Zusammenfassung. 8 Stunden nach Injektion von 25-Hydroxycholecalciferol bei rachitischen Ratten steigerte sich der Kalzium-Transport des Duodenum in vivo wie auch in vitro bedeutend. Während dieser Zeit war Vitamin D_3 wirkungslos. 24 h nach der Injektion stimulierten beide Verbindungen in ähnlichem Masse den Kalzium-Transport.

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Mechanoreceptors on the Antenna of the Tobacco Hornworm Moth (*Manduca sexta*)

Although insect mechanoreceptors appear in many diverse forms, the simplest appears to be the sensilla trichoidea. These structures are innervated by a single bipolar neuron with the cell body just under the cuticle. The dendritic portion of this neuronal arrangement generally terminates within the lumen near the base of the sensory hair. In some instances, there may be three neurons terminating in a single hair, one of which dendritically terminates into a disc and is mechanoreceptive, while the other two have a chemosensory function[1].

Numerous functions have been suggested for the sensilla trichoidea, some of which include 1 proprioceptors in the cockroach leg[2]; 2. sound reception in locusts[3]; 3. contact chemoreception in flies[4], and 4. receptors for humidity changes in the tsetse fly[5].

Preliminary studies have indicated a lack of chemoreceptor function for the trichoid sensilla on antennae of the male tobacco hornworm moth, *Manduca sexta*. In view of this information, these sensilla were then investigated for their mechanoreceptive qualities. Only male moths were used for this study because female antennae are relatively void of any large sensory setae.

Method. Isolated head preparations of male tobacco hornworm moths were securely fastened to a paraffin mounting block by means of 2 dissecting needles. The parietal area of the head cuticle was removed and pharyngeal pump muscles cut away. All antennal musculature was severed, leaving only the antennal nerve as the primary connection.

Afferent responses were recorded by means of a hook stainless steel electrode in contact with the antennal nerve. The indifferent electrode was placed in the contralateral eye, while the ground was placed in the head musculature. The antennal nerve between the recording electrode and the deutocerebrum was severed to eliminate any possible efferent activity.

The antenna of the male tobacco hornworm moth is approximately 'key hole' in cross section with 2 rows of hair sensilla on 2 of the 3 sides. A V-shaped fork was formed from fine wire and sized so as to engage these hairs as the fork was moved along the antenna. The tip of the antenna was waxed to a wire loop holder, stationed in the wax mounting block. The antenna was thus maintained in an extended position, permitting the wire form to displace the hairs in selected areas along its length. The fork was attached to a 4 inch high compliance loudspeaker by means of a plastic cross piece cemented to the speaker cone. The apparatus was mounted on a mechanical stage to permit convenient positioning with respect to the moth antenna.

Stimulus was administered by feeding a square wave signal from a Grass S_4 stimulator to the speaker. By adjusting the voltage and time base of the signal, a stimulus having the desired magnitude and duration could be programmed. The signal to the speaker was paralleled to the lower trace of the oscilloscope to provide a stimulus artifact.

The hair sensilla on one antennal segment were displaced distally followed by an equal proximal displacement. The velocity of displacement was 5.4 mm/sec.

The oscilloscope was operated at a sweep speed of 0.2 sec/cm and sensitivity of 1 mV/cm. In some cases, response traces were superimposed to study form and consistency of interspike interval. A 3 min time interval was maintained between successive stimuli in this study.

Antennal segments for histological examination were fixed in situ in cold (4°C), 4% glutaraldehyde solution buffered to a pH 7.4 with s-collidine buffer[6,7]. The tissue was then excised and placed in a beaker containing the cold buffered fixative for 2 to 3 h. After fixation, the material was transferred to s-collidine buffer at 4°C for a period fo 12 h. Dehydration was carried out through solutions of methyl cellusolve, methanol, ethanol, propanol and embedded in 2-hydroxymethylmethacrylate. Sections were cut at 2 µm with a steel knife, mounted and stained with acid fuchsin and methylene blue.

Results (structural). The difference in external morphologies between antennae of male and female hornworm moths is striking (Figure 1). The male antenna has the shape of a key 'hole' in cross section and is aligned with 2 distinct rows of sensilla trichoidea on 2 of the 3 sides. These sensilla form an arc as they meet at their distal extremities. Between these large sensory hairs, additional smaller sensilla (s. chaetica, s. basiconica) are found. The female antenna is generally shorter and circular in cross section. It does not possess any of the larger s. trichoidea noted in the male, but only setae closely appressed to the antennal surface.

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The trichoid sensilla are innervated by at least 1 bipolar neuron, in which the dendrite tip is capped by a scolopale cell (Figure 2). The length of the scolopale is approximately 5 μm with a maximum width at the midpoint of approximately 1.5 μm. Penetrating through the scolopale tip are 2 dendritic extensions which terminate on the inner wall of the sensillum. 3 pairs of dilations appear along the modified dendritic terminal which may be similar to the ciliary dilations in the tympanic scolopidium of the locust[8]. Similar features were recognized in the dendrites innervating tactile hairs in certain lepidoptera caterpillars (*Pieris rapae*)[9]. The distal 2 pair of thickenings were referred to as the 'epaississement sur les cretes parietales' and the proximal pair as 'points sensoriels'[9].

The axonal protion of this cell extends proximally and aggregates with axons from other neurons which together form the 2 main antennal nerves.

Functional study. The length of antennal segment in the zone of stimulation was approximately 0.25 mm and contained 120 tactile hairs. While most of these hairs were stimulated with the V-shaped fork, the degree of transverse displacement for each hair varied. This information should be considered when examining the traces in Figures 3 and 4.

The response in Figure 3 is to a distal and proximal hair displacement of 1 antennal segment. Figure 4 shows 3 superimposed traces in response to the same deflecting distance noted in Figure 3. Although the response (Figure 4) did not completely coincide, the correlation of interspike interval shown by 3 superimposed traces is somewhat consistent. The first 2 groups of spike potentials indicate a rather high degree of synchronization while the remaining spikes vary somewhat. Similar findings of coincidentral potentials to consecutive stimuli were observed when the antennal pedicel of the blowfly, *Calliphora*, was mechanically disturbed[10].

The monophasic potentials were positive, ranging in amplitude from 0.3 to 1.0 mV, and usually persisted somewhat after cessation of the stimulus. At the onset of stimulus, there was a slight difference in the number of spikes over the first 70 msec. These spikes showed rapid adaptation which developed after 100 msec. The variation noted in spike amplitude is perhaps due to a number of units simultaneously firing. The latency of spike discharge was 0.2 msec which corresponds to latency figures in other mechanoreceptors[11–13]. A slight 'off' effect was noted in Figure 3 and in one of the traces in Figure 4. This portion of the response was not consistently noted throughout the study.

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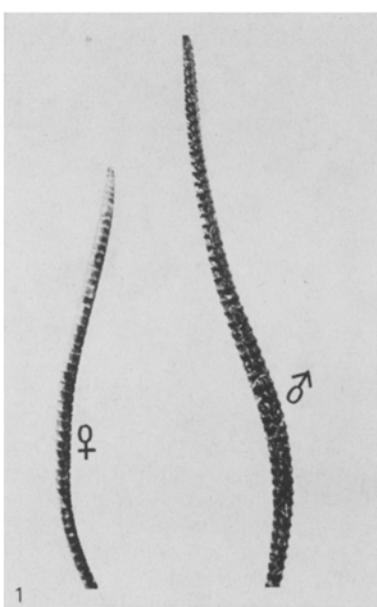

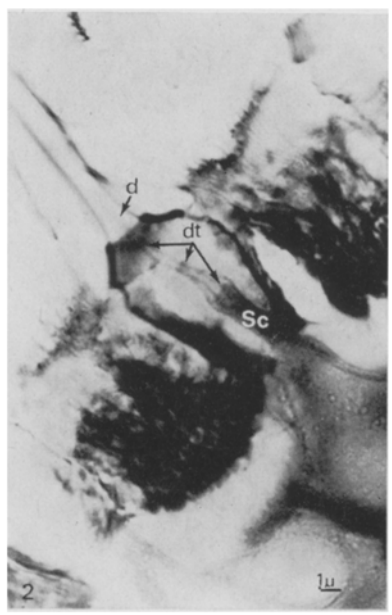

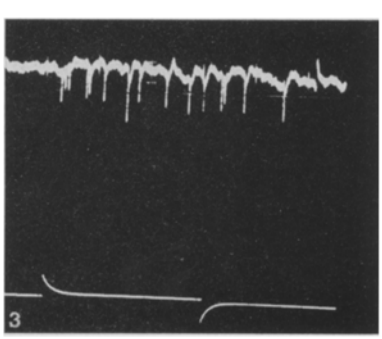

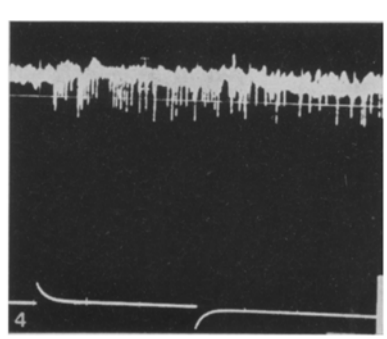

Fig. 1. Photograph illustrating the different morphology existing between antennae of male and female tobacco hornworm moths. ×5.

Fig. 2. Longitudinal section illustrating the innervation of the sensilla trichoidae. Note the modified dendrite (d), and its modifications (dt) as it leaves the scolopale (Sc) to attach to the inner wall of the sensilla. ×4500.

Fig. 3. Deflection response of male antennal hairs (s. trichoidea) to transverse displacement.

Fig. 4. Three superimposed responses to the same transverse displacement as in Figure 3.

Discussion. The mechanoreceptive function of the trichoid sensilla on the male tobacco hornworm moth is suggested by: 1. innervation by at least one bipolar neuron, 2. dendritic distal processes (terminal filament) attached to the hair inner wall, 3. nerve response to transverse displacement of tactile hairs and 4. a latency of 0.2 msec which is similar to latency figures found in earlier mechanoreceptive studies[11–13].

A certain degree of hair displacement is apparently required to obtain a measurable response. Micro-displacement of the hairs by sonic energy at various frequencies in the Hertz and kilo-Hertz range (intensity level of 80 decibles), failed to elicit a response when recording from the antennal nerves or cervical connectives.

The continuing adaptation shown in response to the distal and proximal movements of the stimulating fork does not show a corresponding spike rhythm when the direction of stimulation is reversed (i.e. proximal movement). This might be explained by the hairs not returning to their initial resting position in time for a displacement from the reverse direction. Such unelicited movements might disrupt any similarity in spike rhythm to the initial direction of displacement.

Based on the theory proposed for function of hair cells in the lateral line canal organ, directional sensitivity is apparently lacking for the trichoid sensilla in *Manduca*[14]. Theoretically, a displacement of stereocilia in one direction caused a depolarization, followed by a hyperpolarization when the cilia were stimulated in the reverse direction. The data from this study indicate only a depolarizing response regardless of stimulus direction which continually adapts after a period of 100 msec. This lack of directional sensitivity thus may be explained by the radial symmetry which exists in the cuticular hair joint[15].

The nerve impulses in this study were recorded extracellularly from the antennal nerve as positive going responses. A similar observation was noted while recording from the leg nerve of the blowfly. Another mechanoreceptor study also showed positive going spikes superimposed on a negative going receptor potential, while recording from the cut chemosensory hair on the labellum of *Phormia*[16]. This phenomenon was explained by the pressure from the fluid filled electrode against the membrane which separates the distal process of the receptor from the lumen of the hair. Such a recording situation is apparently responsible for the positive going spikes. Since the method of recording was different in this study, the above explanation does not seem appropriate. Intracellular recordings will have to be accomplished in *Mandua* to determine if the criteria for positive spikes are met[17].

From the obvious mechanoreceptive qualities of these antennal hairs, it is conceivable that these sensilla may serve as air speed indicators. The possibility of a reflex initiated at these antennal hairs (by air speed velocities) with subsequent efferent activities in a descending pathway should be investigated[18].

Résumé. Des études structurales fonctionelles suggèrent une fonction méchanoréceptive pour le S. trichoidea sur l'antenne de la phalène *Manduca sexta*. Les soies de ses antennes sont innervées par un seul neurone bipolaire, dans lequel la dentrite est couronnée par une cellule scolopale. Les pointes positives dont l'amplitude varient ont été enregistrées sur le nerf antennal avec déplacement transversal des soies antennales.

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Effects of CMH on Growth and Alkaloid Formation of *Datura metel* L.

More recently, some hydrazonium salts are the novel group of the growth regulators, such as N,N-Dimethyl(2-chloroethyl)-hydrazonium chloride (CMH), N,N-Dimethyl-(2-bromoethyl)-hydrazonium bromide (BMH), N,N-Dimethyl-isopropyl-hydrazonium bromide (IMH), and N,N-Dimethyl-allyl-hydrazonium chloride (AMH). These derivatives of hydrazonium could cause a reduction in a number of plants in longitudinal growth[1,2]. On the other hand, using CMH under various conditions resulted in a stimulatory effect on stem length and dry matter product of wheat plants while the invertase and amylase activity showed the same tendency[3,4]. Moreover, minerals and pigment contents of wheat plants increased by using the CMH[5]. Whereas treating soyabean plants with CMH and CCC in doses of 50–400 ppm at two different growth stages did not affect the content of N-fractions and oil content in the seeds of treated plants; yet some treatments led to slight increases in total carbohydrates[6]. No information is recorded on the influence of CMH upon medicinal plants. The investigation presented here was made to discover how far the growth and tropane alkaloids synthesis in *Datura metel* L. have been influenced by CMH.

Uniform size seedlings of *Datura metel* L. (45 days old) were transplanted on 10th May 1971 individually into 7.5-inch pots using Nile-Silt of loamy soil, supplimented with 5 g ammonium sulphate, 2.5 g superphosphate and 1.5 g potassium sulphate. The different concentrations of CMH used (0, 1000, 2000 and 4000 ppm in water solution) were sprayed by a small pressure pump at a rate of 10 ml per plant. CMH used was 46% solution obtained from BASF, Limburger Hof, W. Germany, to whom the author is very indebted. The plants received two sprays at 3 and 5 weeks intervals after transplanting. 21 pots were used with 3 replicates for each concentration of CMH as well as con-

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