socket was imposed. The elongation of the pressure distribution in the anterior to posterior direction and the multiple local maxima are typical.

Comparison of the shape of the contours of percentage cartilage compression (Fig. 3B) with the surface pressure distribution (Fig. 3A) shows a strong correlation between the acetabulum geometry and the surface stress on articular cartilage. These data suggest that abnormal hip joint geometry, either congenital or due to trauma, will cause stress concentrations in the cartilage layer. Since abnormal hip joint geometry is often observed in osteoarthritis (*11*), the correlation between geometry and stress contributes to the understanding of the etiology of osteoarthritis.

PAUL D. RUSHFELD*
ROBERT W. MANN
WILLIAM H. HARRIS†
Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge 02139

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* Present address: Perry Oceanographics, Inc., Riviera Beach, Fla. 33404.
† Major affiliation: Hip and Implant Unit, Massachusetts General Hospital, Boston 02114.

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Natural Polyesters: Dufour's Gland Macrocyclic Lactones Form Brood Cell Laminesters in *Colletes* Bees

Abstract. *Bees in the genus* Colletes *make their brood cells in the ground and coat them with a highly resistant, waterproof, transparent membrane. This membrane is a polyester constructed mainly from 18-hydroxyoctadecanoic acid and 20-hydroxyeicosanoic acid, which are stored as their corresponding lactones in the Dufour's gland of the bee. When lining the cells, the bee secretes its glandular content, and the membrane is apparently a product of polycondensation reaction of its contents. This appears to be the first report of a naturally occurring linear polyester. The term laminester (lamina ≃ layer + ester) for this class of compounds is proposed.*

Many of the approximately 20,000 species of solitary and social bees (Apoidea; Hymenoptera) make their nests and brood cells in moist soil. The hygroscopic provision of nectar and pollen is protected against water, fungi, and various soil organisms by a layer of waterproof secretion applied by the nesting female to the soil surrounding the cell (Fig. 1). The chemical composition and origin of this secretion in most bees is unknown (*1*).

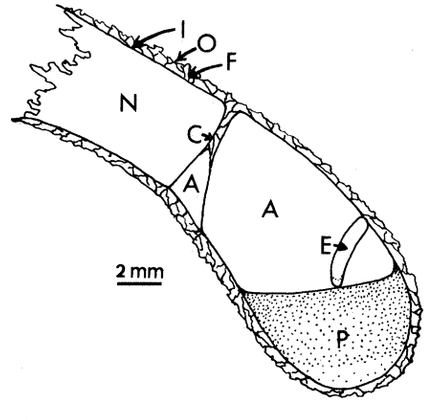

Fig. 1. Brood cell of *Colletes validus*. This semidiagrammatic drawing emphasizes the double-layered structure of this polyester membrane sac. *I*, inner layer of thick membrane; *O*, outer layer of thin, fragile membrane that adheres loosely to surrounding soil; *F*, fibrils that connect the two layers; *A*, air space found in cell cap, in cell, and between layers; *C*, double-layered membranous cell cap; *N*, neck of cell, normally filled with soil after oviposition; *E*, egg adhering to cell by cephalic end; and *P*, provision, consisting of a semiliquid mixture of pollen and nectar. Cells are made in the soil at the ends of tunnels.

As early as 1835, the Dufour's gland of *Colletes* was suspected to be the source of the cell lining (*2*), and the possible role of this gland in cell construction by other bees was also considered (*1*, *3*). These conclusions were mostly drawn on the basis of solubility tests (*1*, *3*), and of morphological (*4*) and behavioral (*5*) evidence. Recent investigations of the fragrant, oily secretion that fills the Dufour's gland in several species of bees have revealed macrocyclic lactones in *Colletes cunicularis* (*6*) and in five genera of halictine bees (*6*, *7*), and farnesyl and geranyl esters in *Andrena* spp. (*8*).

We report here chemical evidence that the cell linings of *Colletes thoracicus*, *C. inaequalis*, and *C. validus* are the polymerized secretions of the hypertrophied Dufour's glands of these bees. In order to find out if the cell lining originates from the glandular content, it was necessary to compare the chemical nature of the Dufour's gland secretion and the cell lining.

Dufour's glands dissected from the living bees were extracted with methylene chloride and analyzed by combined gas chromatography and mass spectroscopy (GC-MS) (*9*). The glands contain a mixture of macrocyclic ω-lactones, hydrocarbons, and aldehydes (Fig. 2A). The two dominant lactones are 18-octadecanolide and 20-eicosanolide, accompanied by 18-octadecenolide (of undetermined double-bond location), 22-docosanolide, a methyl-branched eicosano-

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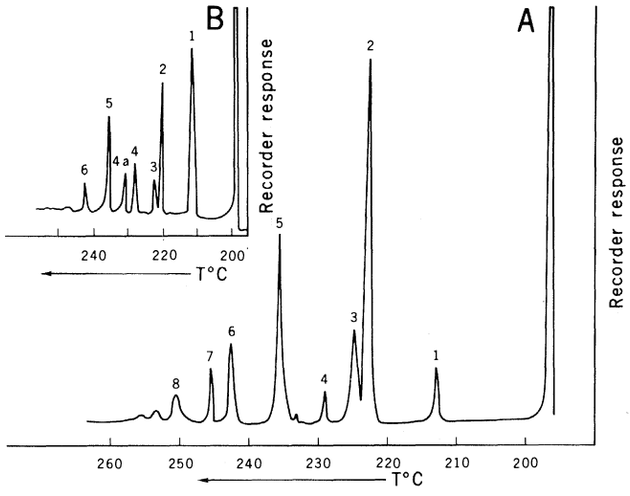

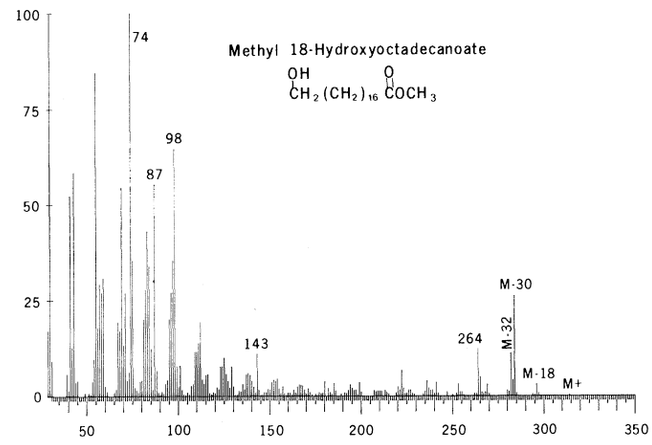

Fig. 2 (left). Gas chromatogram of the Dufour's gland of *Colletes thoracicus* (A) and of the methylene chloride washing of its cell lining (B). Glands were dissected from chilled bees, extracted in methylene chloride and analyzed by GC-MS with an SE-30 capillary column (25 m by 0.2 mm). Cell linings were washed free of dirt with water and then with methylene chloride. The methylene chloride washing was concentrated and analyzed as above. Peaks are as follows: 1, *n*-octadecanal; 2, 18-*n*-octadecanolide; 3, 18-*n*-octadecenolide; 4, *n*-eicosanal; 5, 20-*n*-eicosanolide; 6, methyl-branched 20-eicosanolide; 7, 22-*n*-docosanolide; and 8, tricosane. In chromatogram B, component 4a was not identified. Fig. 3 (right). Mass spectrum of methyl 18-hydroxyoctadecanoate resulting from methanolysis with HCl of the cell lining. The sample was analyzed on an LKB GC-MS with an SE-30 capillary column programmed from 100° to 300°C at 10°C per minute.

lide, and trace amounts of 16-hexadecanolide. The hydrocarbons are present in very low amounts in the gland and ranged from *n*-hexadecane to *n*-tricosane. The two aldehydes present are *n*-octadecanal and traces of *n*-eicosanal. The occurrence of these aldehydes in insects has not previously been reported.

The cell linings are practically insoluble in either aqueous or organic solvents. Sections were washed with water to remove soil contaminants and then extracted with methylene chloride or chloroform. Analysis of the extracts revealed (Fig 2B) the same major lactones and aldehydes present in the glands, but the proportion of lactones is less, because of their consumption by the polymerization process.

After the water and organic solvent extractions, the cell lining was treated with methanolic HCl at 100°C for several hours. The membrane disappeared, and an upper layer formed. This layer was dissolved in methylene chloride and analyzed by GC-MS, revealing two major and two minor components. The two major components showed weak peaks for the molecular ions at *m/z* 314 and 342 as well as fragment ions at $[M - 18]^+$, $[M - 30]^+$, $[M - 32]^+$, *m/z* 143, 87, and 74 characteristic of long-chain ω-hydroxy acid methyl esters, such as methyl 18-hydroxyoctadecanoate (Fig. 3) and methyl 20-hydroxyeicosanoate. Confirmation of their hydroxylic nature was obtained by converting them to their silyl ether derivatives with bis(trimethylsilyl)trifluoroacetamide. The products showed intense $[M - 15]^+$ ions at *m/z* 371 and 399. Similar treatment of authentic 15-hydroxypentadecanoic acid gave products with entirely analogous mass spectra.

The isotope patterns of two minor components from the HCl-methanol treatment indicate that they are chlorinated compounds of molecular weight 332 and 360 respectively. The loss of fragments such as M − 31 (M − CH_3O) and M − 49 (M − CH_2Cl) suggest that they are methyl 18-chlorooctadecanoate and methyl 20-chloroeicosanoate, probably originating from acid-catalyzed exchange of the terminal hydroxyl for chlorine during methanolysis with HCl.

These results show that the cell lining is a polymer derived from the lactones present in the *Colletes* Dufour's glands. How the Dufour's gland secretion in *Colletes* is actually applied remains unknown; however, several genera of Colletidae, including *Colletes* spp., have been observed to coat the cell membrane with the broad, flat glossa (tongue) that is characteristic of this family (*1*, *10*). In Halictidae (*5*), the glossa and legs are used simultaneously to spread the secretions after they have been applied to the soil by the pygidial region. We therefore assume that in *Colletes* spp. a similar mechanism is used for lining the cell.

Once they are deposited, the lactones (or their corresponding ω-hydroxyalkanoic acids) polymerize by an unknown, perhaps enzymatic, mechanism to form a smooth continuous coating of polyester layer on the cells. The two major building blocks are 18-octadecanolide and 20-eicosanolide, but it is very probable that the other lactones present in the gland contribute to the film. We attempted to mimic formation of the natural polyester by heating commercially available 15-hydroxypentadecanoic acid to 200°C for several hours. On cooling, a film, insoluble in methylene chloride or methanol and resembling the natural membrane, appeared in the tube. Infrared spectra of these two substances were similar, both showing broad carbonyl bands at 5.8 μm.

To ensure that the bulk of the cell lining is indeed composed of polyesters we analyzed thoroughly washed sections by the mass spectrometer direct insertion probe. When the sections were heated to ~ 250°C, mass spectra indicated the presence of a mixture of 18-octadecanolide and 20-eicosanolide as well as their dimers and trimers. These results are in agreement with previous observations (*11*) that thermolysis of similar ω-polyesters results in their corresponding lactones.

Pain *et al.* have reported that the lipid extract of honey bee queen larvae contain 10-hydroxydec-2-enoic acid and several of its esters (*12*). They also postulated the existence of di- and oligoesters from the same acid but produced no evidence. Related oligoesters also exist in the wax of the leaves of *Picea pungens*. They have been variously reported as linear tetramers (where they have been termed etholides) and as cyclic tetramers (*13*). In any case, these are reported to be waxy materials greatly differing in solubility from the *Colletes* cell lining. The *Colletes* cell lining is the first case in which a high molecular weight polyester is reported (*14*), and we suggest the name laminester (lamina = layer + ester) for

membranes made of natural polyesters.

The laminester is not soluble in organic solvents like chloroform or benzene, suggesting it has a very high molecular weight. It is so hydrophobic that a fine layer of air forms between the membrane and water. Further indication of its hydrophobicity is the fact that it is untouched by 12*N* aqueous HCl at 100°C overnight, but, as mentioned above, methanolic HCl at 100°C completely hydrolyzes it in 2 hours. Similarly, the polyester was unaffected by treatment with 60 percent aqueous KOH, but addition of methanol to the solution (50 percent by volume) resulted in rapid hydrolysis. These hydrophobic characteristics probably explain how these cell linings resist physical and biological attack and remain intact in soil for more than a year.

Although in ants the Dufour's gland produces secretions that serve as pheromones (*15*), the use of the Dufour's gland products for cell construction in Apoidea is unique. The production and utilization of the lactones provide the bees not only with an efficient protective membrane but also, because of their fragrance combined with the fragrance of the related aldehydes, might serve in nest location and recognition as suggested (*8*). The possible additional function of the lactones as pheromones was not investigated. However, Dufour's gland secretion of *Colletes* spp. retains its distinct musky fragrance for over a year, and the abdomens of actively nesting females smell strongly of these secretions.

ABRAHAM HEFETZ
HENRY M. FALES
Laboratory of Chemistry, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20014
SUZANNE W. T. BATRA
Beneficial Insect Introduction Laboratory, Insect Identification and Beneficial Insect Introduction Institute, Science and Education Administration, Department of Agriculture, Beltsville, Maryland 20705

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Prolonged Inhibition of Neurons by Neuroendocrine Cells in *Aplysia*

Abstract. *In the abdominal ganglion of* Aplysia, *a burst of action potentials in peptide-secreting neuroendocrine cells, the bag cells, produces slow inhibition of two identified bursting pacemaker neurons. The inhibition is due to a slow hyperpolarizing potential that reduces bursting pacemaker activity for 3 hours or more. The slow inhibitory potential results from a large and prolonged increase in membrane conductance to potassium ions as well as a slower ionic process that is relatively independent of membrane conductance.*

The abdominal ganglion of the marine mollusk *Aplysia* is a convenient experimental system for investigating the roles of peptides as chemical messengers in the nervous system. The ganglion contains a group of neuroendocrine cells, the bag cells (1–4), that synthesize and secrete peptides (*5*). One of these is egg-laying hormone, a 6000-dalton peptide that induces egg laying (*6*). An electrically stimulated bag cell discharge produces prolonged augmentation of bursting pacemaker activity in R15, an identified neuron in the abdominal ganglion (*7*). The peptide is thought to mediate this interaction, since local application of the peptide duplicates the response (*8*). The response appears to be induced hormonally rather than transynaptically, for (i) it has a slow, smooth onset and (ii) there is no ultrastructural evidence for synaptic contacts between bag cell axons and other ganglion neurons.

The bag cell axons branch profusely in the sheath surrounding the ganglion, where they end near vascular spaces (Fig. 1A) (*3*). This distinctive anatomical arrangement led us to search for effects on other ganglion neurons (*9*). In this report we describe another type of bag cell action, prolonged inhibition, which lasts for 3 hours or more. Although this response may also be hormonally induced, the effect on electrical signaling and the ionic response mechanism resemble synaptically mediated slow inhibition.

Experiments were performed on abdominal ganglia isolated from sexually mature *Aplysia* (Pacific Biomarine, Venice, California) weighing 300 to 500 g. Animals were usually anesthetized by injecting 120 ml of isotonic $MgCl_2$ to prevent possible premature activation of the bag cells during dissection. The isolated ganglion was pinned to clear resin (Sylgard) in 33 percent isotonic $MgCl_2$ in artificial seawater (ASW) and washed for 30 minutes in ASW before experiments were begun. Experiments were conducted at 20°C in either perfused ASW or 15 ml of filtered standing blood taken from unanesthetized animals. Double-barreled microelectrodes filled with 4*M* potassium acetate were used for intracellular recordings and for passing current.

The bag cells are normally silent (*1*, *10*), but, after being locally stimulated with a brief 1- to 2-second train of electrical pulses (*11*), they fire in a burst of spike activity lasting approximately 20 minutes (*9*, *12*). Since they fire in near unison, their activity was monitored by an intracellular recording from one bag cell. A second intracellular electrode recorded the response of a target cell (Fig. 1A). The target cells are the identified bursting pacemaker neurons L_3 and L_6, which discharge in periodic bursts of action potentials (*4*). These cells lie adjacent to one another and respond identically to bag cell activity.

Figure 1B shows the first few minutes of slow inhibition following the onset of bag cell spike activity. Several seconds after the bag cells began to discharge, the target cell slowly hyperpolarized and all spiking was inhibited for 1 to 2 minutes. Thereafter, burst activity of the target cell resumed, but burst rate and average spike rate remained below baseline rates. The inhibition is due to a slow inhibitory potential (SIP) with an amplitude of 10 to 20 mV, which is superim-

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