Fine Structure of the Tip of Chemosensitive Hairs in Two Blow Flies and the Stable Fly

Although the taste hairs of flies have been studied intensely, the location of the chemosensitive membrane is still unknown. The site of this transducer could be at the membrane of the dendritic nerve fibers that extend to the chemosensitive tip of the hair[1] or at the surface of an adventitious structure. An investigation of the ultrastructure of the tip of the hair should be of assistance in determining the site of chemoreception.

The first electron micrographs of taste hairs were made without sectioning with the black blow fly, *Phormia regina* (MEIGEN)[2]. ADAMS, HOLBERT and FORGASH[3-6] succeeded in sectioning the tip of taste hairs of the stable fly, *Stomoxys calcitrans* (L.) and showed that the dendrites pass within a channel to the tip where this 'inner canal' ('Binnenkanal'[7]) is connected through a pore with the outside.

In one hair[4] a substance was found which apparently had been extruded through the pore. This substance, observed with the electron microscope, seems to be homologous with a viscous material seen with the light microscope extruding from taste hairs of the blow fly, *Calliphora vicina* ROBINEAU-DESVOIDY[8]; however, this material probably had not been expelled from the inner canal since the inner canal is filled mainly with dendritic nerve fibers and its total volume is considerably less than the amount of viscous material observed. In both flies, the viscous substance could have originated from the dendrite-free lumen of the hair – here referred to as the dendrite-free channel – if this channel opened at the tip of the hair. The capacity of the dendrite-free channel is greater than that of the inner canal, judged by cross sections at different heights[4,9,10].

The electron and light microscopic studies briefly reported here were made with the labellar taste hairs of *P. regina* and *C. vicina* as an aid in determining the origin and path of flow of the viscous material. The labellar taste hairs of these flies showed no ultrastructural differences and were therefore considered to be identical. Labellar and tarsal taste hairs of *S. calcitrans* were studied for comparison.

Tarsal taste hairs showed no differences from labellar taste hairs within each species when studied with the light microscope in *P. regina* and *S. calcitrans*. Thus, the tarsal and labellar taste hairs of *C. vicina* probably also have the same structure.

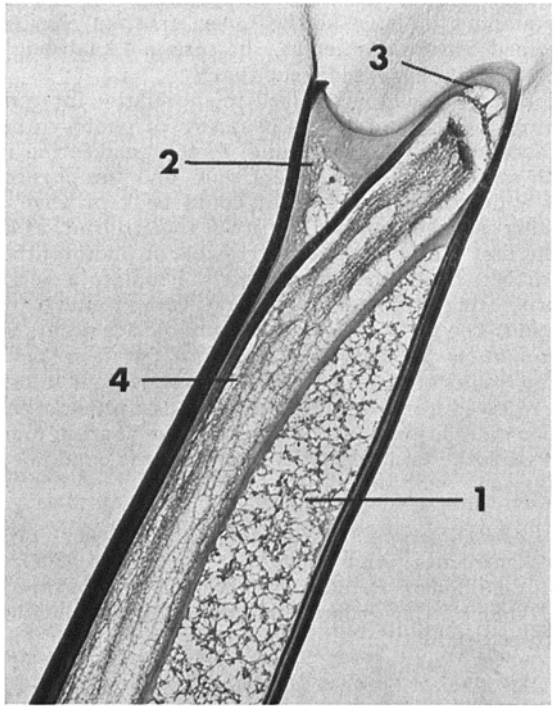

Fig. 1. Longitudinal section through the tip of a labellar taste hair of *Phormia regina*. The dendrite-free channel (1) probably extends into the spongy cuticle within a tooth (2) and possibly also into the complex porous structure (3) distad from the inner canal which contains the dendrites (4). This section was made in close proximity to the opening found in other hairs distad to the dendritic endings. The cuticle is partly rolled. Glutaraldehyde, post-fixed in OsO_4 in MILLONIG's phosphate buffer, methacrylate mixture with 1% benzoyl peroxide and 0.01% uranyl nitrate, × 22,000.

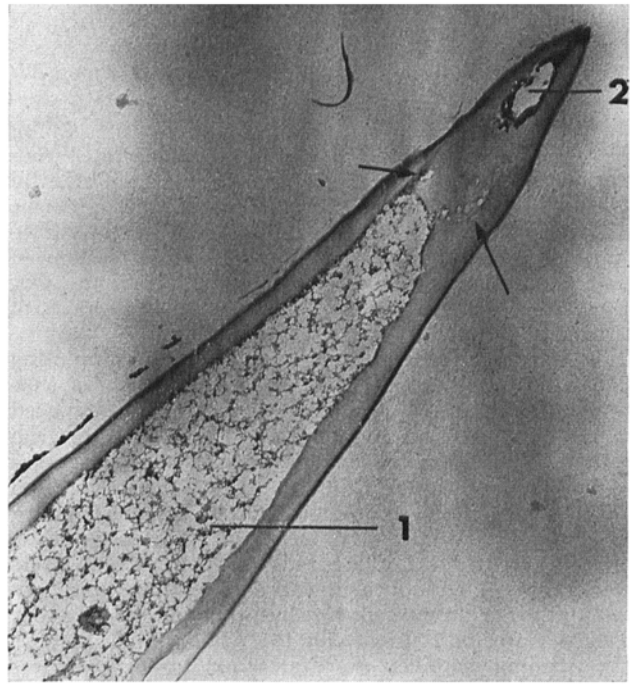

Fig. 2. Longitudinal section through the tip of a labellar taste hair of *Calliphora vicina*. The dendrite-free channel (1) ends with 1 or 2 processes extending into areas of spongy cuticle (arrows). The pore at the tip and the oval opening (2) are probably connected with the inner canal which contains the dendrites (not evident in this slightly oblique section). Fixed in s-collidine buffered OsO_4, methacrylate, × 26,000.

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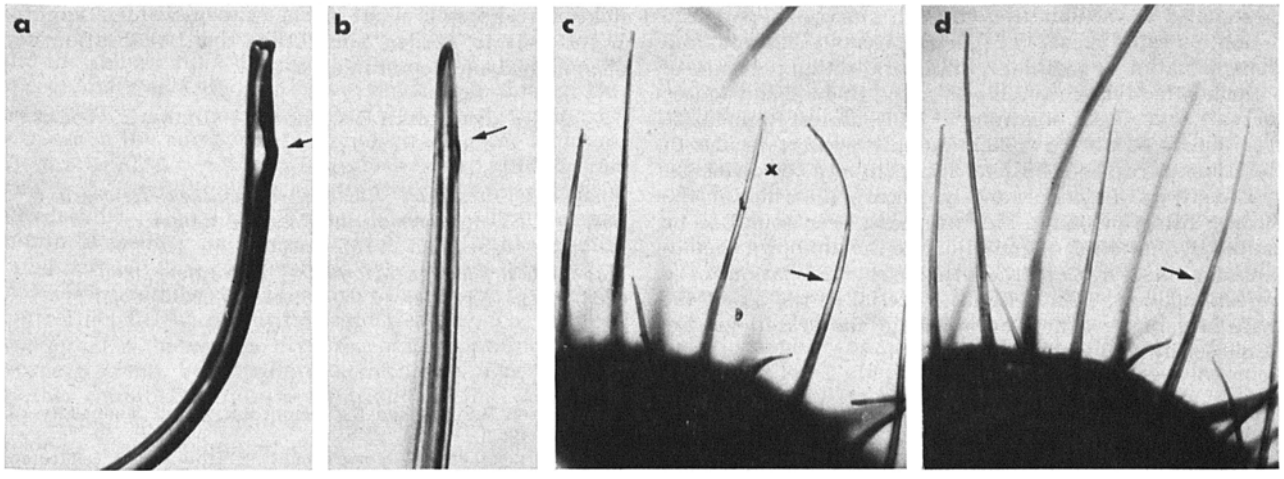

Fig. 3. The tip of the same hair of *Phormia regina* in an open and a closed condition (freshly dissected labellum). (a) Hair fully open in air with viscous substance flowing down the shaft (arrow, accumulation of viscous material); (b) hair closed in 95% alcohol which apparently dissolved the substance except for a small residue (arrow); the relatively wider diameter of the hair may have been caused by different planes of focus or other artifact; (c) hair open in situ (arrow); the tip moved and was bent to point X when picture (a) was taken; (d) hair closed in situ (arrow); the application of alcohol straightened the shaft and closed the tip within a few seconds; (a) and (b) × 1,300 (microscopic magnification × 400), (c) and (d) × 270.

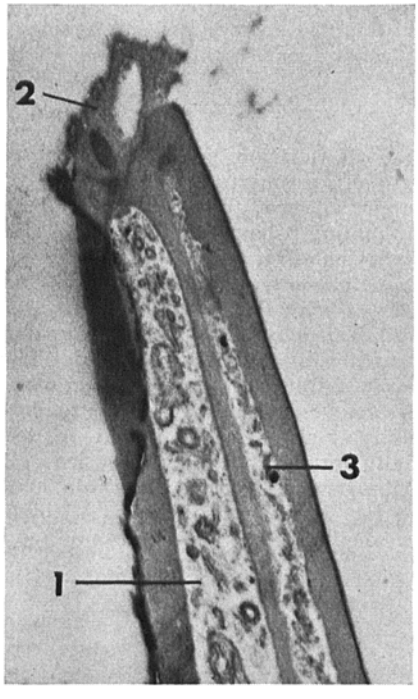

Fig. 4. Longitudinal section through a tarsal taste hair of *Stomoxys calcitrans*. The proximal part of this section (not shown) indicates that canal (1) contains the dendrites which were not fixed well distally. This inner canal ends beneath a material (2) which was apparently extruded and has a surface marked by granulated irregularities; the dendrite-free channel (3) extends into the tip of the hair. Fixed in OsO_4 fumes, methacrylate, × 25,000.

Longitudinal sections through the tip of labellar taste hairs of the blow flies were difficult to make since all hairs were more or less curved. Another difficulty was to obtain a satisfactory fixation.

The electron micrographs of the blow flies appeared to show 2 types of hairs. Of 29 hairs sectioned longitudinally, 17 had a wide blunt tip, and 12 seemed to be pointed. Figures 1 and 2 are examples of these types. Studies with the light microscope, however, revealed that the 2 types represented an open and a closed condition of a single type since the tip of the same hair was observed first in an open and then in a closed condition (Figure 3, a–d). This observation was made with 4 hairs. The photographs of Figure 3 were taken from about the same angle, judged by the position of the accumulated viscous material in Figure 3 (a) compared with the position of the residue in Figure 3 (b) (arrows). Stages of transition between open and closed hairs have also been observed. At higher light microscopic magnification (× 1200), fully open hairs show a funnel-like, three-edged opening.

The mechanism of opening and closing is not yet known in detail. Stimuli such as aqueous solutions of a wide osmotic range and moist air cause the tip to open. Dryness, most of the fixatives, and touch at the very tip will close the hair.

Although labellar and tarsal taste hairs of *S. calcitrans* also exude a viscous substance, they do not show the marked opening and closing when observed with the light microscope. In this fly, the substance is extruded as a thin elastic thread that coils loosely at some distance from the tip when it is exuded in large amounts. In the labellar taste hairs of the blow flies, the substance is extruded as a relatively thick elastic thread or as a droplet; if it is exuded in large quantities, both the thick thread and droplet form a compact ball around the tip of the hair or flow down the shaft.

In the 2 blow flies and in the stable fly, the dendrite-free channel extends into the tip of the hair (Figure 1 and 4) and could be connected with the outer surface by a pore (or pores) which apparently adjoins the pore distad to the end of the inner canal, Figure 1 (3). The isolation of the dendrites from the dendrite-free channel throughout their course to the tip and the proximity of the dendrite-free channel to the dendrites at the opening may indicate that the viscous substance is the chemosensitive material[11]. The reception of a stimulus by this material could

[11] See considerations published during printing, by C. J. C. Rees, Nature *215*, 301 (1967).

be signaled to the dendrites through a change of potential or another rapid event. The shortest latency measured from the application of a stimulus to the first action potential of a chemosensitive sense cell was found to be 1 and 5 msec for salt and sugar solutions[12]. This almost immediate reaction would not be understandable if the molecules of the stimulus reached the dendritic membrane by diffusion.

The effects of alcohols and hydrocarbon amines on the feeding behavior of the blow fly have been found to be caused by inhibition of and injury to the unknown chemosensitive membrane rather than by stimulation of a 'rejection fiber'[13]. The viscous material at the tip of the taste hair offers a new approach in the search for the chemosensitive membrane[14].

Zusammenfassung. Licht- und elektronenmikroskopische Studien der chemorezeptiven Spitze von Geschmackshaaren wurden bei den Fliegen *Phormia regina, Calliphora vicina* und *Stomoxys calcitrans* durchgeführt. Die Geschmackshaare dieser Fliegen ähneln sich strukturell bis auf einen ausgeprägten Öffnungs- und Schliessmechanismus, der für die Geschmackshaare von *Stomoxys calcitrans* lichtmikroskopisch nicht nachgewiesen werden konnte. Die Resultate werden hinsichtlich der Lokalisation der chemosensiblen Membran diskutiert.

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Effects of Isolation on Maternal Aggressiveness and Body Growth Rates of Offspring

As a consequence of the behavioral abnormalities and endocrine imbalances caused by isolation stress, the present study was designed to determine the effects of prior periods of prolonged isolation on maternal fertility and fecundity and maternal-fetal and offspring interrelationships. Various investigators have reported that isolation stress caused behavioral abnormalities such as head-shaking[1-4], heightened locomotor activity[4,5], nervousness and aggressiveness[1,3,6,7] in mice. Physiologically, evidence of hyperadrenocorticalism has been noted in isolated mice[4,7] and rats[8] accompanied by indications of thyroidal imbalances[4,7,8] and reductions in gonadal weights[4,7]. Recent reports[9] claim that isolation caused pituitary-gonadal stimulation and that isolation-induced aggressiveness is dependent on an intact pituitary-gonadal axis with the adrenals exerting a modulating influence. Although other hormonal factors and balances, in addition to sex steroids, are involved in oogenesis as well as fertility and fecundity relationships, if pituitary-gonadal function is stimulated by isolation one might reasonably anticipate, in addition to organ and secondary sex characteristic changes, higher levels of fertility and fecundity in the isolated females.

A total of 60 albino females averaging 19 g were divided into test and control groups. All test or isolated mice were housed singly in stainless steel cages (6.5 · 10 · 7 inches) as opposed to control mice which were maintained in groups of 2/cage. The laboratory recognizes the sensitivity of animal growth and development to such environmental and physical stimuli as temperature[10], noise[11] and handling[12]. Parameters measured were changes in litter size, pup mortality and the developmental growth rates of the young. Body weights and neck-twitch responses were recorded weekly; locomotor activity[13] and aggressiveness at various intervals during the $6\frac{1}{2}$ month isolation period. After observation of consistent significant increases in locomotor activity and aggressiveness, all test and control females were subjected to mating-behavior interaction studies with proven albino males on 4 consecutive days for $\frac{1}{2}$ h intervals. Each female was then mated individually for 6 days. All males were then removed, control females regrouped into the original pairs and then separated shortly before parturition. By this procedure, each test and control female could thus raise and nurse her own litter, and isolated females were only in contact with another animal for the relatively short mating period. All females were checked daily for date of birth and size of litters. En masse weighings and counts were made weekly at the end of the 1st, 2nd and 3rd weeks. All offspring were weighed, weaned and sexed at 4 weeks of age.

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