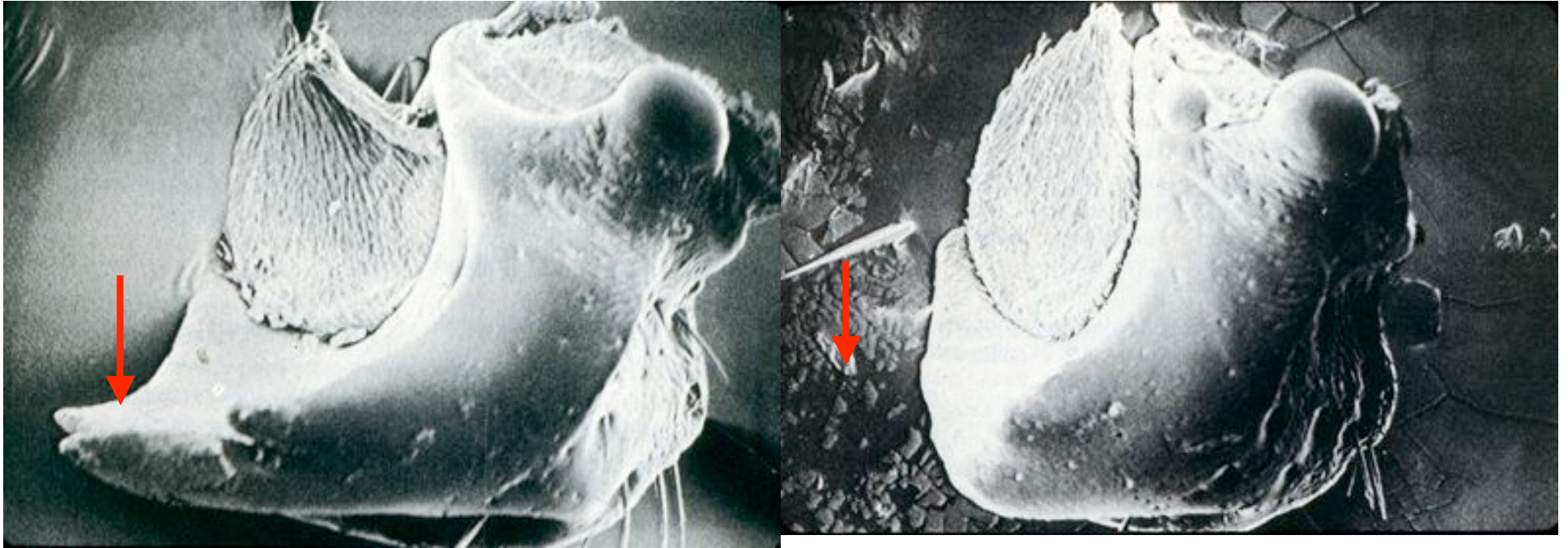
- Hardness
- Flexibility
- Plasticization
- Ability to heal or repair wounds
- Hydrophobicity
- Permeability under certain conditions

Cuticular hardness, strength or flexibility

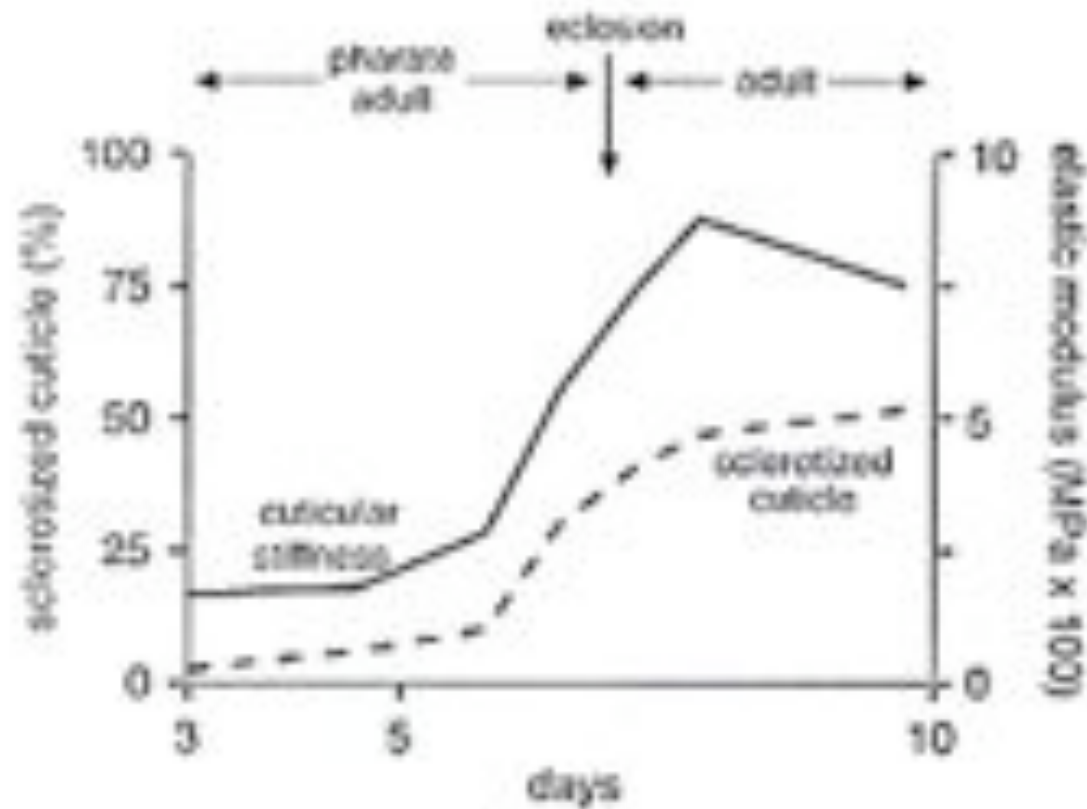
- **Hardness**-Is due to the amount of sclerotization or tanning of the proteins that takes place. This involves cross-linking of the proteins. A process often called tanning or sclerotization. Mainly occurs in the exocuticle.
- **Strength**-Is due to the presence of chitin in the endocuticle.
- **Flexibility** is due to less proteins being sclerotized and is usually due to more endocuticle being present than exocuticle. Also certain proteins, such as resilin provide flexibility. Many 'soft' larvae.



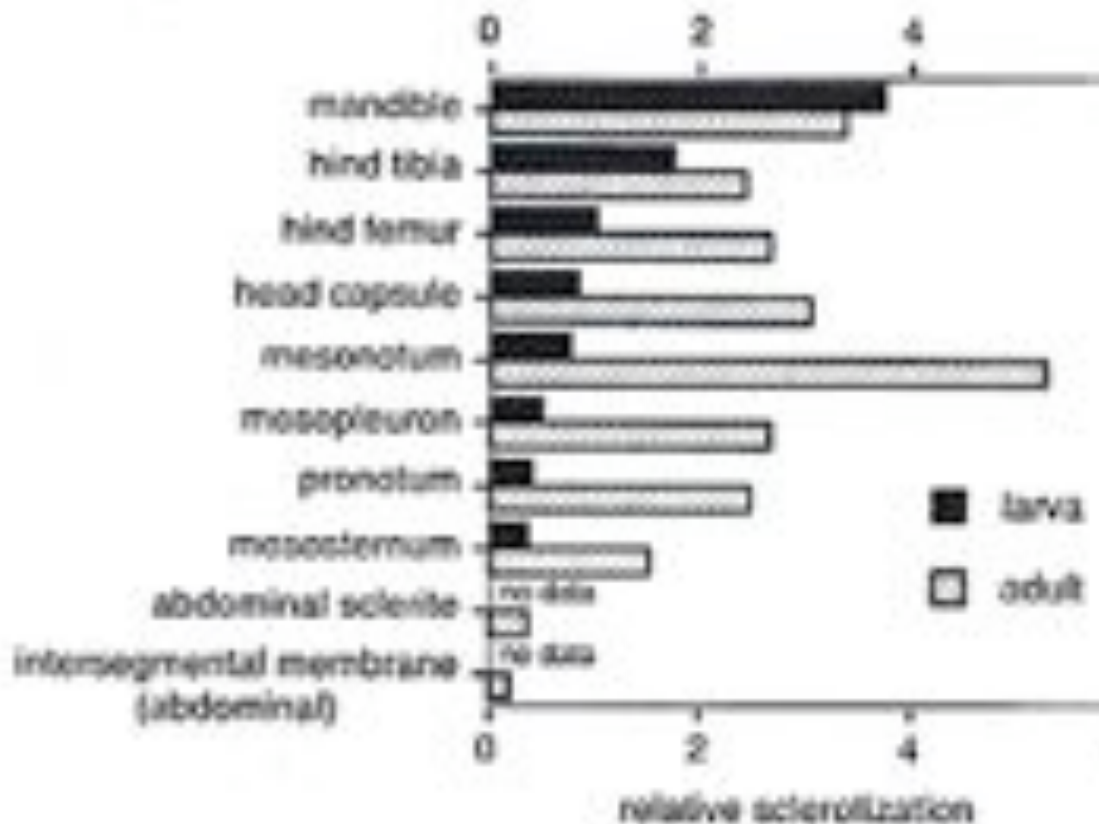
Scanning electron micrograph of the inner surface of a mandible. X-ray microanalysis of the same mandible showing the presence of Zinc in the mandibular cusps, which gives greater strength to the mandible.



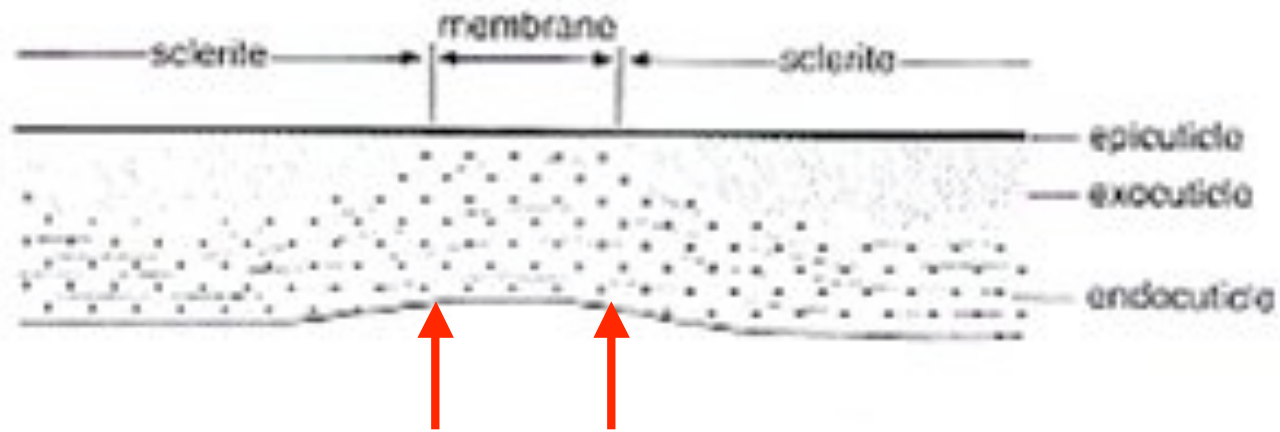
Scanning electron showing the mandibles of a beetle that feeds on willow trees. Note the wearing due to feeding on mature leaves of the tree, which are tougher than newly formed leaves.



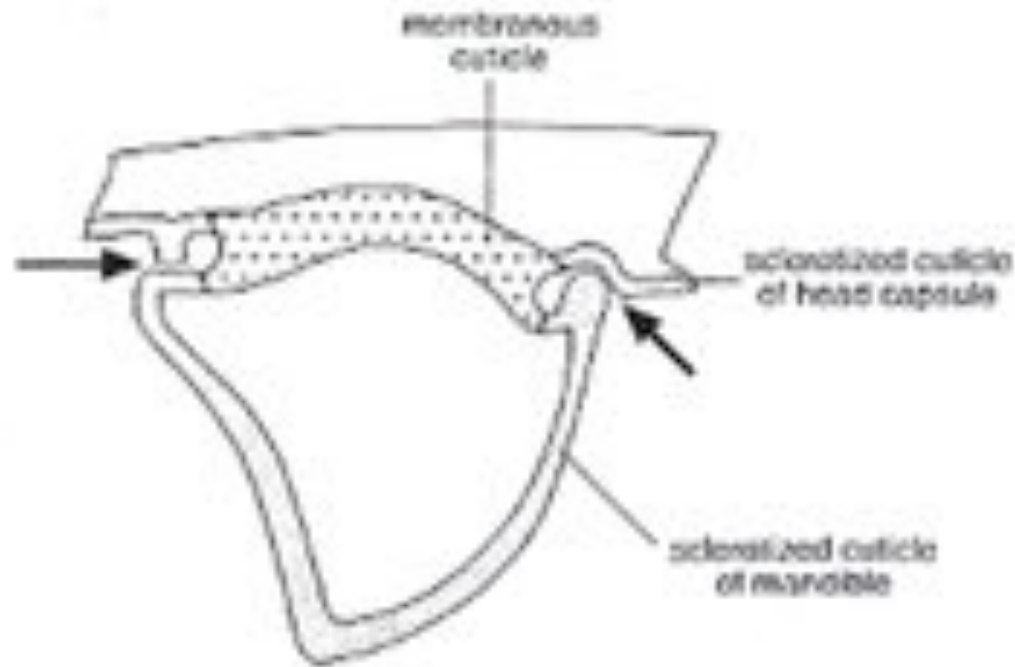
Relationship between sclerotization and cuticular stiffness in the abdominal tergite of the honeybee over the period of the molt, and principally around time of eclosion. Sclerotization is expressed as the inverse of the % of protein that is extractable. Only about half the protein in the cuticle becomes bound. At the same time, the stiffness of the cuticle also increases.



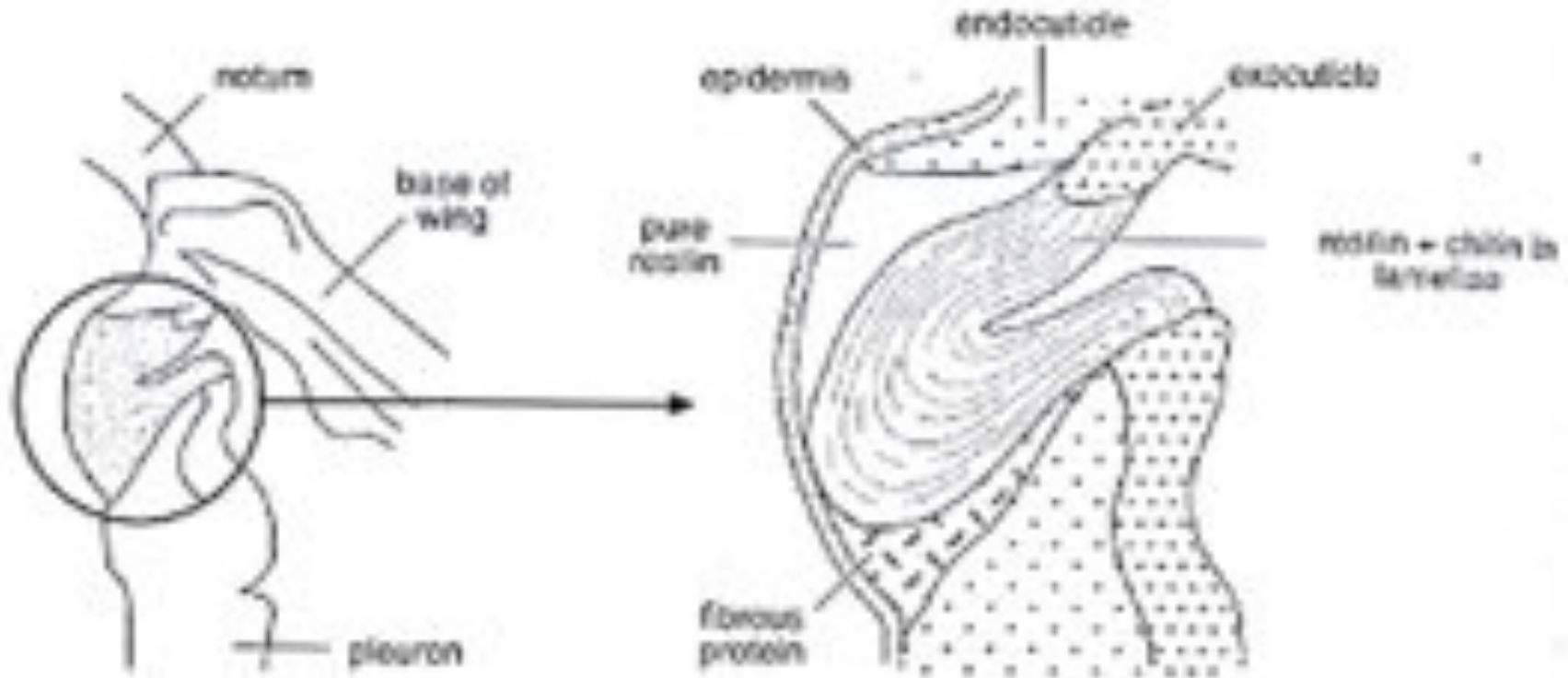
Amounts of sclerotization of the cuticle from different parts of a mid fifth-stage larva (nymph) and a 10-day-old adult locust. Sclerotization is based on the amounts of ketocatechols released when the cuticle is hydrolysed.



Flexibility of the cuticle. Membranous connection of two sclerites forming a membranous area. Which layer of the cuticle is lacking or minimal for the membrane area. Why is this so?

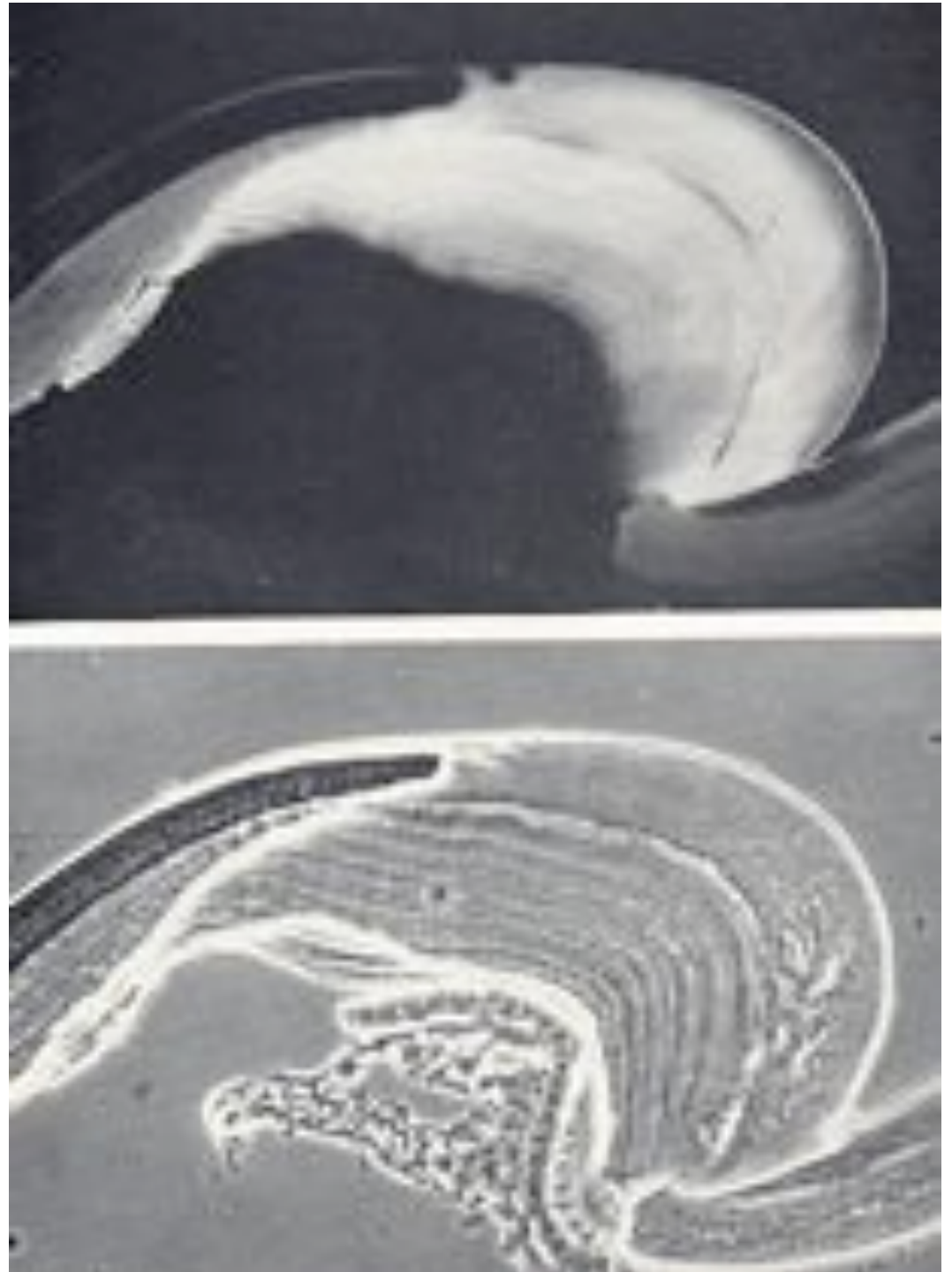


Flexibility of the cuticle. Extrinsic articulations where the sclerotized parts meet outside the membrane.



Transverse section through the thoracic wall and wing base of a grasshopper showing the position of the wing hinge (figure on left). On the right is an enlargement showing the resilin pad.

Section through wing area of desert locust. Top photo was done using UV light to show fluorescence of resilin while lower photo uses phase-contrast microscopy. Endocuticle does not fluoresce nor do the epidermal cells.

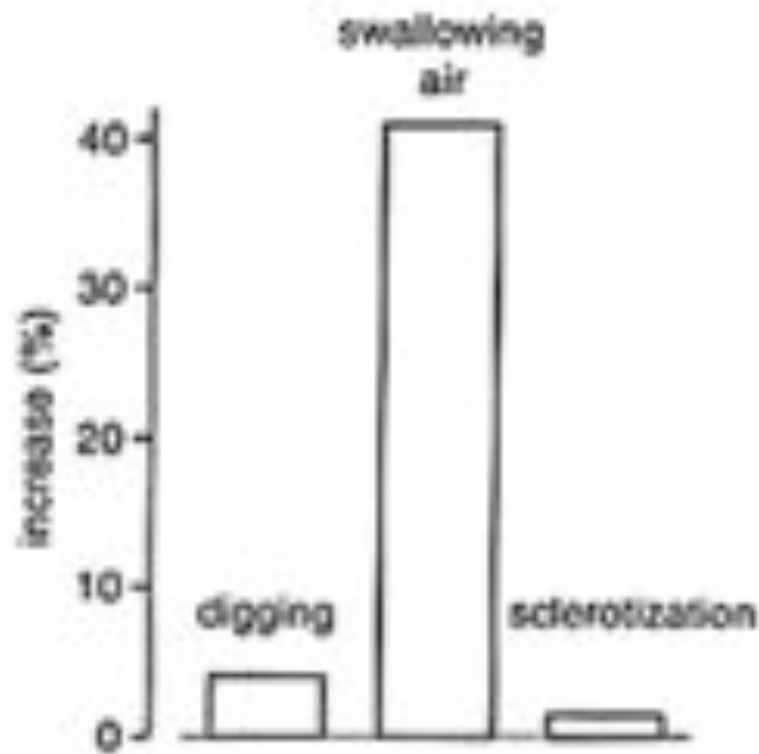


Nahirney, PC (Nahirney, Patrick C.); Forbes, JG (Forbes, Jeffrey G.); Morris, HD (Morris, H. Douglas); Chock, SC (Chock, Susanne C.); Wang, K (Wang, Kuan)

Title: What the buzz was all about: superfast song muscles rattle the tymbals of male periodical cicadas

Source: FASEB JOURNAL, 20 (12): 2017-2026 OCT 2006

Each 50 Hz muscle contraction yielded five to six stages of rib buckling in the tymbal, and a small release of muscle tension resulted in a rapid recovery due to the spring-loaded nature of the stiff ribs in the **resilin-rich tymbal**.



Cuticular plasticity. Extent to which a region on a dorsal sclerite of a fly can be stretched.

- 1. During digging the cuticle is inextensible.**
- 2. Reaches the surface and swallows air to expand new cuticle.
This causes release of bursicon, which initially causes plasticity but later causes sclerotization**
- 3. Cuticle now becomes sclerotized and inextensible.**

Cuticular plasticization

At the time of laying eggs, the desert locust's abdominal cuticle becomes plasticized. This permits the ovipositor to extend further into the soil so that the eggs can be laid as deeply as possible. Note the frothy white material at the bottom of the slide. This is material from the ARG that comes out in bubbles and then hardens like styrofoam. This surrounds the eggs and prevents them from being crushed and also provides a light guide for nymphs to exit from the hole.



Cuticular plasticization in blood feeders.

Rhodnius prolixus-kissing bug
and vector of trypanosome
that is causative agent of Chaga's



Occurs as a result of the action
of hormones or neurohormones.

female *Aedes triseriatus*,
feeding. "The treehole mosquito
(*Aedes triseriatus*) transmits
the virus that causes La Crosse
encephalitis." Courtesy [CDC](#)



Cuticular lipids and their function(s)

- Hydrophobicity of cuticle
- Inter- and intraspecific signal molecules for behavior

Contact Pheromones or cuticular hydrocarbons



"LICKING" AND CONTACT BEHAVIOR



n-alkanes
n-alkenes



Probably common in group insects that antennate or touch a lot.



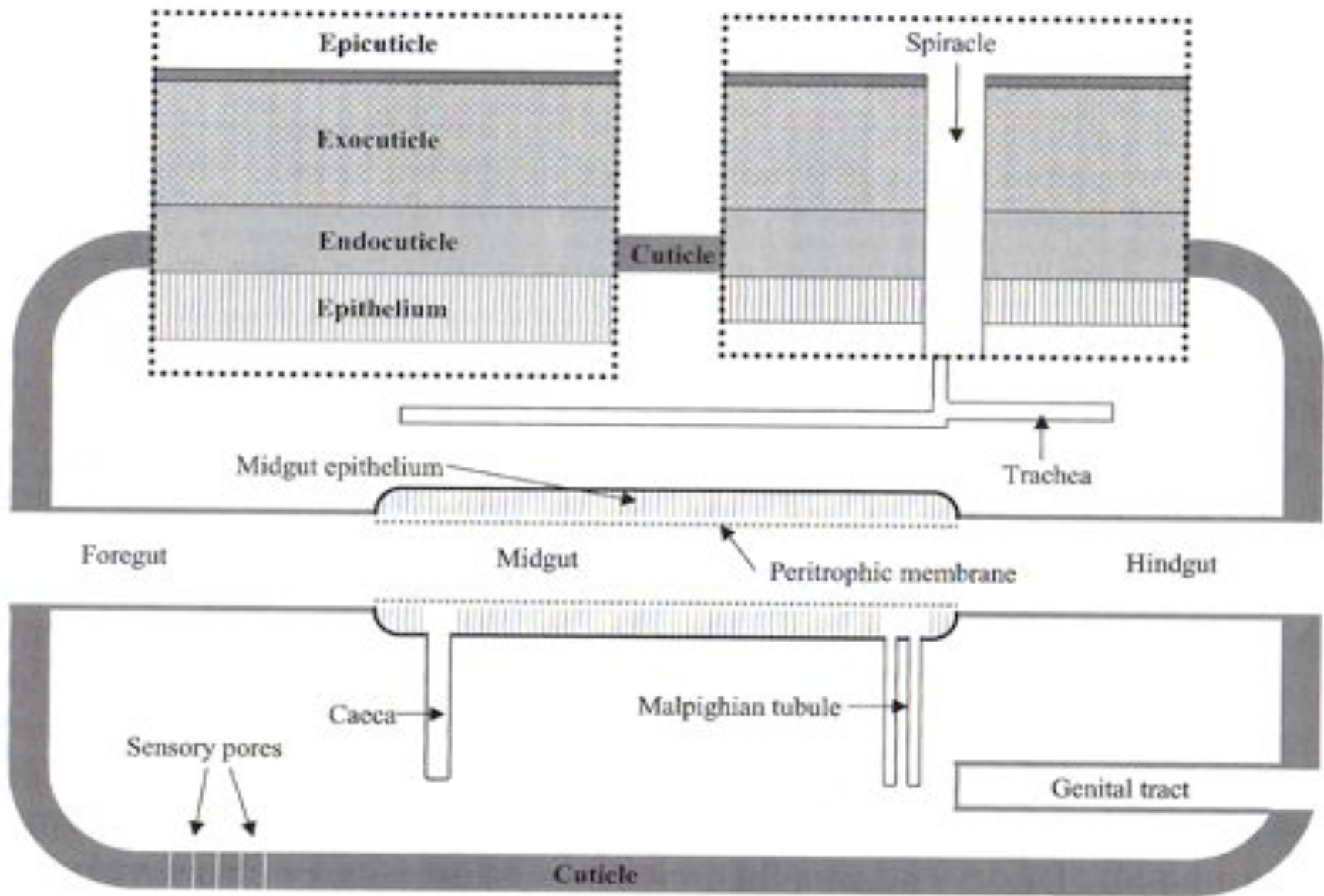
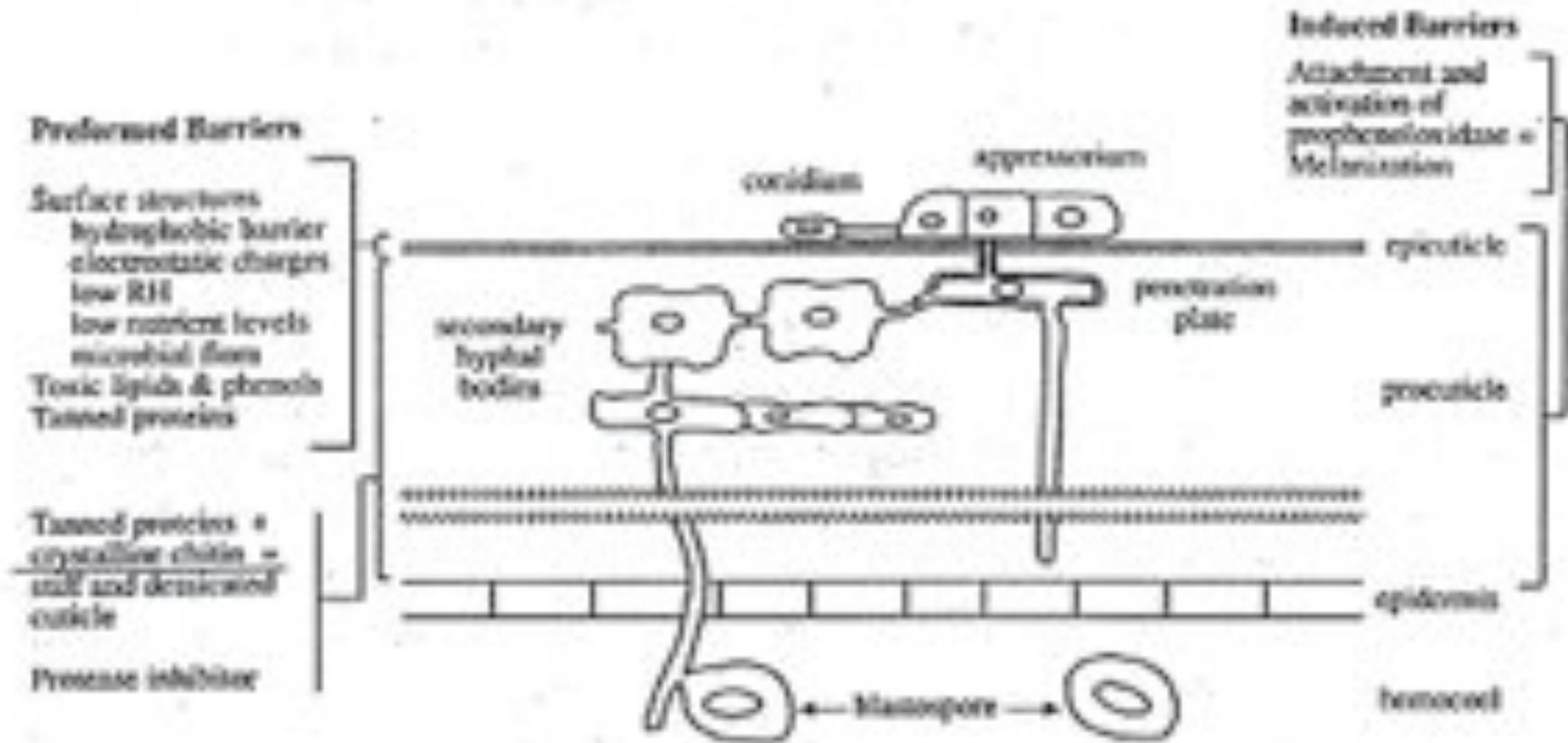


FIG. 2 A schematic figure of the insect's barrier defences, showing the regions where invasion is likely. The dotted boxes reveal detail within the integument.

Taken from Silva-Jothy et al. 2005. *Insect Immunity: An evolutionary ecology perspective*. *Adv. Insect Physiol.* 32: 2-48.

Entry through the integument by pathogens and parasites



What cues does the conidium get to develop into the appressorium and then for the appressorium to send out a penetration structure that penetrates the cuticle.?

Parasitic expulsion

Immature insects are able to encapsulate certain parasites and get rid of them at the molts. Here, a house fly larva is shown with two encapsulated nematodes that are then moved to the area beneath the cuticle. At the molt, these parasites are expelled with the old exocuticle.



WHAT IS AN APHID MUMMIE?



http://www.nysaes.cornell.edu/ent/biocontrol/parasitoids/lysiphlebus_testaceipes.jpg

How do parasites recognize where on the cuticle to penetrate using the ovipositor?

What role has the insect cuticle played in parasite evolution?

What are the consequences of an insect shedding its fore and hindgut lining at each molt? Which came first, cuticle or symbiont association?

6. Coloration and melanization

- Insect colors are due to either:
 - a. **Pigments** in the exocuticle such as melanin and they are usually lacking in endocuticle or
 - b. **Physical structure** of the cuticle to form defraction gradients that defract light in various ways. This results in the iridescence of the blue morpho butterflies and gold of beetles, gold on monarch chrysalis, silver of the fritillaries, and color of tiger beetles, etc.

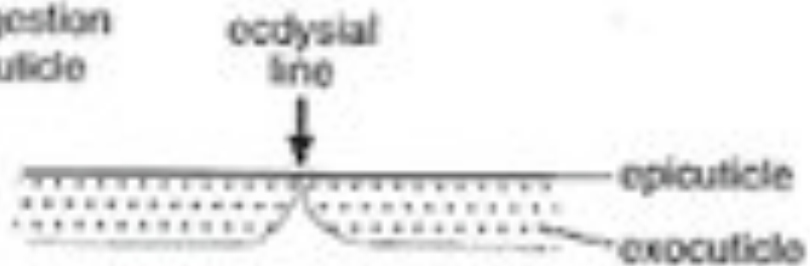


Molting and ecdysial suture

a) mature cuticle



b) after digestion of endocuticle





Note in the photo above that the **ecdysial suture** goes from the head and down the thorax in most insects. It is usually a gravity escape from the old cuticle.

What cuticular layer is absent at the ecdysial suture?

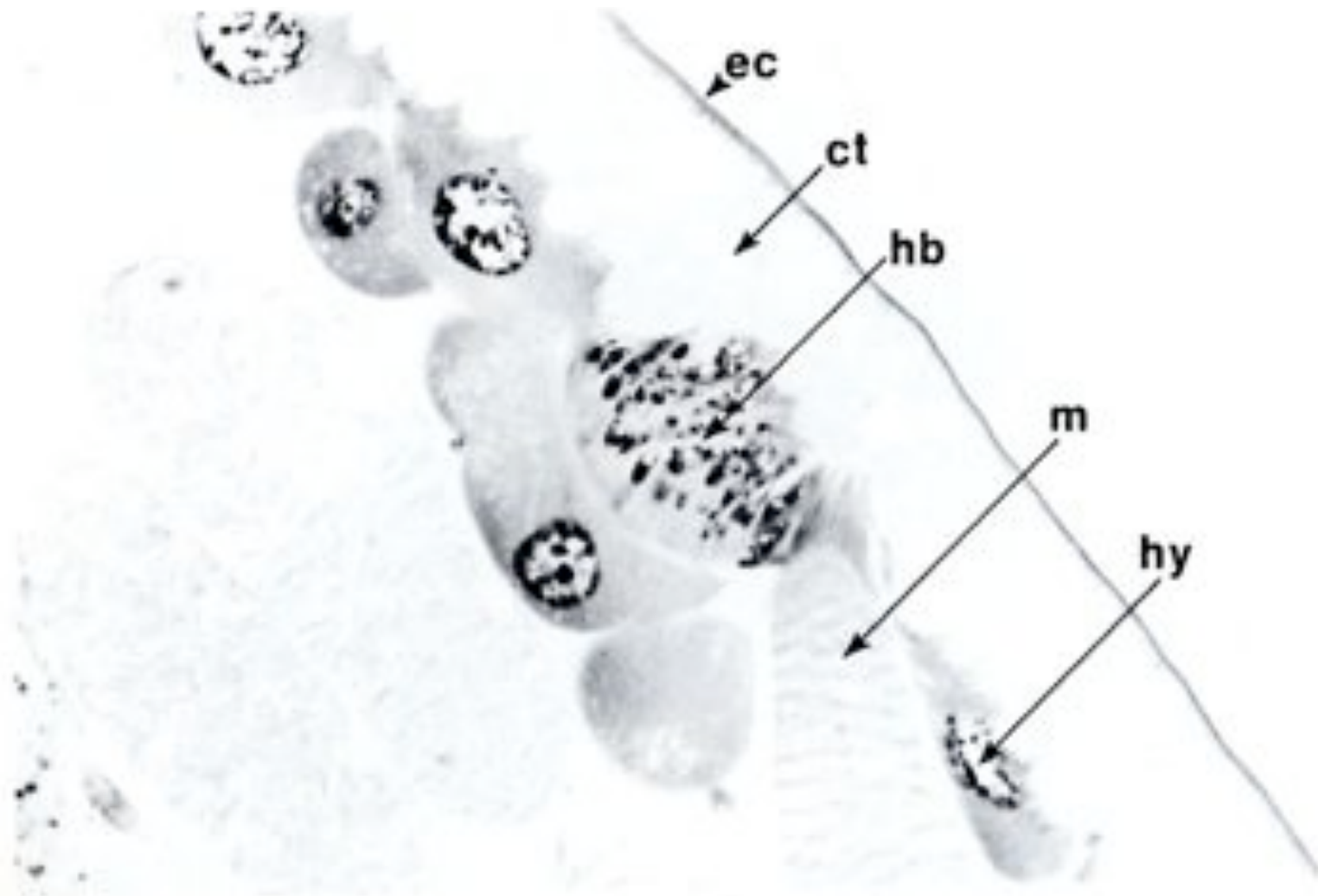


FIGURE 40.—Hypodermal histoblast (*hb*) in body wall of late-instar larva. (*ct*, cuticle; *ec*, epicuticle; *hy*, hypodermis; *m*, muscle)

In the last instar larvae small patches of imaginal hypodermal cells are found in the abdominal segments, two dorsally and two ventrally. These nests, which are called hypodermal histoblasts consist of very small cells and are responsible for the formation of the adult hypoderm. As these cells multiply they spread over the surface between the cuticle and the old hypodermis, displacing the latter into the body cavity, where the old cells are phagocytized.



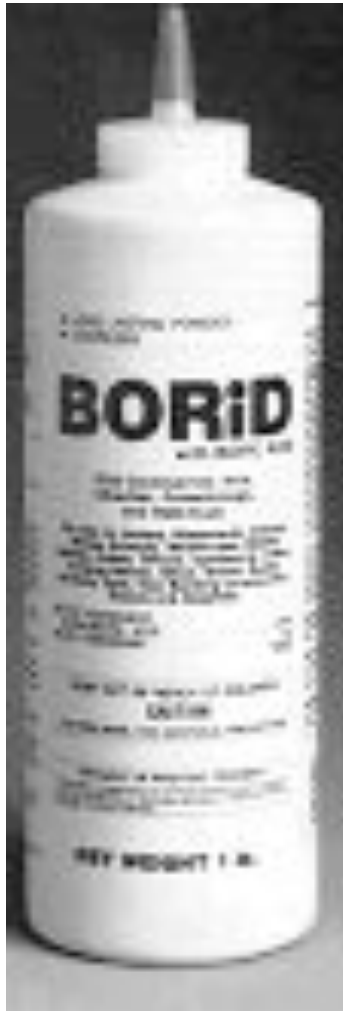
The white out the rear of this fulgorid is wax, which may help deter predators.

7. Techniques and tools used to study the integument

- TEM (transmission electron microscopy) and SEM (scanning electron microscopy)
- Various analytical chemical techniques
- X-ray analyses
- Pressure and strain gauge techniques
- Freeze fracture SEM
- UV light

8. Basic and applied research and questions

- Basic-
 - a. Cuticular diversity, cellular fate and differentiation
 - b. Pattern formation of cuticle
- Applied
 - a. Abrasives and adsorptives
 - b. Chitin-binding proteins
 - c. Chitin polymers for industry
 - d. Chitin synthesis inhibitors-
 - e. Spreaders and stickers for pesticides
 - f. Tsetse control using shoestrings



Abrasives

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**Primitive man and animals
taking dusts baths**



Abrasive

Donkey taking a dust bath

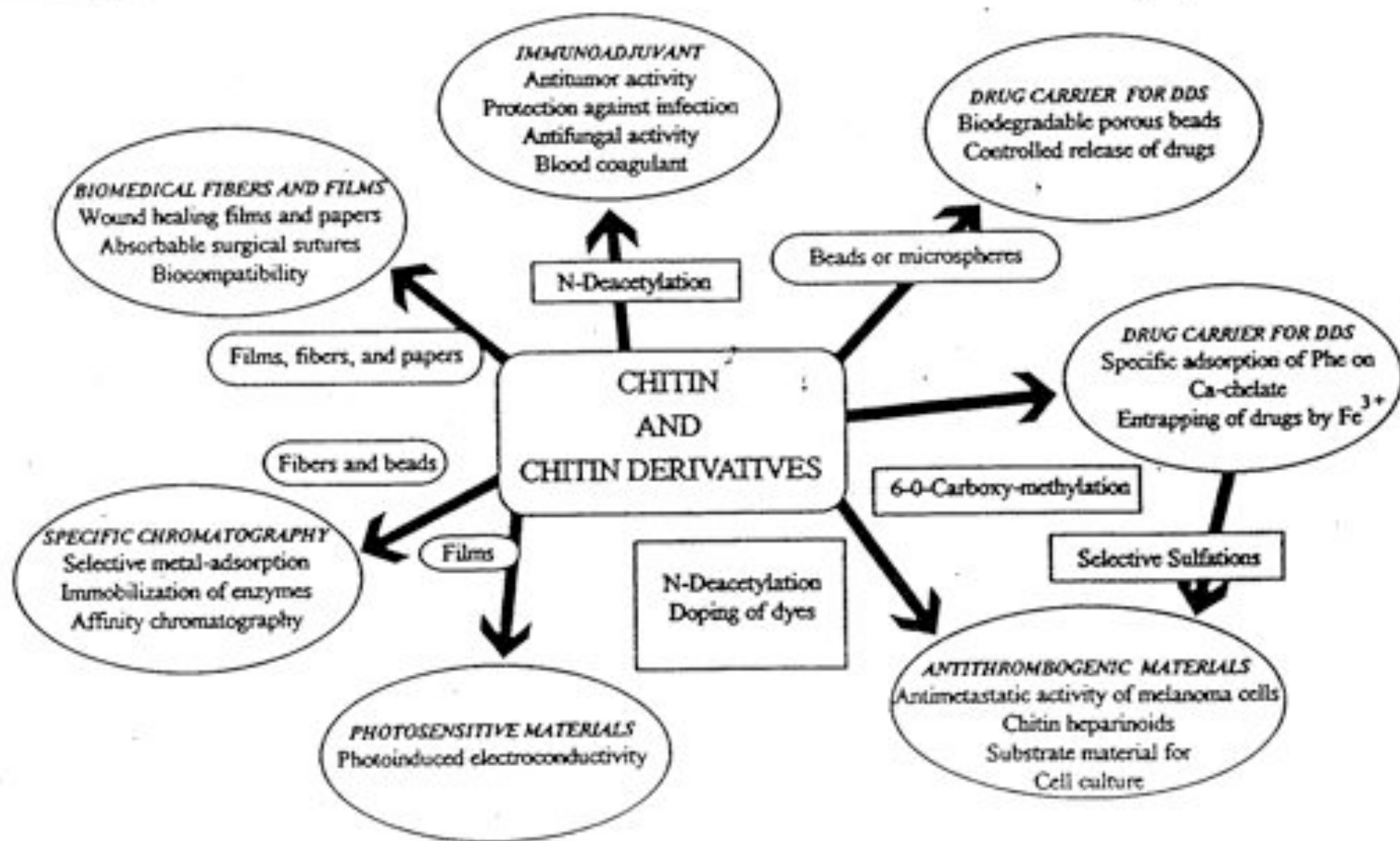
The dominant male bison guards and protects his dusting area

Chitinase or chitin synthesis inhibitors

Alsystin-Triflumeron

Dimilin-Diflubenzuron used in forest and field crops. Stomach and contact poison that acts by inhibiting chitin synthesis so it interferes with formation of cuticle. Ferro used it in combination with *Bauveria* (fungus) released against the Colorado potato beetle. Control went down. WHY? Now removed from market because breakdown products are nasty and chitin now found in bacteria and fungi.

Go to internet and type in chitinase inhibitors to see all that comes up.



Overview of chitin's potential applications.

Key Features

Highly effective against sciarid larvae

- Extremely well tolerated by mushrooms - application does not yield, as may occur with other chemicals
- Non-systemic in mushrooms - residues do not accumulate
- Safe to users

Frequently Asked Questions

[Top](#)

Q: How does Alsystin work on sciarid fly?

A: Triflumuron, the active ingredient in Alsystin, interferes with the formation of chitin "the skin layer" of larvae which causes defective formation resulting in larval death. Alsystin does not control adult sciarids as they do not moult; however, only the larval stage is responsible for effects on yield or quality. Adults do not disappear immediately after treatment with Alsystin.

Q: Is Alsystin safe to beneficial organisms?

A: Since the contact action of Alsystin is fairly limited, the potential for major damage to beneficial organisms is highly unlikely. Extensive tests both in the laboratory and in the field have shown that Alsystin has little or no effect on important beneficials.

Q: Is Alsystin safe to the user and consumer?

A: From acute studies the specific mode of action of Alsystin results in the product being regarded as non-toxic to man, other mammals, birds and fish. The results of all studies demonstrated that Alsytin is a product with extremely low toxicity for users and consumers.



How does this chemical work?

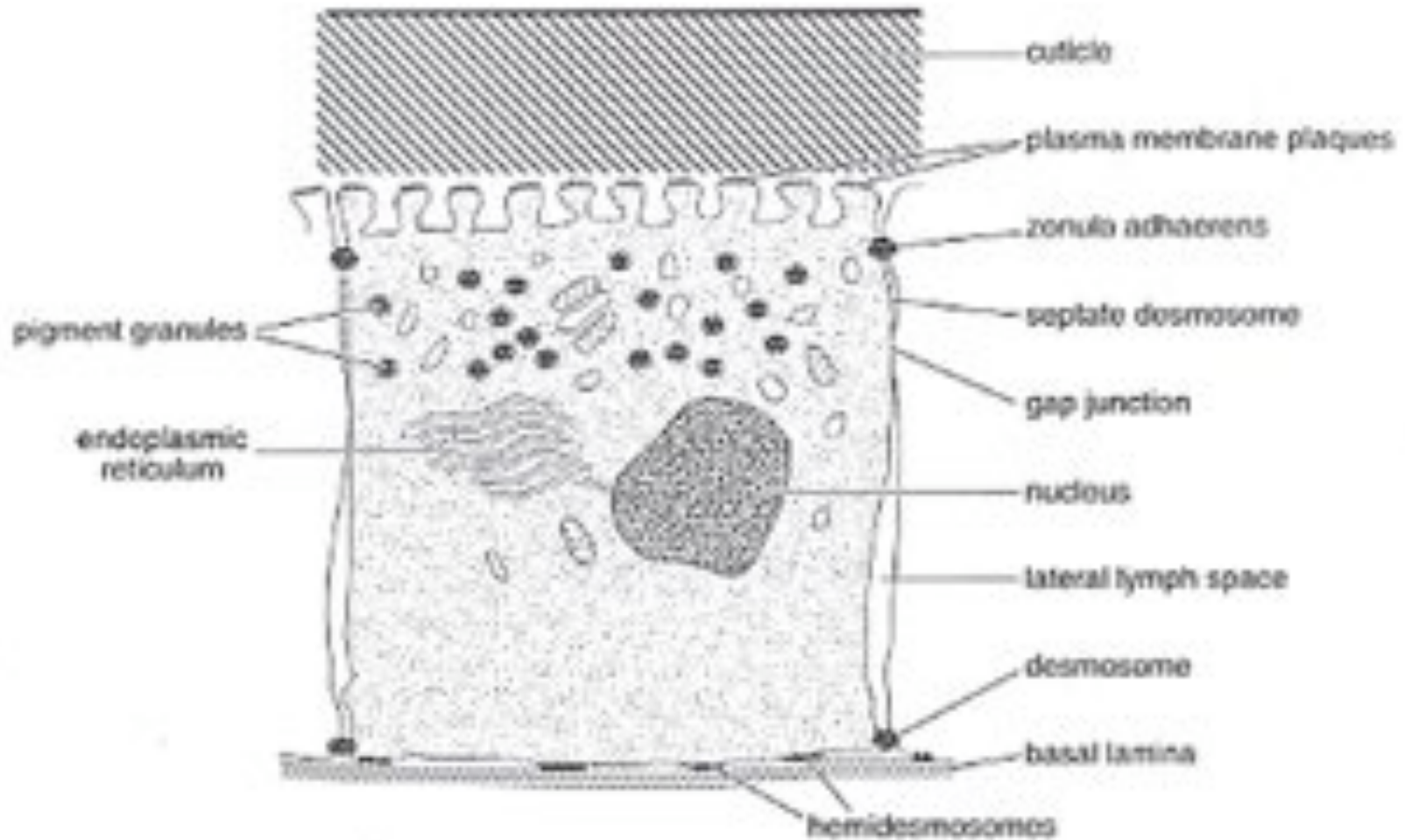
Larval flea has a ‘chitin tooth’ used to break out of the egg shell. The chitinase inhibitor, which the female flea picks up from the host’s blood is put into the embryo. Thus, the ‘tooth’ does not develop and the flea larva can’t emerge from the egg shell and dies.

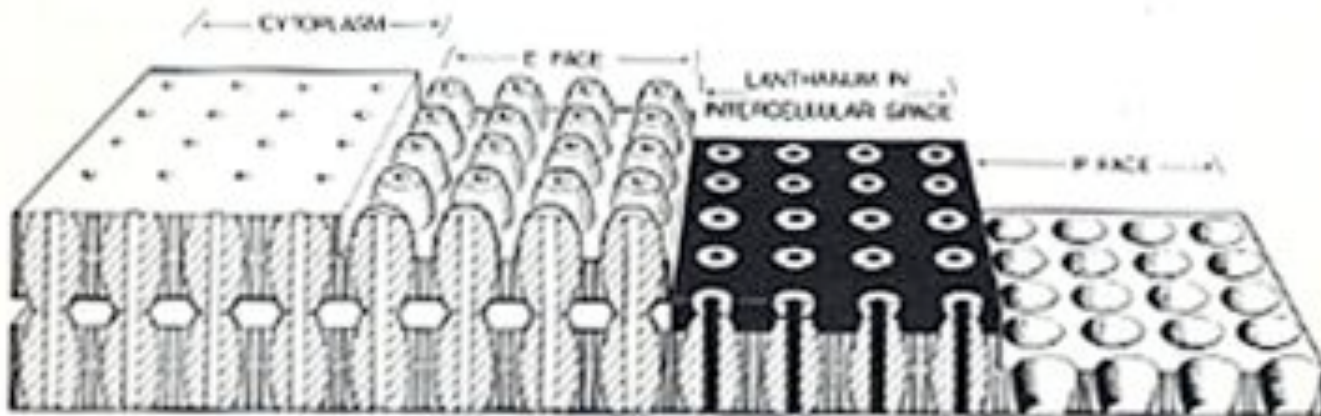


The most common of the canine parasites, fleas create problems for both pets and humans because they multiply so quickly.

The fleas on your dog are in the adult stage. An adult female flea, living on a pet, can lay up to 2,000 eggs in her brief lifetime. PROGRAM® (lufenuron) Flavor Tabs® prevent flea eggs and larvae from developing. This breaks the flea life cycle at its base, and effectively controls the flea population.

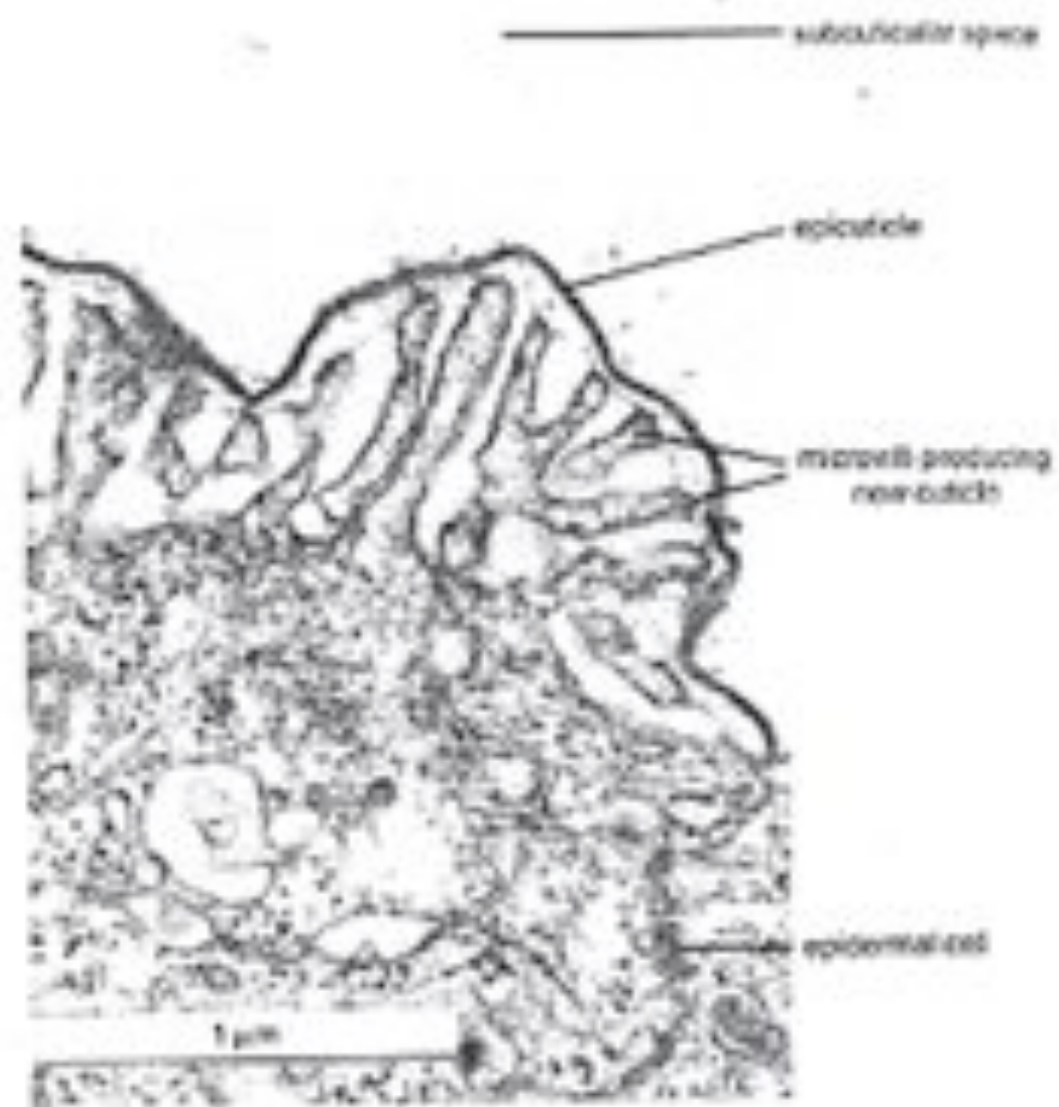
Insect epidermal cell diagram





Model of gap junctions between each epidermal cell. The cells are also electrically coupled so they can act in concert. This permits rapid communication. Model is based on freeze fracture SEM work





Section through nymphal cuticle of *Oncopeltus*.

Pc=pore canals

Cu=old endocuticle

Cu'=old endocuticle is becoming disorganized

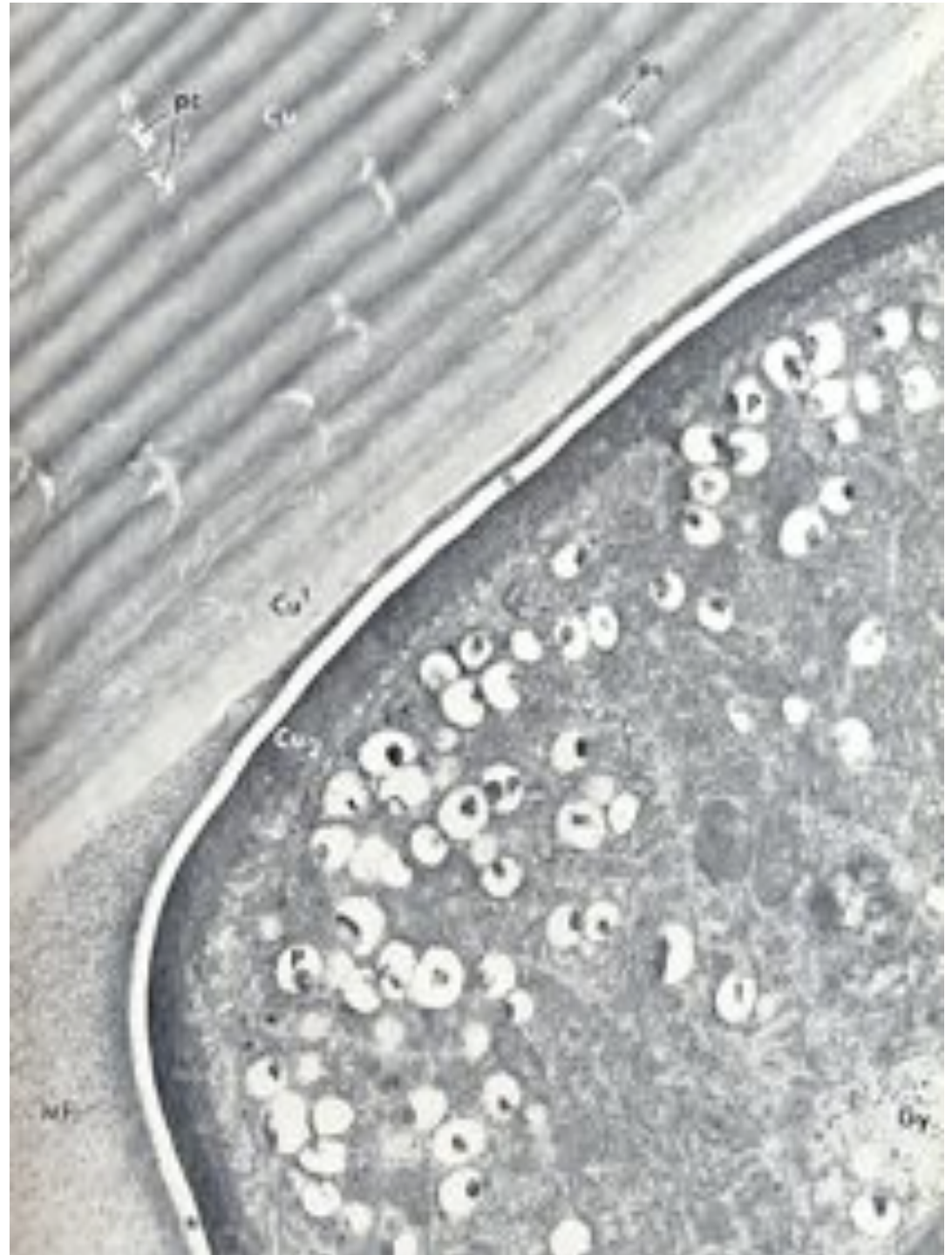
MF=molting fluid

Gy=glycogen

P=pigmented granules

Note lamellar structure of the endocuticle.

Photo taken from pg. 12 of Insect Cells-their structure And function by David Smith. Oliver and Boyd, 1968



What every epidermal cell must know!

- 1. When to begin molting process.**
- 2. What type of cuticle to produce at the molt.**
- 3. What the other cells around it are doing using gap junctions.**

THE END

How do the epidermal cells know to produce the scent pouches of the male, the vein colors and the patterns of this elegant butterfly?

