

ULTRASTRUCTURAL INVESTIGATIONS OF THE CAMPANIFORM SENSILLAE ON THE TRACHAEL GILL OF PALINGENIA LONGICAUDA OLIV. (EPHEMEROPTERA)

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The sensillae of *Invertebrata* that make possible to receive several kinds of stimuli show, however various their appearance is, a high degree of conformity in their structural formation. There are to be found in every sensilla one — and sometimes more — exteroceptive cells and various adjacent supporting or cover cells (trichogen, tormogen, neurilemma cells) (LEWIS, 1970; BLANEY et al., 1971; GNATZY et al., 1971; HAUPT, 1971; MORAN et al., 1971; SCHMIDT et al., 1971; ZACHARUK et al., 1971.) The latter cells are, as supposed by WIGGLESWORTH (1953) and LAWRENCE (1966), as well, the mutated forms of epidermal cells.

The sensillae — and first of all, the campaniform ones — have excited a wide range interest of late years. The aim of this paper is to describe the light and electron microscopic structure of the sensillae taking place on the tracheal gills of *Palingenia longicauda* Oliv., and to try, in the way of morphological investigations, to draw a conclusion concerning their function.

Materials and Methods

The nymphs of *Palingenia longicauda* OLIV. collected freshly from the Tisza and Maros regions in the environs of Szeged were fixed immediately after being collected. The fixation was carried out, in accordance with the aims of the investigations with Carnoy's mixture, ten per cent neutral formalin and osmium tetroxide buffered correspondingly. For surface investigations with light microscope, full tracheal gill plates fixed in 10 p. c. formalin were submitted to shadowing (angle of inclination of the preparation was 60°; silver shadowing; HBA—1 apparatus). After fixation a part of our light microscopic sections were stained or impregnated according to Mallory, respectively with haematein-cosin. After being fixed (Palade-fixative, 2 hours, +4 °C, pH 7.4), the part of the material that was intended for fine structure investigations was dehydrated and embedded in Araldit. Parallel with our thin sections, there were made semi-thin sections of 0.4—0.7 μ , suitable to light-microscope investigations. By means of these, the ultrastructural characteristics could always be exactly localized in different areas of the tracheal gills. Semi-thin sections were stained according to RÜDEBERG (1967) and thin sections was contrasted with REYNOLDS's (1963) lead citrate. The photographs were made with electron microscopes Tesla BS 242 D and JEM 100 B.

Results and discussion

In the course of our light and electron microscope investigations, we could observe trichoid sensillae spinelike from the tracheal gill of *Palingenia longicauda* OLIV., particularly on its dorsal side, and campaniform sensillae deep in the cuticle (Tables I—IV). There are known some literary data relating to sensillae that the place in other *Ephemeroptera* species. Thus in 1936, EASTHAM, having examined the structure of the gill plate of *Caenis macrura* STEPH. and *Caenis horaria* L., was mentioning the some two kinds of sensillae, displaying the site and frequency of their appearance, as well as giving data for recognizing their light microscopic structure.

The distribution of the above-mentioned sensillae in case of our experimental animal, *Palingenia longicauda* OLIV., is considerably differing from the conditions described at *Caenis* species. Trichoid sensillae have been observed by us only in low number in the peripheral area of the dorsal surface of the big gill plate, as well as in the proximal regions of filaments (Table I, Fig. 1). In this paper we do not want to deal with the structure and function of these.

In the course of the light microscope investigation of tracheal gills we have met several small fossulae deep in the cuticle. Studying totally stained or impregnated preparations (Table I; Table II, Figs. 1 and 2), the fossulae may be confounded at first sight with the epidermal and connective tissue cell nuclei lying under and round them, their determination being possible only owing to their difference in depth and their regular circular shape and rather standing diameter (about 6μ). A proof eliminating any doubt has been produced by our unstained preparation shadowed by silver (Table II, Fig. 1), visualizing with that method, due to its nature, only the surface unevenness. From

Table I

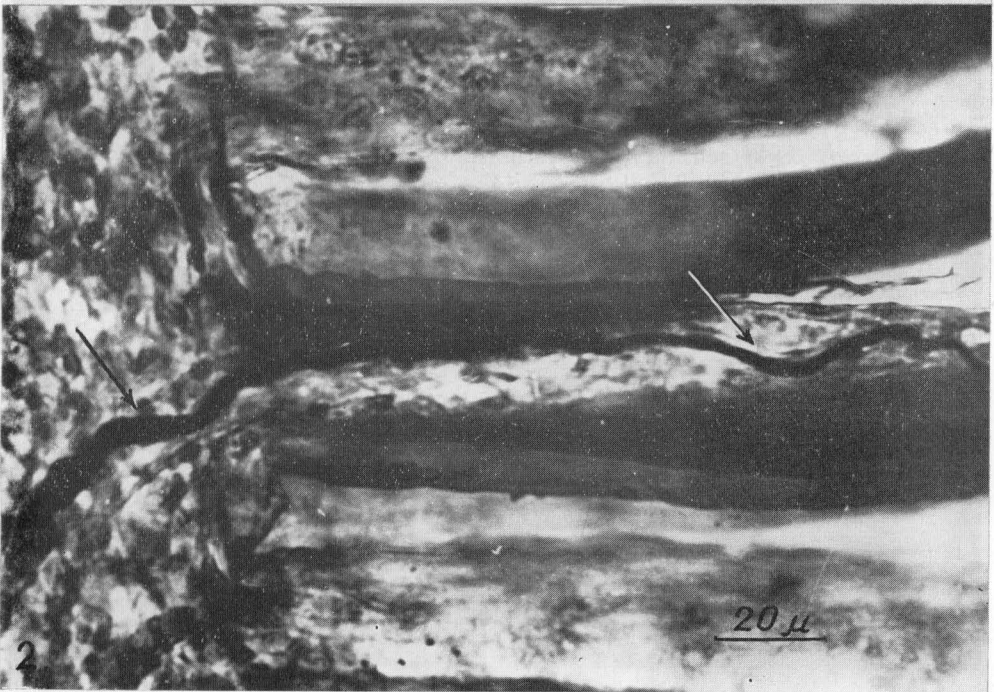
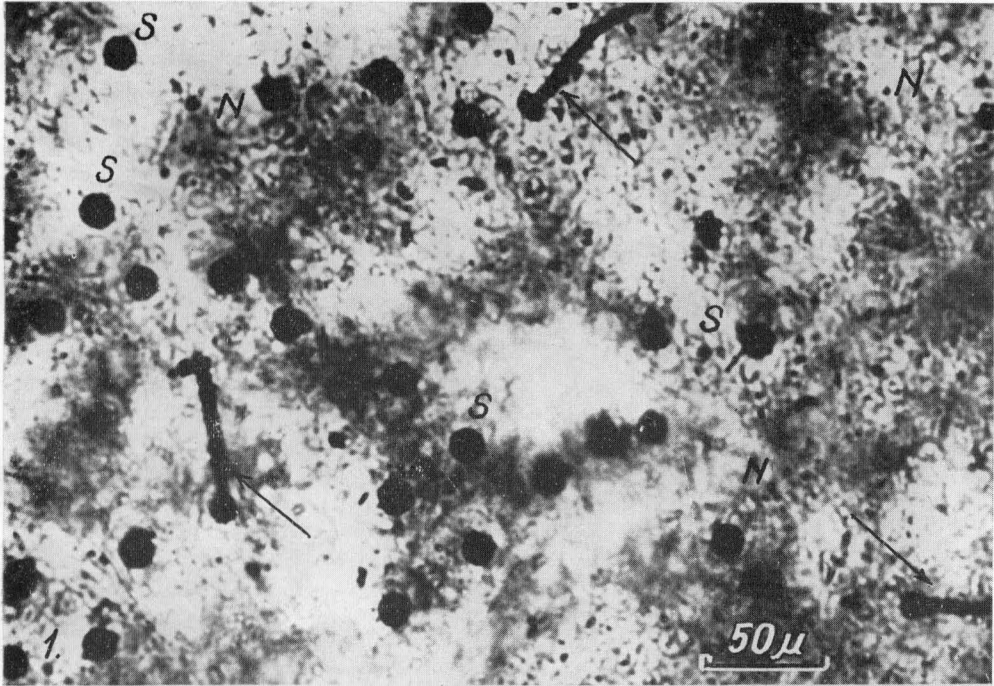
Fig. 1. Peripheral area of the plate of a tracheal gill. The arrows are pointing to the trichoid sensillae. The sensory area of the frequent enough campaniform sensillae is impregnated. The nuclei of deeper epithelial cells become visible. Totally impregnated preparation. $\times 325$

Fig. 2. Plate of a tracheal gill from the zone of beginning of the filaments. A thick nerve bundle (arrow) is penetrating into an filament. Totally impregnated preparation. $\times 720$

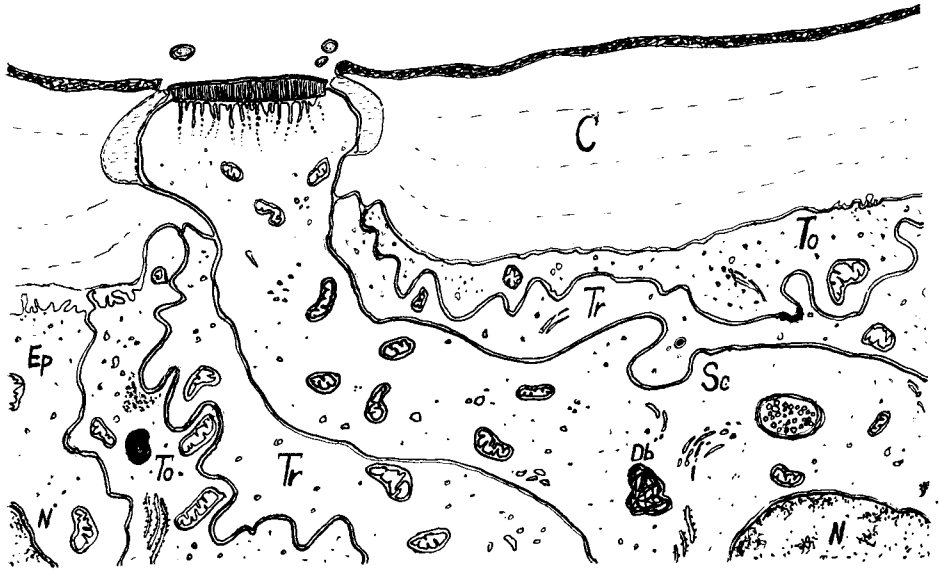
Abbreviations:

bm	— basal membrane	mv	— microvilli
C	— cuticle	N	— nucleus
ci	— cilia-like structures	S	— sensilla
d	— dendrite	Sc	— sensory cell
dcv	— dense-core vesicles	Sch	— Schwann cell
Ep	— epithelial cell	SER	— smooth-surfaced endoplasmic reticulum
g	— glycogen particle	Sp	— sensory process
GER	— granulated endoplasmic reticulum	To	— tormogen cell
Ly	— lysosome	Tr	— trichogen cell
M	— mitochondria	v	— vesicles
mt	— microtubules		

Table I



these facts it also emerges that from the middle of the fossula of sensilla its surface plate protrudes. The distribution of the campaniform sensillae is not homogeneous on the full surface of the gills. They occur, namely, in large numbers (1800—2000 pieces/sq. mm) on the plate itself while they are to be seen more rarely in the area of origin of the filaments and they cannot be found at all in the more distal regions of filaments.

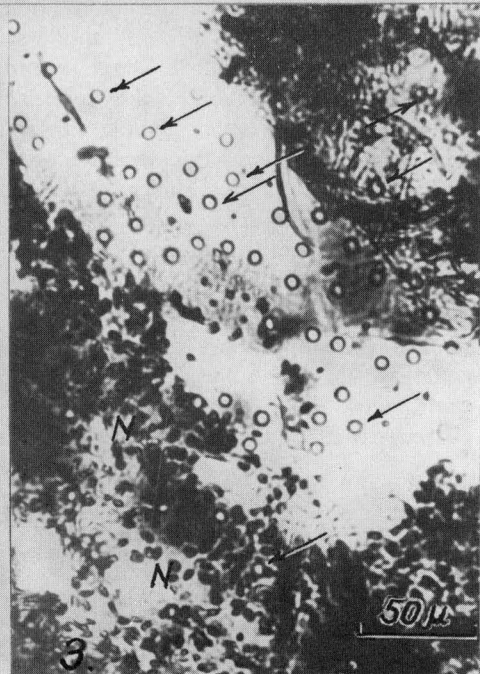
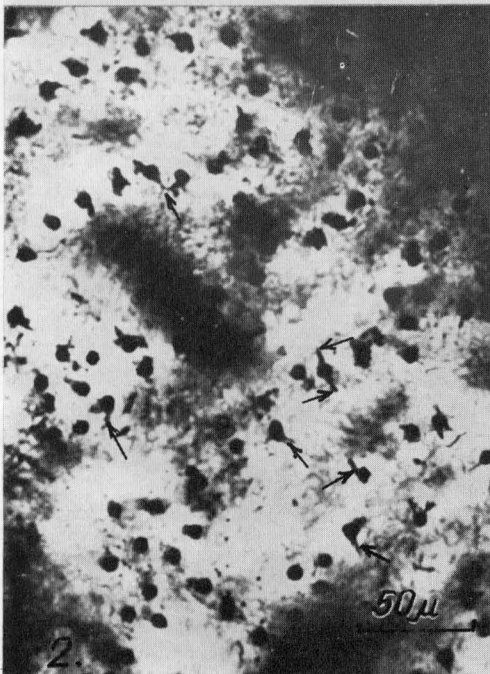
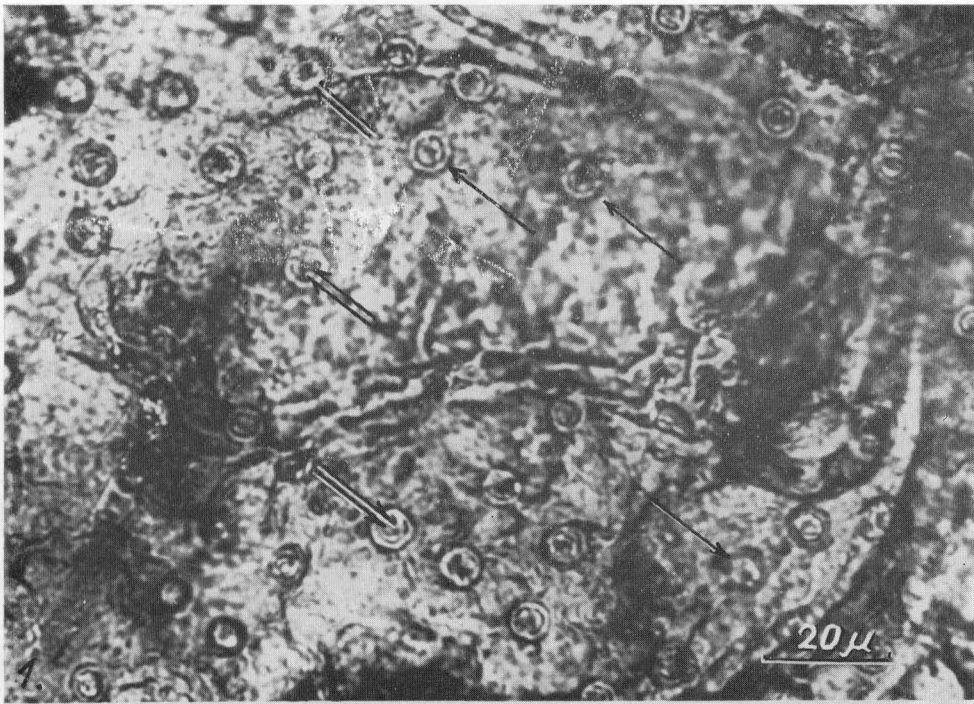


We are introducing the discussion of sensilla with a schematic figure. In its central part the sensory cell takes place, the dendrite of which is declining from the surface of sensilla (in a form similar to letter „L”) and running parallel with surface, it is hardly decreasing in its diameter (4—5 μ). This is the cause of that, while sensillae and dendrites could be investigated in large numbers in electron microscopic photographs, their connection could only be observed very rarely. Above dendrite of sensory cell the „sensorial process” takes place; the cell itself is surrounded by supporting cells. In the following, the main parts of the sensilla are treated of separately.

Table II

- Fig. 1. Plate of a tracheal gill in a silver-shadowed preparation. The border and convex sensory process (double arrows) of the campaniform sensillae (arrows) is set off by the shadowing metal. Total fixed preparation in formalin, Ag-shadowing. x800
- Fig. 2. Campaniform sensillae in the plate of a tracheal gill. Totally impregnated preparation. The impregnated processes (arrows) of the sensory cells can be followed in many places at a short distance. x315
- Fig. 3. Campaniform sensillae in an impregnated section (arrows). The nuclei of the epithelial cells are strongly impregnated. In the epithelial section of the cuticle the apertura of sensillae can be studied well. x315

Table II



The *sensorial process* (Tables III—IV) in the lower part of the cuticular fossula is a somewhat protruding concave body, covered with a continuous, thin exocuticular plate and touching the dendrite at its lower part. It is built up by fine, densely arranged microtubules. Between them, the electron-dense substance may be demonstrated in close connection with them (MORAN et al., 1971). This dense material in the lower part of the microtubular body forms a thin, continuous membrane that seems to be homogeneous, reaches the upper part of dendrite in more places than its line being elongated by vesicles. The microtubular body itself is surrounded by an extracellular cavity containing but a little dense material. It may be supposed that this extracellular substance has been produced by the supporting cells (trichogen cells) that cover the dendrite (BLANEY et al., 1971; GNATZY et al., 1971; MORAN et al., 1971; SCHMIDT et al., 1971).

The external stimuli excite the exocuticular plate covering the top of sensillae and through that the microtubular body that may have an important part in impulse conduction. In addition, according to other ideas, this system might react sensitively to the internal kinetic changes, as well, taking place in the cytoplasm (PORTER, 1966). We could sometimes observe above the cuticular plate some ciliary formations (Table III) in which a tubular pattern could dimly be recognize. We have, anyway, not been able to demonstrate any connection of these formations with cuticular plate or with the dendrite, to be dealt with as follows.

The *dendrite* (Tables III—IV) takes place immediately under the sensory process. Its upper part is cut up, the processes developed reach as far as the lower region of the microtubular body. In the sectional picture prepared in this level (Table IV, Fig. 2) the system looks like a rather regular network. The processes of dendrite have approximately in identical diameters (1500—2000 Å). In this level of the dendrite, the extracellular cavity can still be observed. The processes running towards the interior of the dendrite become united in thicker and thicker branches, then the dendrite end ceases to be divided about 1 μ under the cuticular plate. The following, about half micron, region of the dendrite is characterized by tubules arranged in lines and vesicles containing dense substance (Tables III—IV). The mitochondria of crista structure first appear in that depth, in low numbers. Also some vesicles and sporadically a few free ribosomes may be observed.

In the upper part of the dendrite we could not observe any strict arrangement either of tubules or vesicles from which the presence of a basal body could have been concluded. The presence of the basal body, resp. that of ciliary structures is regarded by more research workers (HAUPT, 1971; MORAN

Table III

Strongly magnified picture of the campaniform sensilla. The sensory process is built up by tubules (mt). In the upper part of the dendrite, the vesicles are arranged in lines, round the upper region a homogeneous material of low density (arrows) takes place. The dendrites are surrounded by supporting cells. In the surface of the cuticle the cross-sections of the cilia (ci) are to be observed. $\times 25\ 000$

Table III



et al., 1971; SCHMIDT et al., 1971; ZACHARUK et al., 1971) as a highly characteristic indication of sensillae (stimulation and impulse conduction). There are known also some data (NADOL and co-worker 1969; LEWIS, 1971) that show that in some of these sensillae (olfactory and stretch-receptors) this structure may be missing. It has been mentioned, at the same time, that the dendrite is full of light vesicles — not coinciding with the synaptic vesicles — and these may have a role in the impulse conduction. On the basis of their morphological peculiarities, the campaniform sensillae found on the tracheal gill of *Palingenia longicauda* OLIV. may be compared with the latter tipe.

Supporting cells: they are cells arranged in two lines round the sensilla cell and taking part in forming the campaniform sensillae of the gill plate (Tables III—IV). They begin in the same level as the epithelial cells, immediately under the cuticle but not all of them spread down the basis of the sensory cell. The bordering cells correspond to the trichogen and tormogen cells known from the literature.

The innermost supporting cells that touch immediately the dendrite are the trichogen cells (LEWIS, 1970; BLANEY et al., 1971; GNATZY et al., 1971; HAUPT, 1971; MORAN et al., 1971) which have a microvillial structure in their surface adjacent to the extracellular cavity. The connection of these cells with the membrane of dendrite is characterized by a mild interdigitation and at places by the zonula occludens, and the connection between the trichogen and the adjacent tormogen cells is indicated by a stronger interdigitation and the desmosomal connection noticed in some places.

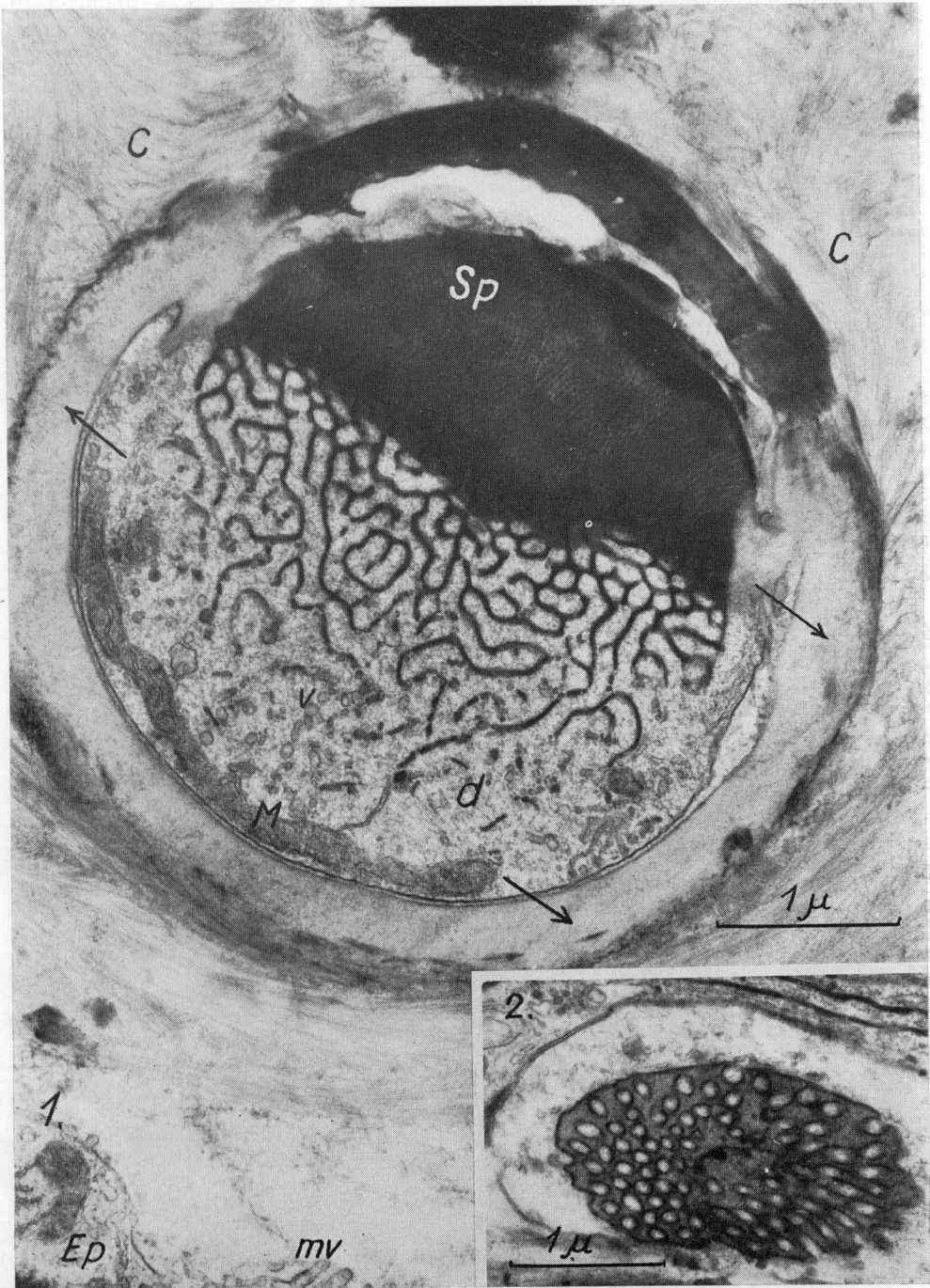
The second layer of supporting cells is formed by the tormogen cells (Table III) (LEWIS, 1970; GNATZY et al., 1971; SCHMIDT et al., 1971; ZACHARUK et al., 1971). According to a part of researches, these have a secretory function, as well. This question is outside the scope of our investigations. The cells contain many glycogen granules in their processes, too. These cells are characterized also by the extremely developed smooth-surfaced endoplasmic reticulum. In these peculiarities they agree — and even can possibly be identified — with the cells covering the centripetal process of the sensory cell.

The body of the *sensory cell* takes place between the epithelial cells or somewhat under their layer, its cytoplasm and nucleus are large, and the nucleus is of central site. The cytoplasm is rich in organelles (Table V). In the cells, the cisternae and vesicles of the Golgi apparatus can be followed interruptedly. Ribosomes could be observed both in free state and connected with the cisternae of the granulated endoplasmic reticulum. Lysosomes, as well as dense bodies with various internal structure are frequent. The fibres observed inside the body of nerve cell in the most part of our electron microscope photographs deserve a particular attention. These fibres are covered often with a membrane

Table IV

- Fig. 1. Strongly magnified picture of an campaniform sensilla cut aslant. A system of microtubules of the sensory process can be studied. Deeper on, the processes and plasma of the dendrite may be observed. The sensilla is surrounded by a homogeneous material of low density (arrows). Below the cuticle, the microvilli of a supporting cell are apparent (mv). x25 000
- Fig. 2. It can be seen in the section parallel with the surface of the cuticle that the processes of the dendrite are embedded in the lower region of the sensory process. x22 500

Table IV



system of loose structure rolled many times. We can observe tubules in the pictures of longitudinal section and clear vesicles of 400—700 Å diameter in certain regions (Table V, Fig. 2). These fibres are to be considered as having a central origin.

In connection of the *nerve fibres* the first thing that needs to be said is that the processes of the nerve cell turn round below the cuticle repeatedly and they course can be followed with light microscope because of the difficult impregnation of the material but in a short region (Table I, Fig. 2, Table II, Fig. 2); but with electron microscope it is practically impossible, the section being too thin. Following the epidermal cells below the cuticle, in a depth of about three to four cell layers, the nerve bundles can again be identified in the thin sections (Table VI).

It is characteristic of these bundles that their fibres are partly embedded in Schwann sheath and partly surrounded by a basal membrane. The diameter of a part of fibres falls short even of the thickness of mitochondria, and they are very poor in organella (Table VI, Fig. 2). In addition, the fibres are not rich in vesicles, either. The dense-core vesicles of some fibres are striking (1000—1200 Å, Table VI) but in the same fibres clear synaptic vesicles can be observed, as well. GUPTA and co-worker (1969) describe similar conditions in other objects. We have managed to observe a part of nerve bundles, particularly the thinner ones, in the area of filaments, too. Quite thin, and in some places even single, fibres run between the epithelial cells covering the filaments and the basal membrane covering their surface (CSOKNYA and co-worker, in press). Thicker nerve bundles have generally been noticed outside the filaments, in a separate basal membrane sheath. A detail of a larger bundle like this is demonstrated in Table IV, Fig. 2.

Knowing the details of the fine structure of sensillae, there comes up also a claim for interpreting their function. Our data are not in contradiction with the results of other authors who had established that the role of these sensillae was to perceive the oxygen and carbon dioxide content of water and have an effect thus indirectly on intensity and frequency of the movement of tracheal gill, i. e., on the volume of respiration. An indisputable solution of the problem needs, anyway, experimental physiological investigations.

Table V

- Fig. 1. Body of a sensory cell with a longitudinal sectioned fibre in it. In the fibre microtubules and heterogeneous vesicles are to be seen. x30 600
 Fig. 2. Body of a sensory cell. Apart from the cysternae of the granulated and smooth-surfaced endoplasmic reticulum the sections of the fibres embedded in the cytoplasm become apparent (arrows). In the cell body there are many free ribosomes. x42 000.

Table VI

- Fig. 1. Nerve bundles under the epithelial cells. The mesaxon bordering the fibres is covered from outside by a basal membrane. Besides the dense-core vesicles, clear synaptic vesicles may be observed. x32 000
 Fig. 2. Picture of the cross-section of a major nerve bundle. The large bundle is divided by the lamellae of basal membrane into smaller fibre groups. x28 000
 Fig. 3. Strongly magnified picture of a nerve fibre containing dense-core vesicles from the area below the epithelial cells. x57 000

Table V

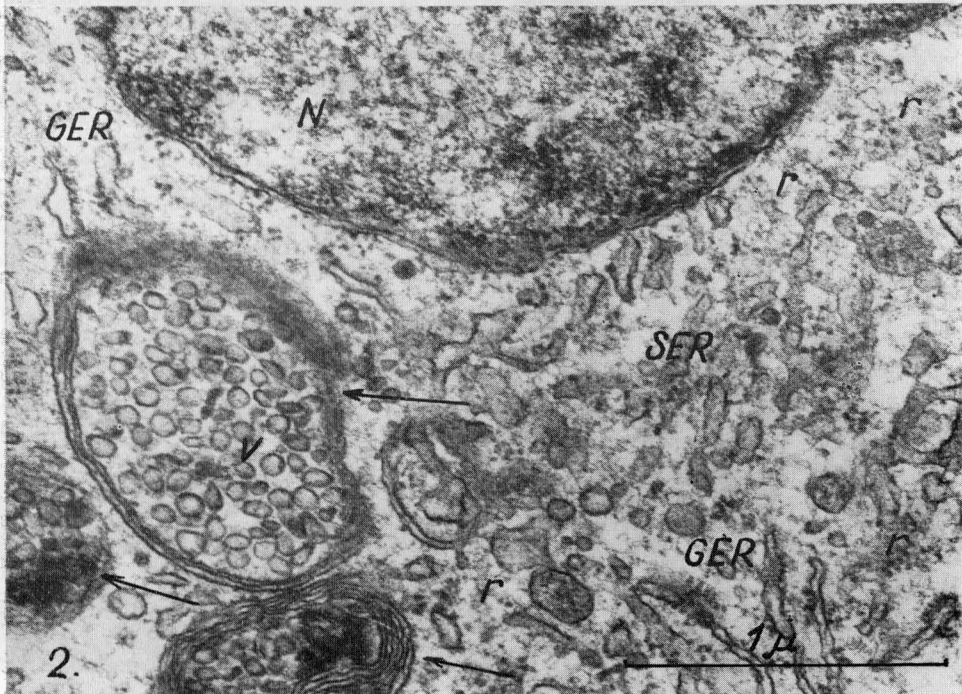
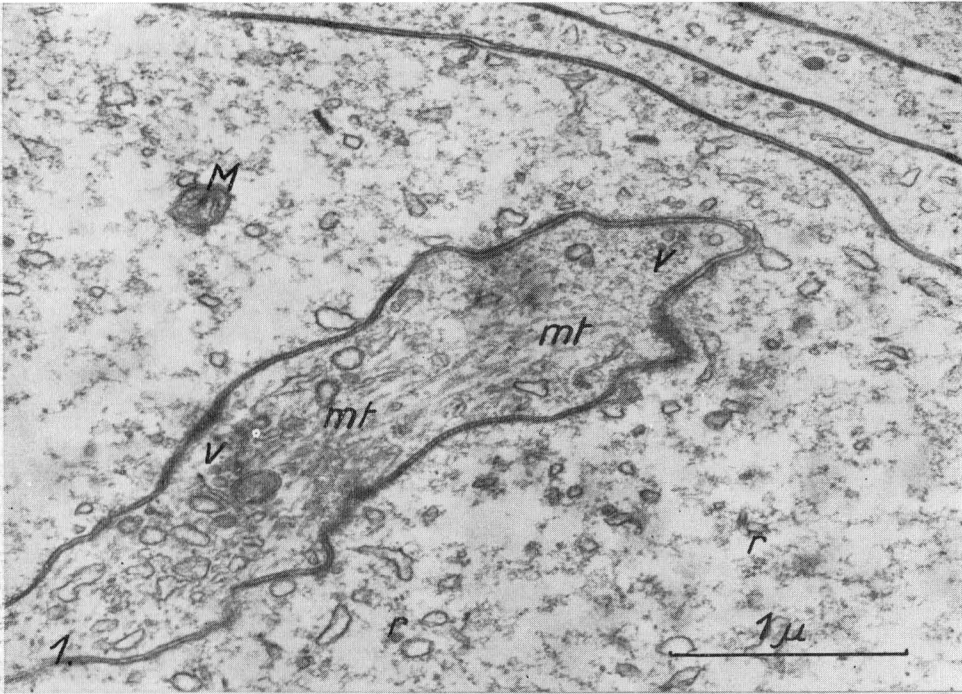
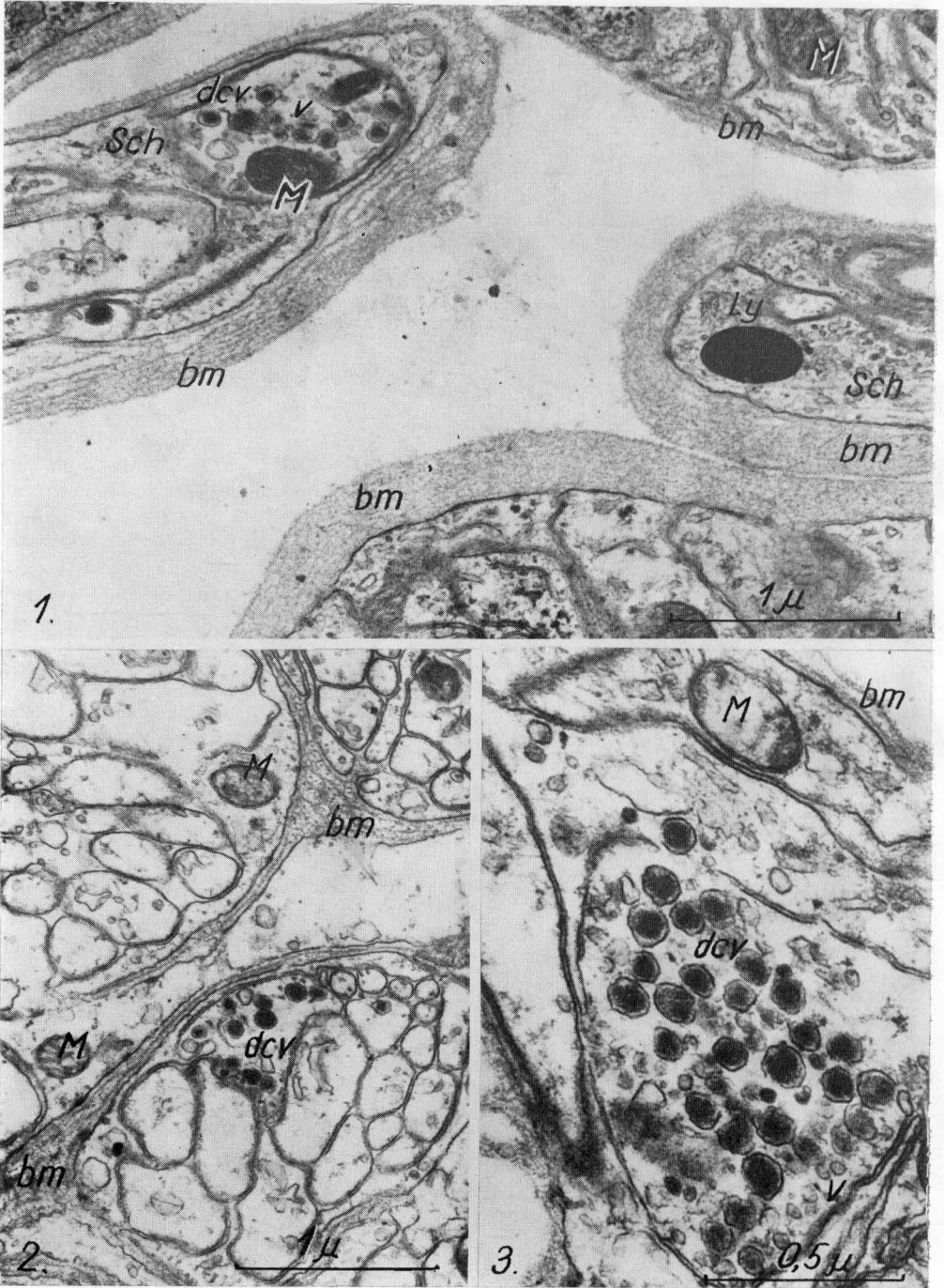


Table VI



Summary

Authors are dealing with the structure of sensory systems taking place on the tracheal gill of *Palingenia longicauda* OLIV. They are describing the structure of the sensilla and supporting cells, the nerve connections of the sensilla cell and the supposed functional role of the sense organ.

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