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15. Coordinates were 0.6 mm posterior to bregma, ± 1.7 mm lateral to the midline, and 8.0 mm from the dura.
16. Before the rats were killed, lesions were made through all electrodes by a direct anodal current of 2 mA for 5 seconds. Rats were then perfused with 0.9 percent saline solution and 10 percent formalin; their brains were removed and immersed in formalin for at least a week before sections (40 μm, stained with Luxol blue and cresyl violet) were made.
17. The threshold for self-stimulation behavior was defined as the lowest current that would maintain a rate of responding higher than the highest rate during any time-out period. Performance was considered stable when the threshold remained constant, and the rates at each current setting varied no more than ± 10 percent from day to day.
18. Eight rats rotated to the left and six to the right. Rotation in response to *d*-amphetamine averaged 39.8 ± 19.8 net rotations per hour preoperatively and 43.6 ± 17.4 net rotations per hour postoperatively (net rotations were determined by subtracting rotations in the nonpreferred direction from those in the preferred direction (*13*). When self-stimulating the side of the brain opposite the preferred direction of rotation, rats averaged 20.3 ± 3.4 net rotations in 25 minutes; when self-stimulating the side of the brain ipsilateral to the preferred direction of rotation, rats averaged 23.2 ± 3.8 net rotations in 25 minutes.
19. If the rotation occurring during self-stimulation sessions had been elicited by the stimulus, the direction would have changed with the side of stimulation; see, for example, G. W. Arbuthnott and T. J. Crow, *Exp. Neurol.* **30**, 484 (1971).
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5 November 1979

Anthophora Bees: Unusual Glycerides from Maternal Dufour's Glands Serve as Larval Food and Cell Lining

Abstract. *The Dufour's gland of* Anthophora abrupta, *a solitary bee, secretes a complex mixture of liquid triglycerides containing one long-chain and two short-chain fatty acids. This is applied inside the earthen brood cells and added to the provision, where it is converted, perhaps by enzymes from the bee's saliva or gut, to solid diglycerides that are later eaten by the bee larvae. This use of Dufour's gland secretion as food and its nutritive function are reminiscent of the royal jelly secreted by honey bees.*

Most bees (Apoidea) eat a diet of nectar and pollen exclusively. Exceptions are the well-known but chemically complex salivary royal jelly of honey bees; the host brood commonly eaten by inquiline bees (*1*); and the ingestion of Thysanoptera (*2*) and floral oils (*3*). The function of the Dufour's gland of the sting apparatus in Hymenoptera is incompletely known; however, in ants it secretes pheromones (*4*), and in various families of bees it produces the waterproof cell lining, which, being fragrant, may include pheromones (*5*). Several species of *Anthophora* have white, waxy cell linings with a cheesy odor (*6*), which long have been hypothesized to originate from the Dufour's gland (*7*). In this report we characterize the contents of Dufour's gland as an unusual group of triglycerides, show their conversion to diglycerides for construction of the cell lining, and demonstrate their ultimate use as larval food. This combination of features has apparently not been reported heretofore.

Anthophora abrupta Say is a univoltine solitary bee that usually nests gregariously in clay banks (*8*), and its subterranean cells are lined internally by a layer (0.1 mm) of waxy, white, waterproof substance. A group of females constructed 36 such cells in narrow, clay-filled, acrylic plastic chambers where their behavior was observed. During cell construction, the bees moistened the clay with regurgitated water, manipulated it by the mandibles and legs, and compacted it by the pygidial plate to form a smooth-walled earthen cell. A colorless, oily, faintly fragrant liquid was secreted onto the compacted soil from the sting chamber. Provisioning with pollen and nectar with addition of a transparent liquid from the sting chamber, oviposition, and cell capping followed. A few hours after provisioning began, the cell lining became opaquely white and developed a cheesy odor. The provisions also developed this odor and contained white flocculi. Larvae consumed the provisions within 3 weeks, and for the next 2 days they ate the cell lining (Fig. 1) before transforming into diapausing prepupae. Cells (500) opened in the field revealed that those with prepupae lacked the white lining but those with young larvae retained it, an indication that cell lining is normally eaten by larvae.

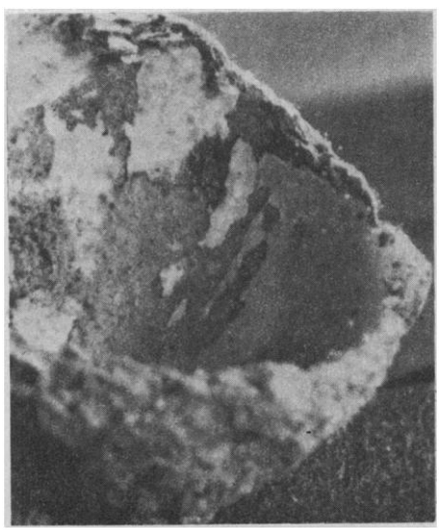

Fig. 1. Brood cell of *A. abrupta* showing grooves in the white lining that were made by the feeding larva.

The hypertrophied Dufour's gland of this bee occupies about half of the abdomen (Fig. 2). It is surrounded by numerous tracheoles, indicating a high metabolic rate. The lumen contains a copious, transparent oily liquid that solidifies after several weeks to a white, waxy, microcrystalline solid when smeared on glass and exposed to air.

The fresh Dufour's gland secretion (3.4 mg) was collected by capillary tube and dissolved in 1 ml of methylene chloride, and 1 μl was injected onto a 3-m column (packed with 1 percent Hi-Eff 3BP; Applied Sciences) of an LKB-9000 gas chromatograph–mass spectrometer system. Five main components eluted at 250°C (Fig. 2). Their electron ionization mass spectra show many ions related by a 2-carbon homology (*m/z* 43, 71, 99) suggesting acetyl, butyroyl, and hexanoyl groups, and all show a common palmitoyl ion at *m/z* 239. Unfortunately, no molecular ions (MH^+ ions) were observed, even with the use of chemical ionization with isobutane (Finnigan 4023). However, with the latter technique, all chromatographic peaks do show abundant ions for the loss of various acids from hypothetical MH^+ ions. Thus, in the mass spectrum of peak 4, assuming an MH^+ ion at *m/z* 499, fragment ions appear at *m/z* 243 (MH^+ − palmitic acid, 100 percent), 383 (MH^+ − hexanoic acid, 12 percent), and 411 (MH^+ − butyric acid, 8 percent). Subtraction of the acids from the molecular weight (498) leaves only 38 atomic mass units (C_3H_2), and hence the compound is simply the triglyceride butyroylhexanoylpalmitin. Its electron ionization mass spectrum shows ions as expected (*9*) at *m/z* 243 (M − palmitoyloxy), *m/z* 383 (M − hexanoyloxy), and *m/z* 411 (M − butyroyloxy). Spectra of the other chromatographic peaks show homologous ions that allow assignment of their structures as isomers of the re-

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lated triglycerides, acetylbutyroylpalmitin, dibutyroylpalmitin, acetylhexanoylpalmitin, and dicaproylpalmitin. Small amounts of the diacylstearin glycerides were also observed although they were not chromatographically resolved.

To decide which isomer of dibutyroylpalmitin was present, 1,2- and 1,3-dibutyroylpalmitin were prepared from the corresponding 1- and 2-monopalmitins (Supelco, Bellefonte, Pennsylvania) with the use of butyric anhydride and pyridine. The isomers are indistinguishable by gas chromatography; however, their mass spectra show reproducible differences and a diagnostic ion at *m/z* 201 ($M - C_{15}H_{31}COOCH_2\cdot$) is present only in the 1,2-isomer. Peak 2 shows a mass spectrum identical in all respects with this isomer and is therefore 1,2-dibutyroylpalmitin. The spectrum of peak 5 shows a similar diagnostic ion at *m/z* 257, establishing its structure as 1,2- rather than 1,3-dihexanoylpalmitin. The very low intensity of a related ion at *m/z* 229 ($M - C_{14}H_{29}COOCH_2\cdot$) has been used recently to elucidate the structures of 1,3-diisovaleroylpentadecanoin (*10*) and 1,3-diisovaleroyltetradecanoin (*11*). These compounds were found in the pilot whale and porpoise, respectively, where they may serve as an acoustic lens in echolocation. In both cases, their spectra were compared with synthetic 1,2-triglycerides only [1,2-dipentanoyltetradecanoin (*10*) and 1,2-diisovaleroyltetradecanoin (*11*)]; our results, based on the spectra of both synthetic isomers entirely corroborate their conclusions.

The triglycerides represented by peaks 1, 3, and 4 contain three different acids, and the spectra are more complex. However, all have ions from $M - C_{15}H_{31}COOCH_2\cdot$ (*m/z* 173, 229, and 269, respectively) and are thus 1,2-diacylpalmitins. Furthermore, in all cases at least one important ion from the alternative 1,3-diacylpalmitin structure is missing or very weak: peak 1, *m/z* 341 ($M - C_3H_7COOCH_2\cdot$); peak 3, *m/z* 369 ($M - C_5H_{11}COOCH_2\cdot$); peak 4, *m/z* 369 ($M - C_3H_7COOCH_2\cdot$).

The above absences combined with ions in peak 1 at *m/z* 369 ($M - CH_3COOCH_2\cdot$), peak 3 at *m/z* 397 ($M - CH_3COOCH_2\cdot$), and peak 4 at *m/z* 397 ($M - C_3H_7COOCH_2\cdot$) allow the tentative formulation of the compounds as 1-acetyl-2-butyroylpalmitin, 1-acetyl-2-hexanoylpalmitin, and 1-butyroyl-2-hexanoylpalmitin, respectively. The compounds all appear to have their longest chain in the 3 rather than the 2 position in contrast to the above examples of naturally occurring mixed short- and long-chain triglycerides.

An infrared spectrum of the Dufour's gland constituents shows no hydroxyl absorption and only one intense band in the carbonyl region at 1735 cm^{-1}, typical of glycerides. Its spectrum, is, in fact, nearly superimposable on that of 1,2-dibutyroylpalmitin.

To further confirm that the Dufour's gland contents consisted almost exclusively of these substances, a small amount was placed on the direct insertion probe of the spectrometer. A transient mass spectrum of palmitic acid was observed; then the bulk of the sample was evaporated at ~100°C, showing superimposed spectra of the above constituents. On heating to ~200°C small amounts of long-chain triglycerides were observed.

By contrast, a sample of the cell wall, extracted from adhering earth with ether, consisted of a mixture of diglycerides, mostly dipalmitin, with some palmitostearin and a little distearin, when inspected by direct insertion probe ($M^{+\cdot} - CH_3$ ions at *m/z* 550, 578, and 606, respectively). Their presence was confirmed by gas chromatography–mass spectrometry after derivatization with bistrimethylsilyltrifluoroacetamide; $M^{+\cdot} - CH_3$ ions appeared at *m/z* 625, 653, and 681, respectively. This treatment also disclosed the presence of considerable quantities of free palmitic and stearic acid as well as a very small amount of the Dufour's gland constituents. Ether extracts of the provision materials gave approximately the same result.

Since the Dufour's gland appears to be the only source of the bulk of both materials, we suggest that the lipid is biosynthesized by the bee in the less viscous triglyceride form (both synthetic dibutyroylpalmitins are mobile oils at 23°C) to facilitate storage and transfer from the Dufour's gland. After secretion, the glycerides appear to be rapidly hydrolyzed and transesterified, perhaps by enzymes present in the bee's saliva or gut, as the contents are spread on the walls of the cells. Supporting this view is the fact that fresh Dufour's gland contents extracted by microcapillary lack the cheesy odor (butyric acid) characteristic of the cell lining.

The higher-melting diglycerides (dipalmitin is a solid at room temperature) resulting from this process appear to be better suited for cell wall construction, but their ultimate function is to serve as a source of easily digested food for the larvae (diglycerides are generally consid-

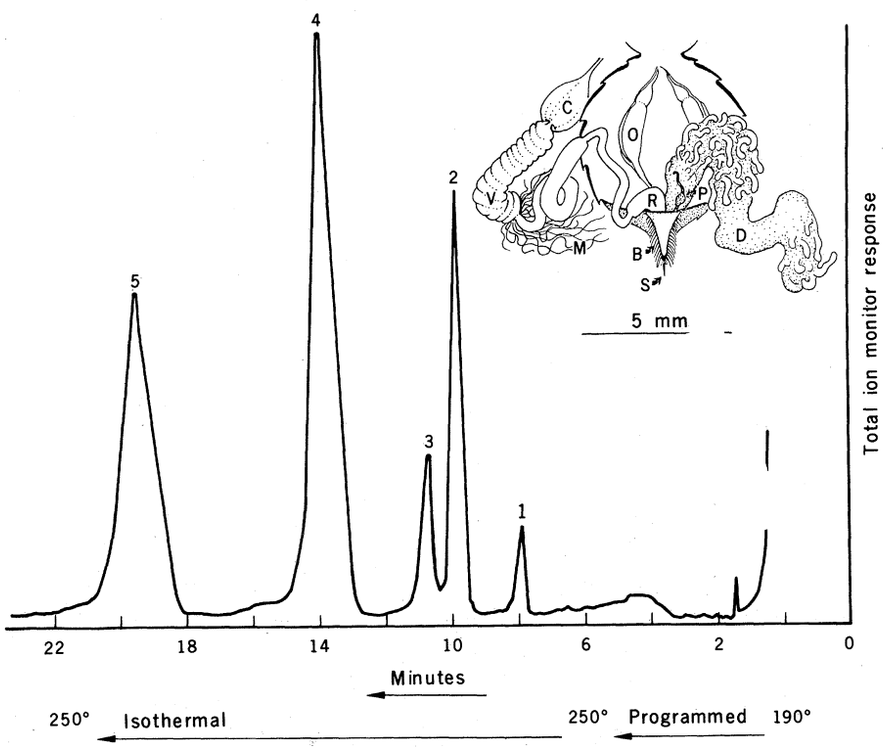

Fig. 2. Gas chromatogram of Dufour's gland constituents. See text for conditions. Inset shows abdomen of female revealing relative sizes of Dufour's gland (*D*), poison gland (*P*), ovaries (*O*), digestive system, including stomach or crop (*C*), ventriculus (*V*), Malpighian tubules (*M*), and rectum (*R*). The pygidial brush (*B*) that surrounds the bare pygidial plate is used with the legs for applying the Dufour's gland secretion that emerges from the sting (*S*) chamber.

ered more digestible than triglycerides). It seems likely that the odoriferous short-chain acids also serve some purpose, such as a feeding stimulant or to identify the locations of cells, but we have no evidence on this point. The microbial production of this odor in Anthophoridae has often been assumed (*1*), but we isolated no odorifactant yeasts or other such microorganisms from cells or provisions.

Analyses of the Dufour's gland contents of related species, *A. bomboides* Kirby and *Clisodon furcata terminalis* Cresson, reveal that they also contain triglycerides. In contrast, the cell linings of many other species of *Anthophora* and related genera are thin transparent films (*5, 6*) and probably not used as larval food. Most of the secretion may be deposited instead in the provision, which often has a cheesy or rancid odor similar to that of *A. abrupta*. The use of a glandular secretion as larval food indicates a high level of specialization in these solitary bees, comparable to the production of royal jelly by honey bees.

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Binocularity in the Cat Visual Cortex Is Reduced by Sectioning the Corpus Callosum

Abstract. *In the normal cat, most cells in area 17 can be binocularly driven. Sectioning the corpus callosum results in a significant reduction in binocularly driven cells. Normal binocular vision is thus dependent on the corpus callosum.*

Integrating information from the two eyes is essential for binocular single vision and for binocular depth perception. The neural substrate for this integration is assumed to be cells that receive binocular input. In the visual pathway from the retina, information from each eye remains segregated in separate laminae of the lateral geniculate nucleus, and binocular cells first occur in the visual cortex. It has always been assumed that convergence of the input from cells in two adjacent lateral geniculate laminae onto single cortical cells is the primary means of achieving binocular integration (*1*). We have found that the corpus callosum also plays an important role in this system of binocular integration.

The corpora callosa of 11 adult cats were sectioned (*2*). In one sham-operated control animal, the skull was opened and the right hemisphere retracted to expose the corpus callosum, but no lesion was made. Between 6 and 51 days after surgery, extracellular recordings were made from single neurons in area 17 of the left hemisphere (*3, 4*). Recordings were made from the medial bank of the lateral gyrus to ensure sampling across ocular dominance columns. Receptive fields were classified as simple (types I and II) or complex, and each of these types was further subclassified as hypercomplex if the unit did not respond to long lines (*5, 6*).

Ocular dominance was estimated according to the 1 to 7 scale of Hubel and Wiesel (*7*). The receptive field size and location of each cell was marked on a tangent screen, and its position relative to the optic disks and vertical meridian was measured. We used a fiber optics system to project the retinal landmarks onto the tangent screen (*8*). After the final recording sessions, each animal was killed, the brain sectioned, and completeness of the lesion confirmed. Fourteen cells were recorded from the sham-operated cat, 66 cells from normal cats, and 304 cells from cats in which the corpus callosum had been sectioned (CCX cats). Of the 304 cells recorded in the CCX cats, 29 were not responsive to visual stimulation and 10 were geniculate afferent fibers. These 39 units have not been included in the data analysis. The ocular dominance distribution of the remaining 265 cells recorded in CCX cats is shown in Fig. 1. Sixty-three percent of the cells responded to input from one eye only (ocular dominance groups 1 and 7), and 40 percent of all neurons were driven only by the contralateral eye. The distribution of cells driven by the left or right eye was not random but was clustered, suggesting a columnar organization (*9*). In the sham-operated cat, only 16 percent of the cells were monocularly driven; this proportion did not differ from that of the five normal cats, and the data were thus combined (Fig. 1). In this normal ocular dominance distribution, only 20 percent of the cells are monocularly driven. The ocular dominance distribution in cats with corpus callosum section is significantly different from normal [χ^2 (3) = 45.5, $P < .001$].

Figure 2 shows the ocular dominance distribution in CCX cats for simple type I and type II cells and for complex cells, with receptive fields more than 4° from the area centralis. We have excluded

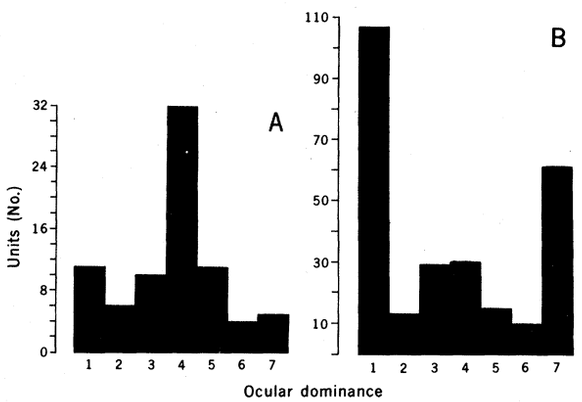

Fig. 1. Ocular dominance histogram compiled from single unit recordings in area 17 of five normal and one sham-operated cat (A) and 11 cats in which the posterior corpus callosum had been sectioned (B). In normal cats, 80 percent of cortical neurons can be binocularly activated (ocular dominance columns 2 to 6) compared with 37 percent in CCX cats.

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