Circular lumens in the tubules of the setal shaft, as well as in its ornamentations, are interpreted as tracks of dynamic microvilli[4,8,13].

If the apical surface of the chaetoblast secretes material near one margin at a greater rate than near the opposite one, then a curved seta might result. A helical seta might result if such a gradient of secretion rate were appropriately asymmetrical, or if it rotated about the setal axis. The diameter of the seta in figures, A and B is about 4 µm, while each turn of the helix is about 250 µm long and about 50 µm across. This geometry would be expected from a gradient only 4% greater on one side than on the other.

On the other hand, the arrangement of scales imposes a structural heterogeneity across the seta at any point. A physical change in the setal material after deposit on the chaetoblast template, such as shrinkage, would perhaps be reflected in warping of the seta. Because the scales are asymmetrical and arranged in a series which itself takes a spiral course about the setal axis, such a warp would be expected to assume a helical form. We have no direct evidence that such a physical change occurs. The hypothesis might be tested by comparison of the arrangements of spines on the straight, or only slightly helical, long, thin setae of other maldanids.

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Cell surface proteolytic activity of suspended embryonic cells isolated with and without proteolytic enzyme

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Summary. Although trypsin-disaggregated embryonic chick neural retina cells are incapable while EDTA-disaggregated cells are capable of immediate aggregation in culture, cells from both populations exhibit equally negligible levels of cell surface proteolytic activity as measured by substrate assay. The trypsin-induced lag does not appear, therefore, to depend upon adsorbed enzyme.

Embryonic chick cells isolated by proteolytic treatment are not immediately adhesive[1,2]. Suspensions of cells in rotation culture are capable of aggregation only after a variable period of time, depending upon cell type and enzyme preparation. On the other hand, cells isolated without enzymatic treatment (e.g. with EDTA) aggregate immediately without a lag. One possible explanation for the aggregation lag is that active enzyme is adsorbed to cell surfaces and interferes with subsequent cell-cell interactions. Trypsin can be adsorbed to various substrates[3] and cells treated with trypsin are reported to digest extracellular protein[4]. However, it has been demonstrated that although trypsin activity is eliminated by either soybean trypsin inhibitor or serum, neither treatment abolishes the aggregation lag of enzymatically isolated cells[1].

We wished to test directly for the presence of proteolytic activity on the surfaces of isolated cells. EDTA-isolated cells, capable of immediate aggregation, and trypsin-isolated cells, requiring time to become aggregation competent, were assayed for surface proteolytic activity. Neural retina cells were isolated from 7 day White Leghorn chick embryos by standard procedures used in our laboratory[5], using either 0.1% crude trypsin (1:250 Difco) or 1.0% EDTA, each prepared in calcium and magnesium-free Hanks' balanced salt solution. Isolated cells were washed in 10% horse serum and in Hanks' solution.

Azocoll (Calbiochem), an insoluble, powdered cowhide containing assorted peptide linkages, to which a red dye is attached, served as substrate. 25 mg Azocoll was suspended in 5 ml phosphate buffered saline (pH 7.0) in test tubes containing 1×10^6 cells per ml. The mixture was incubated for 15 min at 37 °C in either a stationary water bath or on a test tube rotator at 60 rpm. After incubation the tubes were centrifuged and the supernatant, free of cells and Azocoll particles, was examined spectrophotometrically at 520 nm for the presence of released dye. Azocoll plus 0.1% crude trypsin, with and without soybean trypsin inhibitor (Sigma), served as controls.

The table gives the results. Crude trypsin alone, as expected, easily digested the Azocoll substrate as indicated by the release of red dye. The supernatant was deep red and absorbance at 520 nm was high. Soybean trypsin inhibitor reduced proteolytic activity in the assay system.

Cells isolated by either crude trypsin or by EDTA showed

Optical density readings at 520 nm for Azocoll assay

Constituents added to Azocoll	Stationary water bath, 37 °C	Test tube rotator 37 °C, 60 rpm
0.1% Crude trypsin	2+	2+
0.1% Crude trypsin and 0.05% soybean trypsin inhibitor	1.4	1.8
0.1% Crude trypsin and 0.2% soybean trypsin inhibitor	-	0.48
Cells isolated with 0.1% crude trypsin	0.01	0.02
Cells isolated with 1.0% EDTA	0.01	0.01

no significant differences in the amounts of residual proteolytic activity on their surfaces as indicated by the Azocoll assay. It appears that the aggregation lag of enzyme-dissociated cells (i.e. return of adhesiveness) is not due to a necessary inactivation of adsorbed enzyme; EDTA-treated cells aggregate immediately even though they exhibit the same negligible surface proteolytic activity as the trypsin-treated cells. This interpretation conforms with earlier reports[1] and with our own unpublished observations that the addition of soybean trypsin inhibitor to trypsin-dissociated cells does not abolish the aggregation lag.

Enzymatic disaggregation procedures are known to produce cellular injury[6,7] and it seems likely, therefore, that the aggregation lag represents a 'recovery' period during which cells disrupted by enzymatic action regenerate or repair certain surface components.

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Olfactory-induced muscle potentials in *Dendroctonus frontalis:* Effects of trans-verbenol and verbenone[1]

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Summary. Potentials from antennal muscle movement induced by stimulation with the pheromone frontalin were recorded simultaneously with the electroantennogram (EAG) from *Dendroctonus frontalis.* The beetle-produced pheromones verbenone and trans-verbenol were found to decrease the muscle potential activity elicited by frontalin.

In recent years numerous insect pheromones have been referred to as attractants because they elicit positive orientation behavior from insects in laboratory bioassays and field tests[4-6]. More recently, additional chemicals have been indentified which, although unattractive when presented alone, modify the response of insects to attractants. These chemicals have been classified primarily into 2 groups: those which increase and those which decrease the response of insects to the attractants. However, until recently neither the behavioral[7] nor neurophysiological[8] roles of any of these compounds were known.

Frontalin (1,5-dimethyl-6,8-dioxabicyclo [3.2.1] octane) was identified as an attractant for *D.frontalis* and found to be moderately attractive in field tests[5]. However, the host tree terpene α-pinene and the pheromone trans-verbenol[9] were found to increase trap catches substantially when combined with frontalin[10,11]. When the pheromone verbenone[9] was added to attractant mixtures the response of beetles was significantly reduced[10].

Payne[12] recorded muscle potentials from electroantennogram (EAG)[13] preparations of *D.frontalis* stimulated by the bicyclic ketals, frontalin and exo-brevicomin (exo-7-ethyl-5-methyl-6,8-dioxabicyclo [3.2.1] octane[4]) and the host tree terpenes α-pinene and 3-carene. Muscle potential activity was considerably greater in response to pheromone stimulation than to stimulation by the terpenes. These muscle potentials were thought to originate from the antennal muscles which are presumably involved in antennal raising and orientation.

The purpose of this investigation was to determine the effects of verbenone and trans-verbenol on these muscle potentials.

Materials and methods. Adult beetles newly-emerged from brood material collected in East Texas were used. EAG's were recorded using Ag-AgCl capillary electrodes. The recording electrode was implanted in the antennal club. The indifferent electrode was placed either in the antennal scape or head capsule[14]. Muscle potentials were recorded in a similar manner with the preamplifier in the AC recording mode. In some cases a high pass filter was also used. Recordings were also made from single sensilla basiconica within the sensory bands[15,16], by implanting tungsten electrodes in the sensillum base. Muscle potentials were induced by stimulating the antenna with frontalin or frontalin + α-pinene, and the effects of verbenone and trans-verbenol on these potentials were studied. 9 male beetles and 7 female beetles were used.

Stimulus compounds were delivered as 5-µl samples placed on filter paper and inserted into glass cartridges. The muscle potential stimulus (e.g., frontalin) was placed in 1 cartridge; trans-verbenol or verbenone in the other. Presentation of stimuli was facilitated by 2,3-way solenoid operated air valves[14].

Results. Figure 1 shows the relationship between the EAG and the muscle potentials during frontalin and α-pinene stimulation. Stimulation with verbenone and trans-verbenol immediately (i.e., with msec) following muscle potential induction resulted in reduction of the potentials in both male and female beetles (figure 2).

The delay between the beginning of sensory activity and the onset of muscle potential activity was determined by recording the EAG and the muscle potentials simultaneously but on separate channels (figure 3). The response on 1 channel was recorded in the DC mode with a low-pass

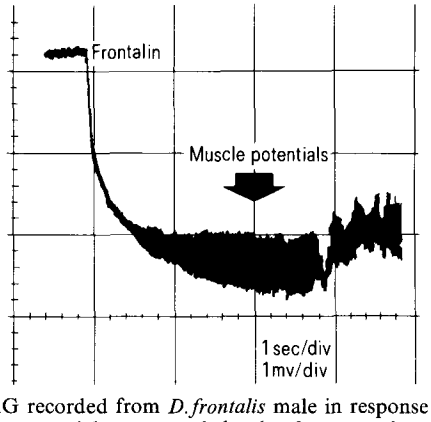

Fig. 1. EAG recorded from *D.frontalis* male in response to frontalin. Muscle potentials are recorded only after some degree of EAG depolarization.