THE ANGULAR ACCELERATION RECEPTOR SYSTEM OF THE STATOCYST OF *OCTOPUS VULGARIS*: MORPHOMETRY, ULTRASTRUCTURE, AND NEURONAL AND SYNAPTIC ORGANIZATION†

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CONTENTS

† This paper is dedicated to Professor Hermann Schöne, Seewiesen, on the occasion of his 65th birthday.

The angular acceleration receptor system (crista/cupula system) of the statocyst of *Octopus vulgaris* has been thoroughly reinvestigated, and detailed information is presented regarding its morphometry, ultrastructure, and neuronal and synaptic organization. In each of the nine crista sections, some receptor hair cells are primary sensory cells with an axon extending from their base. Also, there are large and small secondary sensory hair cells without axons, which make afferent synapses with large and small first-order afferent neurons. The afferent synapses are of two morphologically distinct types, having either a finger-like or a flat postsynaptic process; both can be seen in the same hair cell. In addition to the afferents, there is a dense plexus of efferent fibres in each crista section, and efferent synapses can be seen at the level of the hair cells and of the neurons. The morphometric analysis of the nine crista sections shows obvious differences between the odd-numbered (C1, C3, C5, C7, C9) and the even-numbered (C2, C4, C6, C8) crista sections: they differ in length, in the number of the small primary sensory cells and in the number of the small first-order afferent neurons. Centrifugal cobalt filling of the three crista nerves revealed a disproportionate innervation of the nine crista sections: the anterior crista nerve innervates section C1 and the first half of section C2, the medial crista nerve innervates the second half of section C2, sections C3, C4, C5, and the first half of section C6, and the posterior crista nerve innervates the second half of section C6, and sections C7, C8 and C9. In each of the three crista nerves, only 25 % of the total number of axons are afferent fibres, the remaining 75 % are efferent. To each of the nine crista sections a cupula is attached. In the form and size of the cupulae there is again a conspicuous difference between the odd and the even crista sections: a small wide-based cupula is attached to each of the odd crista sections, whereas the even crista sections each have a large narrow-based cupula with a small area of attachment. The results are discussed with reference to their functional consequences.

1. INTRODUCTION

Cephalopods, competing with fish during the course of evolution (Packard 1972), have developed equilibrium receptor systems (statocysts) of sophistication and complexity that show striking parallels in structure and function with the vertebrate counterpart, the vestibular system (Budelmann 1980). Thus, the cephalopod receptor systems have become of increasing interest in recent years (see, for example, Stephens & Young 1982; Chichery 1983; Messenger 1983; Budelmann & Young 1984; Maddock & Young 1984; Young 1984; Williamson 1985 a, b; Williamson & Budelmann 1985 b). Much is known about their gravity receptor systems (see, for example, Young 1960; Barber 1966 a; Vinnikov *et al.* 1967, 1971; Budelmann 1970, 1975, 1978, 1979; Colmers 1977, 1981); however, there is a considerable lack of information

concerning their angular acceleration receptor systems, and in a number of basic structural properties controversies and uncertainties still exist (compare Young 1960; Barber 1966a; Budelmann 1977).

The aim of this present work is to make the understanding of the morphometry, ultrastructure, and neuronal and synaptic organization of the angular acceleration receptor system of *Octopus* as complete as possible as a basis for the physiological experiments now in progress (Williamson 1985a, b; Williamson & Budelmann 1985a, b). These experiments, in turn, are part of a more detailed analysis of the uptake and central processing of information in the cephalopod statocyst-oculomotor system.

1.1. *Angular acceleration receptor system*

The *Octopus* angular acceleration receptor system is composed of a sensory epithelium (crista) with cupulae attached to it. Like a ridge, the crista runs half-way round the inside of the statocyst sac over three, almost perpendicular, planes. It is subdivided into nine sections C1–C9 (figure 97). Sections C1–C6 run almost horizontally in a transverse (C1–C2 in front), longitudinal (C3–C4 at the lateral side), and again transverse direction (C5–C6 at the back), and sections C7–C9 run almost vertically (at the back) (for a review see Budelmann 1977).

Each crista section is composed of various types of large and small hair cells, supporting cells, and large neurons underneath and small neurons at the side (Young 1960; Barber 1966a; Vinnikov et al. 1967; Budelmann 1977). The large hair cells are arranged in regular rows parallel to the course of the crista ridge. The odd-numbered crista sections (C1, C3, C5, C7, C9) are characterized by a double row of large hair cells, whereas the even-numbered sections (C2, C4, C6, C8) have only a single row (Young 1960). Each hair cell bears numerous kinocilia, but it is morphologically polarized in only one direction, as defined by the orientation of the basal foot structure and the arrangement of the internal $9 \times 2 + 2$ tubuli contained in each kinocilium (figure 97) (Barber 1966b; Budelmann 1977).

The nature of the hair cells has been a subject of controversy (compare Young 1960; Budelmann 1977): they were earlier considered to be primary receptor cells with an axon running from their base (Hamlyn-Harris 1903; Young 1960; Barber 1966a; Vinnikov et al. 1967); but electron micrographs of complete serial sections have shown that at least the large hair cells in the middle of the crista ridge are secondary sensory cells (Budelmann 1977). Uncertainty also exists about the nature of the neurons: they have been described as being neurosensory (Young 1960) or glial cells ('dark cells'; Barber (1966a)).

According to Young (1960), the nine crista sections are connected with the CNS in three portions by three crista nerves, every three crista sections (C1–C3, C4–C6, C7–C9) by one nerve. However, some doubt has been shed on this description (Budelmann 1977). At the level of light microscopy, the number of fibres within each crista nerve has been determined in size classes from 2–22 μm, with the suggestion that the distributions are bimodal (Young 1965). However, as has already been shown at the level of electron microscopy for the macula nerve (Budelmann et al. 1973), the number of fibres will probably run much higher, because there are many fibres considerably smaller than 2 μm (Young 1965; Budelmann 1977).

Only very little is known about the delicate cupula structures attached to each crista section (Young 1960; Budelmann 1977). It has been briefly reported that they vary in form and size in the odd-numbered and even-numbered crista sections (Budelmann 1977).

This investigation deals with all these uncertainties and open questions and makes the morphological understanding of the crista/cupula system more clear, by using various techniques: light microscopy, scanning electron microscopy, transmission electron microscopy of serial sections, silver impregnations, and centrifugal cobalt fillings of the three crista nerves.

2. MATERIAL AND METHODS

All *Octopus vulgaris*, both males and females, and 3 *Eledone moschata* for comparison, were obtained from the Gulf of Naples. Some of the cobalt filling experiments were performed at the Stazione Zoologica di Napoli, Italy. All other experiments were done at the University of Regensburg, where the animals were kept separately in a closed system of recirculating artificial seawater at 15–18 °C.

In all experiments isolated head preparations, obtained by decapitation without prior anaesthesia, were used. Except for the cobalt filling experiments, the statocysts, with the surrounding cartilage, were cut out rapidly and kept in seawater during the further treatment. For fixation, the statocyst cavity was opened widely and in most specimens a small window was cut ventrally into the statocyst sac, but for comparison some were left unopened. In both preparations care was taken to minimize damage to the strands and vessels that hold the statocyst sac in a fixed position within the cartilaginous capsule and prevent collapsing during fixation. For the cupula preparations extreme care was taken in handling the tissue to prevent disconnection of the cupula from the crista sections. The data are based on tissues obtained from left and right statocysts, but no differences were observed between sides.

Note that as shown diagrammatically in figure 97, the nine crista sections run half-way around the statocyst sac, and then turn sharply upwards, i.e. sections C1–C6 run almost horizontally and sections C7–C9 almost vertically. Consequently, any structure being 'dorsal' in the horizontal crista sections C1–C6 correspond to those being 'lateral' in the vertical crista sections C7–C9 (similarly 'ventral' corresponds to 'medial'). However, throughout the text for the general description of the crista sections (which include the sections C7–C9) only the terms 'dorsal' and 'ventral' are used.

The term 'primary hair cell' is used synonymously for those hair cells that are primary sensory cells (i.e. with an axon), and 'secondary hair cell' is used for those that are secondary sensory cells (i.e. with afferent synapses but without an axon).

2.1. *Light microscopy*

Twenty-four statocysts from 16 *Octopus* (100–250 g) were used for this study. The statocysts were fixed in 2% OsO_4 (Zetterquist, buffered at 7.6 pH) for 1.5 h at 4 °C. Thereafter the nine crista sections (C1–C9) were dissected from the statocyst sac in 70% ethanol and subsequently embedded individually in Durcupan (Fluka, Buchs, Switzerland). Serial semi-thin sections (3 μm) of the crista ridge were cut transversally or tangentially, with a glass knife on a LKB Ultrotome III. For the investigation of the cupula morphology thicker sections (4–8 μm) were used. Sections were stained with Richardson blue and covered with Durcupan. For the nerve cell counts, the neurons were viewed with a Reichert Visopan microscope and superimposed on transparencies. Silver impregnations followed the standard methods of Holmes (1943) or Fraser-Rowell (1963).

2.2. *Transmission electron microscopy*

Thirty-two statocysts from 22 *Octopus* (80–320 g) were used for this study. For fixation and embedding see § 2.1. Serial ultra-thin cross sections of the crista were cut by using a diamond knife with a Reichert OmU 3 ultramicrotome. Sections were double-stained (uranyl acetate, lead citrate) and viewed with a Siemens Elmiskop 101.

Counts of axons in the crista nerves were made from montage electron micrographs at magnifications of up to 7200 times. They were placed in diameter-size categories with the aid of transparent stencils with squares corresponding in size to the class boundaries at the appropriate magnifications. Irregularly shaped axons were fitted into one of the squares by rotation.

2.3. *Scanning electron microscopy*

Sixty-eight statocysts from 46 *Octopus* (90–440 g) were used for this study. Fixation was either in 70 % alcohol by volume or 10 % formaldehyde/seawater solution by volume with a few drops of glacial acetic acid added; but best fixations were obtained with 2 % OsO_4 by mass (Zetterquist, buffered at 7.6 pH) for 1.5 h at 4 °C. The statocysts were dehydrated in graded alcohol solutions, transferred to acetone and critical-point dried with liquid carbon dioxide in a CPA II unit (Technics, Alexandria, Virginia, U.S.A.). The dried statocyst sacs were 'spot mounted' on specimen stubs with a double-sided tape and then cut open and flattened out to expose the various crista sections. The mounted tissues were gold-coated with a Hummer II sputter unit (Technics, Alexandria, Virginia, U.S.A.) and viewed with a Cambridge Stereoscan S4–10 scanning electron microscope at an accelerating voltage of 10 kV.

2.4. *Centrifugal cobalt filling of the crista nerves*

Sixteen statocyst preparations from nine *Octopus* (110–360 g) and four preparations from three *Eledone moschata* (390–480 g) were used for this study. The isolated head preparations were kept in seawater and split sagittally into a right and a left preparation with the supra-oesophageal mass of the brain removed. The crista nerve in question was carefully dissected from its surrounding connective tissue and cut close to its entry into the CNS. The sub-oesophageal mass of the brain was then removed. The cut end of the nerve was repeatedly treated with distilled water and then drawn into a polyethylene suction electrode of matching size, filled with a 1.0 M $CoCl_2$ solution. The Co^{2+} ions were injected iontophoretically into the nerve fibres by passing positive current pulses (6–7 s^{-1}, 13–20 µA, 100 ms in duration) for 10–20 h at a temperature of 3–5 °C. The statocyst cavity was left unopened.

After iontophoresis, the statocyst cavity was opened ventrally and a small window was cut into the statocyst sac allowing careful washing out of the endolymph with a fine jet of seawater. The Co^{2+} ions were then precipitated as black cobalt sulphide by treating the tissue with saturated ammonium sulphide (about one drop per millilitre of seawater) for about 5 min. After washing with seawater for about 15 min, the tissue was fixed in either 70 % alcohol or 10 % formaldehyde/seawater solution, with a few drops of glacial acetic acid added, for several hours, dehydrated in graded ethanol solutions and cleared in cedar wood oil. Thereafter the relevant crista sections were cut out of the statocyst sac and mounted in cedar wood oil as flat whole-mount preparations under a cover slip.

For the limits of evidence of the cobalt filling technique, especially for the very fine efferent fibres, see Budelmann & Young (1984).

3. RESULTS

For general data on the gross morphology and fine structure of the *Octopus* crista, see Young (1960), Barber (1966a,b, 1968), Vinnikov *et al.* (1967), Budelmann *et al.* (1973), and Budelmann (1977).

3.1. *Morphometry of the crista* (*tables* 1, 2 *and* 4)

The average length of the crista, encompassing all nine crista sections C1–C9, is 4.03 mm; in the six specimens with complete C1–C9 data, it varies between 3.53 mm and 4.23 mm (table 1). The average length of a single crista section is 0.43 mm, with section C9 having the smallest value (0.26 mm) (table 1). As is shown in figure 1, the average lengths of the nine crista sections differ, and note that there is an increase in length from sections C1–C4 to sections C5–C8 (in figure 1, this is shown separately for the odd-numbered and even-numbered crista sections; see §3.1.1 and §3.1.2). For all nine crista sections C1–C9, the average number of large hair cells in one row is 31. However, differences were found between individual crista sections, with C8 having the largest average of 35 cells per row, and C9 the smallest, with an average of 19 cells per row (table 1).
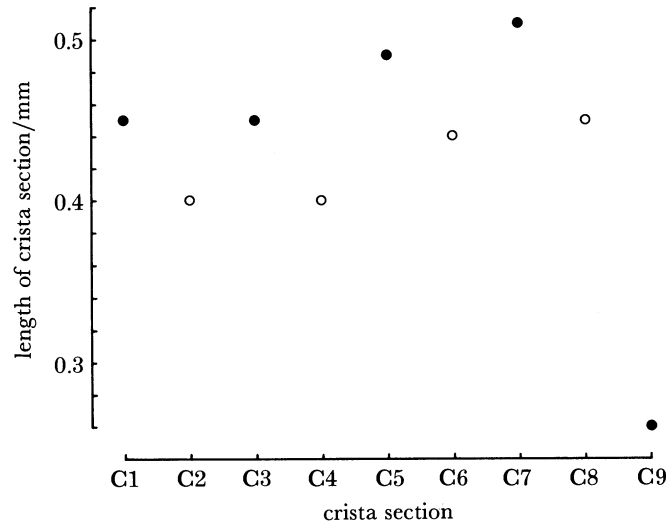

FIGURE 1. Differences in the length of the nine crista sections C1–C9 of *Octopus*, with the odd-numbered sections shown as (●) and the even-numbered sections as (○). For data see table 1.

Looking at the data from individual crista sections (table 1), the variations in the number of large hair cells per row, and in the crista length respectively, are even larger, but they are intelligible from the fact that the data come from specimens of different sizes and body masses (90–440 g). Figure 2 shows that the average length of a single crista section increases with increasing mass of the animal; however, the correlation ($r = 0.62$; $p = 0.01$) is low (see §4.1.1). On the other hand, a strong correlation is to be seen between the average number of large hair cells per crista row and the average length of a single crista section (figure 3; $r = 0.88$, $p < 0.001$). These data imply that the increase in length of a single crista section is most probably due to a formation of additional hair cells (rather than to an increase in their
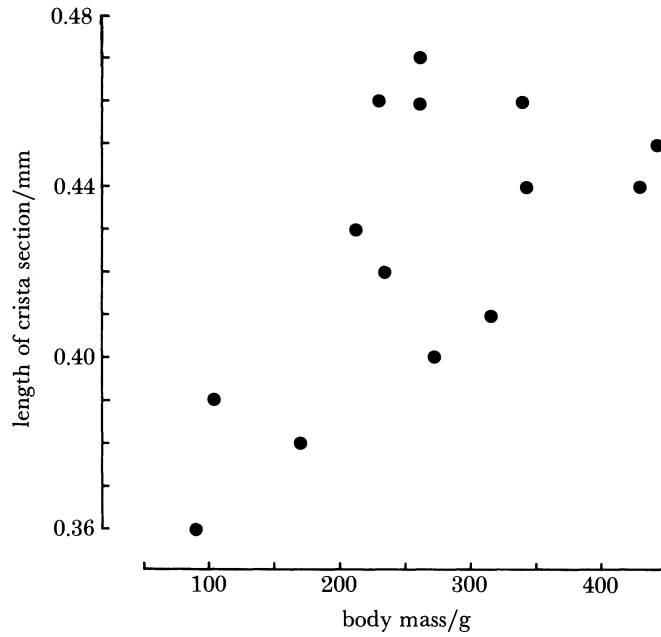
FIGURE 2. Correlation between the average length of a single crista section and the body mass of *Octopus*. For data see table 1.
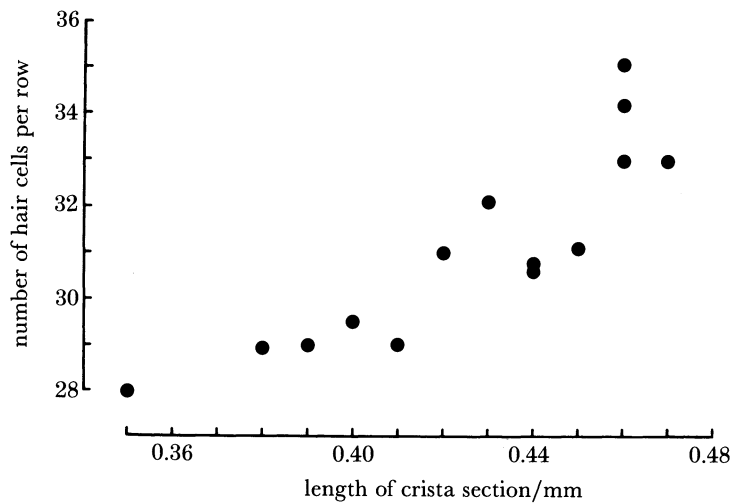

FIGURE 3. Correlation between the average number of large secondary hair cells per row and the average length of a single crista section of *Octopus*. For data see table 1.

size). The zones of hair cell formation ('growth zones') are at both ends of a crista section, where the formation of new hair cells – as indicated by ciliary groups with much shorter kinocilia could be observed (figures 8 and 9).

The above morphometric data about length, and number of large hair cells per row, of an average crista section have taken all nine sections as being uniform. However, as can be seen from figure 1 and tables 1, 2 and 4, in the nine crista sections, there are obvious differences in some parameters that alternate regularly and this alternation correlates with the double-row

and single-row crista section arrangement described by Young (1960). Consequently, the double-row crista sections C1, C3, C5, C7, C9 and the single-row crista sections C2, C4, C6, C8 will be discussed separately in the following paragraphs; they can easily be identified as such by the arrangement of the kinociliary bundles of the hair cells on the surface of the epithelium (§3.1.1 and §3.1.2).

From the fact, however, that the data come from specimens of different sizes (body masses 90–440 g) and size does not necessarily correlate with age (e.g. owing to starvation the animals can reduce their mass to 50% without obvious debilitation; Mangold & Boletzky (1973), Wells (1978); see also figure 2 and §4.1.1), it becomes difficult, in a limited number of specimens that vary largely in size, to demonstrate small differences that are statistically significant. From the data of one specimen, however, or data of several specimens but of a given size category only (e.g. 210–230 g or 315–340 g of the specimens of table 1) these differences are very obvious and also significant. Therefore, in the following paragraphs §3.1.1 and §3.1.2 the morphometric data will be presented without a statistical analysis and small differences will be stated as such only when they can be deduced from specimen of comparable statocyst size.

3.1.1. *Double-row crista sections* C1, C3, C5, C7, C9

The morphology of the double-row crista sections is quite uniform. The only exception is section C9 which is much smaller; its length and numbers of hair cells and neurons lie between one third and two thirds of those of the other double-row crista sections (see table 1). Therefore C9 data were not included in the following calculations.

A double-row crista section has an average length of 0.48 mm (C9, 0.26 mm), with the sections C5 and C7 being about 10% larger than sections C1 and C3 (figure 1; table 1). With the scanning electron microscope a double-row crista section can easily be identified by the appearance and arrangement of the kinociliary bundles of the hair cells. Each bundle represents a single hair cell (Barber 1966a, b), and in a double-row crista section all bundles are characterized by compactness and a clear separation from their neighbouring bundles (figures 4 and 6†).

Large hair cells. There are two rows of large hair cells in the middle of the crista ridge and these cells are very regularly arranged along the crista length (figures 4, 6, 11 and 12). The cells are about 30 μm in height, with a diameter at the base of 20–25 μm (figures 11 and 12). Their kinociliary bundles are the largest (13 μm in length) and most compactly arranged of all hair cells of the crista section (figures 4 and 6). There are an average of 28 cells in the dorsal row of large hair cells and 31 cells in the ventral row (13 and 18 in C9, respectively; tables 1 and 4). In serial light microscopical sections, no axons have been found leaving these cells and it will be shown in §3.2.1.2 and §3.4, that the large hair cells are secondary sensory cells.

Small dorsal hair cells. Dorsal to the two rows of large hair cells are three to five rows of smaller hair cells (figure 4; upper outer hair cells, Young 1960), with an average of 94 cells per section (C9, 67 cells; table 4). They again are 25–30 μm in height, but their diameter is only 5–15 μm (figures 10 and 16). These cells are not as regularly arranged in rows as the large hair cells and their ciliary bundles consist of fewer kinocilia (figures 4 and 6). The distance between the neighbouring ciliary bundles of the small dorsal hair cells varies from 4–6 μm (figure 6); it is the largest gap between ciliary bundles of all types of hair cells in the crista sections and is very

† Figures 4–55 and 57–96 appear on plates 1–15.

TABLE 1. MORPHOMETRIC DATA FROM THE CRISTA SECTIONS OF *OCTOPUS VULGARIS* OF DIFFERENT MASSES (90–440 g)

(For each of the nine crista sections, the length (mm) and the number of the large ventral secondary hair cells per row are given. All data are from osmium-fixed material. The data averages of the odd-numbered crista sections are given separately with, and without, C9 because of its unusual characteristics (compare §3.1.1 and table 4).)

animal	mass/g	C1	C2	C3	C4	C5	C6	C7	C8	C9	C1–C9 $\bar{x}$	C2, C4 C6, C8 $\bar{x}$	C1, C3 C5, C7 $\bar{x}$	C1, C3 C5, C7 C9 $\bar{x}$
171 re	90	—	0.31	0.40	0.37	0.40	0.33	0.40	0.40	0.23	0.36	0.35	0.40	0.36
		26	30	28	30	28	30	30	35	18	28.33	31.25	28.00	26.00
173 re	105	0.38	0.41	0.35	0.44	0.41	0.41	0.44	0.47	0.22	0.39	0.43	0.40	0.36
		26	32	28	30	29	33	32	33	17	28.89	32.00	28.75	26.40
162 li	170	—	0.36	0.38	0.40	0.43	0.41	—	0.48	0.18	0.38	0.41	0.41	0.33
		—	32	27	31	28	32	—	36	16	28.86	32.75	27.50	23.67
161 re	210	—	0.34	0.49	0.40	0.51	0.42	—	0.41	—	0.43	0.40	0.50	0.50
		—	31	31	33	31	33	—	35	—	32.30	33.00	31.00	31.00
246 li	225	0.51	0.37	0.56	0.37	0.52	0.47	0.54	0.45	0.31	0.46	0.42	0.53	0.49
		33	33	40	35	42	35	39	37	22	35.10	35.00	38.50	35.20
172 re	230	—	—	0.52	0.39	0.48	0.40	0.48	0.44	0.23	0.42	0.41	0.49	0.43
		—	—	34	33	35	32	33	32	18	31.00	32.33	34.00	30.00
249 li	260	0.49	0.49	0.43	0.43	0.51	0.52	0.54	0.48	0.34	0.47	0.48	0.49	0.46
		31	36	29	37	36	37	32	37	22	33.00	36.75	32.00	30.00
249 re	260	0.47	0.49	0.40	0.43	0.48	0.52	0.52	0.49	0.33	0.46	0.48	0.47	0.44
		29	39	—	37	30	39	32	37	22	33.00	38.00	33.33	28.25
166 re	270	—	0.41	0.41	0.38	0.46	0.43	0.43	0.43	0.24	0.40	0.41	0.43	0.39
		—	32	27	30	32	33	33	33	16	29.50	32.00	30.67	27.00
164 li	315	—	—	—	0.39	0.51	0.45	0.44	0.42	0.24	0.41	0.42	0.48	0.40
		—	—	—	31	31	34	—	32	17	29.00	32.33	31.00	24.00
251 re	335	0.43	0.45	0.51	0.39	0.56	0.47	0.59	0.47	0.28	0.46	0.45	0.52	0.47
		28	38	36	36	35	39	37	39	20	34.20	38.00	34.00	31.20
170 li	340	—	0.38	0.44	0.40	0.56	0.43	0.61	0.41	0.29	0.44	0.41	0.54	0.48
		—	33	31	35	32	31	33	33	19	30.90	33.00	32.00	28.75
163 re	430	—	0.43	0.43	0.40	0.47	0.49	0.55	0.49	0.25	0.44	0.45	0.48	0.43
		—	34	31	33	31	33	32	35	16	30.60	33.75	31.33	27.50
167 re	440	0.39	0.41	0.53	0.40	0.53	0.44	0.58	0.45	0.30	0.45	0.43	0.51	0.47
		26	30	32	33	34	34	34	35	22	31.10	33.00	31.50	29.60
mean length crista section		0.45	0.40	0.45	0.40	0.49	0.44	0.51	0.45	0.26	0.43	0.43	0.48	0.43
mean number of large hair cells per row		28.4	33.3	31.2	33.1	32.4	33.9	33.4	34.9	18.9	31.12	33.79	31.47	28.47

characteristic for the double-row crista sections. In serial light-microscopical sections all the small dorsal hair cells were found to have an axon, giving the cells a pear-shaped cross-section at their base when cut tangentially to the crista ridge (figures 12 and 13). Thus, these cells are clearly primary sensory cells (see also §3.2.1.1 and §3.4).

Small ventral hair cells. Ventral to the two rows of large hair cells are two to three fairly regularly arranged rows of smaller hair cells (figure 4; lower inner and lower outer hair cells (Young 1960)). Together these rows have an average of 53 cells per section (C9, 31 cells; table 4). The

sizes of the cell bodies are comparable to those of the small dorsal hair cells, the sizes of their kinociliary bundles lie in between those of the small dorsal and the large hair cells, and their ciliary bundles are clearly separate from those of its neighbouring cells (figure 6). In serial light microscopical sections no axons have been found leaving these cells and it will be shown in §3.2.1.3 and §3.4 that these small ventral hair cells – contrary to the small dorsal hair cells – are secondary sensory cells.

Large neurons. Somata of large neurons occur proximally in the epithelium, underneath and between the bases of the large hair cells, or ventrally to the side. They can easily be distinguished from any other cell in the crista ridge by their cytoplasm which is less dense (figures 10, 11 and 15). Their somata are rounded, with diameters between 20 μm and 35 μm, and do not extend more than 50 μm ventrally from the middle of the crista ridge. They are arranged almost regularly in a row but, because of occasional gaps in-between of up to 25 μm, they do not form a continual band (figure 15). The nuclei of the neurons are often surrounded by dark granules (figures 12 and 15). Their axons are large (up to 10 μm in diameter) and run underneath the hair cells nearly parallel to each other in a dorsal direction, but some may also cross over each other (figure 10, 12, 15 and 16; see also §3.4). In serial light-microscopical sections an average of 17 large neurons (C9, 9 large neurons) were found in a double-row crista section (table 4). It will be shown in §3.2.1.2 and §3.4 that these large neurons are the first-order afferent neurons to the large hair cells only, which are all secondary sensory cells.

Small neurons. A second, smaller type of neuron is also found in the crista ridge. Their somata again are rounded but have a diameter of only 5–15 μm. They always occur ventral to the large neurons, underneath and ventral to the small ventral hair cells (figures 10 and 17). They do not have a regular arrangement but are scattered and may extend up to 75 μm ventrally from the middle of the crista ridge. Their axons are small (up to 3 μm in diameter) and run underneath the hair cells in an almost dorsal direction, but they are less regularly arranged than those of the large neurons. Because of the slope of the crista ridge, the small neurons are

TABLE 2. COMPARISON OF THE MORPHOMETRIC DATA FROM THE ODD-NUMBERED (C1, C3, C5, C7) AND EVEN-NUMBERED (C2, C4, C6, C8) CRISTA SECTIONS OF *OCTOPUS VULGARIS*

(†, The data from the odd-numbered crista section C9 are not included in this analysis (compare with table 4). ‡, Data from Williamson & Budelmann (1985 *a, b*).)

cupula	crista sections	
	C1, C3, C5, C7†	C2, C4, C6, C8
	small with large area of attachment	large with small area of attachment
length of crista section/mm	0.48	0.43
number of small dorsal primary hair cells	94	166
number of fairly large/large secondary hair cells in the dorsal row	28	35
number of large secondary hair cells in the ventral row	31	34
number of large first-order afferent neurons	17	17
number of small ventral secondary hair cells	53	56
number of small first-order afferent neurons	86	115
resting activity‡	no	yes
sensitivity to linear acceleration‡	no	yes
threshold‡	high	low

difficult to count in serial light microscopical sections, but it will be shown in §3.4 that there is an average of 86 small neurons (C9, 29 small neurons) in a double-row crista section (table 4). Moreover, it will be shown in §3.2.1.3 and §3.4 that the small neurons are the first-order afferent neurons to the small ventral hair cells only, which are all secondary sensory cells.

3.1.2. *Single-row crista sections* C2, C4, C6, C8

The morphology of the single-row crista sections is uniform throughout all the four sections. They have an average length of 0.43 mm, with sections C6 and C8 being about 10 % larger than sections C2 and C4 (figure 1, table 1). Using a scanning electron microscope, a single-row crista section can easily be distinguished from a double-row crista section in that there is only a small gap between the neighbouring ciliary bundles of the small dorsal hair cells (figures 5 and 7).

Large hair cells. In a single-row crista section there is only one row of large hair cells in the middle of the crista ridge and this corresponds with the ventral row of large hair cells of the double-row crista sections. The large hair cells are very regularly arranged along the crista length (figures 5, 7 and 14). The cell somata have the same dimensions as those of the large hair cells of the double-row crista sections, and again their kinociliary bundles are the most compact ones of those of all the hair cells of the single-row crista sections (figures 5 and 7). Each single-row section has an average of 34 cells in the row of large hair cells (table 1). In serial light microscopical sections, no axons have been found leaving these cells, and it will be shown in §3.2.1.2 and §3.4 that the large hair cells are secondary sensory cells.

Fairly large hair cells. Dorsal to the row of large hair cells lies another regular row of hair cells, the somata of which are somewhat smaller than those of the large hair cells. Their cross-sections are often rectangular, with a diameter of 10–15 μm along the crista length. This row of fairly large hair cells (upper inner hair cells (Young 1960)) corresponds with the dorsal row of large hair cells in the double-row crista section. Using the scanning electron microscope, the fairly large hair cells can be distinguished from the large hair cells in that their kinocilia are less compactly arranged in bundles that stand almost perpendicular to the surface of the epithelium (figure 7); this differs from the ciliary bundles of all the other types of hair cells in the single-row and double-row crista sections, which are inclined towards the plane of the crista surface, forming an angle of 40–60° with it (figures 7 and 10). Counts give an average of 35 fairly large hair cells in a single row (tables 2 and 4). In serial light microscopical sections, no axons have been found leaving these cells. It will be shown in §3.2.1.2 and §3.4 that the fairly large hair cells are secondary sensory cells.

Small dorsal hair cells. Dorsal to the row of fairly large hair cells are four to five rows of smaller hair cells (figure 5; upper outer hair cells (Young 1960)), with an average of 166 cells per section (table 4). They do not form very distinct rows (figure 7). Their average size is comparable with that of the small dorsal hair cells of the double-row crista sections, but their ciliary bundles are less compactly arranged and almost in contact with those of their neighbouring cells along the length of the crista ridge. Thus, there is little, or no, gap between bundles and this is very characteristic for the single-row crista sections (figure 7). In serial light microscopical sections all the small dorsal hair cells were found to have an axon. Thus, these cells – as the corresponding ones in the double-row crista sections – clearly are primary sensory cells (see also §3.2.1.1, figure 19 and §3.4).

Small ventral hair cells. Ventral to the row of large hair cells are two to three rows of smaller

hair cells that are fairly regularly arranged in rows along the crista length (figure 5; lower inner and lower outer hair cells (Young 1960)). Together these rows have an average of 56 cells per section (table 4). The average somata size is comparable with that of the corresponding cells of the double-row crista sections, but – as for the small dorsal hair cells in this section – many of their ciliary bundles are almost in contact with those of their neighbouring cells along the length of the crista ridge.

Large neurons. Somata of large neurons are also present in the single-row crista sections. They are rounded and have a diameter of 15–25 μm (figures 14 and 20); they are thus about 25% smaller than the corresponding ones in the double-row crista sections. The neurons occur proximally in the epithelium, underneath and between the bases of the large hair cells, or ventrally to the side. Again, they can be distinguished from any other cell in the crista ridge in that their cytoplasm is less dense (figure 14). They do not form distinct rows and are less regularly arranged than the large neurons in the double-row crista sections (compare with figure 71). Their nuclei are also surrounded by dark granules (figure 14). Their axons have a diameter of up to 7.5 μm and run almost parallel in a dorsal direction underneath the hair cells; again, some may also cross over each other. Serial light microscopical sections gave an average of 17 large neurons in a single-row section (table 4). It will be shown in §3.2.1.2 and §3.4 that these large neurons are the first-order afferent neurons to the large and fairly large hair cells only, which all are secondary sensory cells.

Small neurons. In addition to the large neurons, there are also smaller ones with somata diameters of 5–15 μm. They are scattered ventrally to the large neurons and lie underneath and ventrally beside the small ventral hair cells. Compared with their corresponding neurons in the double-row crista sections, they extend further ventrally, up to 100 μm from the middle of the crista ridge (compare with figure 58). Because of the slope of the crista ridge, these small neurons are also difficult to count in serial light microscopical sections, but in §3.4 it will be shown that there is an average of 115 small neurons in a single-row crista section (table 4). Moreover, it will be shown in §3.2.1.3 and §3.4 that these small neurons are the first-order afferent neurons to the small ventral hair cells only, which all are secondary sensory cells.

3.2. *Neuronal and synaptic organization of the crista sections*

In the crista sections C1–C9 the nature of the different types of receptor cells and of the neurons, as well as their synaptic organization, has been investigated on the basis of serial electron microscopical reconstruction. No differences have been observed between the single-row and the double-row crista sections; thus the following results are valid for both.

3.2.1. *Afferent system*

The afferent system of the crista sections consists of different types of receptor cells: the small dorsal hair cells, the large hair cells, the fairly large hair cells (which occur only in the single-row crista sections) and the small ventral hair cells.

The different types of hair cells can easily be distinguished in cross-sections of the crista according to the hair cells' size (see §3.1) and the direction of inclination their kinocilia form with the crista surface. As is shown in figure 16, the large hair cells, together with the small ventral hair cells, all have kinocilia inclined in the same direction which is dorsal, whereas the small dorsal hair cells are the only ones that have kinocilia inclined in the opposite direction

which is ventral. Another item is the position of the supporting cells: in cross-sections of the crista there is only one, normally very small, supporting cell in between two neighbouring hair cells (at least distally), but there are three to five supporting cells between the small dorsal and the large (and fairly large) hair cells (figure 16). In addition, the position of the somata of the neurons helps to orient since they occur mostly ventrally, or occasionally medially, in the crista ridge but never dorsally (figure 10).

3.2.1.1. *Small dorsal hair cells.*

In ultrathin serial reconstructions, representatives of all five rows of small dorsal hair cells were found to carry an axon that originated from the base of these cells and ran in an almost dorsal direction (figure 19; compare also with figures 12 and 13). In none of these cells were signs found of an afferent type of synapse, with the synaptic vesicles inside the receptor cell. Thus, these data clearly demonstrate that the small dorsal hair cells are primary sensory cells (see also §3.1 and §3.4).

3.2.1.2. *Large and fairly large hair cells, and their large first-order afferent neurons.*

Twenty-two of the large and fairly large hair cells have been investigated and no axons were found to originate from any of these cells. They normally have smooth, rounded bases (figures 10, 16 and 35), but occasionally these may be irregular with short processes of different shapes and sizes that interdigitate with protrusions from the supporting cells or elements of the neuronal plexus (figures 21 and 36). The hair cell processes are usually densely filled with mitochondria and lack neurofilaments (figure 21); both criteria facilitate their distinction from any other plexus elements such as efferent profiles and axonal segments (e.g. figure 27).

In every large and fairly large hair cell at least one afferent synapse was found, as indicated by the position of the synaptic vesicles inside the hair cell. The afferent synapses were of two distinct types, one with a postsynaptic finger-like process that invaginates into the hair cell (figures 22–26), and another that makes a flat synaptic contact (figures 22, 23 and 27). The synapses with the finger-like postsynaptic process are more common. The process invaginates into the hair cell to a depth of up to 1.5 μm, but no branching of the process within the zone of synaptic contact was observed. The synaptic cleft is about 20 nm wide, has symmetric membranes, and often contains electron dense cleft material (figures 22 and 25). The synaptic vesicles cluster presynaptically around almost the entire length of the finger-like invagination. The vesicles vary in diameter from 20–40 nm (figures 22 and 23); most of them are agranular, others have a dense core or dense content (figures 22, 23, 26 and 27). All types of vesicles can occur together in the same hair cell at the same synapse (figure 26). The synapses that make a flat synaptic contact are less common. They are usually seen in direct contact with an axon cylinder (figures 22, 23 and 27) and reach a maximum of about 0.5 μm in length. The synaptic cleft is approximately 20 nm wide and often contains electron dense cleft material. The synaptic vesicles, both agranular and dense-cored, cluster presynaptically along the synaptic cleft; sometimes they are arranged regularly in a row (figures 22, 23 and 27).

Both types of synapse can occur in the same hair cell, sometimes side by side (figures 22 and 23). No synapses of transitional form have been observed. The number of afferent synapses per hair cell may vary. On a single section, a maximum of three synapses with a finger-like postsynaptic process have been seen in one hair cell, and a maximum of two synapses with a flat postsynaptic process. However, the total number of afferent synapses in a single hair cell might be higher, because no quantitative analysis has been performed.

The postsynaptic processes of the large and fairly large hair cells investigated, whether finger-like or flat, were all seen to originate from axons of only the large neurons (figures 22 and 23). The finger-like processes can therefore be considered as axodendrites; they sometimes emerge as fine processes from the axon cylinder and run for some distance in the neuronal plexus before invaginating the hair cell (compare figure 24). When two or more finger-like processes make synaptic contacts with one hair cell, they have been seen originating either from the same or from different axons. Finger-like and flat synaptic contacts have been seen between a hair cell and its postsynaptic axon (figures 22 and 23).

No afferent synaptic contacts of either type were found between the large and fairly large hair cells, and the axons of the small neurons. Moreover, lateral synaptic connections between the hair cells were not observed. These data, therefore, demonstrate that both the large and the fairly large hair cells are secondary sensory cells (see also §3.4) and in addition that these cells are in afferent synaptic contact with the axons of only the large neurons, which have thus to be considered as the first-order afferent neurons to these hair cells.

3.2.1.3. *Small ventral hair cells, and their small first-order afferent neurons.* In single cross sections of the crista ridge, two to three small ventral hair cells can be seen. Their somata often appear to be pressed ventrally aside by the somata of the large hair cells and the large neurons (figure 16). Thus, the bases of almost all somata of the small ventral hair cells are found ventrally to those of the large neurons; only a few may occur in-between. Eighteen of the small ventral hair cells have been investigated. In none of these cells has an axon been found to originate. Their bases are usually quite smooth, but may also be irregular, with processes that intermingle with elements of the neuronal plexus (i.e. processes of other hair cells or supporting cells, axons and efferent profiles; figures 17 and 18).

As was found for the large and fairly large hair cells, on every small ventral hair cell at least one afferent synapse was present, with the synaptic vesicles inside the hair cell. Again, the afferent synapses were of the two distinct types already described, with either a finger-like or a flat post-synaptic process. Their morphologies, including those of their synaptic vesicles, are in all respect similar to those of the large and fairly large hair cells. However, the number of afferent synapses in a single small hair cell is obviously less. Again, no lateral synaptic connections between the hair cells were observed. In contrast to the large and fairly large hair cells, the postsynaptic processes of the afferent synapses of the small ventral hair cells were all seen to originate from axons of only the small neurons. Therefore, the data together clearly demonstrate that the small ventral hair cells are secondary sensory cells (see also §3.4) and that these cells are in afferent synaptic contact with the axons of only the small neurons, which have thus to be considered as the first-order afferent neurons to these hair cells.

3.2.2. *Efferent system*

3.2.2.1. *The course of the efferent fibres.* The efferent fibre system of the crista sections originates from somata in the anterior and posterior lateral pedal, the posterior pedal, and the ventral and anterior dorsal magnocellular lobes of the ipsilateral CNS; only very few fibres originate contralaterally in the posterior pedal and posterior lateral pedal lobes (Budelmann & Young 1984). The course of the efferent fibres in the statocyst to and within the crista sections can be visualized with Holmes's and Fraser Rowell's silver impregnations. These techniques reveal

an elaborate plexus of very fine interweaving fibres dorsal to, and in-between, the crista hair cells and neurons (plate 5); but no cell bodies have ever been seen within the crista sections associated with the fibres of this plexus. It is not known why none of the various receptor hair cells and only occasionally a few of the large and small first-order afferent neurons, including their axons, were stained by these techniques (figures 29, 33 and 34). Also, lesion of the statocyst nerves inside the brain capsule results in a degeneration of the plexus of fine fibres, indicating their somata's location inside the brain (Auerbach & Budelmann 1986). Thus, together with the ultrastructural data described here, the analysis of the numbers and diameters of the fibres of the crista nerves (see §3.3 and §4.1.7) and of the centrifugal cobalt fillings of these nerves (which showed axons and cells of only the afferent system; see §3.4), almost certainly the elaborate plexus of fine fibres reveals the efferent fibre system of the crista sections (compare also Young 1960; Stephens & Young 1982).

This plexus of efferent fibres derives from bundles of very fine fibres that run beside the afferent fibres (see plates 9–11) towards the various crista sections. From these bundles larger and smaller ones diverge about 200 μm dorsal to the crista sections. Some bundles of efferent fibres run long distances parallel to the crista ridge: bundles of the anterior crista nerve run along C3, C4 and C5 (figures 28 and 29) and bundles of the posterior crista nerve along C7, C6 and C5. In some preparations, a few single efferent fibres of C1 could be followed through the adjacent macula into the macula nerve.

About 100 μm dorsal to the small dorsal hair cells the efferent fibres form a dense meshwork of interweaving fibres (figures 29 and 30) which is continuous all along the nine crista sections. The meshwork extends into the region of the somata of the small dorsal hair cells (figure 31), the large hair cells and fairly large hair cells (figure 32), and the small ventral hair cells together with the somata of the large and small first-order afferent neurons (figure 33). Often the finest fibres could be seen surrounding individual cell bodies (figure 33), but it is difficult to decide whether the fibres terminate there; some may proceed to other hair cells or neurons. In the few preparations in which axons of the large and small first-order afferent neurons were stained, many varicosities could be seen associated with these axons (figure 34). The varicosities may be close to the synaptic contacts of the efferent fibres to these axons (compare with figure 41).

3.2.2.2. *Efferent contacts with the hair cells and the first-order afferent neurons.* A large number of vesicle-filled profiles occur in the neuronal plexus (figures 35 and 36). Many of these profiles can be seen in presynaptic contact with either the hair cells (plate 6) or the first-order afferent neurons (plate 7). Because no evidence has been found for lateral synaptic connections between the hair cells or the neurons, the vesicle-filled profiles have to be considered as terminals of efferent fibres, whose somata lie in the CNS.

The synaptic vesicles are mostly agranular and about 15–30 nm in diameter (figures 24–26, 37, 38, 43–48). A few larger ones occur, with diameters of 40–60 nm (figures 25, 38 and 45), some of these have a dense core or dense content (figures 26, 35 and 41). At the zone of synaptic contact, the vesicles cluster presynaptically at the membrane (figures 24, 25, 43 and 44). The synaptic cleft is about 20 nm wide, with the membranes being symmetric. Electron-dense cleft material can often be seen in a striated arrangement (figures 24, 25, 38 and 48).

The efferent endings are very numerous on the hair cells and neurons, and often occur in palisades. In a single section, 15 efferent endings have been seen in contact with the base of

a single large secondary hair cell (figure 35), and 30 efferent endings have been found, over a length of 15 μm, in synaptic contact with an axon of a large first-order afferent neuron. However, the total number of efferent endings on the soma of a single hair cell, or a given length of an axon cylinder, is not known, since no quantitative analysis has been performed.

In the sections, the great majority of the efferent endings have been seen in synaptic contact with only one hair cell, or one neuron. Occasionally, however, a single ending was found to be in contact with two hair cells simultaneously (figure 38), with the axons of two neurons, or with one hair cell and an axon of a neuron, and some efferent endings have also been observed in synaptic contact with other efferent profiles. However, the complete arrangement of synaptic contacts of a single efferent neuron is even more complex. Because there are many more efferent synaptic contacts in the crista epithelia than efferent fibres in the relevant crista nerves (see §3.4.1, §3.4.2 and table 5), there is either a considerable branching of the efferent fibres, or a single fibre, without branching, makes *en passant* synaptic contacts with several hair cells and/or neurons.

Most of the efferent endings form a flat or slightly curved synaptic contact with either the somata of the primary or secondary hair cells (figures 35 and 38), or with the axons of the first-order afferent neurons (figures 41–44). Occasionally, efferent endings make such a flat synaptic contact with small processes originating from the base of a hair cell (figures 36 and 37).

Some efferent profiles have been observed distally between the hair cells, usually between the two large hair cells in the middle of the crista ridge, or between the large and fairly large hair cells respectively. The profiles are always densely filled with vesicles, most of which are

DESCRIPTION OF PLATE 1

Arrangements of hair cells, as shown by their kinociliary groups, in the two types of crista sections of *Octopus*. The cupulae have been removed. (Figures 4–7 are of a small animal of 90 g; see table 1.)

FIGURES 4 AND 6. Crista C3, showing the arrangement of the kinociliary groups of the different hair cells of the odd-numbered crista sections (C1, C3, C5, C7, C9; characterized by a double row of large hair cells) with small dorsal primary hair cells (dhc), two regular rows of large secondary hair cells (lhc), and small ventral secondary hair cells (vhc).

FIGURES 5 AND 7. Crista C6 (figure 5) and C2 (figure 7), showing the arrangement of the kinociliary groups of the different hair cells of the even-numbered crista sections (C2, C4, C6, C8; characterized by a single row of large hair cells) with small dorsal primary hair cells (dhc), one regular row of fairly large (flhc) and one of large (lhc) secondary hair cells, and small ventral secondary hair cells (vhc).

FIGURES 8 AND 9. Elongated groups of short kinocilia of presumably young large secondary hair cells (lhc) at the ends of an odd-numbered and even-numbered crista section. Crista C3 adjacent to C2 (figure 8); crista C8 adjacent to C7 (figure 9).

DESCRIPTION OF PLATE 2

Cellular organization of the crista sections of *Octopus*, as seen with the light microscope.

FIGURE 10. Cross section of crista C3, with small dorsal primary hair cells (dhc), two large secondary hair cells (lhc), small ventral secondary hair cells (vhc), and large (ln) and small (sn) first-order afferent neurons. Dorsal is to the right of the figure, ventral is to the left.

FIGURES 11 AND 12. Tangential sections of crista C1, showing the double-row of large secondary hair cells (lhc), small dorsal primary hair cells (dhc) with axons and a large first-order afferent neuron (ln).

FIGURE 13. Small dorsal primary hair cells (dhc) in a tangential section of crista C7.

FIGURE 14. Tangential section of crista C4, with one row of large secondary hair cells (lhc), and several large first-order afferent neurons (ln) on the right.

FIGURE 15. Large first-order afferent neurons (ln) in a tangential section of crista C3.
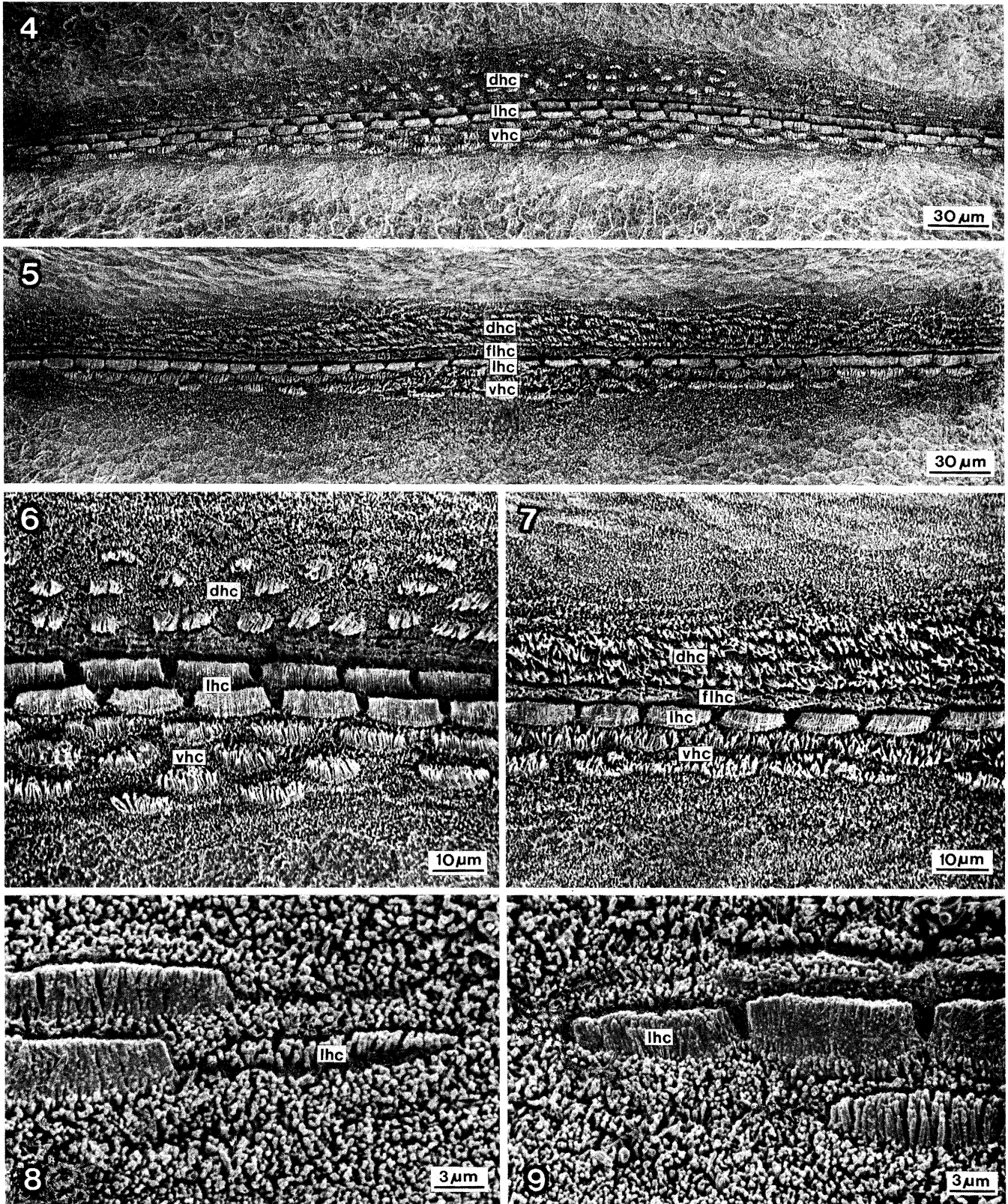
FIGURES 4–9. For description see opposite.
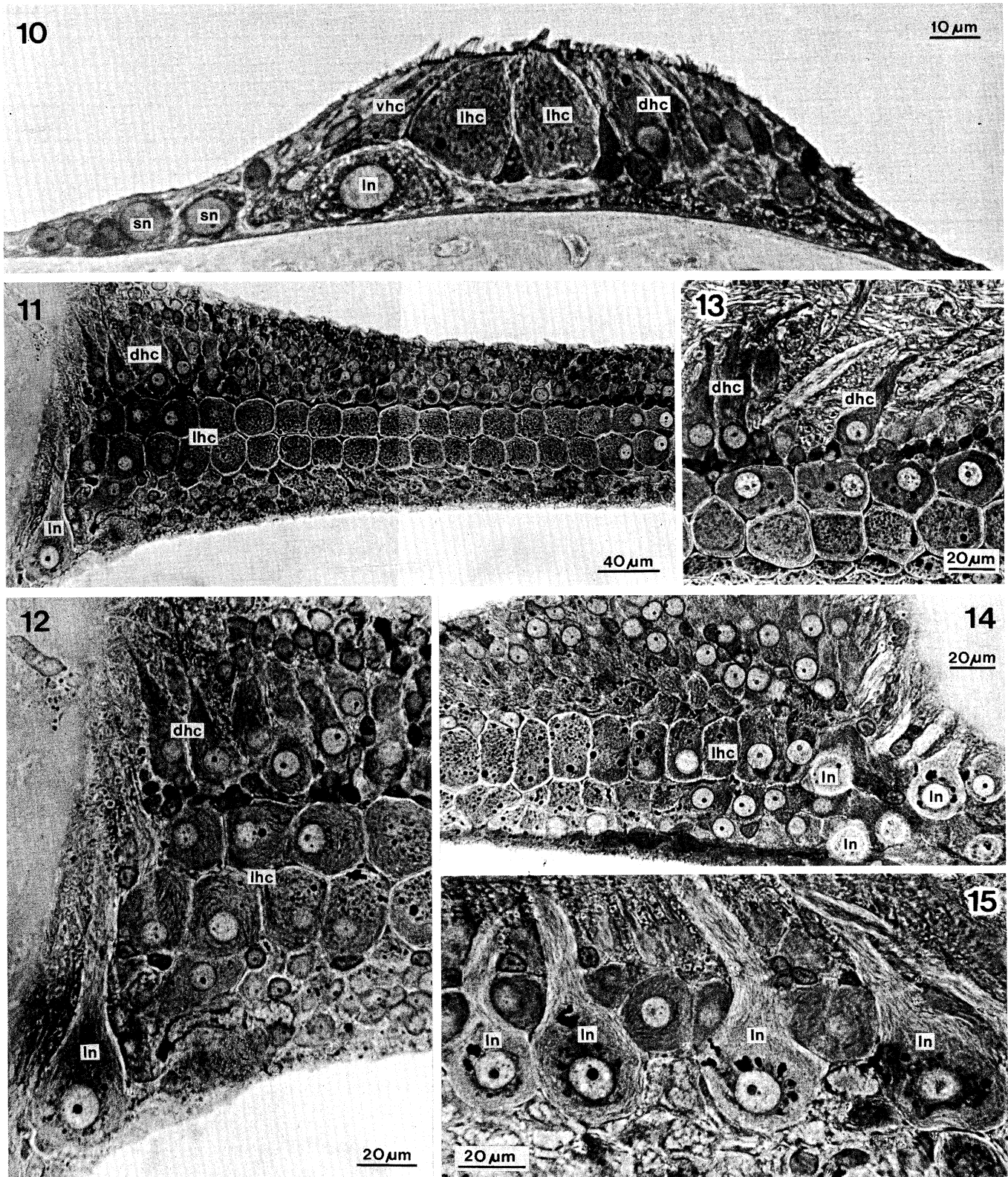
FIGURES 10–15. For description see page 320.
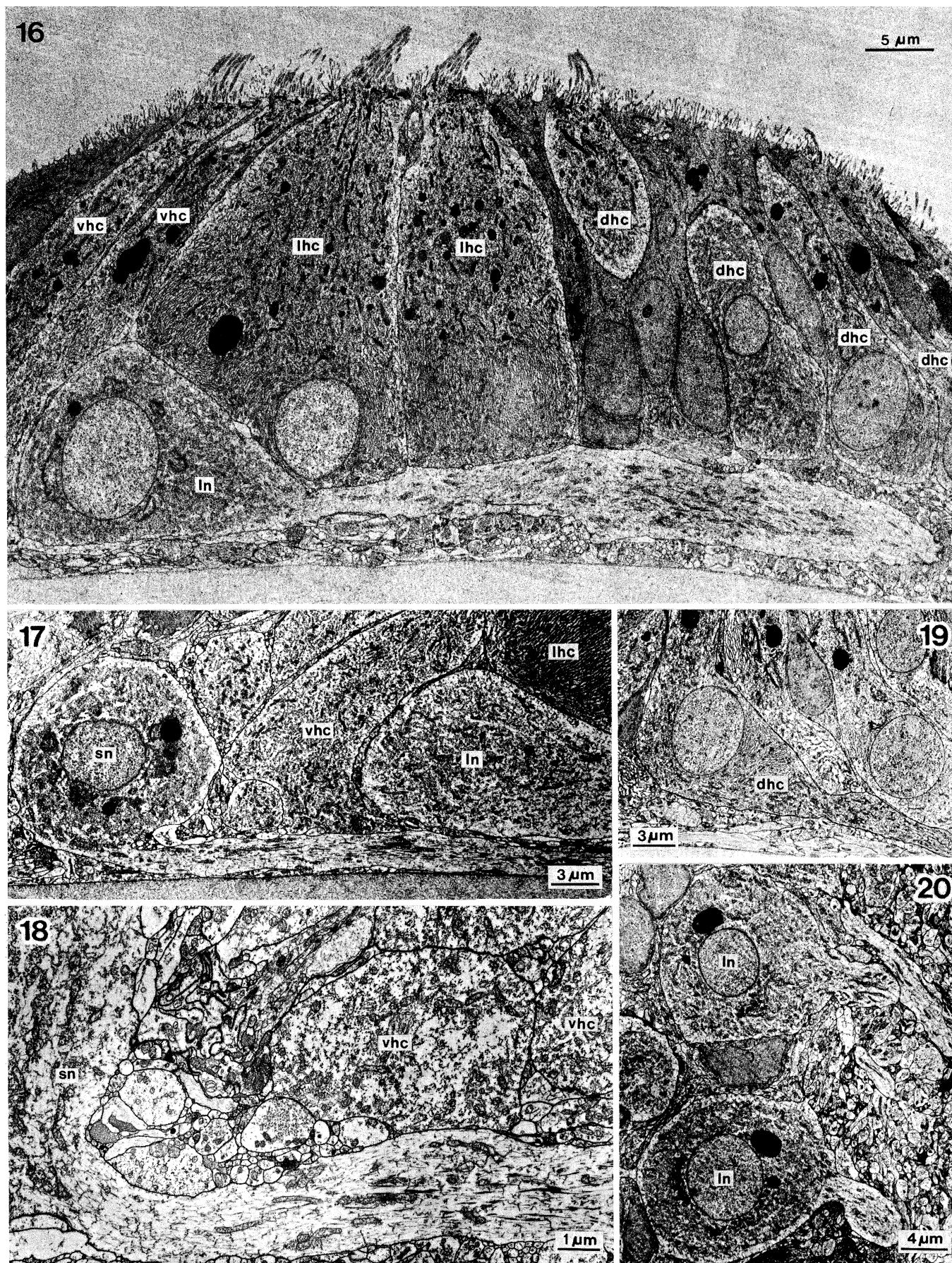
FIGURES 16–20. For description see facing plate 4.
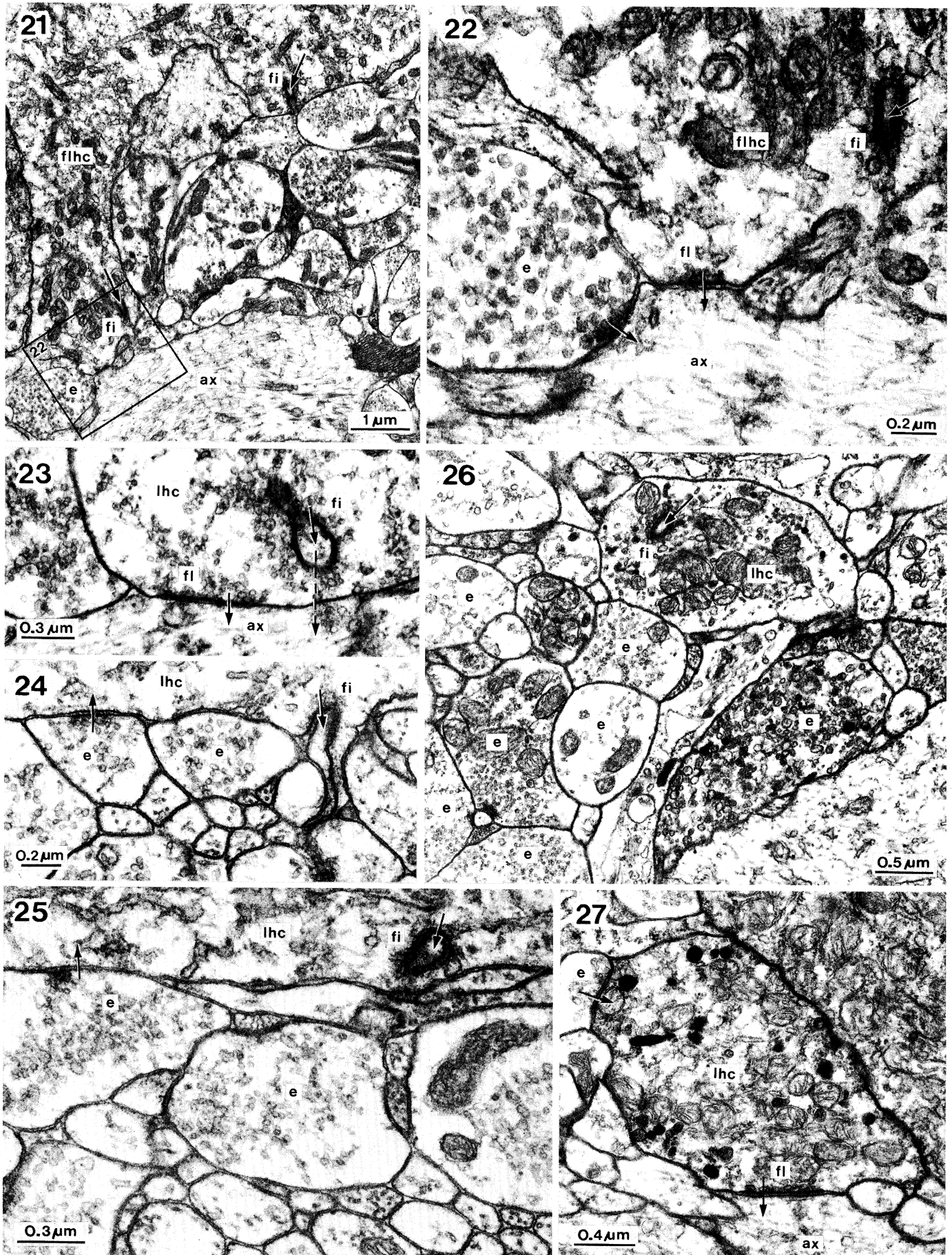
FIGURES 21–27. For description see opposite.

DESCRIPTION OF PLATE 3

Cellular organization of the crista sections of *Octopus*, as seen with the transmission electron microscope.

FIGURE 16. Cross section of crista C3, with two large secondary hair cells (lhc), small dorsal primary hair cells (dhc), small ventral secondary hair cells (vhc), supporting cells in between, and large first-order afferent neurons (ln). Dorsal is to the right of the figure, ventral is to the left. The cupula has been removed.

FIGURES 17 AND 18. Large (ln) and small (sn) first-order afferent neurons below and beside the large (lhc) and small ventral (vhc) secondary hair cells. Cross section of crista C3.

FIGURE 19. Small dorsal primary hair cell (dhc, slightly retouched). Cross section of crista C2.

FIGURE 20. Two large first-order afferent neurons (ln). Tangential section of crista C6.

DESCRIPTION OF PLATE 4

Afferent synapses in the secondary sensory hair cells of the crista of *Octopus*, and their connections to the axons of the first-order afferent neurons. Arrows indicate the direction of information flow across the synapses.

FIGURES 21 AND 22. Flat (fl) and finger-like (fi) afferent synaptic contacts between a fairly large secondary hair cell (flhc) and the axon (ax) of a large first-order afferent neuron. To the left, an efferent profile (e), in synaptic contact with the same axon. Crista C4.

FIGURE 23. Flat (fl) and finger-like (fi) afferent synaptic contacts between a large secondary hair cell (lhc) and the axon (ax) of a large first-order afferent neuron. The connection of the finger-like postsynaptic profile to the axon was confirmed by examining serial sections. Crista C3.

FIGURES 24 AND 25. Finger-like (fi) afferent synapses at the base of a large secondary hair cell (lhc). To the left of the figure, efferent profiles (e) in synaptic contact with the hair cell. Crista C2 (figure 24) and C8 (figure 25).

FIGURE 26. Cross section of a profile of a large secondary hair cell (lhc) with a finger-like afferent synapse (fi). The efferent profiles (e) show various kinds of synaptic vesicles. Crista C8.

FIGURE 27. Flat afferent synaptic contact (fl) between a large secondary hair cell (lhc) and an axon (ax) of a large first-order afferent neuron. To the left of the figure, an efferent profile (e) in synaptic contact to the hair cell. Crista C2.

DESCRIPTION OF PLATE 5

Silver impregnations of efferent nerve fibres (compare text) innervating the crista sections of *Octopus*. Flattened whole-mounts, dorsal is top of plate.

FIGURE 28. Plexus of efferent nerve fibres dorsal to crista C1–C3. Note bundles of very fine fibres running long distances dorsal and parallel to the crista sections. Holmes's stain.

FIGURE 29. Dense plexus of efferent nerve fibres dorsal to crista C3. Note bundles of fine fibres running parallel to the crista section. Ventrally, faintly stained somata (s) of first-order afferent neurons can be seen, together with their thick axons running parallel in a dorsal direction. Fraser-Rowell's stain.

FIGURE 30. Plexus of fine efferent nerve fibres in the wall of the statocyst sac, dorsal to the small primary hair cells (dhc). Crista C3. Fraser-Rowell's stain.

FIGURES 31 AND 32. Plexus of efferent nerve fibres intermingling with the dorsal primary hair cells (dhc) (figure 31, crista C3; enlargement of figure 28) and surrounding the large secondary hair cells (lhc) (figure 32, crista C1). Bracket indicates double row of large secondary hair cells. Ventrally are nuclei (s) of large first-order afferent neurons. Holmes's stain.

FIGURE 33. Efferent fibres surrounding the somata (s) of large first-order afferent neurons. Crista C7. Fraser-Rowell's stain.

FIGURE 34. Thick axons of large first-order afferent neurons (ax), in contact with efferent varicosities (compare with figure 41). Crista C1. Fraser-Rowell's stain.
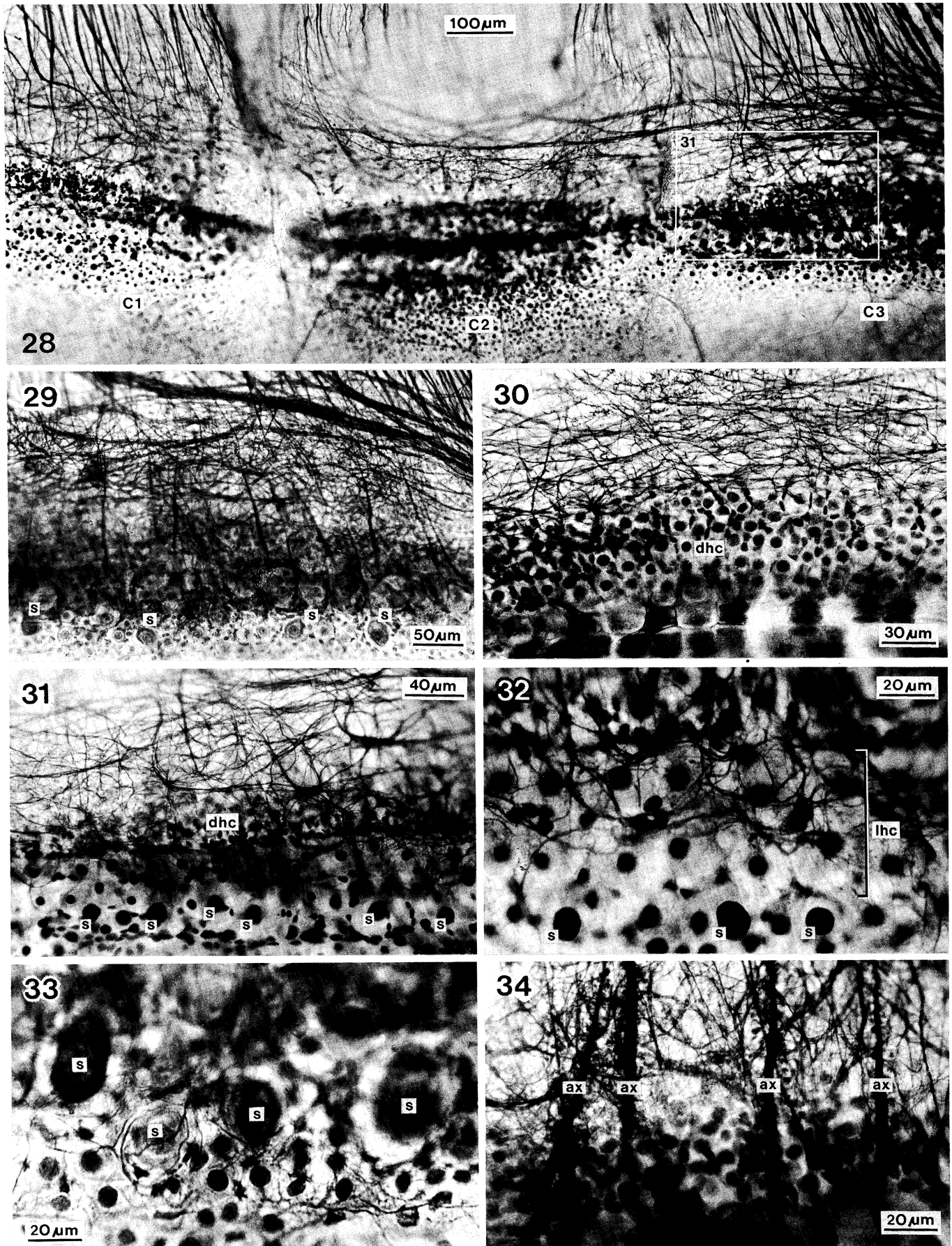
FIGURES 28–34. For description see facing plate 4.
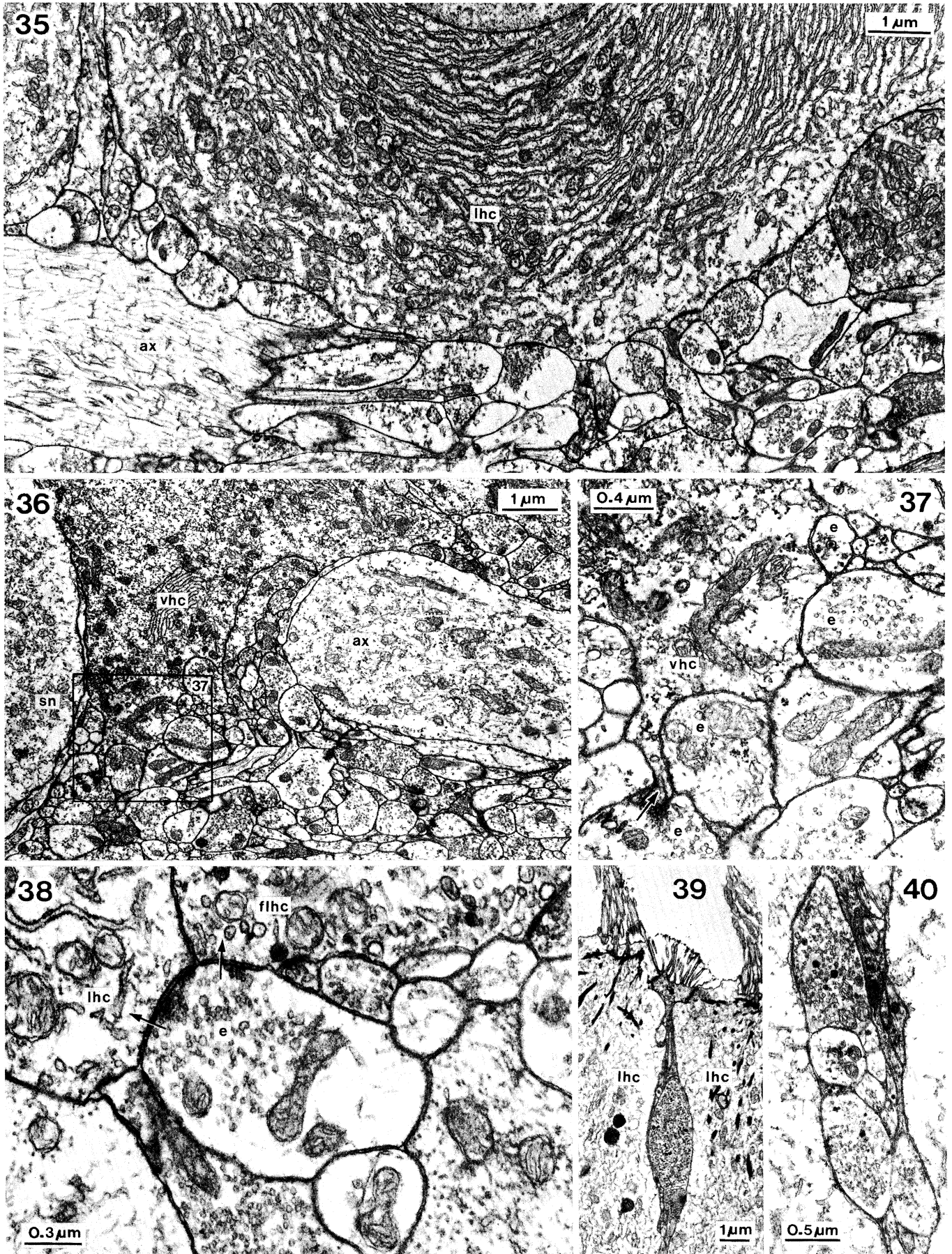
FIGURES 35–40. For description see page 321.
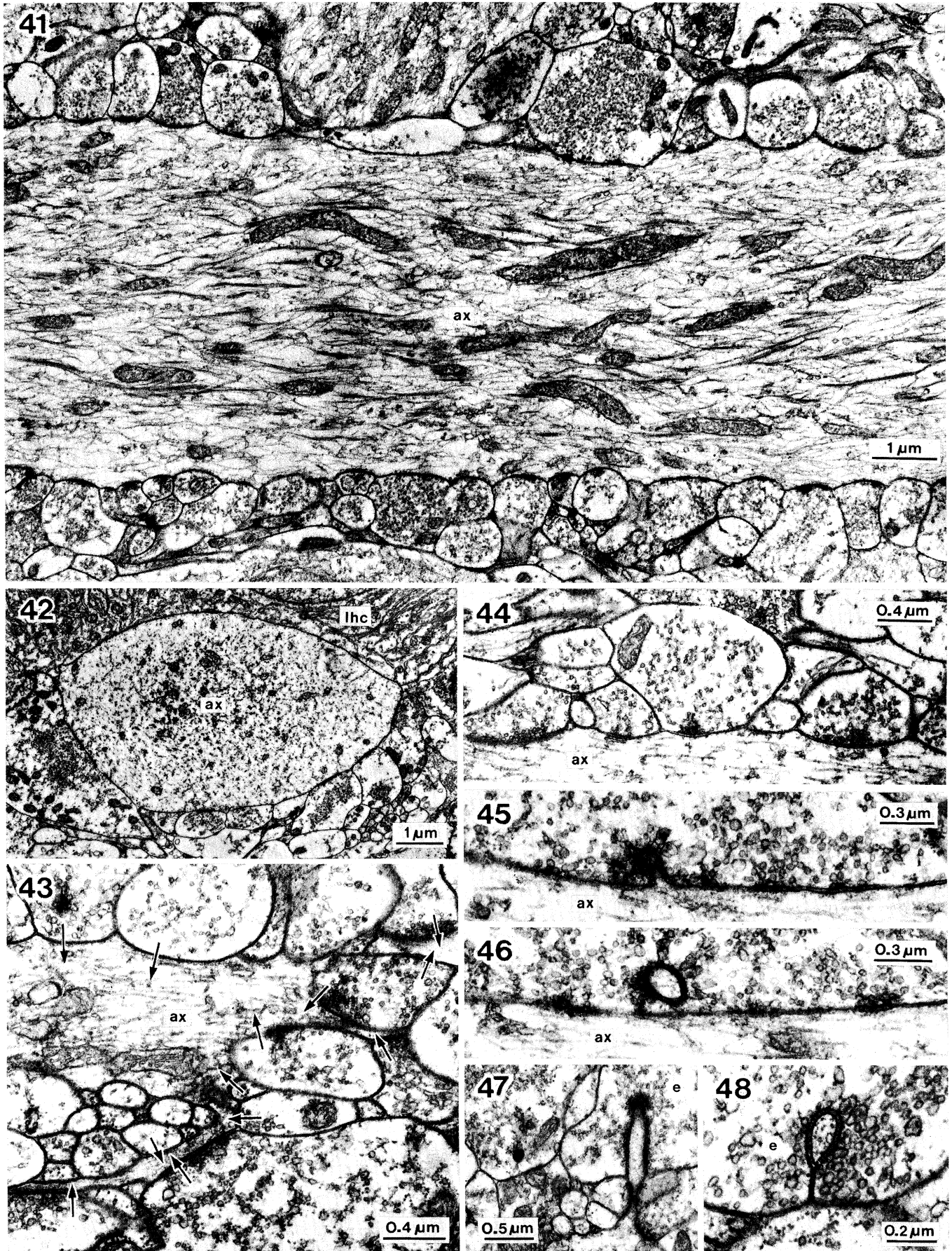
FIGURES 41–48. For description see opposite.

agranular and small (15–30 nm in diameter), but a few dense core and dense content vesicles have also been observed in these profiles (figures 39 and 40).

In addition to the flat synaptic contacts there are a few others in which one efferent profile covers a small dendritic process of an axon cylinder (figures 45–48); this finger-like synaptic contact can occur in close proximity to flat ones, and even in the same efferent ending (figures 45 and 46). Some axodendritic processes can be seen synaptically surrounded by two or three efferent endings (figure 26).

3.3. *Numbers and diameters of the fibres of the crista nerves*

Three nerves connect the nine crista section with the cns: the anterior, the medial and the posterior crista nerves. They all contain afferent and efferent fibres that project to, and come from respectively, various parts of the cns (Budelmann & Young 1984). The numbers and diameters of the fibres have been measured so far only at the level of light microscopy and amounted to 3085 fibres in all three crista nerves (Young 1965). However, this technique gave no detailed data about the fibres below 2 μm. At the level of transmission electron microscopy, cross-sections have now shown that the three crista nerves, at the point where they pass separately through the cranial cartilage, contain about 8700 fibres, with 7400 fibres (85%) less than 1 μm in diameter (figure 56; table 3).

The anterior crista nerve is the smallest (0.005 mm²) and contains roughly 1200 fibres

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DESCRIPTION OF PLATE 6

Efferent profiles in synaptic contact with the hair cells of the crista of *Octopus*.

FIGURE 35. Base of a large secondary hair cell (lhc), in contact with a large number of efferent profiles. To the left, axon (ax) of a small first-order afferent neuron. Crista C3.

FIGURE 36. Section through the nerve plexus below a small ventral secondary hair cell (vhc), with many efferent profiles, and the somata (sn) and axon (ax) of a small first-order afferent neuron. Crista C4.

FIGURE 37. Efferent profiles (e), one in synaptic contact with a small process of a small ventral secondary hair cell (vhc). Enlargement of figure 36. Crista C4.

FIGURE 38. One efferent profile (e) in synaptic contact with a large (lhc) and a fairly large secondary hair cell (flhc). Crista C8.

FIGURES 39 AND 40. Profiles, densely packed with synaptic vesicles, distally between two large secondary hair cells (lhc). Crista C7 (figure 39) and C1 (figure 40).

DESCRIPTION OF PLATE 7

Efferent profiles in the crista of *Octopus*, in synaptic contact with axons of first-order afferent neurons.

FIGURE 41. Axon (ax) of a large first-order afferent neuron, in contact with a large number of efferent profiles. Crista C6.

FIGURE 42. Cross section of an axon (ax) of a large first-order afferent neuron (below a large secondary hair cell (lhc)), surrounded by efferent profiles. Crista C5.

FIGURE 43. Several efferent profiles in synaptic contact with an axon (ax) and a small collateral of a first-order afferent neuron. Arrows indicate the direction of information flow across serially confirmed synapses. Crista C4.

FIGURE 44. Palisades of efferent profiles in synaptic contact with an axon (ax) of a large first-order afferent neuron. Crista C4.

FIGURES 45 AND 46. Efferent profile in synaptic contact with an axon (ax) of a first-order afferent neuron. Two successive sections showing one finger-like and several flat synaptic contacts in one and the same profile. Crista C5.

FIGURE 47 AND 48. Finger-like efferent synapses. The efferent profile (e) is invaginated by a postsynaptic axonal spine of a first-order afferent neuron. Crista C5 (figure 47) and C3 (figure 48).
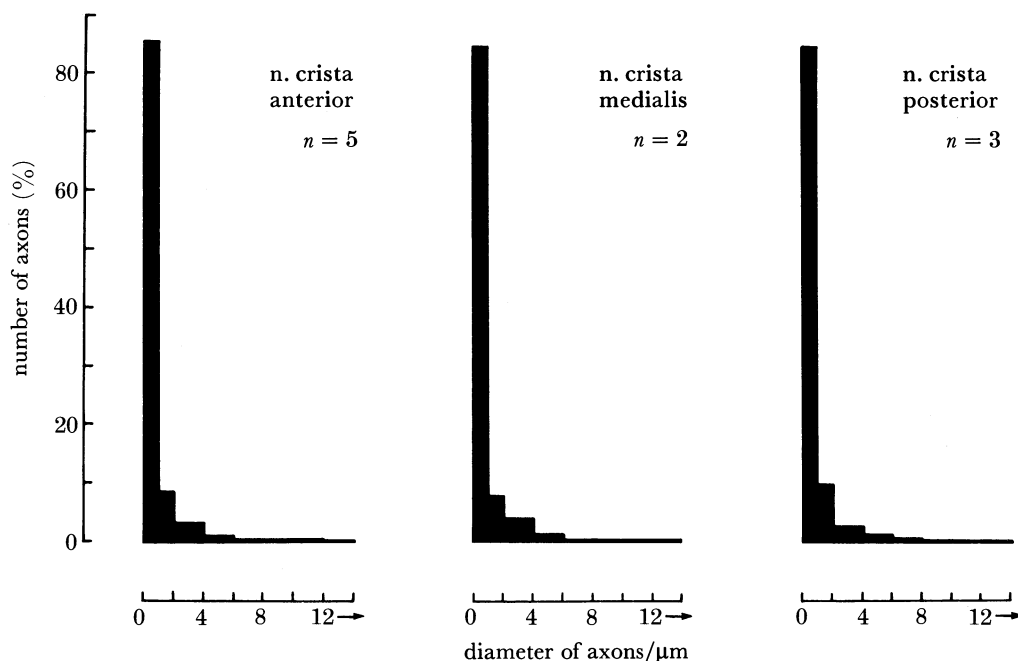
FIGURE 56. Histograms showing the fibre-size composition of the three crista nerves of *Octopus*.
For data see table 3.

(figures 50 and 54–56; table 3); the medial crista nerve is the largest (0.018 mm^2) and contains roughly 4300 fibres (figures 49 and 56; table 3); and the posterior crista nerve is intermediate in size (0.009 mm^2) and contains roughly 3200 fibres (figures 51 and 56; table 3). A few small-diameter fibres of the posterior crista nerve do not innervate the relevant crista sections, but branch off from the main trunk of the nerve as fine bundles and spread out into the posterior sac epithelium (figure 52). There, at least some of them, make efferent synaptic contacts with the somata of the scattered, ciliated cells, the ciliary tufts of which are known to beat (B. U. Budelmann & R. Starkmann, unpublished observations).

The fibres in all three crista nerves range from 18 μm in diameter to less than 1 μm (table 3). Their spatial distribution within the nerve is rather uniform with fibres of all sizes mixed (figures 53–55). No clear segregation into distinct bundles has been observed. Only the posterior crista nerve contains a separate component (about one third of the cross-sectional area of the nerve) with mostly small-diameter fibres (figure 51). There is no evidence of bimodality in fibre size-composition in any of the crista nerves (figure 56), as has previously been suggested (Young 1965).

In the cross sections of the nerves no distinction could be made between the afferent and the efferent fibres. However, because no branching of the fibres has been observed, the number of afferent fibres in a given nerve corresponds to the number of the primary sensory cells, plus the number of the first-order afferent neurons in the relevant crista sections (see §3.1 and §3.4). The number of efferent fibres in the nerve can then be calculated from the total number of fibres in the nerve, minus the number of afferent fibres. Such calculations have shown that in each of the crista nerves, roughly 75 % of the fibres are efferent, and only 25 % are afferent (tables 4 and 5).

TABLE 3. COUNTS OF AXONS IN SIZE CLASSES SHOWN FOR THE THREE CRISTA NERVES OF OCTOPUS VULGARIS

(† Data by courtesy of Dr W. F. Colmers.)

diameter of axons/μm	n. crista anterior							n. crista medialis				n. crista posterior				
	O123	O128	O138	O142	WFC†	x̄	%	O109	WFC†	x̄	%	O111	O109	WFC†	x̄	%
>12	1	0	0	0	20	4	0.3	7	25	16	0.4	3	3	10	5	0.2
10–12	1	0	0	7	8	3	0.3	11	11	11	0.3	15	6	11	11	0.3
8–10	4	2	4	5	12	5	0.4	15	21	18	0.4	21	11	14	15	0.5
6–8	7	3	4	3	14	6	0.5	19	37	28	0.7	26	22	31	26	0.8
4–6	13	3	17	9	28	14	1.1	35	85	60	1.4	32	44	48	41	1.3
2–4	21	19	42	40	86	42	3.4	73	301	187	4.4	69	60	146	92	2.8
1–2	115	49	62	88	206	104	8.4	115	597	356	8.3	256	295	393	315	9.7
<1	1529	1161	887	1115	603	1059	85.6	3719	3511	3615	84.2	2556	2615	3003	2725	84.4
total number of axons	1691	1237	1016	1267	977	1237	100	3994	4588	4291	100	2978	3056	3656	3230	100

TABLE 4. MORPHOMETRIC DATA OF THE NINE CRISTA SECTIONS C1–C9 OF OCTOPUS VULGARIS

(For each section, the average number (n = 2) of the various types of the primary and secondary hair cells, and of the first-order afferent neurons are given, as obtained with scanning electron (SEM) and light-microscopical (LM) as well as cobalt staining (cobalt) techniques. Note that the data of the large secondary hair cells (ventral row) are slightly different from those of table 1 because of the different numbers (n = 2, n = 14) of specimen included in the calculations. † For the data averages of the odd-numbered crista sections, C9 has not been included in the calculation because it has unusual characteristics (compare text).)

method		SEM	SEM	SEM	SEM	SEM	LM	cobalt	cobalt
type of cell		primary hair cells	secondary hair cells				afferent neurons		
		small dorsal	large (dorsal row)	fairly large	large (ventral row)	small ventral	large first-order	large first-order	small first-order
crista section									
n. cr. ant.	C1	77	23		25	45	11	14	67
	C2	179		34	30	57	18	17	127
	C3	99	29		31	49	18	19	112
n. cr. med.	C4	138		35	31	50	16	17	123
	C5	90	28		30	51	20	18	94
	C6	167		35	33	59	16	16	104
n. cr. post.	C7	109	30		33	66	15	17	71
	C8	178		36	36	59	19	15	107
	C9	67	13		18	31	9	8	29
total C1–C9		1104	263		267	467	142		834
x̄ C1–C9		123	29		30	52	16	16	93
x̄ C1, C3, C5, C7†		94	28		30	53	17	17	86
x̄ C2, C4, C6, C8		166		35	33	56	17	17	115

TABLE 5. THE NUMBERS AND PERCENTAGES OF THE EFFERENT AXONS IN THE THREE CRISTA
NERVES OF *OCTOPUS VULGARIS*

(These were obtained by the total number of axons in the nerve (A) minus the sum of the number $(=$ axons$)$ of the small dorsal primary hair cells (B) and the number $(=$ axons$)$ of the large and small first-order afferent neurons (C) in the relevant crista sections. The data of A are taken from table 3, those of B and C are based on table 4.)

nerve and crista sections innervated	A	B	C	$A-(B+C)$‡	$p(\%)$†
n. crista anterior C1, C2/2	1237	167	152	918	74
n. crista medialis C2/2, C3, C4, C5, C6/2	4291	500	515	3276	76
n. crista posterior C6/2, C7, C8, C9	3230	438	309	2483	77

† p is the percentage of efferent axons in the nerve.   ‡ The number of efferent axons in the nerve.

3.4. Centrifugal cobalt filling of the crista nerves

By centrifugal cobalt filling, the courses of the crista nerves can easily be traced to the relevant crista sections. Also, by this technique, the somata of only those crista cells that send their axons directly into the nerve will be stained, i.e. the primary hair cells and the first-order afferent neurons, but no filling of somata of the secondary hair cells will occur (see §3.1 and §3.2). From the data obtained it is obvious that only the afferent fibres in the crista sections were stained, and not the efferent ones (most probably for technical reasons; compare with Budelmann & Young (1984)). In addition to *Octopus*, a few fillings have also been performed in *Eledone moschata*; the results were identical and will be described together.

3.4.1. Afferent innervation of the crista sections

Anterior crista nerve. The afferent fibres of the anterior crista nerve do not form a compact bundle on the statocyst sac. After passing through a hole of the cartilaginous cranium, the fibres spread out, like a fan, and then run almost parallel straight down to the first crista sections. As shown by the distribution of the stained somata, the anterior crista nerve, contrary to Young (1960), innervates only crista section C1 and the first half of C2, which is adjacent to C1 (figures 57, 60, 61 and 71).

Medial crista nerve. This nerve splits into two main divisions on its way to the crista sections. The majority of its afferent fibres form a compact bundle that runs posteriorly in a curve to crista sections C4, C5 and C6; but some of the afferent fibres branch off sequentially from the main trunk of the nerve and run in an almost parallel course straight down to crista sections C2 and C3 (figures 58 and 74). As shown by the distribution of the stained somata, the medial crista nerve, again contrary to Young (1960), innervates the second half of crista section C2, which is adjacent to C3, sections C3, C4, C5 and the first half of C6, which is adjacent to C5 (figures 58 and 62–66).

Posterior crista nerve. The afferent fibres of the posterior crista nerve approach medially, as a compact bundle, the vertical crista sections, cross the crista ridge at the very end of section C9 (figures 59 and 70), and then run downwards to innervate successively sections C9, C8 and C7 laterally, and some fibres proceed to the horizontal section C6. The distribution of the stained somata again show that, contrary to Young (1960), the posterior crista nerve innervates the second half of crista section C6, which is adjacent to C7, and sections C7, C8 and C9 (figures 59 and 67–70).

3.4.2. *Cellular organization of the crista sections*

The results obtained from the centrifugal cobalt fillings of the crista nerves agree with those obtained at the level of light and electron microscopy (see §3.1 and §3.2).

In all crista sections, the somata of the small dorsal hair cells were stained, thus again indicating that these cells are primary sensory cells (figures 72 and 73; see also figures 60–71). Over the length of a single crista section, the pear-shaped somata of these cells, as well as their axons, are not arranged in a parallel manner, but diverge fan-like from the crista section (e.g. figures 63, 68 and 73)

No signs of staining occurred in the somata of the large and fairly large hair cells, or in those of the small ventral hair cells (figure 72; see also figures 60–71). This again indicates that these cells are secondary sensory cells.

The somata of the large and small first-order afferent neurons were deeply stained in all crista sections (figures 60–72). Many of them look like unipolar neurons; however, there are others which have an irregular shape and might be bipolar (e.g. figure 67) or multipolar (e.g. figures 60 and 61), with short dendrites (but see §4.1.4). In the first crista sections C1–C4 the axons of the majority of the neurons run almost straight in a dorsal direction (figures 60–64). In the sections C5–C9, however, they all turn to one side when leaving the epithelium to join the relevant crista nerve (figures 65–70). Only in section C6 which is innervated by two crista nerves (see §3.4.1), do they turn in different directions, depending on which of the nerves they join (figures 66 and 67). In all crista sections there are a few axons of large as well as small, mostly neighbouring, neurons that cross (figures 61, 66, 68 and 70).

As has already been described at the level of light and electron microscopy (§3.1 and §3.2), the cobalt staining technique shows differences in the cellular organization of the double-row (C1, C3, C5, C7, C9) and the single-row (C2, C4, C6, C8) crista sections. These differences concern three of the six types of cells: the small dorsal hair cells, and the large and small first-order afferent neurons.

Small dorsal hair cells. In all cobalt filling experiments the number of the small dorsal hair cells that were stained was roughly twice as great in the single-row crista sections, with an average of 166 hair cells per section, compared with the double-row ones, with an average of 94 hair cells per section (figures 57–69). For additional data see §3.1 and table 4.

Large first-order afferent neurons. In both types of crista sections the numbers of the stained large neurons were the same and totalled 17 (C9: 9) (table 4). The diameter of the somata, however, were smaller in the single-row than in the double-row sections (15–25 μm, and 20–35 μm respectively) (e.g. figures 60, 61, 68, 69 and 71).

Small first-order afferent neurons. In the single-row crista sections an average of 115 small neurons were stained, whereas in the double-row sections there were only 86 (C9: 29); those in the double-row sections extended further ventrally from the middle of the crista ridge (e.g. figures 58, 63, 64 and 71; table 4). In both types of crista sections, the diameters of the small neurons were the same (5–15 μm).

3.5. *Structure of the cupula*

A cupula is attached to each of the nine crista sections. It is transparent in a living preparation and still invisible in alcohol or formalin-fixed material that is unstained (figure 74). After osmium fixation, however, provided that the statocysts were handled with extreme care during all steps of preparation, the cupulae can clearly be seen attached to the crista sections. The

cupulae appear as flap-like structures that project towards the middle of the cyst cavity. They are connected to the crista ridge along the whole length of the crista section.

The nine cupulae differ remarkably in form and size. A small cupula, type I, is attached to each of the odd-numbered crista sections C1, C3, C5, C7 and C9, whereas a large cupula, type II, is attached to each of the even-numbered crista sections C2, C4, C6 and C8 (plates 12 and 13); thus, small and large cupulae alternate regularly (figures 75–82).

The small cupula type I has an average length of 0.48 mm, which is equal to the length of the related crista section. Its height increases from both lateral edges towards the centre (figures 76 and 79), reaching a maximum of about 0.165 mm in the middle of the crista section. Its width similarly increases (figures 79–81), reaching a maximum of about 0.275 mm in the middle of the crista. Its area of lateral projection, as it is seen in figure 76, is about 0.065 mm^2, and its cross-sectional area (figure 77) is about 0.024 mm^2.

The large cupula type II has an average length of 0.43 mm and is more complicated in form. It has a tapered base and bulges in width and length (plate 13) at the centre of the cupula. It reaches its maximum height of about 0.480 mm at the middle of the crista section and, owing to its central bulge, its maximum width of about 0.230 mm at half its height. Its area of lateral projection, as is seen in figures 76 and 83, is about 0.13 mm^2, and its cross-sectional area (figure 78) is about 0.052 mm^2, i.e. both areas are about twice as large as those of cupula type I.

Both cupulae, type I and type II, are composed of a dense meshwork of long fibrils (figures 84–90). These vary in diameter from 10–50 nm in specimens fixed for transmission electron microscopy (figures 93–96), and from 100–300 nm in specimens fixed for scanning electron microscopy (figures 88–90), with some fibres being densely packed with varicosities (figure 90). The fibrils are tube-like and often show a dense core (figures 94–96). Many of the fibrils are interconnected or even branch (figures 88 and 90). In material fixed for scanning electron microscopy, the cupula fibrils sometimes appear to be embedded in a homogeneous matrix (figures 85 and 86), but this is not seen in all preparations. Towards its base, the cupula is characterized by distinct cupula particles which are composed of cupula fibrils embedded in a homogeneous matrix of medium electron density (figures 95 and 96). At a constant distance of 3 μm from the luminal surface of the crista cells, and 1.5 μm from the tips of their microvilli respectively, these cupula particles form a clear line of demarcation to a subcupular space (figures 91 and 92).

Both cupulae, type I and type II, are connected to the receptor cells and to the adjacent tissue of the crista ridge in two ways: in a direct connection to the receptor cells, and an indirect connection to the supporting cells (plate 15). The direct connection to the receptor cells is achieved by the cupula particles such that they directly contact the distal tips of a few, or all, kinocilia of the receptor cells (figures 93 and 95). An additional direct connection is made laterally to the distal ends of those kinocilia of the ciliary group that face into the direction of its morphological polarization, which is away from that of its inclination (figure 95). No cupula particles have ever been seen in lateral contact with the kinocilia of the inclined side of the ciliary group. On the other hand, the indirect connection of the cupula to the supporting cells is achieved by a meshwork of fine filaments that traverse the subcupula space and connect the cupula particles to the distal ends of the microvilli of the supporting cells, in-between and lateral to the receptor cells (figures 92–94). These fine filaments can also be seen linking the various kinocilia of a single receptor cell, the microvilli of the supporting cells, and the kinocilia and microvilli of adjacent cells (figures 93 and 94). In some preparations the filamental

connections between the kinocilia and microvilli of a receptor cell are especially dense within a zone about 0.3 µm wide and 0.5 µm above the surface of the cell (figure 93).

The whole area of cupula attachment to the crista ridge differs for the two types of cupulae. The small wide-based cupula type I has a large area of attachment, with an asymmetric, more dorsally positioned cupula relative to the crista ridge (figure 84), and with cupula fibres that extend far out from the crista hair cells (figures 84 and 88). In contrast, the large narrow-based cupula type II has a small area of attachment (about one quarter of that of cupula type I), with a symmetric position on the crista ridge (figure 85), and cupula fibres that extend only a few micrometres from the outermost groups of kinocilia (figures 86 and 87).

4. DISCUSSION

The present investigation improves our knowledge of the structural organization of the *Octopus* angular acceleration receptor system in many details of morphometry, ultrastructure, and neuronal and synaptic organization. This invertebrate receptor system, in its level of complexity, rivals, and in some aspects even exceeds, its vertebrate counterpart, the semicircular canal system.

The striking division of the crista into differently organized sections, with alternately large and small cupulae, is the basis of the recent physiological finding of two crista sub-systems differing in their sensitivity ranges by an order of magnitude. This has been interpreted as an adaptation to the animal's two forms of locomotion, slowly crawling and fast swimming by jet propulsion (Williamson & Budelmann 1985 *a, b*). The paper describes many structural details of the crista and the cupula that have to be known for the performance and interpretation of physiological experiments on this unique system.

4.1. *Crista*

From the data presented it is evident that the *Octopus* crista is a highly complex sensory epithelium, with both primary and secondary sensory receptor cells, the latter being of two types and in contact with two types of first-order afferent neurons. Although no ultrastructural data of a crista system are available for any other cephalopod (with the exception of the polarization pattern of the crista hair cells in the statocysts of *Sepia* and *Loligo*, Budelmann (1977)), the light microscopical data of the crista of *Loligo* and *Vampyroteuthis* already point to a similar level of complexity (Stephens & Young 1976, 1982). Thus, the cellular organization of the crista of probably all cephalopods (with the exception of the nautiloids, which have no crista/cupula system) reaches the highest level of organization known for any angular acceleration receptor epithelium in either vertebrates or invertebrates (see, for example, Iurato *et al.* 1972; Wersäll & Bagger-Sjöbäck 1974).

4.1.1. *Crista dimensions*

In vertebrates, measurements of the dimensions of the angular acceleration receptor systems allow inferences about their functional properties (see, for example, Jones & Spells 1963; Lindeman 1969; Melvill Jones 1974; Ramprashad *et al.* 1984), and a comparable attempt has recently been made for the cephalopod systems (Maddock & Young 1984). Any such comparative study is complicated by the fact that cephalopods, during almost their whole life, show a continuous and rapid growth. In *Octopus vulgaris* the growth rate varies between 1.14 %

DESCRIPTION OF PLATE 8

Crista nerves of *Octopus*.

FIGURES 49–51. Cross sections of the medial (n.cr.med.; figure 49), anterior (n.cr.ant.; figure 50) and posterior (n.cr.post.; figure 51) crista nerves, at the point where the nerves pass through the cartilaginous brain capsule.

FIGURE 52. Fine bundles of efferent nerve fibres branching from the posterior crista nerve (n) into the posterior sac of the statocyst. Whole-mount preparation. Fraser-Rowell's stain.

FIGURES 53–55. Cross sections of the posterior (figure 53) and anterior (figures 54 and 55) crista nerves, showing the wide range of axon diameters. The axons are wrapped individually, or in bundles, by glia cell processes. Several nuclei (*) of glia cells are to be seen.

DESCRIPTION OF PLATE 9

Centrifugal cobalt fillings of the three crista nerves of *Octopus*. Flattened whole-mount preparations.

FIGURE 57. Cobalt filling of the anterior crista nerve, with somata stained in the crista sections C1 and C2 (first half, adjacent to C1).

FIGURE 58. Cobalt filling of the medial crista nerve, with somata stained in the crista sections C2 (second half, adjacent to C3), C3, C4, C5 and C6 (first half, adjacent to C5). Note the alternating differences between the odd and even crista sections concerning the number of the small dorsal primary hair cells, the diameter of the somata of the large first-order afferent neurons, and the number of the small first-order afferent neurons (compare figures 71–73).

FIGURE 59. Cobalt filling of the posterior crista nerve, with somata stained in the crista sections C6 (second half, adjacent to C7), C7, C8 and C9.

DESCRIPTION OF PLATE 10

Stained somata in the nine crista sections C1–C9 of *Octopus* after centrifugal cobalt filling of the three crista nerves. In all crista sections, only the small dorsal primary hair cells and the large and small first-order afferent neurons are stained, but not the large, fairly large or small secondary hair cells (for orientation see figure 72).

FIGURE 60. Stained somata in crista C1.

FIGURES 61 AND 62. Stained somata in crista C2. Its first half (adjacent to crista C1) is innervated by the anterior crista nerve (figure 61), its second half (adjacent to crista C3) is innervated by the medial crista nerve (figure 62).

FIGURE 63. Stained somata in crista C3.

FIGURE 64. Stained somata in crista C4.

FIGURE 65. Stained somata in crista C5.

FIGURES 66 AND 67. Stained somata in crista C6. Its first half (adjacent to crista C5) is innervated by the medial crista nerve, its second half (adjacent to crista C7) is innervated by the posterior crista nerve.

DESCRIPTION OF PLATE 11

FIGURE 68. Stained somata in crista C7.

FIGURE 69. Stained somata in crista C8.

FIGURE 70. Stained somata in crista C9.

FIGURE 71. Stained somata in crista C1 and C2, showing the differences of the diameter of the large first-order afferent neurons in the odd and even crista sections.

FIGURE 72. Stained somata in crista C5 (same preparation as in figure 65), photographed with a more closed condensor to show the unstained secondary hair cells: the double row of large hair cells (lhc) and the small ventral hair cells (vhc). (The somata of the large and small first-order afferent neurons are out of plane of focus.)

FIGURE 73. Stained somata in crista C5 (enlargement of figure 63), showing the small dorsal primary hair cells.

FIGURES 49–55. For description see opposite.

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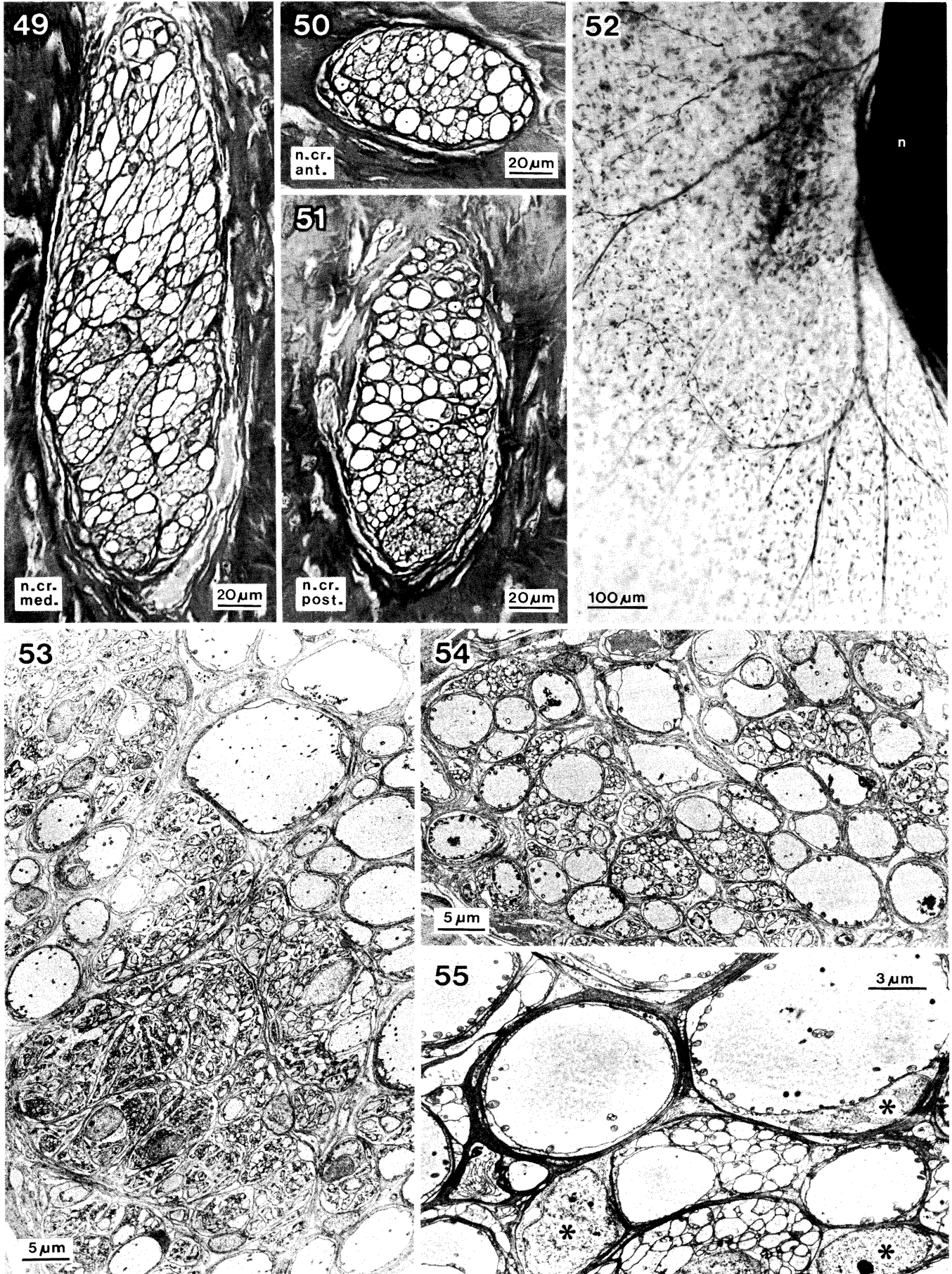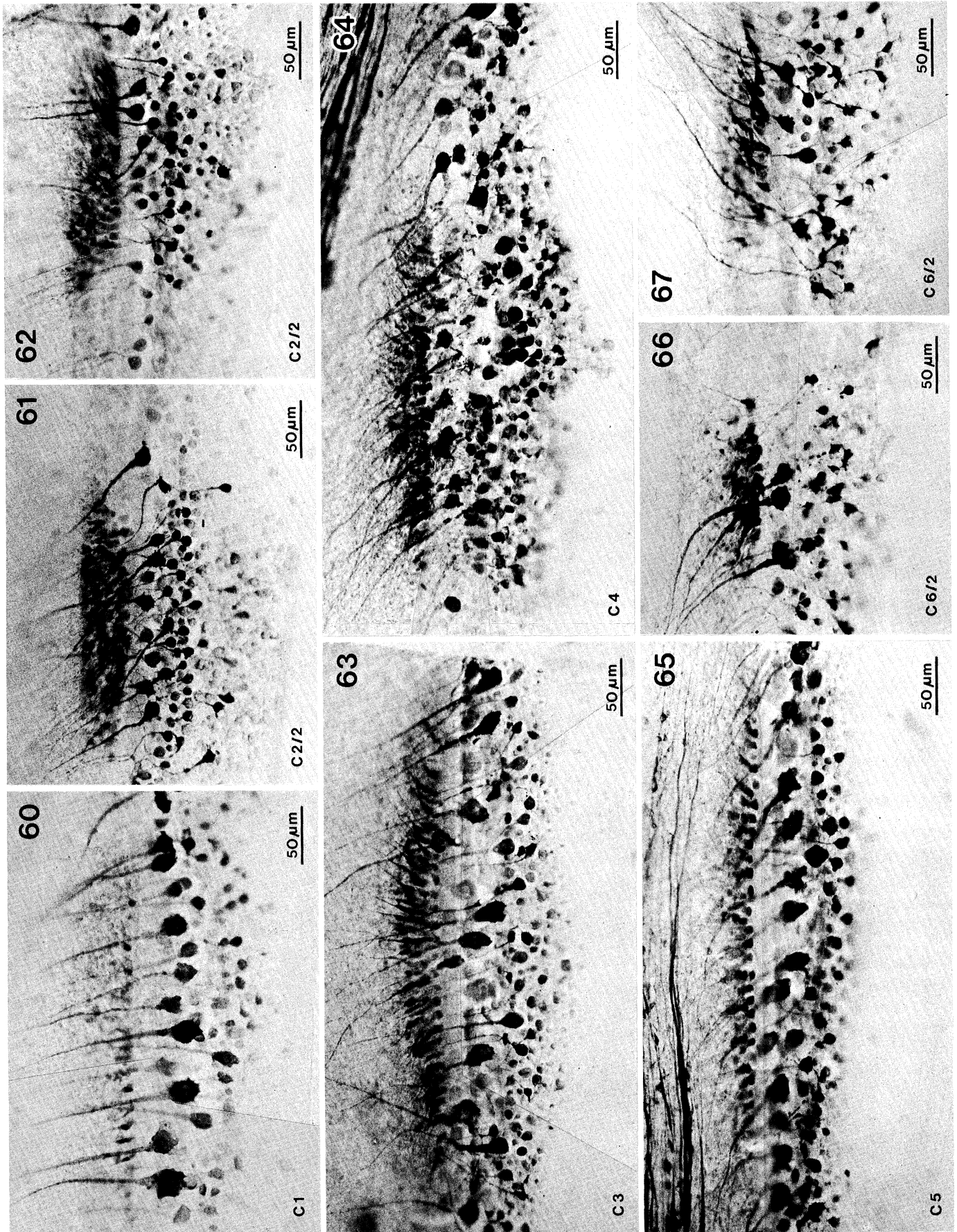
FIGURES 57–67. For description see page 328.

FIGURES 68–73. For description see page 328.

DESCRIPTION OF PLATE 12

Alternating differences in form size of the cupulae attached to the nine crista sections of *Octopus*. Small cupulae are attached to the odd-numbered crista sections (C1, C3, C5, C7, C9) and large cupulae are attached to the even-numbered crista sections (C2, C4, C6, C8).

FIGURE 74. Lateral view of the left statocyst, showing the orientation of the nine crista sections C1–C9. Formalin-fixed preparation; part of the statolith (st) and of the mucus layer attaching it to the sensory epithelium is decalcified. Crossbar indicates dorsal (d), ventral (v), frontal (f) and caudal (c).

FIGURES 75 AND 76. Ventral view into the opened right (figure 75) and left (figure 76) statocyst sac, showing the cupulae attached to the horizontally arranged crista sections. Figure 75 shows the cupulae attached to the cristae C1–C6, the statolith (st) and the cartilaginous anticrista (ac). Figure 76 shows the cupulae of the cristae C1–C5. Crossbar indicates medial (m), lateral (l), frontal (f) and caudal (c.). Osmium-fixed preparations.

FIGURES 77–83. For description see page 329.

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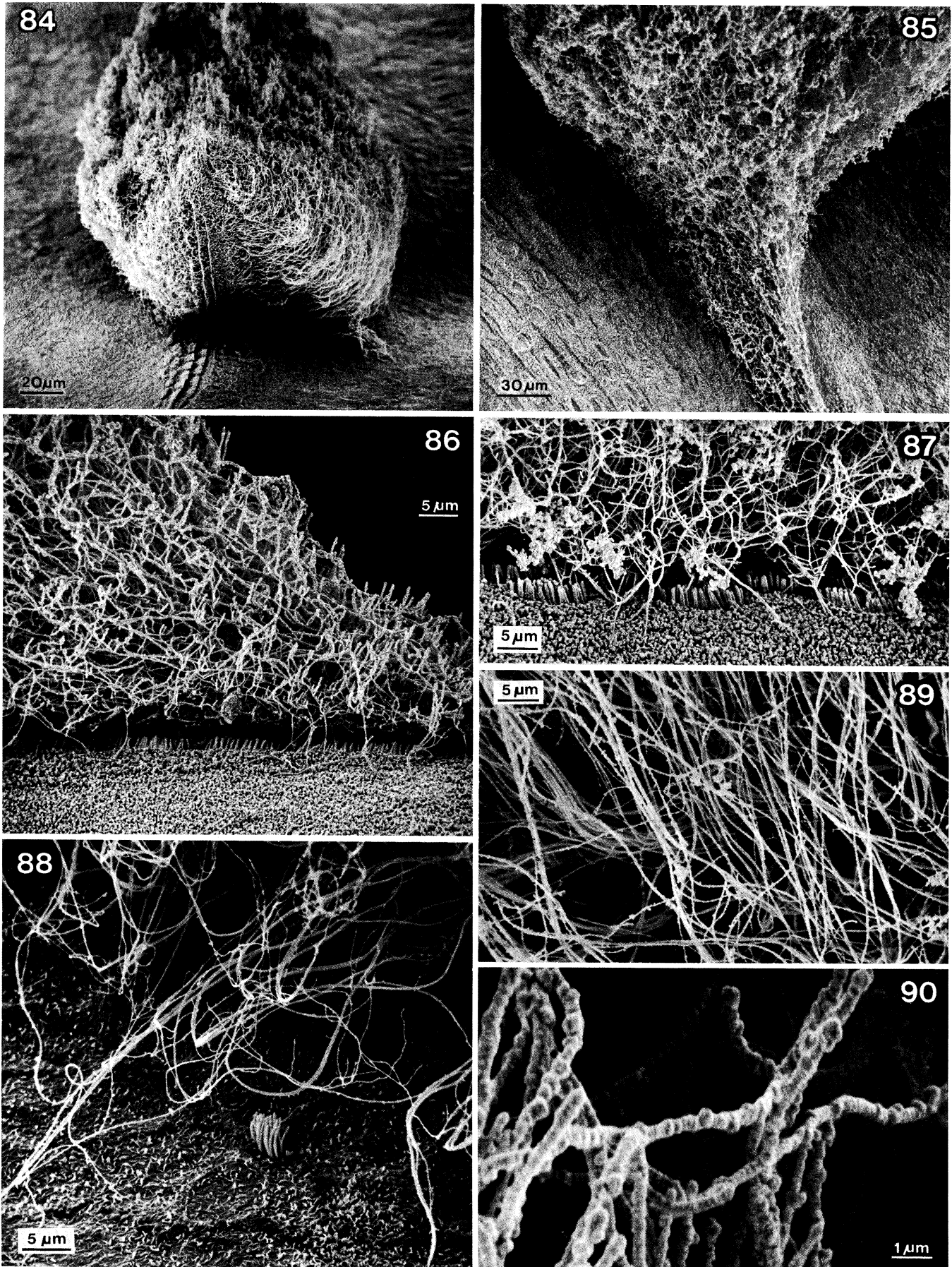
FIGURES 84–90. For description see page 329.
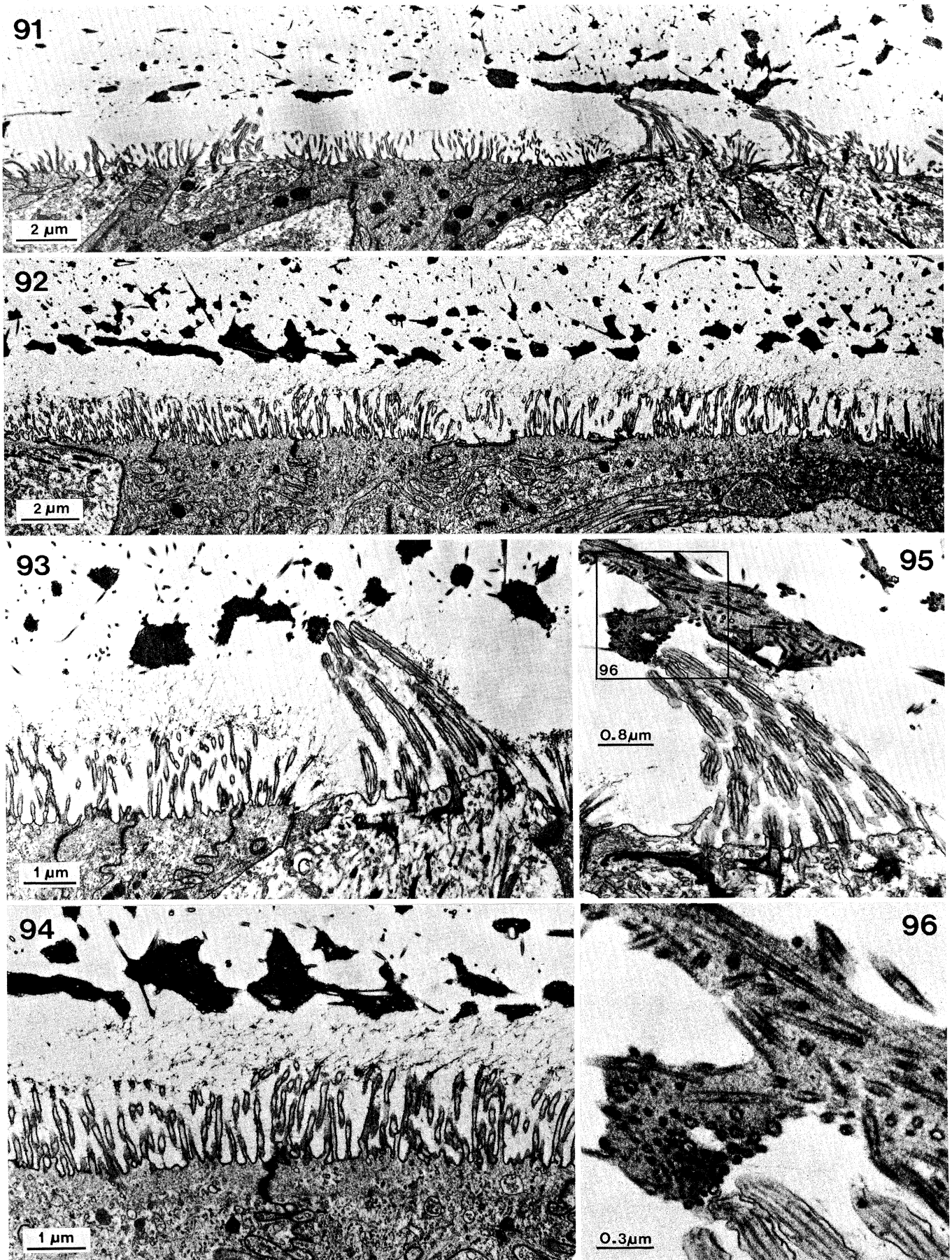
FIGURES 91–96. For description see opposite.

and 10.6% daily weight increase, with smaller animals having the higher growth rates (for references see Forsythe 1984). Thus, for comparative studies, dimensional data necessarily need the animal's size (mantle length in decapods; mass in octopods) as a reference system. But in octopods, the mass does not necessarily correlate with the animal's age, because there are large individual differences in the growth rate, and starvation can reduce their mass to about 50% without debilitation (Nixon 1966; Mangold & Boletzky 1973). This may explain the low correlation ($r = 0.62$) between the average length of a single crista section and the animal's mass (figure 2).

DESCRIPTION OF PLATE 13

The two types of cupulae attached to the crista sections of *Octopus*. Cupula type I is small and is attached to the odd-numbered crista sections (C1, C3, C5, C7, C9), cupula type II is large and is attached to the even-numbered crista sections (C2, C4, C6, C8).

FIGURES 77 AND 78. Cross sections, as seen with the light microscope, of the small cupula type I of crista C7 (figure 77) and of the large cupula type II of crista C4 (figure 78).

FIGURES 79–82. Small and large cupulae as seen with the scanning electron microscope. Dorsal is to the left of the figure.

FIGURE 79. Small cupula type I (crista C3) and large cupula type II (crista C2).

FIGURE 80. Large and small cupulae of the cristae C2–C5, showing the alternating occurrence of cupula type I and type II.

FIGURE 81. Small cupula type I (crista C3 and C5) and large cupula type II (crista C4), seen from the top.

FIGURE 82. Cupulae of cristae C3 and C4, showing the narrow attachment of cupula type II (crista C4) and the wide attachment of cupula type I (crista C3) to the crista ridge.

FIGURE 83. Large cupula type II (crista C8), seen from the side.

DESCRIPTION OF PLATE 14

The structure of the cupulae of *Octopus* and their attachment to the crista and surrounding epithelium.

FIGURE 84. Small cupula type I of an odd-numbered crista section (C3). Its wide base is in contact with the crista hair cells and the surrounding tissue. The cupula has become detached at one end and bends upwards. Note the asymmetric more dorsal position of the cupula relative to the crista ridge. Dorsal is to the right of the figure.

FIGURE 85. Large cupula type II of an even crista section (C4). Its small tapered base covers just the crista ridge.

FIGURE 86. Lateral view (from ventral) of the tapered end of a large cupula type II (crista C6). Note its regular distance from the kinocilia of the hair cells (compare figure 92) and the homogenous matrix in-between the cupula fibrils.

FIGURE 87. Basal part of a large cupula type II (crista C8) with fibrils in contact with microvilli of the tissue just beside the hair cells.

FIGURE 88. Fibrils of a small cupula type I (crista C7) extending far out from the crista ridge into the posterior sac, where ciliated cells occur.

FIGURES 89 AND 90. Fibrils of cupulae (figure 89, crista C8; figure 90, crista C3).

DESCRIPTION OF PLATE 15

Cupula attachment to the crista of *Octopus*.

FIGURE 91. Cross section of the apex of crista C1. Dorsal is to the left of the figure.

FIGURE 92. Longitudinal section dorsal and parallel to crista C5. Note the regular distance of the cupula from the epithelial surface.

FIGURES 93 AND 94. Attachment of the cupula to the kinocilia of a hair cell and to the microvilli of supporting cells. Note the fine filaments between the particles of cupula material and the microvilli of the supporting cells. Crista C5.

FIGURES 95 AND 96. Particles of cupula material in contact with the tip of the kinociliary bundle of a hair cell. The cupula particles show fibrils, some with a dense core, embedded in a matrix of medium electron density. Crista C1.

Moreover, in comparative dimensional studies the mode of fixation has to be taken into account, since most fixations are known to cause shrinking of the tissue to differing extents. In the osmium-fixed material used, the shrinking of the length of a single crista section was about 15%, compared with that of formalin-fixed material.

The crista dimensions, and all other morphometric data given in this paper, are of those animal sizes that are mostly used in laboratory research, and they correspond to data that were previously described for a more limited number of animals and less criteria (Young 1960; Budelmann et al. 1973). For the first time, however, the data of the individual crista sections C1–C9 were handled separately, and this revealed that the odd-numbered crista sections (except C9) are about 10% longer than the even-numbered crista sections, although the number of large hair cells per row in these latter sections is 10% less (table 1).

Also, in both the odd-numbered and even-numbered crista sections, there is an obvious step-like increase of 10% in the section length between the cristae C1–C4 and C5–C8 (figure 1). This increase is difficult to understand, but one explanation could be the presence of the cartilaginous anticrista lobe in the territory of the sections C5–C8, since the anticrista certainly influences the direction and intensity of the movement of the endolymph fluid across those crista sections (see Young 1960).

4.1.2. Crista growth

In octopods (figure 2), and decapods (Maddock & Young 1984), there is a clear increase in the length of the crista sections with increasing mass, or size, of the animals. This increase is due to a formation of additional hair cells (figure 3), rather than to an increase in their size. In hatchlings of Sepia officinalis the numbers of the various types of hair cells, and neurons, in each crista section is about one fifth compared with adults (B. U. Budelmann & J. Forkel, unpublished observations). Such post-embryonic hair cell addition is well-known in the vertebrate lateral line systems and anamniote inner ears (see, for example, Alfs & Schneider 1973; Corwin 1981, 1983; Popper & Hoxter 1984); however, there is no evidence for it in birds and mammals (see, for example, Ruben 1967; Rosenhall 1973; Cohen & Fermin 1978).

The site of formation of new hair cells is at both ends of each crista section (figures 8 and 9). There, in Loligo, patches of small round cells occur which have been suggested to include those that differentiate (Stephens & Young 1982). Thus, these terminal areas of the crista sections offer interesting material for a study of differentiation processes in a sensory tissue, including the formation of the primary and secondary hair cells and the first-order afferent neurons.

4.1.3. Crista innervation and fibre diameters

The centrifugal cobalt fillings of the three crista nerves (see §3.4) showed an obvious disproportionate innervation of the nine crista sections (figure 97). The cobalt-filled fibres are mostly of large diameter and all could easily be traced to somata of primary hair cells or first-order afferent neurons in the crista sections (plates 9–11); this clearly characterizes them as afferent fibres. On the other hand, efferent fibres are abundant in the crista nerves (plates 5–7; table 5), but they have not been seen stained with cobalt, most probably because they are very small in diameter and thus difficult to fill. The innervation of the nine crista sections by the efferent fibres most probably differs from that of the afferent fibres, because small bundles of efferent fibres run long distances parallel to the crista ridge (figures 28 and 29) and these are difficult to trace in their relation to particular crista sections.
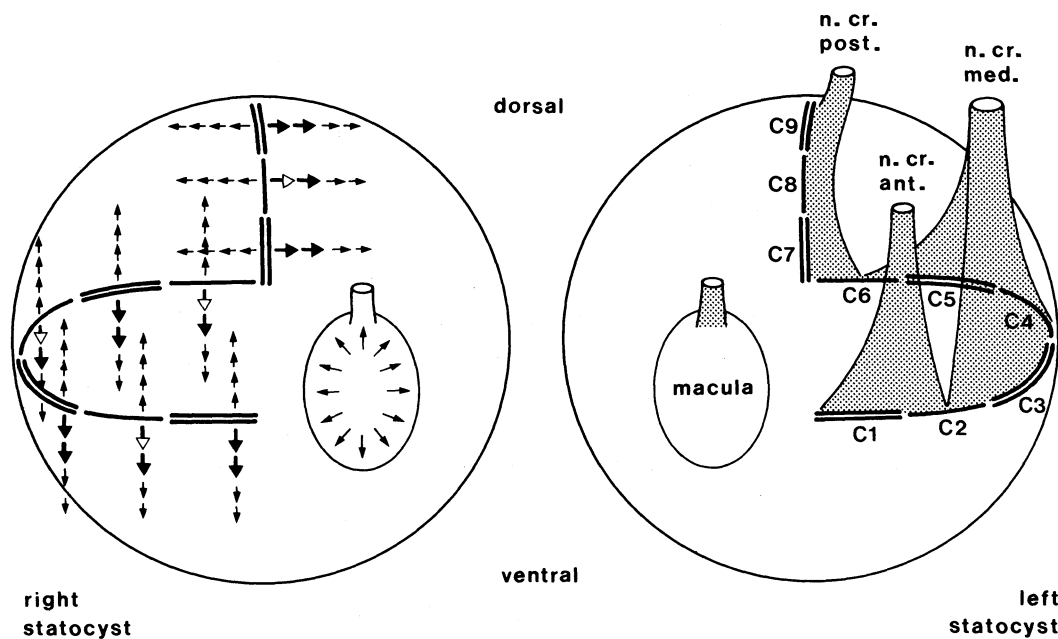
FIGURE 97. Diagram of the two statocysts of *Octopus vulgaris* (frontal view). To the left: pattern of morphological polarization of the hair cells in the nine crista sections C1–C9. Each arrow stands for a row of hair cells and indicates the direction of polarization, i.e. the direction in which the basal feet of all their kinocilia point; large, dark arrows refer to the large secondary hair cells, large, light arrows refer to the fairly large secondary hair cells, and small arrows refer to the small dorsal (C7–C9: lateral) primary and small ventral (C7–C9: medial) secondary hair cells (from Budelmann 1977). To the right: pattern of afferent innervation of the nine crista sections by the anterior (n.cr.ant.), medial (n.cr.med.) and posterior (n.cr.post.) crista nerves (compare figures 57–59).

For physiological experiments it is essential to know that the crista sections C2 and C6 are both innervated by two crista nerves (plate 9; figure 97). This is particularly important for the posterior crista nerve, which innervates not only the vertically oriented crista sections C7–C9, which are sensitive to yaw movements, but also part of section C6, which is horizontally oriented and thus sensitive to pitch.

In *Octopus*, the innervation of the crista sections is clearly different from that of decapods and *Vampyroteuthis* where only two and one crista nerves respectively innervate the four crista sections (Stephens & Young 1976, 1982). In *Sepia*, the small crista nerve (n. crista minor) even contains some fibres that innervate the inferior macula neglecta (Klein 1932).

The numbers of fibres in the three crista nerves of *Octopus* vary, according to the number of crista sections each nerve innervates (tables 3 and 5). All nerves together contain about 8700 fibres; this is about 1000 fibres per crista section. The total number of crista fibres is much greater than previously described by light microscopy (Young 1965); it is equal to that of the statocyst macula nerve (8900 fibres: Budelmann *et al.* (1973); W. F. Colmers, unpublished observations) and comparable to the three ampullary nerves of the vertebrate labyrinth (pigeon: 4950, guinea pig: 4900, cat: 7300, monkey: 11200; Gacek & Rasmussen (1961); Landolt *et al.* (1973); see also §4.1.4). There is a considerable difference (70%) in the total number of fibres in the anterior, compared with the medial (10%) and posterior (20%), crista nerves (table 3). To some extent, this may be explained by the different size, or age, of the animals used in this study (see also §4.1.2). But similar unexplained high differences in the

numbers of fibres (70–95%) were also found in the vertebrate vestibular and cochlear nerves (Rasmussen 1940; Wersäll 1956; Gacek & Rasmussen 1961).

In *Octopus*, the diameters of the fibres in the crista nerves vary from 18 μm to less than 1 μm (table 3), and this variation, of course, affects the conduction velocity of the fibres. The range of diameters is quite large, compared with that of the vertebrate ampullar, and all vestibular, nerves, where the average diameter is 1–4 μm and the largest reach about 12 μm (Wersäll 1956; Gacek & Rasmussen 1961; Landolt *et al.* 1973); however, the latter contain myelinated fibres.

The number of fibres (148) that have a diameter larger than 6 μm corresponds well to the number (142) of the large first-order afferent neurons (tables 3 and 4). Most of the fibres (85%), however, have a diameter of less than 1 μm and these most probably are all efferent fibres (compare with table 5).

The fibre size-distribution is equal for the three crista nerves, and there is no indication for a bimodality as previously suggested (Young 1965). The same holds true for the two crista nerves of *Sepia officinalis* (B. U. Budelmann & C. Mauerer, unpublished observations).

4.1.4. *Crista cellular organization*

The cellular organization of the crista of *Octopus* is the most differentiated so far described for any angular acceleration receptor system; it is even more differentiated than that of mammals (see, for example, Wersäll 1972; Lowenstein 1974).

Hair cells. There are four types of hair cell: small dorsal primary hair cells; fairly large, large and small ventral secondary hair cells. Their kinocilia are inclined towards the apical cell surface, with the exception of those of the fairly large hair cells in the even crista sections. This inclination is one of the features that determines the direction of the cell's morphological as well as physiological polarization (figure 97; Budelmann 1977, 1979; Williamson & Budelmann 1985*b*). There seem to be small areas of a close membrane contact at the tips of the kinocilia (figures 93 and 96), as well as filamental interconnections between the kinocilia, some in a position similar to those that have recently been described for the vertebrate labyrinthine hair cells (Ross 1974; Osborne *et al.* 1984; Neugebauer & Thurm 1985; Thurm 1984; Little & Neugebauer 1985). This is of particular interest, since the cephalopod hair cell differs from that of the vertebrates in that it has no stereovilli (=stereocilia; Neugebauer & Thurm (1985)), which are known to play a key role in the transduction process of the vertebrate hair cell (Hudspeth 1983); however, further cephalopod material, fixed in a more appropriate way (Neugebauer & Thurm 1985) is necessary to confirm these kinociliary interconnections.

One of the most interesting findings is the presence of both primary and secondary sensory hair cells in the *Octopus* crista epithelium, and this, indeed, solves the previous contradictory results (see Budelmann 1977). The presence of secondary sensory cells in invertebrates is quite unusual in itself, but it has been described in several sense organs, including the *Octopus* statocyst (Budelmann 1977; Budelmann & Thies 1977, also for references; Colmers 1977, 1981). This paper, however, gives the first detailed description of an epithelium in which primary and secondary sensory cells occur together. It has convincingly been proved by serial reconstruction of the hair cells at the level of light and electron microscopy (see §3.1 and §3.2.1) as well as by the centrifugal cobalt filling of the primary, but not the secondary, sensory hair cells via the crista nerves (see §3.4.2). With the same three techniques, primary and secondary hair cells have also been found in the crista of *Sepia officinalis* (B. U. Budelmann, unpublished results),

and light microscopical investigations indicate that both cell types may also occur in the crista of *Loligo* and *Cirrothauma* (Stephens & Young 1982; Aldred *et al.* 1983). It is still unclear however, how primary and secondary cells develop side by side in the same epithelium and it remains to be investigated whether the secondary sensory cells start as 'primaries' but then loose their axons, as has previously been suggested (Vinnikov 1985). An investigation of the growth zones of the crista sections (compare with §4.1.2) may solve this question.

Of the total of 2100 hair cells in the nine crista sections about half are the dorsally polarized primary hair cells, and half the ventrally polarized secondary hair cells (table 4). All the types of hair cells are more or less evenly distributed in the crista sections, with a clear line of separation between the oppositely polarized hair cells. This is comparable, to some extent, to the situation in the crab angular acceleration receptor system (Sandeman & Okajima 1972), but it is different from the situation in the vertebrate crista, where all hair cells are polarized in the same direction and where regional differences in density as well as in distribution, of the type I and type II hair cells occur (see, for example, Lindeman 1969; Flock 1971; Lindeman *et al.* 1981). Furthermore, in vertebrates the two types of hair cells in each of the three cristae are very numerous, between 4500 (rabbit; Watanuki *et al.* (1983)) and 7600 (man; Rosenhall (1972)).

Neurons. There are about 1000 first-order afferent neurons in all nine crista sections, 85 % of which are small and 15 % are large (table 4). The nature of these neurons has been a subject of much controversy (Owsjannikow & Kowalevsky 1867; Young 1960; Barber 1966*a*; Vinnikov *et al.* 1967; Aldred *et al.* 1983). Our data now clearly show that the neurons are postsynaptic to the large and small ventral secondary sensory hair cells; this characterizes the neurons as being first-order afferent neurons to these hair cells (see §4.1.5). There is strong evidence that the various types of neurons in the cristae of *Sepia* (Klein 1932) again are first-order afferent neurons to secondary hair cells (B. U. Budelmann, unpublished results).

The majority of the neurons are unipolar. Some somata appear to have short dendrite-like processes which could designate them as bipolar or multipolar (see §3.4.2); but these processes have never been seen in synaptic contact with hair cells (which, by definition, characterizes a dendrite; Bullock & Horridge (1965)). A similar situation has recently been described for the peri- and intra-macular first-order afferent neurons of the *Octopus* gravity receptor system (Colmers 1981), but more data are necessary to clarify this point for the crista system.

4.1.5. *Crista synaptic organization*

The large number of profiles and specialized membrane structures, found in the *Octopus* crista associated with synaptic vesicles, point to a highly developed and complex system of chemical information transfer already at the level of the receptor epithelium. No indications for electrical synapses have been found.

In the cephalopod nervous systems a variety of synaptic vesicles of different size and content are well-known and are possibly associated with different transmitter substances (for references see Ducros 1979; Tansey 1979). In the *Octopus* crista, most vesicles are small and agranular, but there are also others which are larger and either agranular or dense-cored. Sometimes these types of vesicles have been seen in the same profile or receptor cell. For the possible transmitters involved see §4.1.6 and §4.1.7.

In the crista, the afferent synaptic contacts between the secondary hair cells and the first-order afferent neurons were of the same two types that have been described for the *Octopus* macula

(Colmers 1977). No transitional forms of synapse have been found and no equivalent structures to the presynaptic bodies, bars, or rods, of the vertebrate vestibular hair cell (Wersäll & Bagger-Sjöbäck 1974). In a given receptor cell, both types of synapse probably transmit identical information; they may, however, differ in their transduction properties and the transmitters used.

The number of afferent synapses a single hair cell possesses has not been analysed in detail, but it is, most probably, less than the average of nine (six finger-like, three flat) which were found in the macula hair cell (Colmers 1977), and furthermore less in a small ventral, compared with a large, hair cell. For further details of the afferent system see §4.1.6.

In the crista, the majority of vesicle-filled profiles are efferents which pass to the primary and secondary hair cells, and to the neurons. The synaptic contacts are mostly flat and contain a large number of agranular vesicles. Only a few profiles cover short axonal spines of the neurons. No postsynaptic specializations, such as membrane sacs (Wersäll & Bagger-Sjöbäck 1974), were found.

There are many efferent endings on each hair cell, or a single neuron: up to 30 or 40 on a large hair cell, but certainly less on a small dorsal primary, or ventral secondary, hair cell. Because there are about twice the number of efferent fibres in the crista nerves as of all the hair cells and neurons (tables 4 and 5), and since there are many more than two endings on a single hair cell, or single neuron, there must be a considerable branching of individual efferent fibres.

4.1.6. *Crista afferent system*

The crista afferent system contains three distinct components: (i) primary hair cells, (ii) large secondary hair cells and their associated large neurons, and (iii) small secondary hair cells and their associated small neurons. All primary hair cells are dorsally polarized and have a direct connection (via their axons) to the cns, whereas all secondary (large and small) hair cells are ventrally polarized and have an indirect connection (via their first-order afferent neurons) to the cns.

The somata of the neurons are prolonged into axon-like trunks. Because these are often surrounded by numerous efferent endings, up to a distance of at least 100 μm from the somata, this raises the question of the site of spike generation. No other morphological criteria were found that indicate the point from which the trunk should be considered as a spike-propagating axon. Spikes can already be recorded at a distance of about 200 μm from the somata of the neurons (Williamson & Budelmann 1985b).

There is an interesting difference in the relations of the numbers of large secondary hair cells and large neurons, and of small secondary hair cells and small neurons (table 4). Four large secondary hair cells relate to each large neuron, clearly indicating a convergent information flow; in contrast, one small secondary hair cell relates to two small neurons, clearly indicating a divergent information flow.

The convergence of several hair cells upon a single neuron is not surprising for hair cells that are polarized in the same direction (compare with Colmers (1981), for the macula hair cells) and this is also well-known for the vertebrate vestibular hair cells (see, for example, Landolt *et al.* 1973). A divergence, however, from one sensory cell upon, at least, two neurons already peripherally is quite unusual for any sensory epithelium. It has been described for the *Octopus* macula (Colmers 1981) and resembles to some extent the situation in the vertebrate retina (see,

for example, Dowling & Boycott 1966). An explanation for the divergence in the crista could be that the neurons run to, and terminate in, different areas of the cns, four of which have been found to receive crista afferent fibres (Budelmann & Young 1984). It is difficult to understand, however, why the divergence occurs already at the level of the sensory epithelium, and not at that of the cns.

Recent electrophysiological recordings from afferent units in the crista nerves of *Octopus* during sinusoidal oscillations showed that the units in the even crista sections are an order of magnitude more sensitive than those in the odd crista sections (Williamson & Budelmann 1985 *a, b*). This difference can be explained by the differences in cupula size and cupula attachment to the hair cells, rather than by differences in the properties of the hair cells themselves. In addition, within each crista section there are secondary hair cells which, with their neurons, have differing sensitivity as well (Williamson & Budelmann 1985 *b*). It remains to be investigated whether these differences can be attributed to the two types (large and small) of secondary hair cell. Moreover, the secondary hair cells were found to be more sensitive than the dorsal primary ones (Williamson & Budelmann 1985 *b*). Such differences in sensitivity are also known for the type I and type II hair cells of the vertebrate crista (Goldberg & Fernández 1977; Henn *et al.* 1980).

In contrast to the efferent system (see §4.1.7), it is still not known which substance(s) act as neurotransmitters in the crista or macula afferent fibre system. Possible candidates might be octopamine, histamine, tyramine, and some amino acids, such as L-glutamate, or neuro-peptides (Ducros 1979; Tansey 1979; Martin *et al.* 1981; Auerbach & Budelmann 1986; Florey *et al.* 1985).

4.1.7. *Crista efferent system*

Young (1960) mentioned an elaborate supracristal, cristal and infracristal nerve plexus at the level of light microscopy and discussed the fibres as most probably being efferent. Later, ultrastructural findings of vesicle filled profiles, some of which clearly being presynaptic, supported his idea (Barber 1966 *a*; Vinnikov *et al.* 1967). The results of this paper (see §3.2.2), and recent neuroanatomical and electrophysiological findings, now leave no doubt that the elaborate plexus of fine fibres is efferent in nature:

(i) there are 6700 small-diameter fibres in the crista nerves that do not originate from the receptor cells, or first-order afferent neurons, in the crista sections (tables 4 and 5);

(ii) centripetal filling of the crista nerves with cobalt showed many stained somata in various parts of the cns (Budelmann & Young 1984);

(iii) lesion of the crista nerves inside the brain capsule caused a degeneration of the plexus of fine fibres in the crista sections (Auerbach & Budelmann 1986);

(iv) a large number of efferent profiles were found synapsing onto the hair cells and neurons (see §3.2.2.2);

(v) stimulation of the efferent fibres clearly influence the electrical activity of the crista afferent units (Williamson 1985 *a*); and

(vi) efferent fibre activity can be recorded from the central ends of cut crista nerves, and shown to be driven by afferents from the contralateral statocyst (Williamson 1985 *b*).

In all three crista nerves, the majority of fibres (85%) are very small in diameter (figure 56). Since many of the large fibres could be traced to their somata in the crista sections (plates 9–11), the small ones, most probably, represent the efferents (see §4.1.3). Although the fibre

size categories are arbitrary, there is a striking correspondence of the number of efferent fibres (6700; table 5) to the number of all those less than 1 μm (7400; table 3). This, in turn, indicates that the conduction velocity is much lower in the efferent than in the afferent fibres.

The data presented point to a considerable branching of a single efferent fibre and indicate, as do recent electrophysiological experiments (Williamson 1985a), that multiple efferents synapse onto a single afferent unit. Sometimes the axons of the first-order afferent neurons are almost completely covered by efferent endings (figures 41–44).

Very fine, presumably efferent, fibres have recently also been described in the cristae of *Loligo* (Stephens & Young 1982). In the macula nerve of *Octopus*, as in the crista nerves (table 5), about 70 % of the fibres originate from efferent somata in the cns (see Budelmann & Young 1984). Moreover, efferent fibres may be present in the statocysts of some gastropods (Wolff 1970; Jong & Wilgenburg 1975; Wolff 1975); but they are well-documented in the vestibular systems of all major vertebrate groups, at the level of both the sense organs (maculae and cristae) and the cns (Gacek & Lyon 1974; Klinke & Galley 1974; Precht 1978; Goldberg & Fernández 1980; Hartmann & Klinke 1980; Schwarz et al. 1981). The numbers and percentages of the efferent fibres in the vertebrate vestibular nerves, however, are much smaller than those of *Octopus*: in vertebrates, only 10 %, or less, depending upon the species, are efferent fibres (see Schwarz et al. 1981).

In *Octopus*, the functional significance of the crista efferent system might be a feed-forward mechanism (Williamson 1985a; compare with Klinke & Schmidt 1968 and Klinke & Galley 1974). Electrophysiological experiments have recently shown that most of the efferents (77 %) have an inhibitory, and a few (16 %) an excitatory, influence on the resting activity of the crista afferent units (Williamson 1985a); comparable results have been obtained in vertebrate systems (Hartmann & Klinke 1980; Henn et al. 1980; Rossi et al. 1980). In *Octopus*, recordings from efferent units showed that their activity could be synchronized with imposed oscillations, with the response of each limited to one direction of oscillation (Williamson· 1985b).

The transmitters involved in the crista and macula efferent fibre systems include acetylcholine, dopamine and/or noradrenaline (Budelmann & Bonn 1982; Auerbach & Budelmann 1986). Whether other substances are involved as well and whether the cholinergic and catecholaminergic fibres stem from the two different areas of the cns that give rise to the efferent fibres (pedal lobe, magnocellular lobe; Budelmann & Young 1984) remains to be investigated.

4.1.8. *Differences in the organization of the crista sections*

The crista/cupula sections differ in a number of criteria (table 2) and can thus be classified into two types: type I (the odd-numbered crista sections C1, C3, C5, C7, C9) and Type II (the even-numbered crista sections C2, C4, C6, C8). The most conspicuous difference occurs in the form and size of the cupulae attached to the sections. In the type I crista sections, the cupulae are small and have a large area of attachment to the crista ridge, whereas in the type II crista sections the cupulae are large, but their area of attachment is only small (figures 75–85). These morphological differences are connected with differences in sensitivity of the type I and type II sections (see §4.2).

In the type II crista sections, with the large cupulae, there are about 75 % more dorsal primary hair cells, and about 35 % more small first-order afferent neurons than in the type I crista sections with the small cupulae; however, the numbers of their associated small ventral secondary sensory hair cells are almost the same. The numbers of the large first-order afferent

neurons are identical; but the diameters of their somata are about 25 % smaller in the type II crista sections.

In summary, the type II crista sections, with the large cupulae, contain more receptor cells and more first-order afferent neurons; the functional significance of this, however, remains unclear. One explanation could be that these more sensitive crista sections have additional, or other, pathways in the brain.

4.2. *Cupula*

The size and form of the cupula, and its relationship to the crista hair cells and adjacent tissue, are critical parameters for understanding the functioning of the angular acceleration receptor system. Although recent investigations have described the morphology of the cephalopod statocysts, very little information is available concerning the cupulae of their angular acceleration receptor system (Young 1960; Budelmann *et al.* 1973; Budelmann 1977; Stephens & Young 1976, 1978, 1982). The present investigation, for the first time, describes these cupulae in detail.

The most striking finding is the regular alternation of the two different types of cupulae, type I and type II. Although cupulae, including those of the vertebrate semicircular canals, are generally considered to be easily deformed by preparation, fixation and subsequent dehydration procedures in conventional histological techniques (Hillman 1974; Barber & Emerson 1979; Dohlman 1980), it is almost certain, that in *Octopus* the differences in cupula form and size are not artefactually produced, for the following reasons:

(i) the differences occur in specimens prepared by a variety of fixation methods (see §2) for light, transmission and scanning electron microscopy;

(ii) the differences are very clear, with no transitional forms between type I and type II;

(iii) the differences alternate regularly along the nine crista sections, as does the cellular and neuronal organization of the crista (see §4.1.8), with the small cupula type I, and the large cupula type II respectively, being attached always to the same type of crista section;

(iv) if the cupulae were all large (type II), then there would not be enough space to allow their undisturbed movement inside the statocyst sac (see figure 75, which shows the cristae/cupulae in their original half-circular arrangement); and

(v) recent electrophysiological experiments support the dualism of cupula type, showing a dual sensitivity, with sensitivity thresholds an order of magnitude lower in crista section C2, having the large narrow-based cupula type II, compared with crista section C1, having the small wide-based cupula type I (Williamson & Budelmann 1985 *a, b*).

The structural composition of the *Octopus* cupulae shows close parallels to similar cupula structures found in the semicircular canals of mammals, where the cupulae have been described as a meshwork of crossing fibrils, with an interposed homogeneous substance (see, for example, Wersäll 1956; Dohlman *et al.* 1959; Igarashi & Alford 1969). It has been proposed that in fixed preparations the fibrils may arise from the fixation of a structure that is gelatinous in the unfixed state (Wersäll 1956). Although preparational artefacts have to be taken into account, the fibrillar structure of the *Octopus* cupula is unlikely to arise during fixation. This is because the cupulae show (i) a well organized structural arrangement at the bottom of cupula type I (figure 84), and most probably also of type II, which is different from that of the rest of the cupula, and (ii) a distinct ultrastructural organization, such as tube-like fibrils, with a dense core (figure 96).

The present account gives no data about the chemical components of the *Octopus* cupula. Thus, it remains to be seen whether it contains, like the cupula of mammals, neutral and acid mucopolysaccharides, as well as mucoproteins (Dohlman *et al.* 1959; Jensen & Vilstrup 1960; Dohlman 1969; Igarashi & Alford 1969).

Electrophysiological experiments showed an additional sensitivity of the *Octopus* crista/cupula type II system to linear acceleration, from which it has been concluded that the *Octopus* cupula type II has a higher specific gravity than its surrounding endolymph fluid (Budelmann & Wolff 1973; Williamson & Budelmann 1985 *b*). This should also be true for the cupula type I, since no basic structural differences have been found between both types. The fact that the crista/cupula type I system shows neither a sensitivity to linear acceleration nor a level of resting activity in its normal upright position (Williamson & Budelmann 1985 *b*) could be explained in terms of the cupula's much smaller size and larger area of attachment to the crista ridge. The asymmetric, more dorsal position of cupula type I on the crista ridge (figure 84) may also serve to prevent it from a pendulum function.

The morphological data give no indication of the attachment of the cupula to tissue other than that of the crista ridge. Therefore, its mode of movement during angular acceleration can best be described as that of a swinging door with the crista ridge as a fulcrum. This movement is different from the diaphragm-like movement of the cupula within the vertebrate semicircular canals, where the cupula is attached to the ampulla wall along its circumference (see, for example, Igarashi 1966; Hillman 1974; Lim 1975).

Several parameters are of special importance for the mechanics of cupula deflection: (i) cupula height (small in type I, large in type II), (ii) cupula form (as seen in cross-sections; see figures 77 and 78), (iii) area of resistance presented to the fluid movement (small in type I, large in type II), (iv) cupula base (wide in type I, narrow in type II), and (v) area of cupula attachment to the crista ridge (large in type I, small in type II). It is clear that cupula type II has more flexibility on the crista ridge, and more freedom for movement, than cupula type I. Consequently, in cupula type II, compared with type I, any given angular acceleration should result in a larger cupula deflection and thus should exert a larger mechanical force (i.e. stimulation) on the kinocilia of the receptor hair cells. Recent electrophysiological experiments indeed showed that during a given angular acceleration the sensitivity of afferent units of crista section C2, with the large cupula type II, is an order of magnitude greater than that of units of crista section C1, with the small cupula type I (Williamson & Budelmann 1985 *a, b*).

For a proper movement of the cupula on the crista ridge, a subcupular space is probably important since it is present in *Octopus* and in vertebrates (see, for example, Wersäll 1956; Igarashi 1966; Igarashi & Alford 1969; Hillman 1974; Barber & Emerson 1979). In both, a meshwork of fine filaments traverses this space, attaching the cupula to the surface of the crista cells (figures 92–94; Hillman 1974). These filaments are not seen in specimens prepared for scanning electron microscopy, most probably because of differences in the preparational procedures (Barber & Emerson 1979).

A unilateral contact between the ciliary bundle and the accessory structure (cupula or statoconial membrane) is thought to be essential for the mechano-electrical transduction by the hair cells (Hudspeth 1983; Thurm 1984). In this context it is interesting to note that, contrary to the situation in the vertebrate system where the kinocilia of the hair cells deeply insert into cupula microcanals (Wersäll 1956; Igarashi & Alford 1969; Hillman 1974, 1977; Lim 1975), in *Octopus* the bundles of kinocilia just contact the cupula from below but do not

insert into it; however, because of the inclination of the ciliary bundle, the cupula contact to the kinocilia is always unilateral and, as in vertebrates, is from the side of morphological polarization of the kinociliary bundle, and of the hair cell respectively (plate 15).

4.3. *Information processing in the crista/cupula system*

The present morphological account shows that in the statocyst of *Octopus* there is a highly developed crista/cupula system for the reception of angular acceleration and deceleration. Such angular movements result, because of inertia, in a movement of the endolymph fluid relative to the crista. This endolymph movement, in turn, deflects the cupula which is attached to the crista ridge, and thus causes the kinocilia of the receptor hair cells to shear in one or the other direction, depending on the direction of angular acceleration. The shearing of the kinocilia is the adequate stimulus for the receptor hair cells and either excites or inhibits these cells, depending on their polarization (Budelmann & Wolff 1973; Williamson & Budelmann 1985*b*).
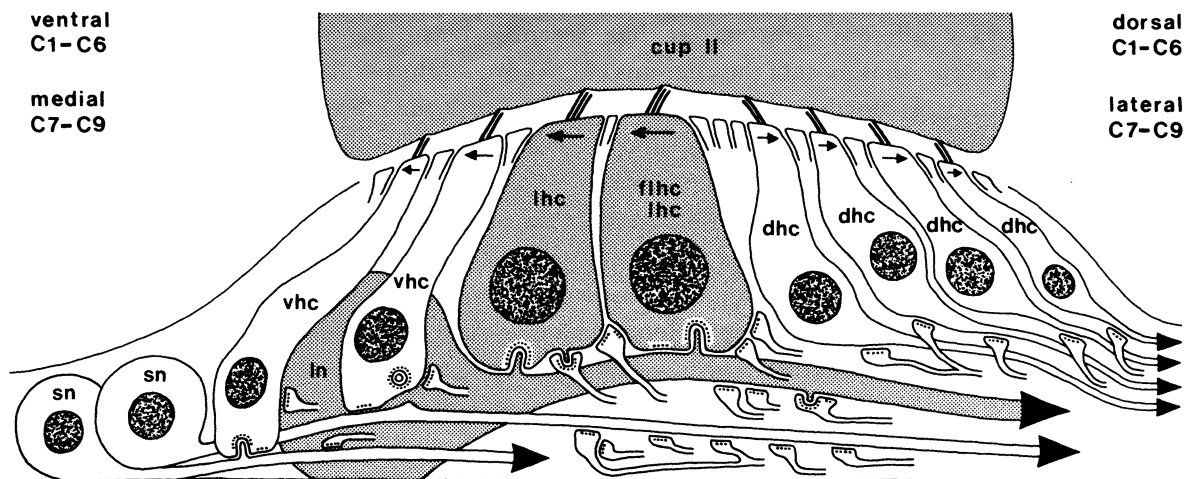
FIGURE 98. Diagram of a cross section of the crista of *Octopus vulgaris*. It represents both the odd-numbered (C1, C3, C5, C7, C9) and the even-numbered (C2, C4, C6, C8) crista sections, and demonstrates the different types of hair cells and first-order afferent neurons, as well as their afferent and efferent synaptic organization with finger-like and flat synaptic contacts. Some details have been enlarged for emphasis. In the middle (shaded): the large (lhc), and large or fairly large (flhc) secondary hair cells, together with their large first-order afferent neuron (ln). To the left: the rows of small ventral (C7–C9: medial) secondary hair cells (vhc), together with their small first-order afferent neurons (sn). To the right: the rows of small dorsal (C7–C9: lateral) primary hair cells (dhc). The arrows in the distal ends of the hair cells indicate the direction of their morphological polarization (compare figure 97). Only the basal part of the large cupula type II (cup II) is shown; the small cupula type I has a larger area of attachment and extends further to the right.

The information about angular movements can travel three different pathways to the CNS (figure 98):

(i) directly via the primary sensory hair cells;

(ii) indirectly via the large (and fairly large) secondary sensory hair cells and their associated large first-order afferent neurons; and

(iii) indirectly via the small ventral secondary sensory hair cells and their associated small first-order afferent neurons. In the two indirect pathways, the information is first transformed by the secondary sensory hair cells into a signal that causes a change in the release of a

transmitter at two morphologically different types (finger-like or flat) of chemical synapses. The transmitter(s) of these afferent synapses is not yet known. But it is clear that they relay the information to the first-order afferent neurons, which then carry it to the CNS in terms of an angular velocity code (Williamson & Budelmann 1985 b).

The three kinds of afferent fibres (those of the primary hair cells and those of the two types of first-order afferent neurons) proceed to various parts of the CNS: ipsilaterally to the anterior and posterior lateral pedal, the median basal, and the peduncle lobes, and in both directions along the brachial to pallivisceral lobe connective, and contralaterally, to the lateral pedal and median basal lobes (Budelmann & Young 1984). However, it is not yet known whether each of the three types of afferent fibres proceed to all of these various parts of the CNS, or only to some of them.

Before the information enters the CNS it is, or can be, influenced by efferent information that comes from three parts of the CNS: the anterior lateral pedal, the posterior pedal, and the magnocellular lobes (Budelmann & Young 1984). The sites of interaction, where the efferent fibres synapse peripherally with the afferent units, are at the level of the hair cells and at that of the first-order afferent neurons. It has recently been demonstrated physiologically (Williamson 1985 a), that most of the efferents (77 %) are inhibitory, but there are a few (16 %) that are excitatory. Histochemical studies have shown that the efferent transmitters involved are acetylcholine as well as dopamine and/or noradrenaline (Budelmann & Bonn 1982; Auerbach & Budelmann 1986).

In comparison with the crista/cupula systems of the vertebrate semicircular canals (compare Wersäll & Bagger-Sjöbäck 1974), it is apparent that the morphological organization of the *Octopus* system is different in gross morphology and is more complex, in that there are two different types of cupulae and three different types of hair cells with only kinocilia, and no stereocilia. But despite these differences striking analogies have also been found between the *Octopus* and the vertebrate system, both in morphological and physiological details (Budelmann & Wolff 1973; Budelmann & Young 1984; Williamson 1985 a, b; Williamson & Budelmann 1985 a, b).

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FIGURES 4–9. For description see opposite.

FIGURES 10–15. For description see page 320.

FIGURES 16–20. For description see facing plate 4.

FIGURES 21–27. For description see opposite.

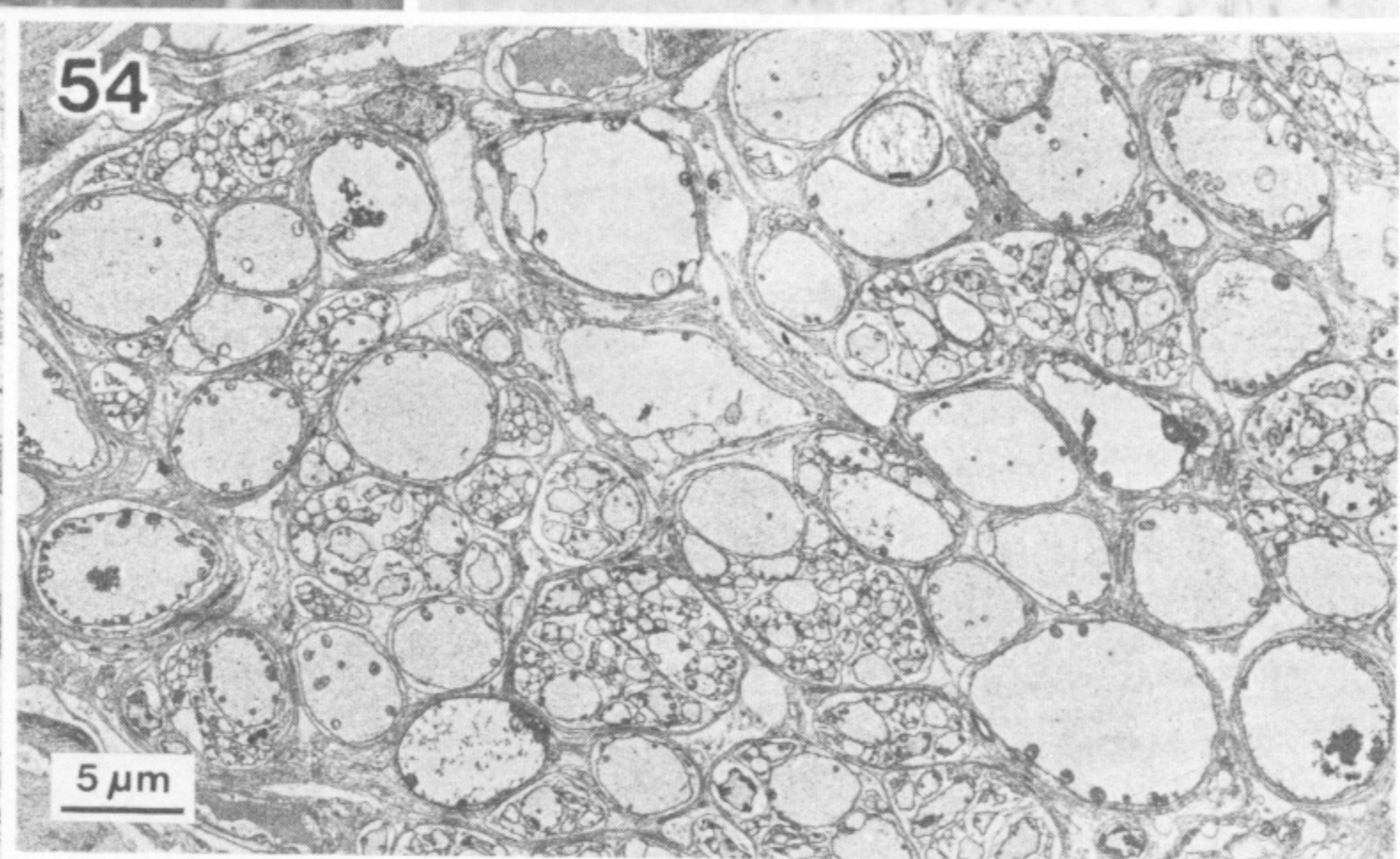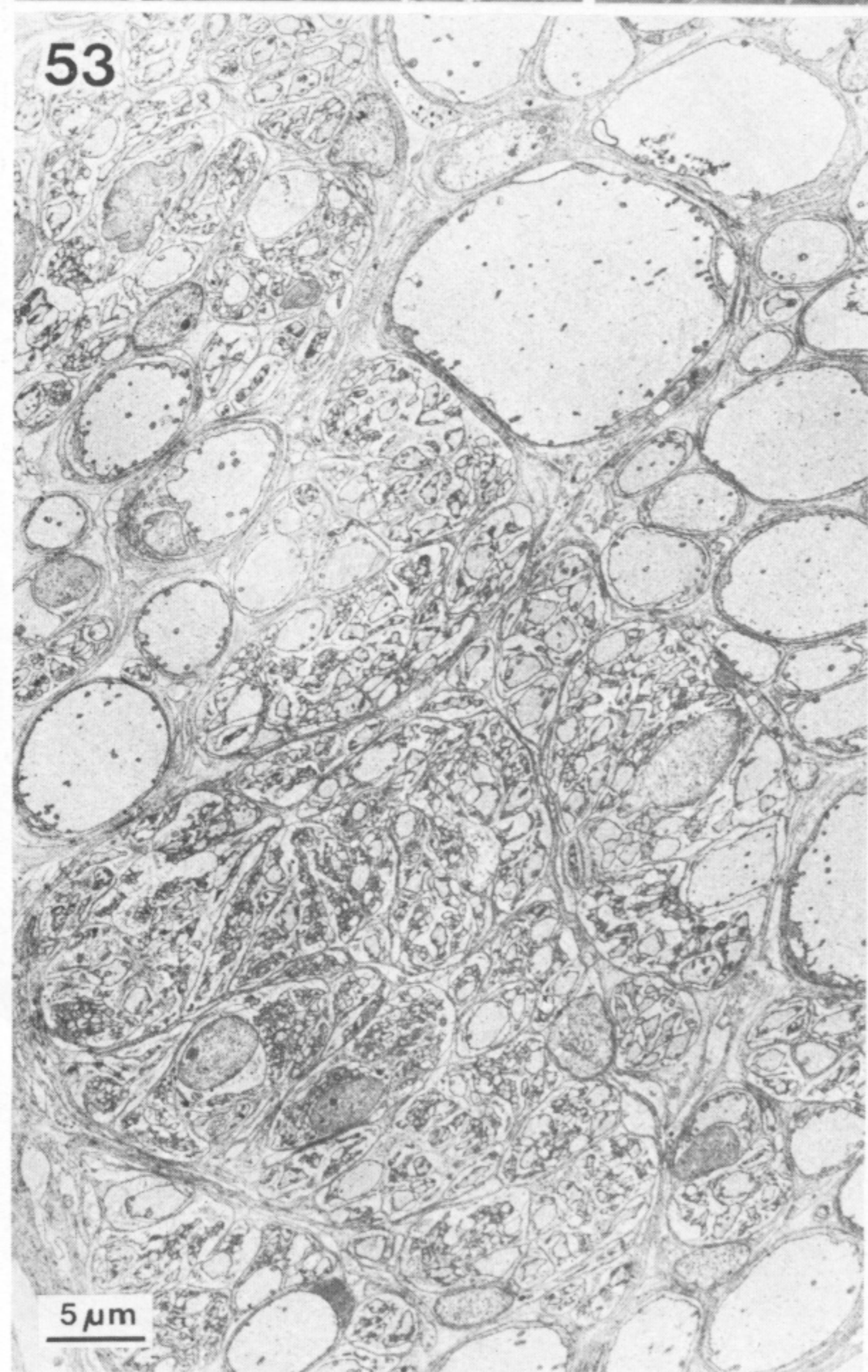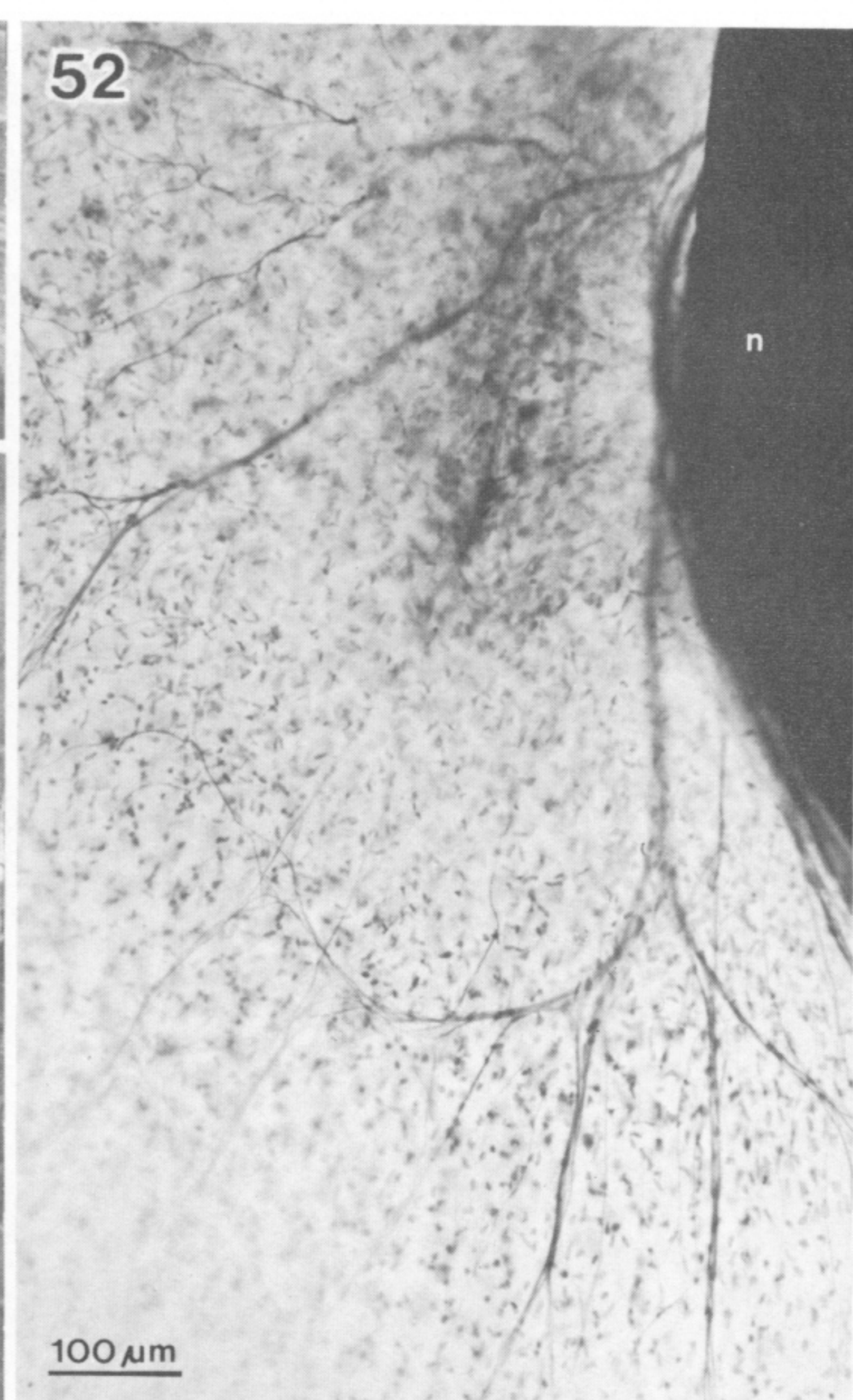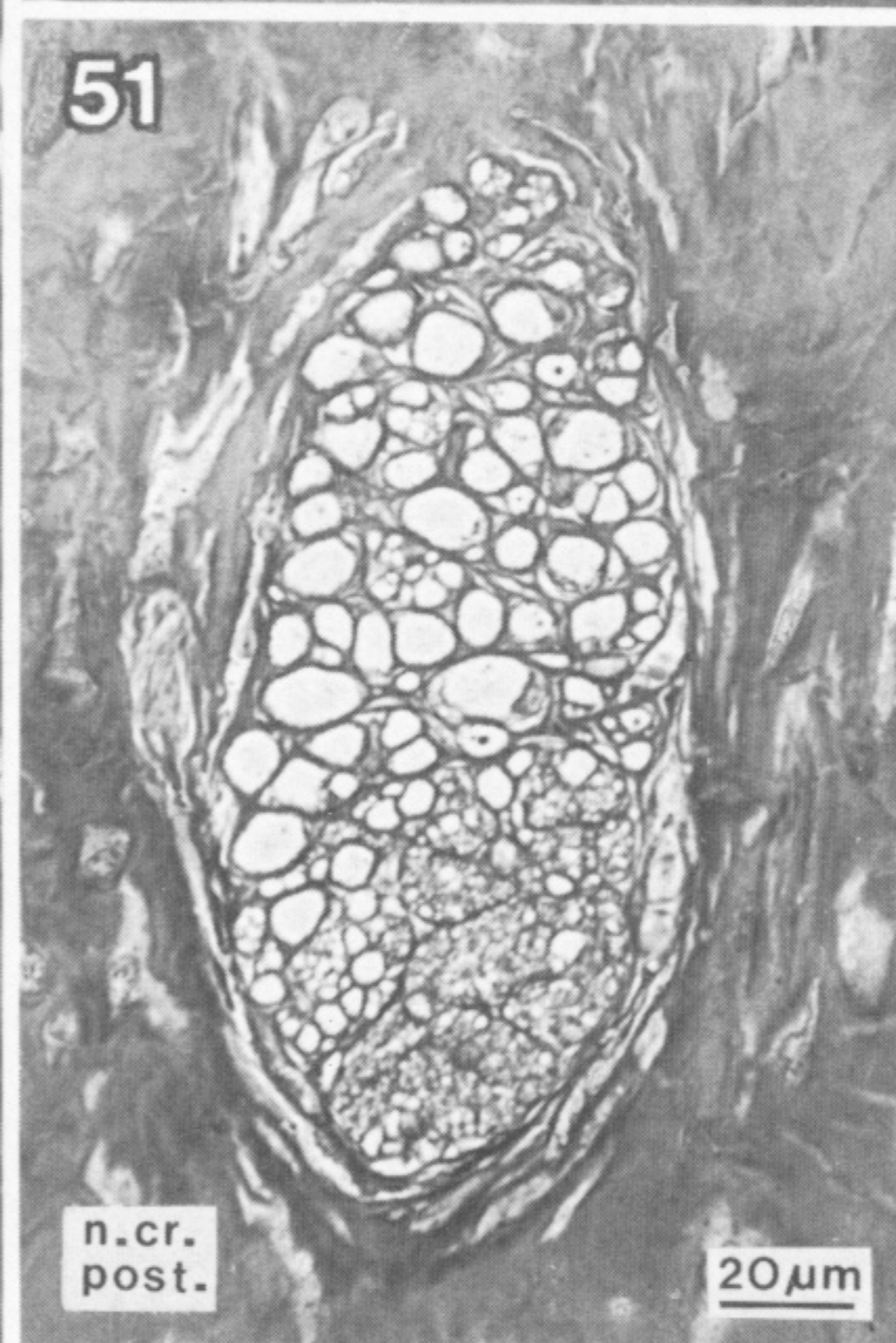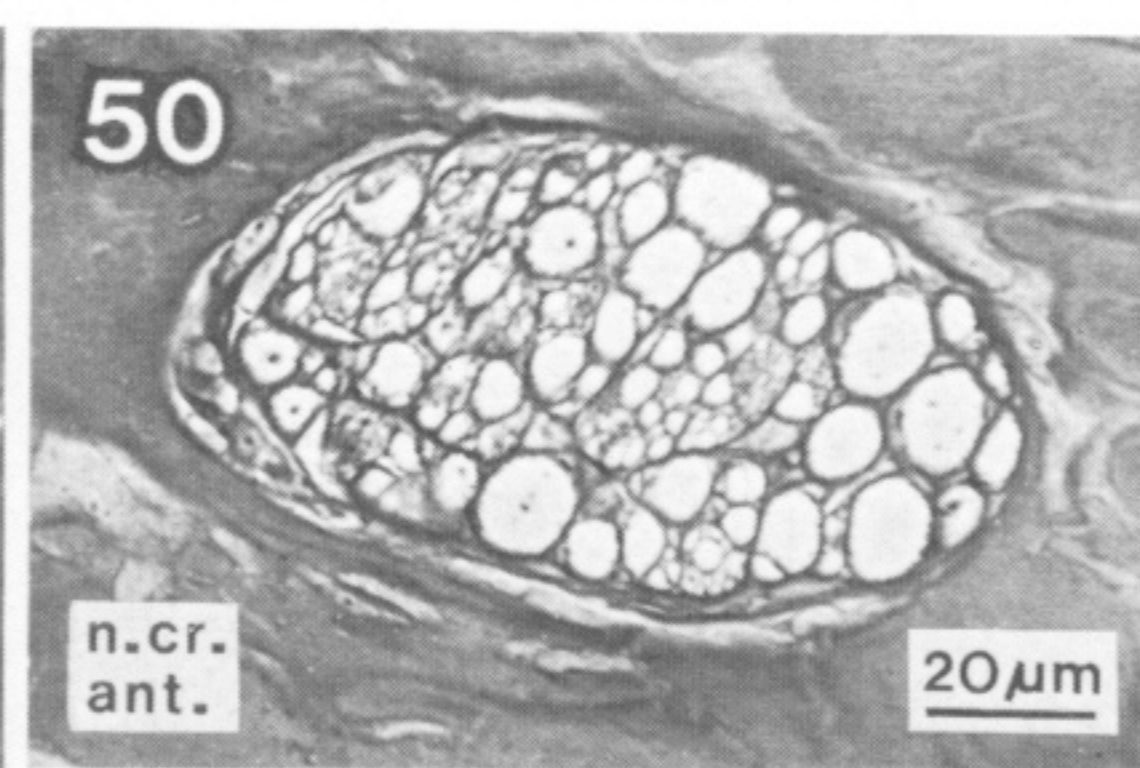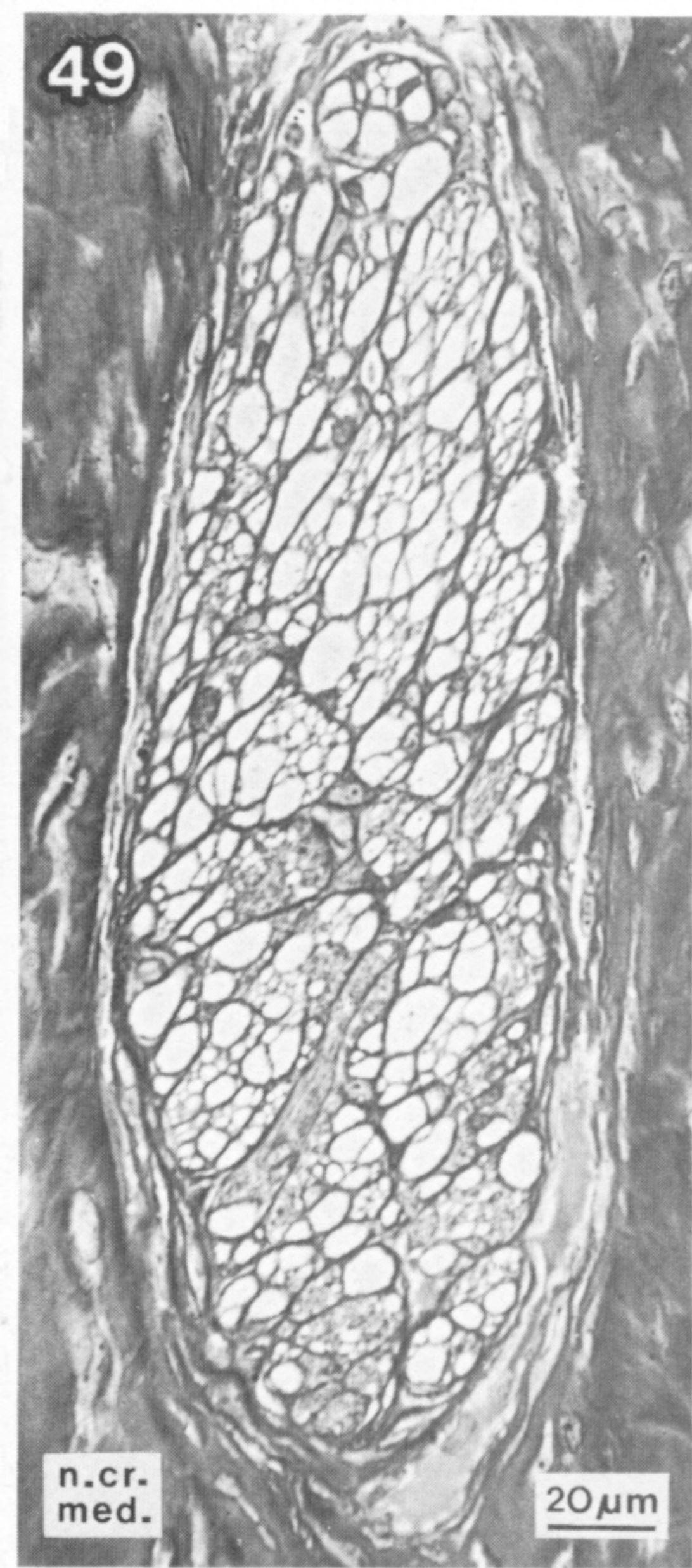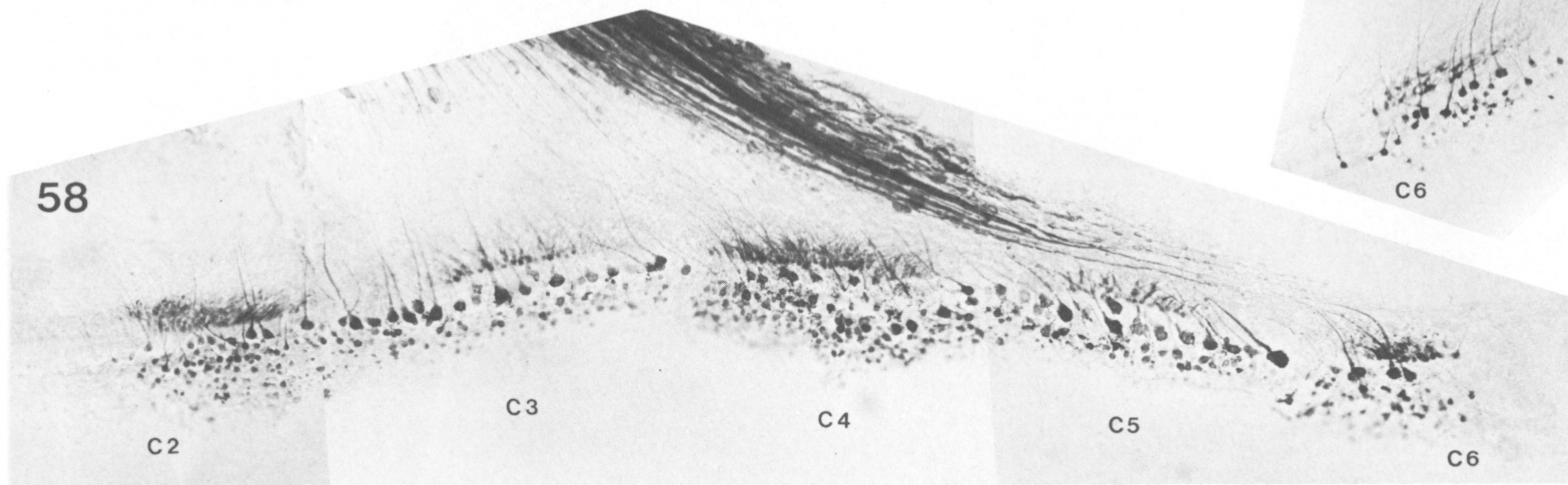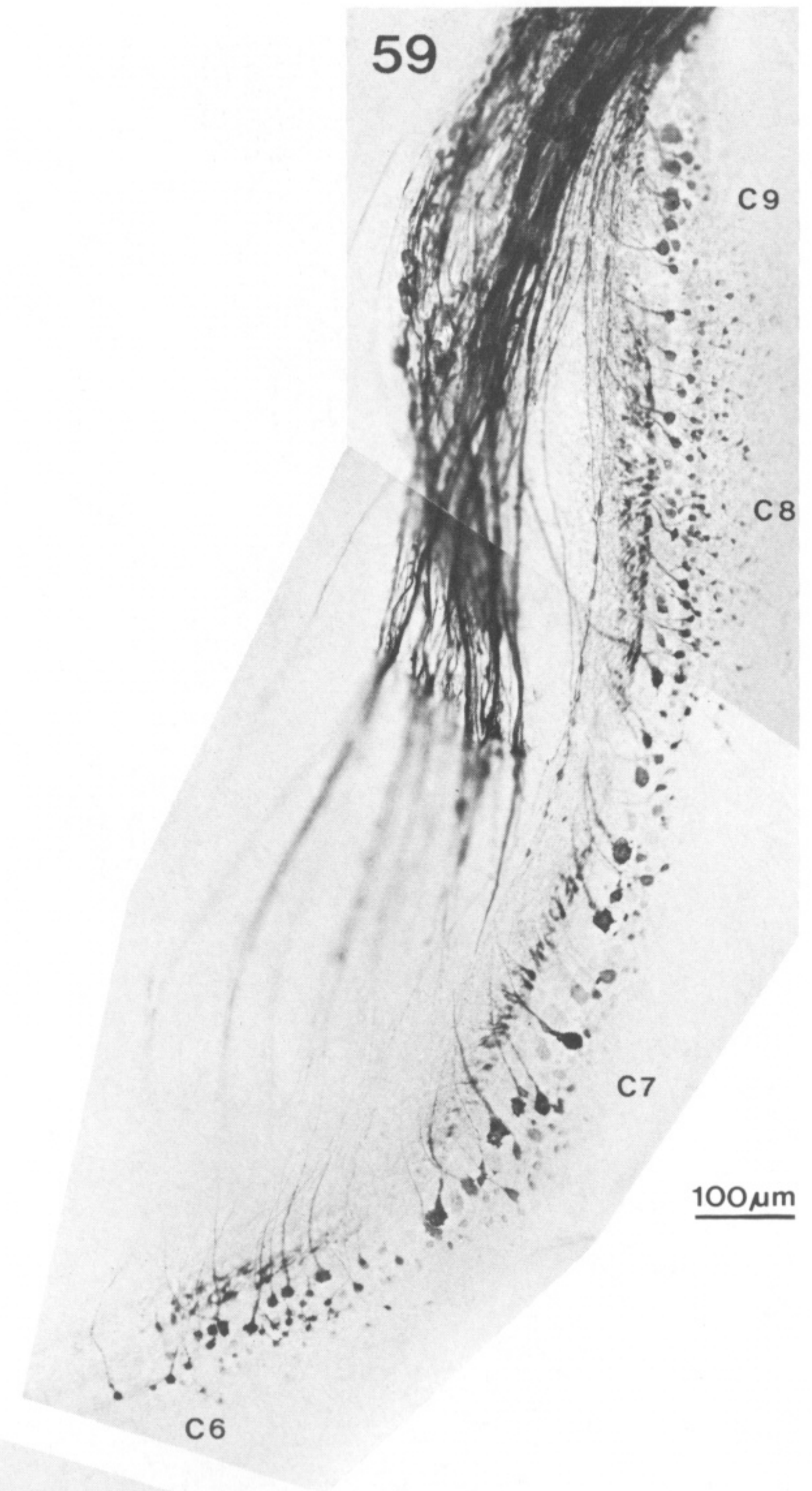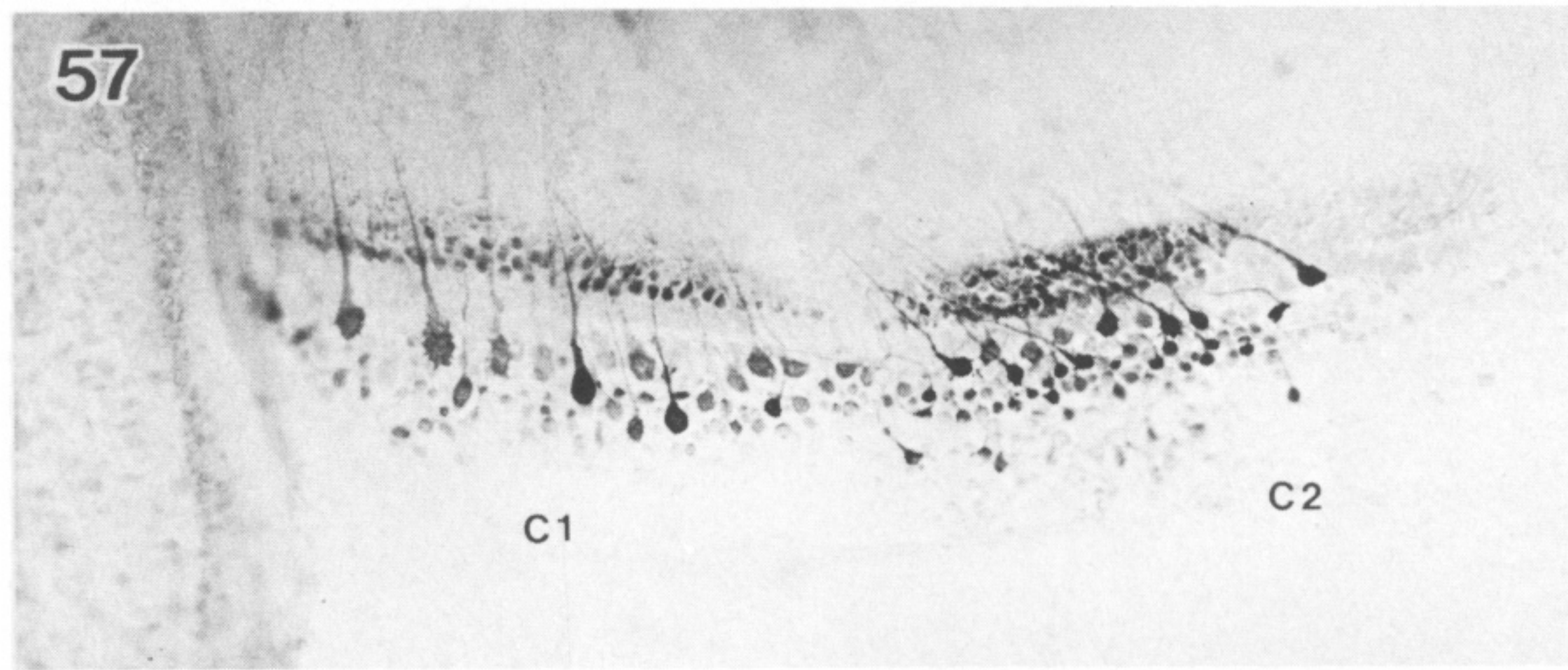FIGURES 28–34. For description see facing plate 4.
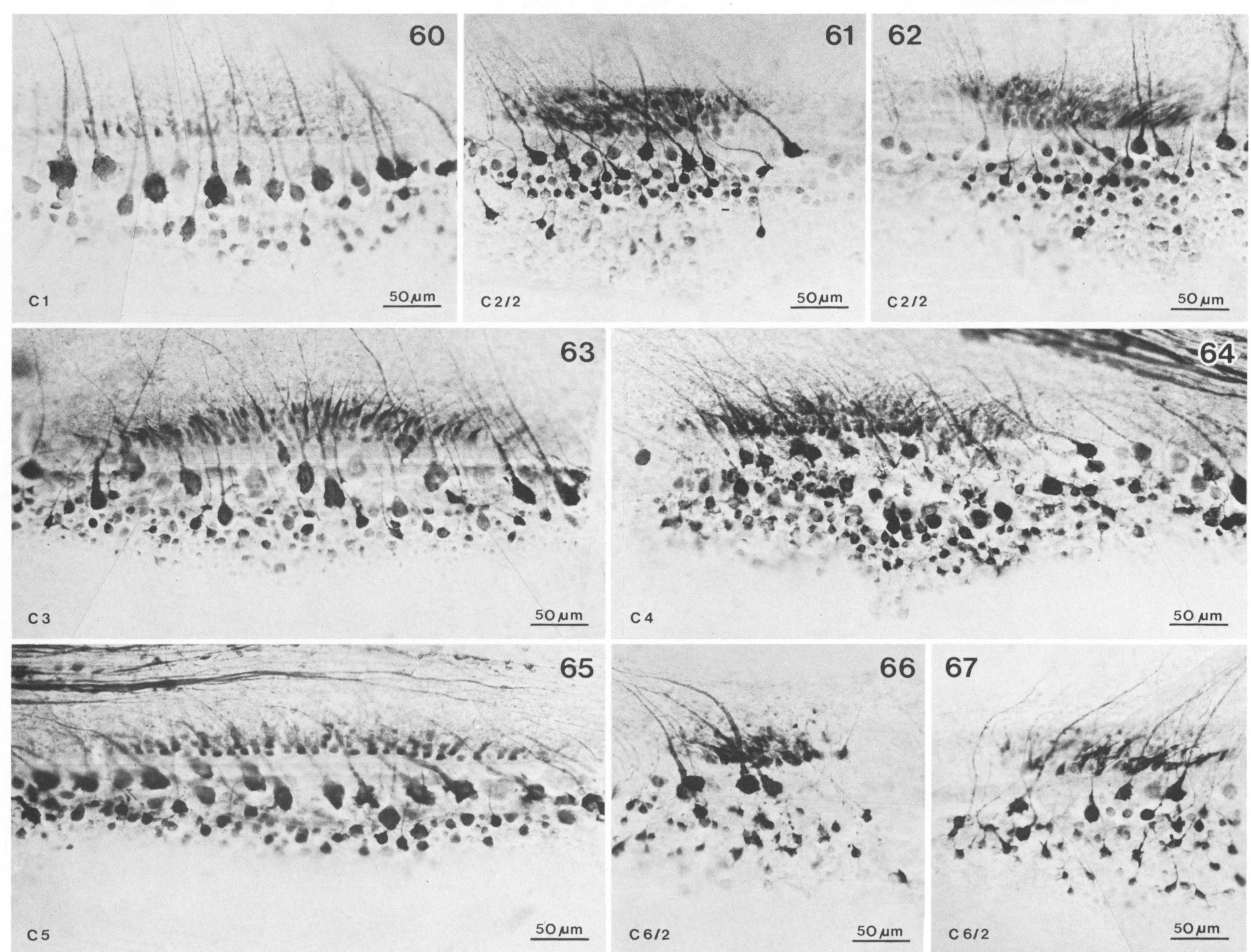
FIGURES 35–40. For description see page 321.

FIGURES 41–48. For description see opposite.

49

n.cr. med.

20 μm

50

n.cr. ant.

20 μm
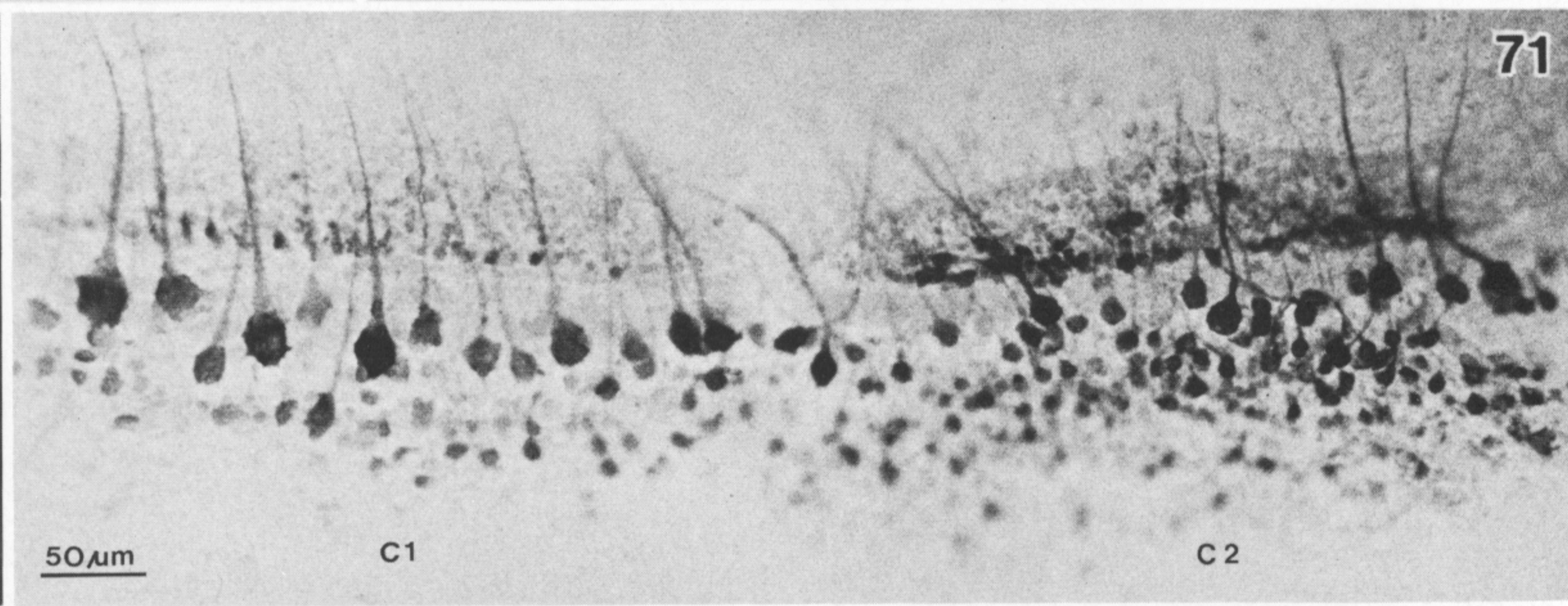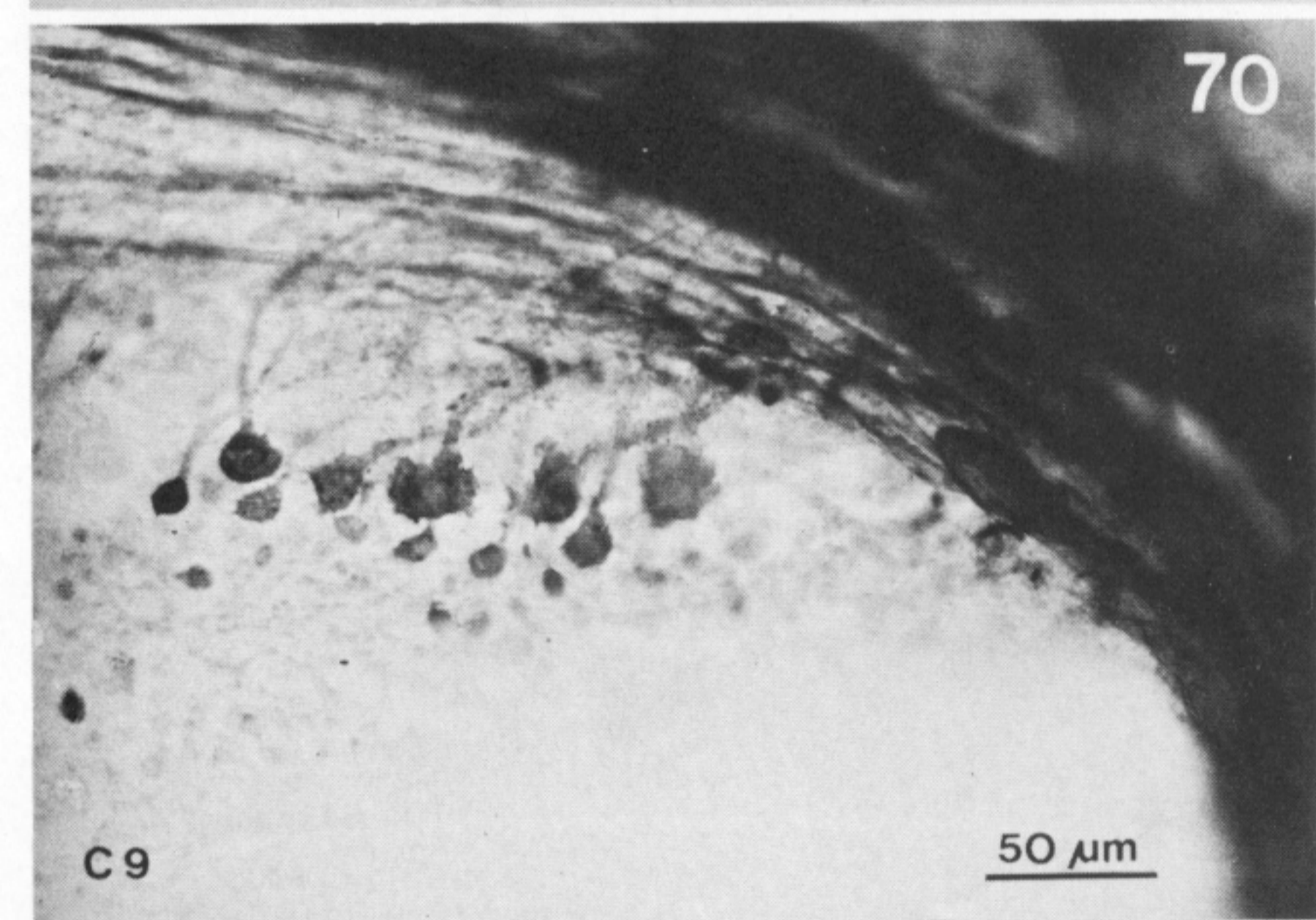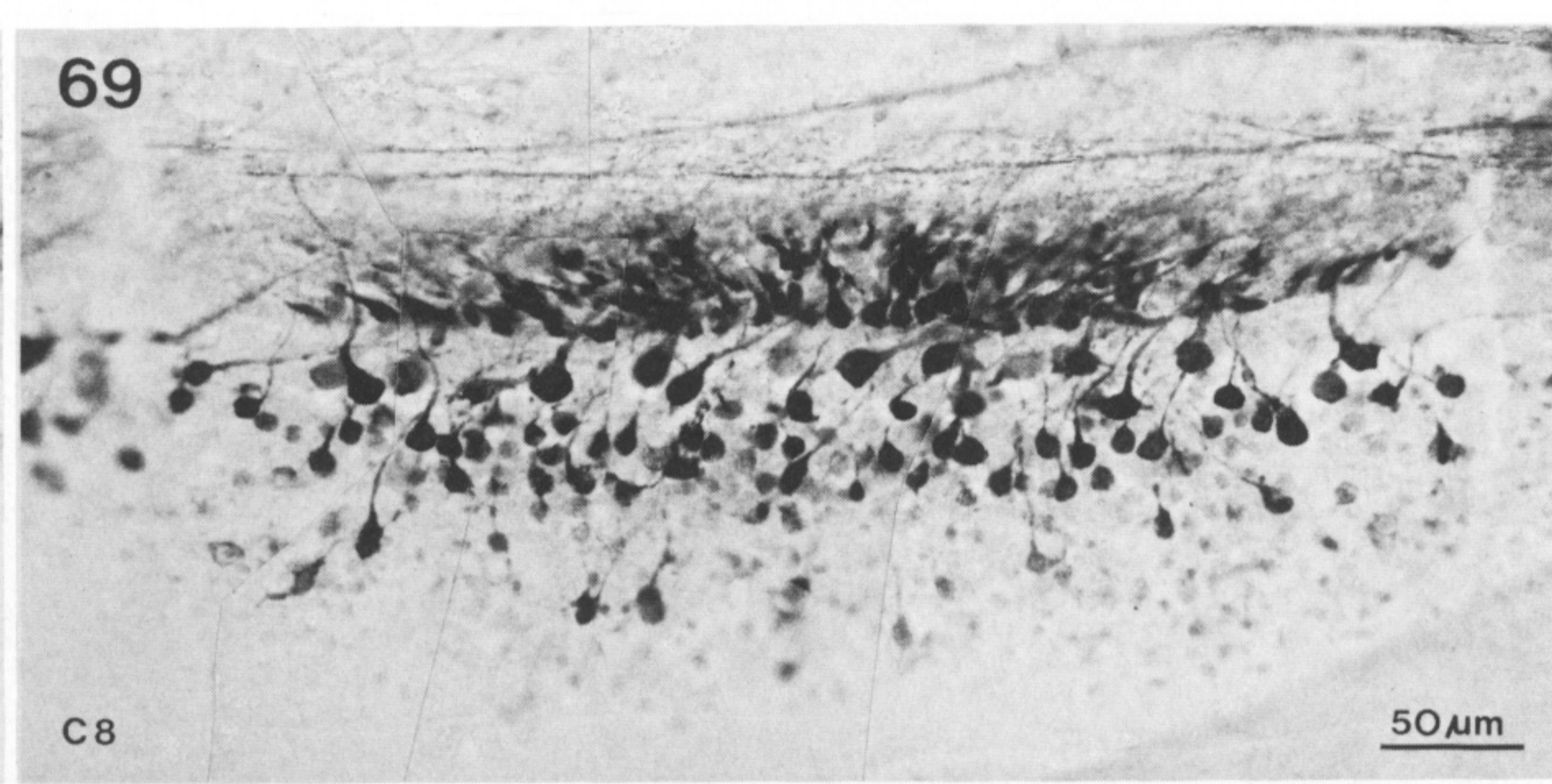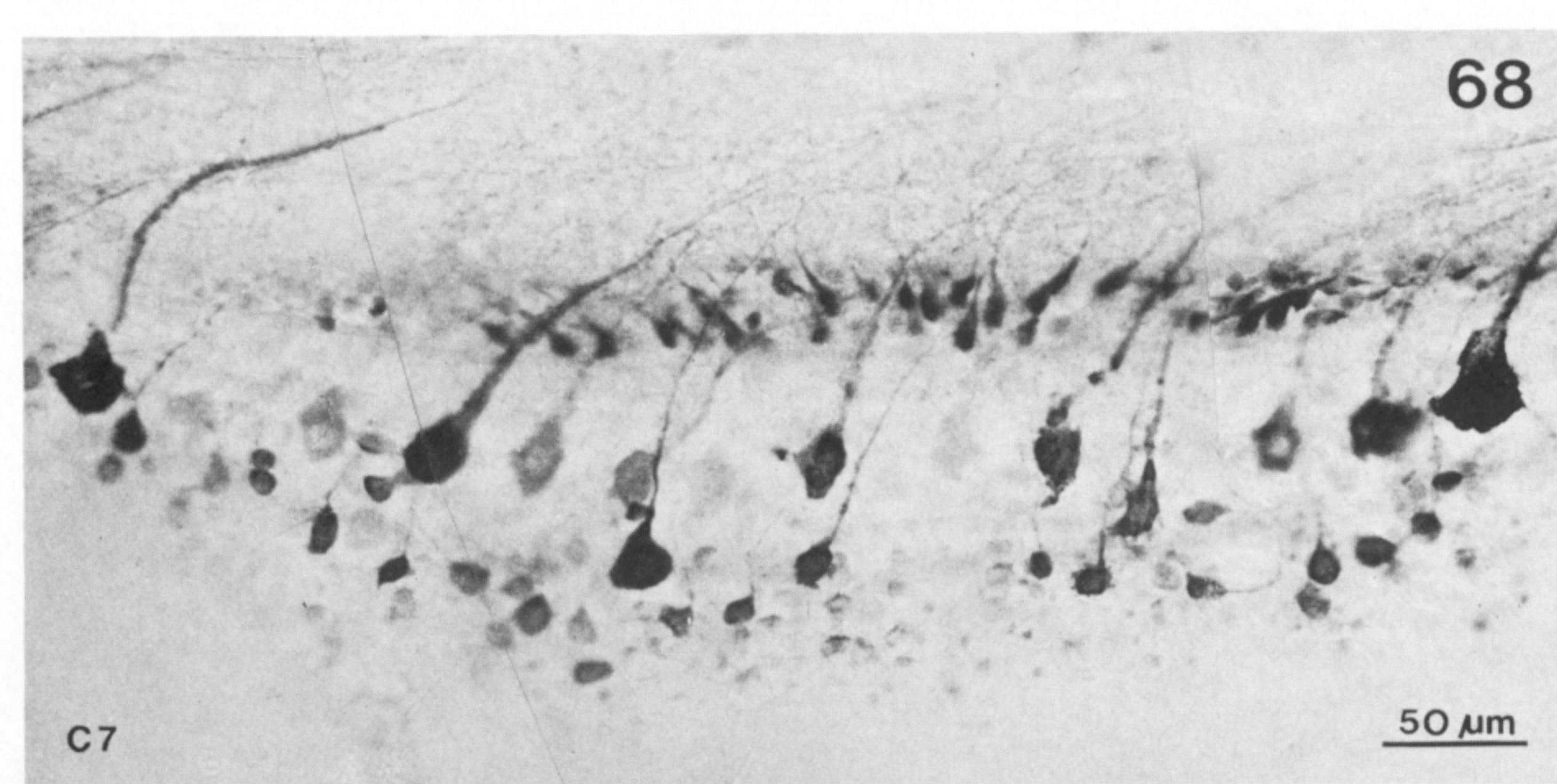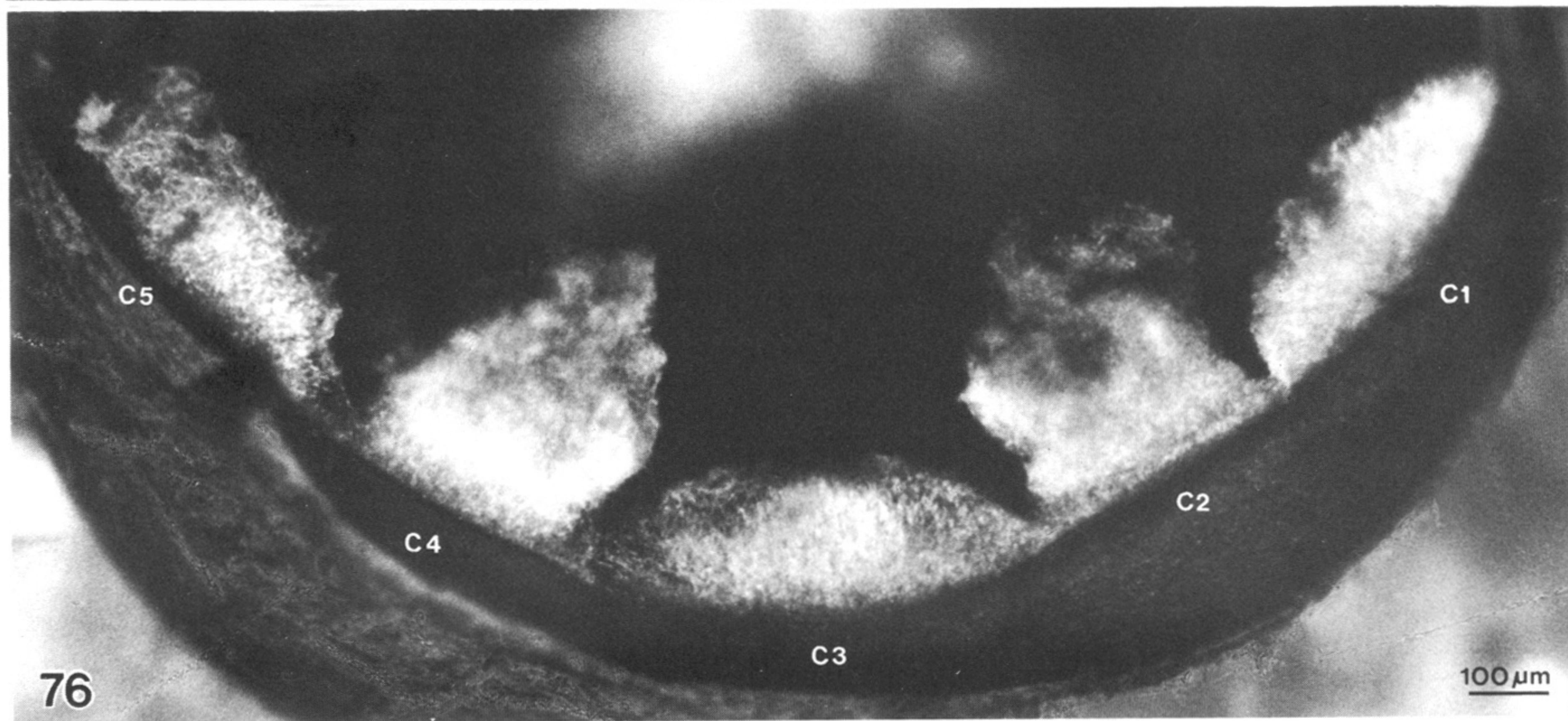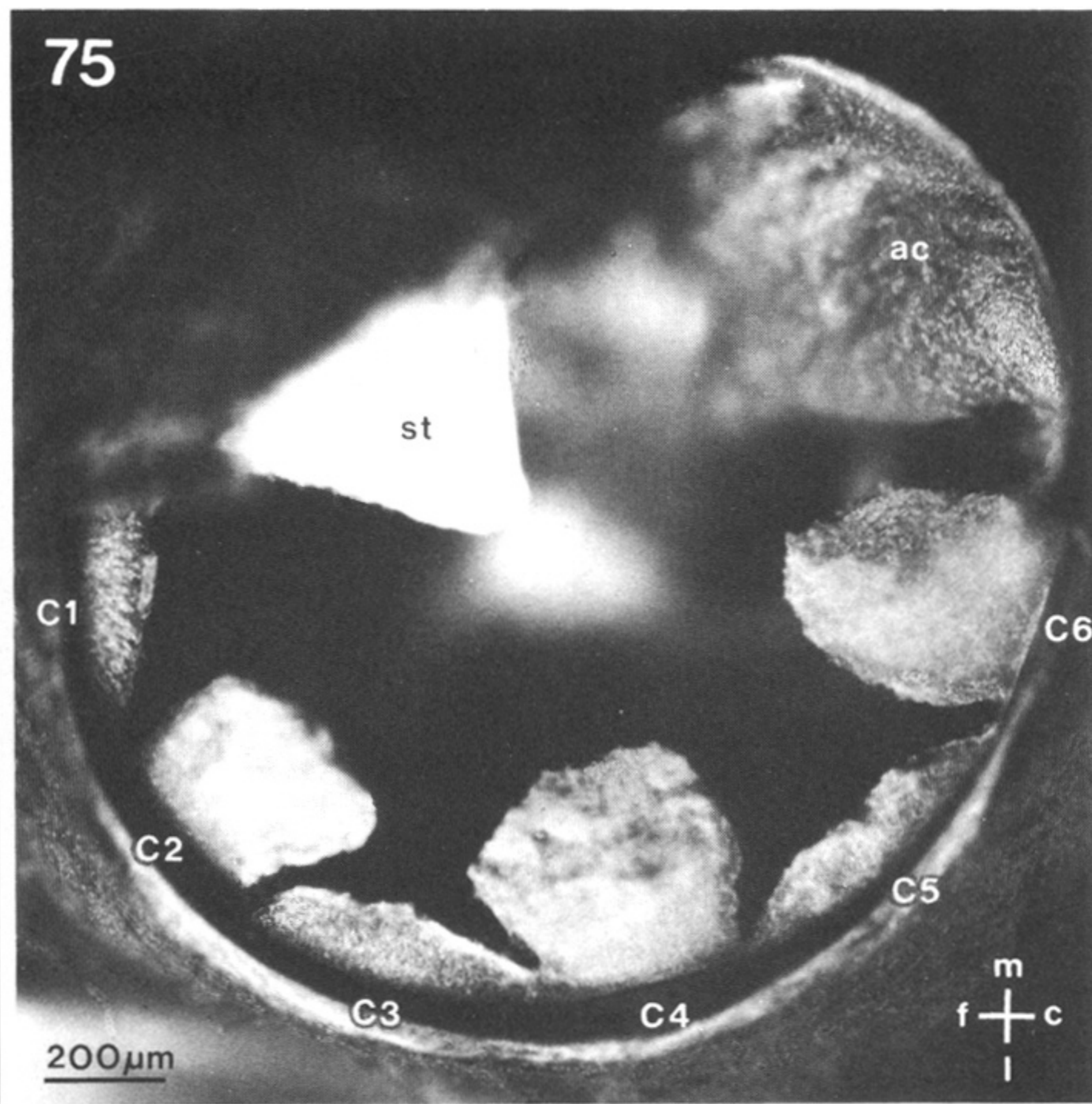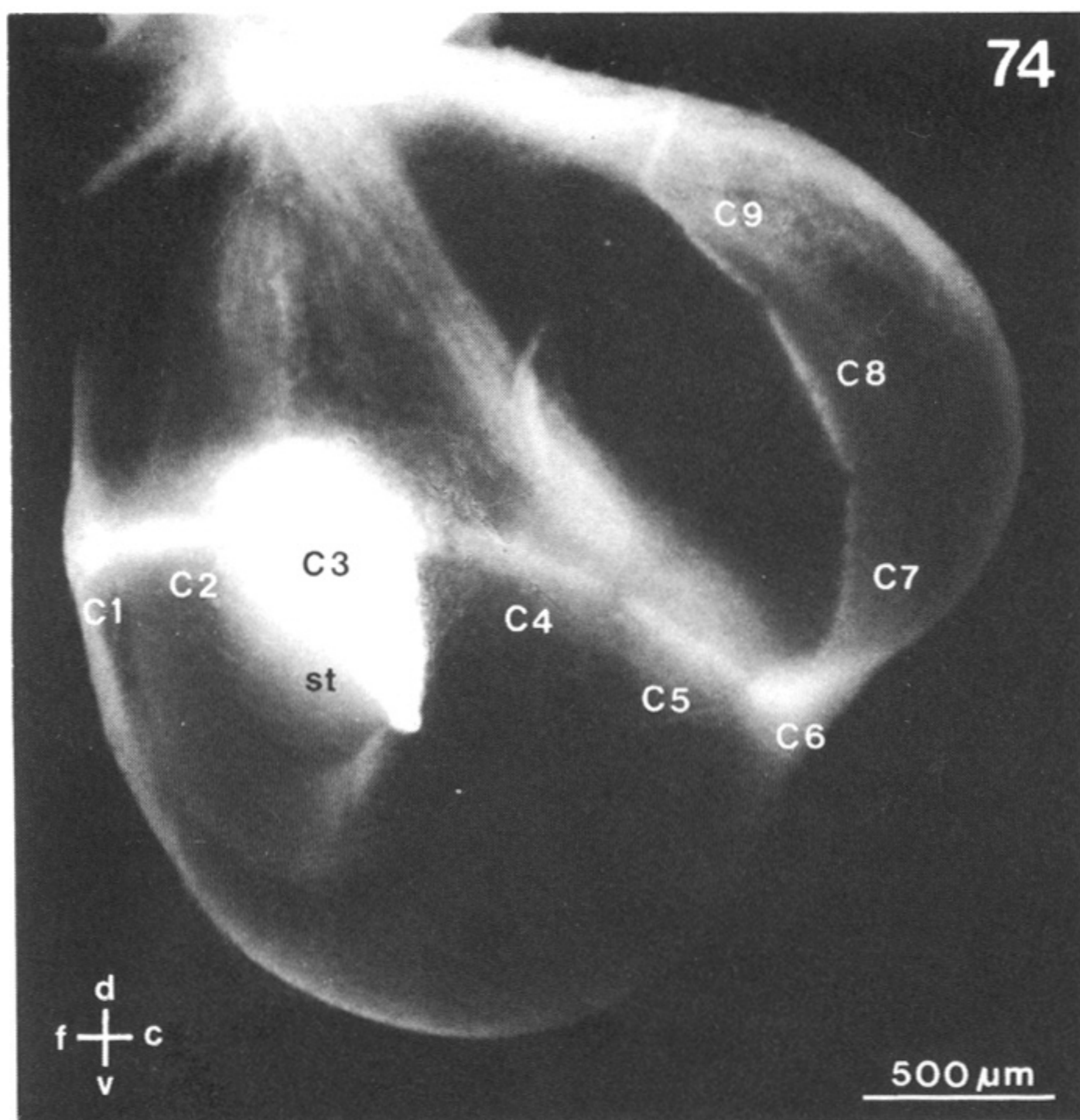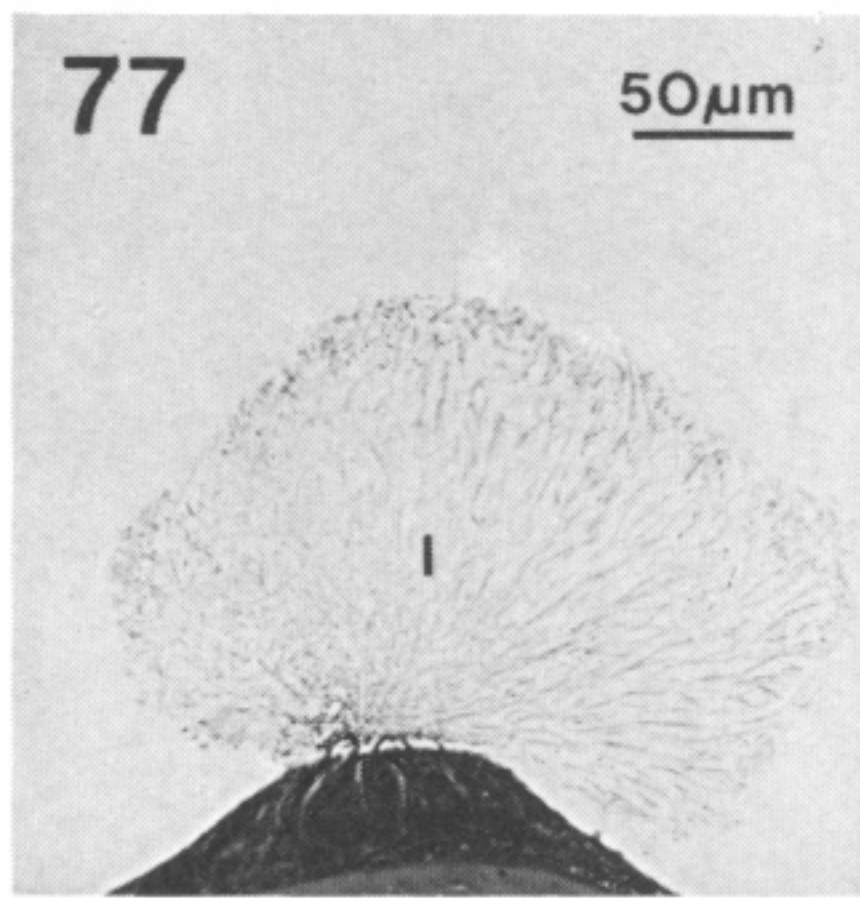
51

n.cr. post.

20 μm

52

n

100 μm

53

5 μm

54

5 μm

55

3 μm

FIGURES 49–55. For description see opposite.

57

C1 C2

100μm

58

C2 C3 C4 C5 C6

100μm

59

C9 C8 C7 C6

100μm

60 C1   50 μm

61 C2/2   50 μm

62 C2/2   50 μm

63 C3   50 μm

64 C4   50 μm

65 C5   50 μm

66 C6/2   50 μm

67 C6/2   50 μm

FIGURES 57–67. For description see page 328.

68

C7

50 μm

69

C8

50 μm

70

C9

50 μm

71

50 μm

C1

C2

72

lhc.

lhc

vhc

40 μm

73

20 μm

FIGURES 68–73. For description see page 328.

Description of plate 12

Alternating differences in form size of the cupulae attached to the nine crista sections of *Octopus*. Small cupulae are attached to the odd-numbered crista sections (C1, C3, C5, C7, C9) and large cupulae are attached to the even-numbered crista sections (C2, C4, C6, C8).

Figure 74. Lateral view of the left statocyst, showing the orientation of the nine crista sections C1–C9. Formalin-fixed preparation; part of the statolith (st) and of the mucus layer attaching it to the sensory epithelium is decalcified. Crossbar indicates dorsal (d), ventral (v), frontal (f) and caudal (c).
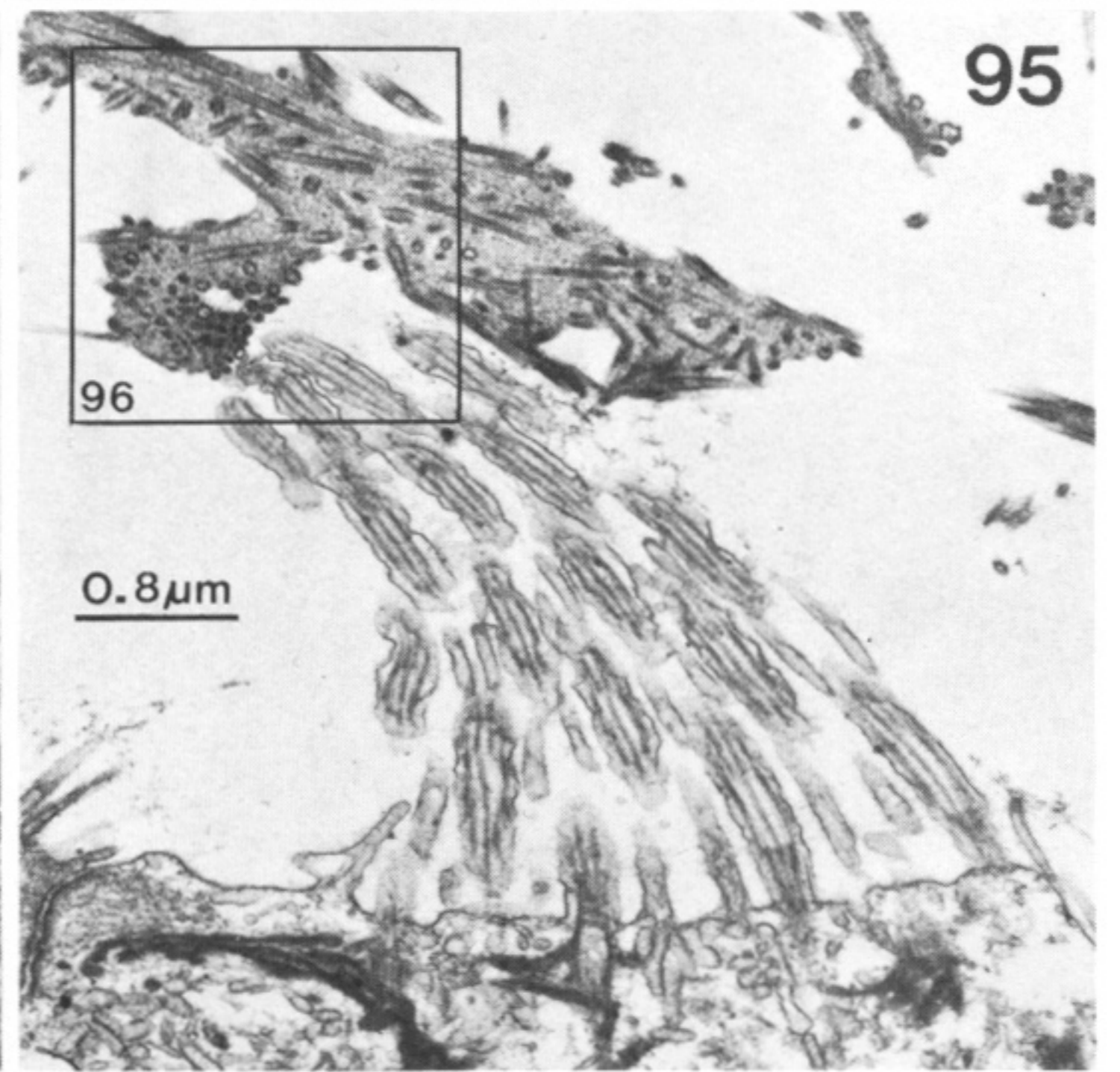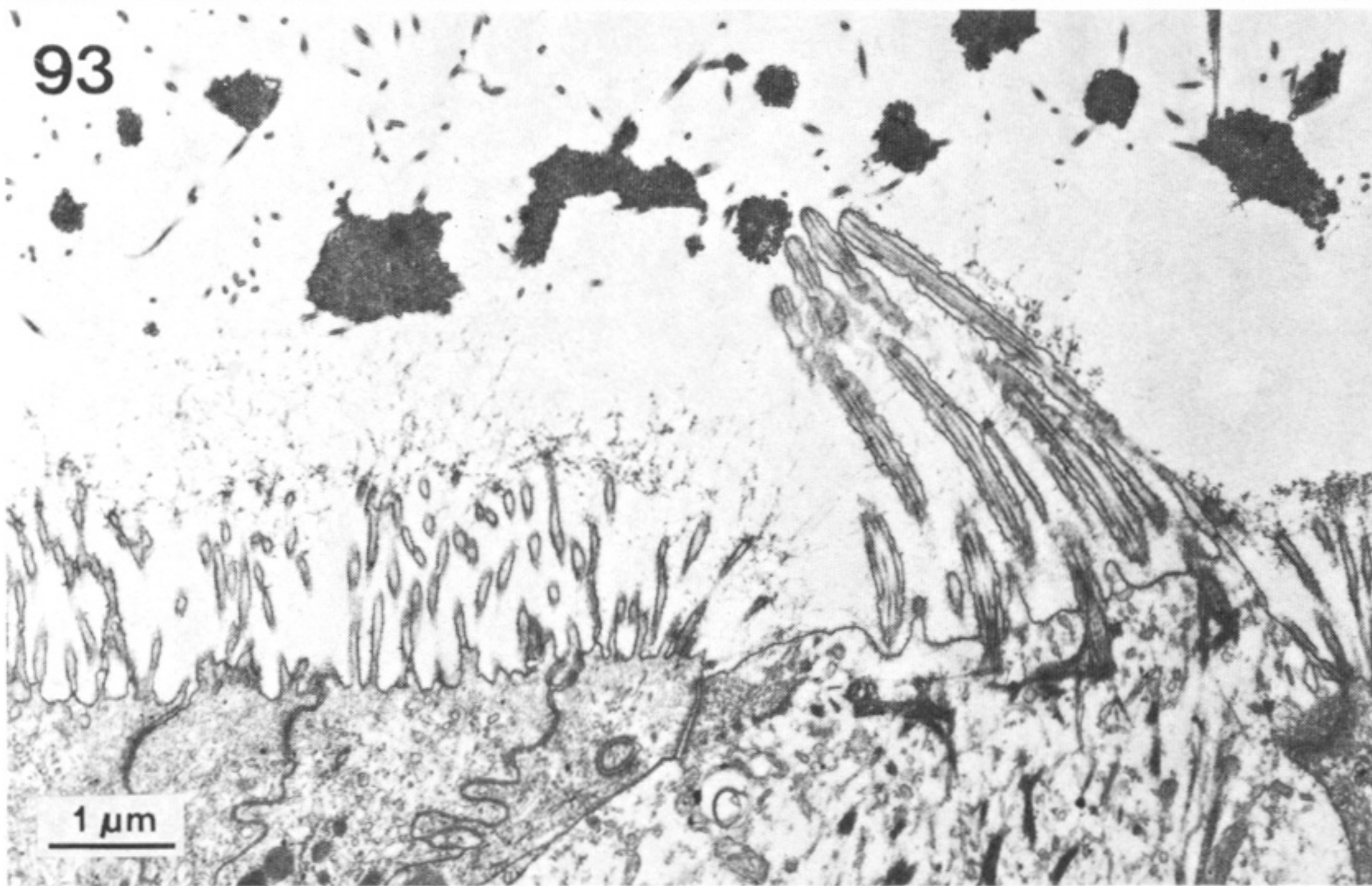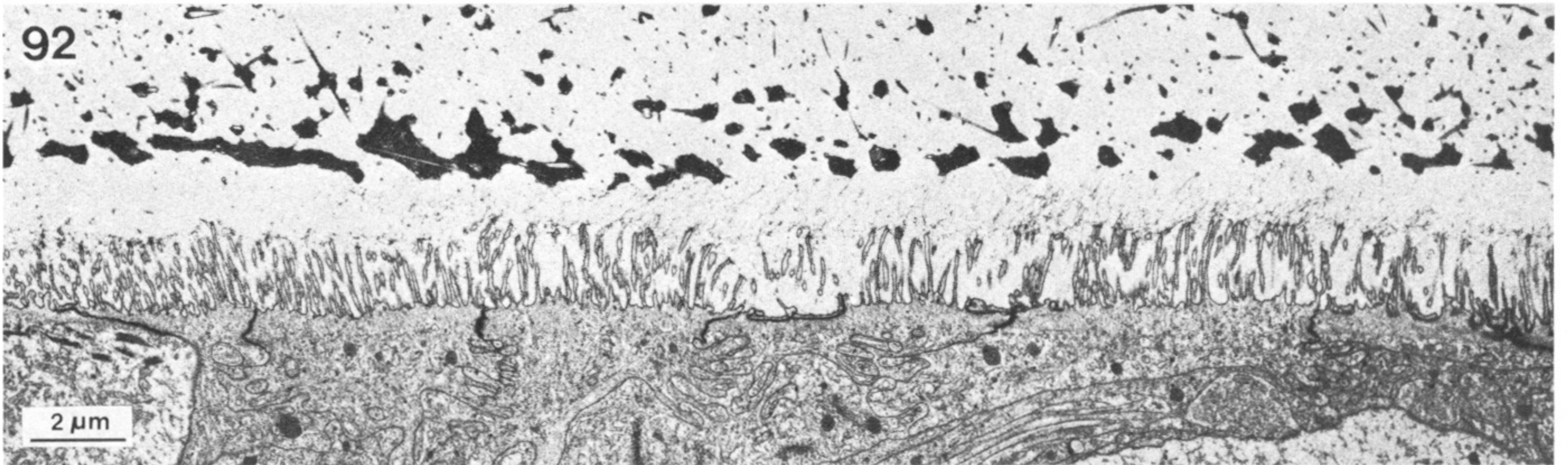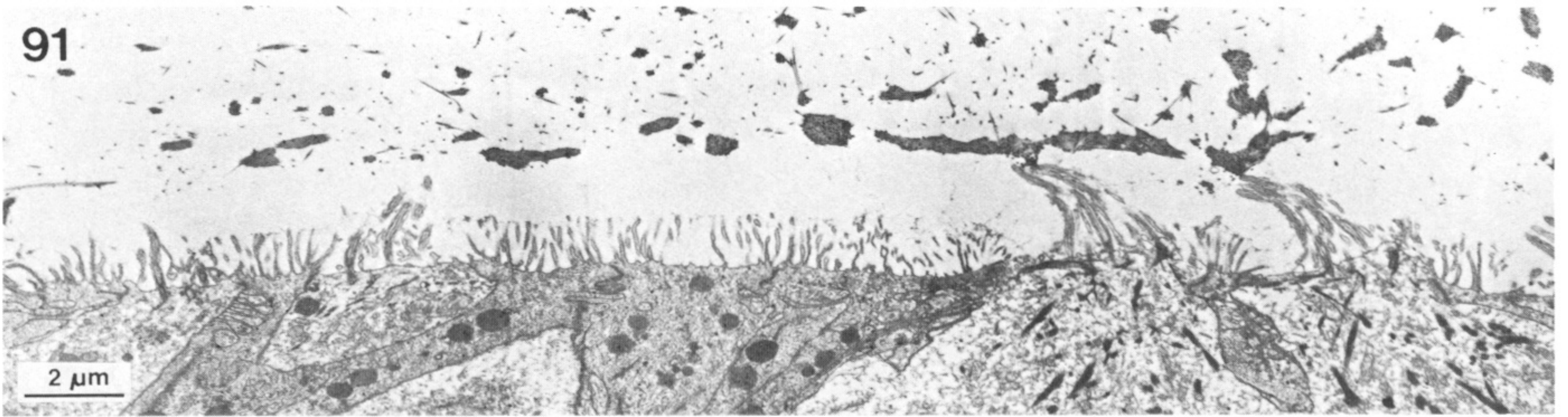
Figures 75 and 76. Ventral view into the opened right (figure 75) and left (figure 76) statocyst sac, showing the cupulae attached to the horizontally arranged crista sections. Figure 75 shows the cupulae attached to the cristae C1–C6, the statolith (st) and the cartilaginous anticrista (ac). Figure 76 shows the cupulae of the cristae C1–C5. Crossbar indicates medial (m), lateral (l), frontal (f) and caudal (c.). Osmium-fixed preparations.

79 II C2 I C3 100μm

80 C2 C3 C4 C5 100μm

81 C3 C4 C5 100μm

78 II 50μm

77 I 50μm

82 C3 I II C4 50μm

83 50μm

FIGURES 77–83. For description see page 329.

FIGURES 84–90. For description see page 329.

FIGURES 91–96. For description see opposite.