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THE LOBSTER OPTIC LAMINA
IV. GLIAL CELLS

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SUMMARY

There are 3 distinct types of glial cells in the optic lamina of the lobster: dark, light, and sheet cells, all distinguished from the neurons by being multipolar and not having dictyosomes. Dark cells are surrounded by intercellular material and together with light cells constitute a structural support for the groups of nerve cells. Light cells are also sheath cells for the neuron somata. The sheet cells have numerous flat processes which together form the 2 glial layers in which the synaptic region is sandwiched. An extensive system of extracellular cisterns between the sheet processes may serve for fluid transport towards the fibres and synapses, and the numerous vesicles in the sheet cells may represent an extension of the extracellular transport system.

INTRODUCTION

While investigating the fine structure of the neurons and synapses of the lobster lamina (Hámori & Horridge, 1966*a*, *b*) we found a number of glial structures which are so peculiar that they deserve separate attention. The glial cells and their processes encapsulate the nerve cells, fibres and the synaptic areas known as the cartridges, but do not penetrate the latter. Also, the synaptic layer of the lamina is closed off from surrounding spaces and layers by a well-developed glial layer on each side. The ultrastructure and possible functional significance of these glial layers are the topic of this paper.

MATERIALS AND METHODS

Small pieces of optic lobes of *Homarus vulgaris* containing the optic lamina were dissected in glutaraldehyde fixative; the fixation and embedding procedure is described in a previous paper (Hámori & Horridge, 1966*a*).

RESULTS

Three types of glial cells have been found. All have numerous processes, whereas the nerve cells are unipolar. The glial cells never contain the large dictyosomes which are a characteristic feature of the ganglion cells. One never encounters intermediate types of cell. All glial cells are relatively large, with a very large surface area (Figs. 2–5).

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None of the types of glial cell found here corresponds with those found by Trujillo-Cenóz (1965) in the lamina of the fly. In the lobster there are none of the capitate projections which are peculiar to the glial cells of the fly optic cartridge, and there is no layer of special 'epithelial' glial cells between the optic cartridges.

Dark cells

These are usually situated between the nerve cell bodies, or between the peripheral glial layer and the nerve cells. These cells (Figs. 2, 4) usually have several very thin processes. Chromatin in the nucleus is mostly aggregated along the nuclear membrane, which is commonly slightly folded. The perinuclear cytoplasm is densely filled with ribosomes, vesicles of 60–150 mμ diameter, dense bodies and small mitochondria. The appearance of the cytoplasm is characteristic, so that there is no difficulty in recognizing even small parts of these cells in sections. The cell body and the processes are separated from other cells by an extracellular space, 100–300 mμ thick, which is filled with a dense, homogeneous cement-like substance. This substance is composed of very fine, osmiophilic granular material.

Light cells

These are more numerous than the dark cells and occur round them (Fig. 4). They have a round nucleus which is surrounded by a scanty perinuclear cytoplasm. Several thick processes emerge from the cell body (Fig. 5). Both the cell body and its processes are relatively free of inclusions, with only a few elements of endoplasmic reticulum and free ribosomes scattered throughout the whole cell. This cell type characteristically has few light mitochondria. If found at all, the Golgi apparatus is poorly developed and situated close to the nucleus. It consists of 3 or 4 small curved cisternae, the margins of which are dilated to bulbous structures. Some of the cell processes are 'fixed' to the cement substance of the dark cells (Figs. 4, 5) in a way that suggests they contribute to it. Fine granules are commonly gathered at the contact with the cement substance. Elsewhere processes of the 2 cells interdigitate in deep folds. Occasionally desmosomes are found where 2 interdigitating processes meet. Other processes of these cells closely invest the nerve cells, as described in the first paper of this series.

Sheet or spindle-shaped cells

Both dark and light glial cells may have bordering on them numerous thin processes which are distinguished by their many large empty vesicles (Figs. 4, 5). These are processes of the spindle-shaped cells, which make up the bulk of the two glial layers, 15–25 μ thick, on each side of the columnar synaptic region (Fig. 3). The ovoid nucleus is surrounded by a thin cytoplasmic layer, 1·0–1·5 μ thick, which contains a few small mitochondria of 0·3–0·5 μ diameter, vesicles 1–3 μ in diameter, and free ribosomes. The Golgi apparatus, if present, is meagre, consisting of 1 or 2 cisternae and a few vesicles (Figs. 6, 8). The processes of these cells are long flat sheets with dilations at intervals along them (Figs. 6, 9). They contain vesicles 1–3 μ in diameter which appear empty and are so numerous that they give the cell a frothy appearance.

There are also free ribosomes and frequently another class of smaller vesicles of 15–70 mμ diameter. In some places the sheets of many cells are pressed closely together, separated from each other by an intercellular gap of only 6–10 mμ. The intercellular spaces, or cisternae, are obvious between the processes of the spindle-shaped cells, in size from 40 to 400 mμ (Figs. 6–9 and 11). These cisternae are sometimes closed off by well-developed desmosomes between the processes (Figs. 7, 11) or between the cell body and a process (Fig. 8). Desmosomes can be found elsewhere, however, not

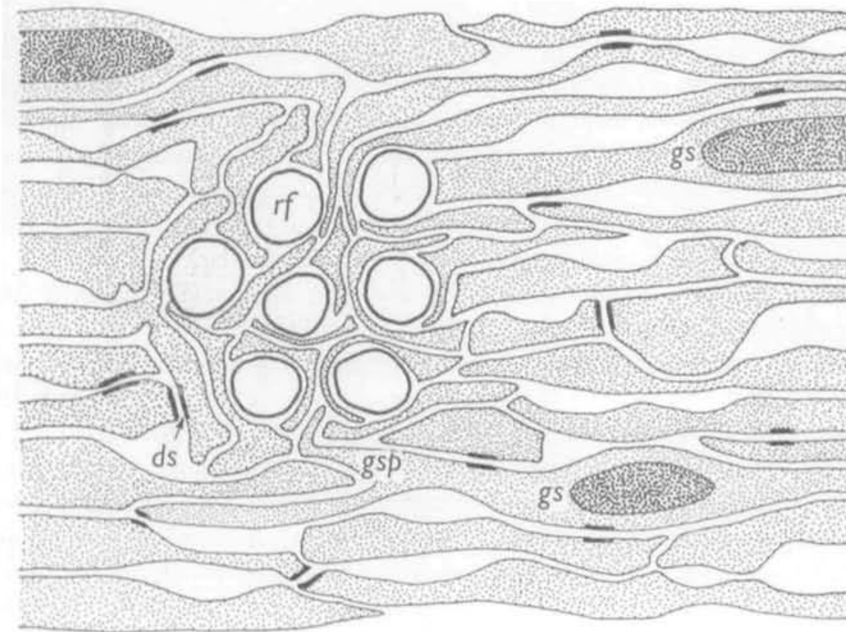

Fig. 1. Schematic surface view of the glial layer where it is pierced by a bundle of retinula cell axons (*rf*), showing the distribution of cisternae, desmosomes (*ds*), and glial cell bodies (*gs*) distant from the axons. (*gsp*, glial sheet cell process.)

particularly related to an extracellular cistern; only by three-dimensional reconstruction could it be determined that all the desmosomes play a part in closing off some cistern. The cisterns are cavities of the haemocoele but they have never been observed to have contents, which would indicate that they are a functional part of the vascular system, although this is a possibility. In each desmosome the 2 cell membranes are thickened symmetrically, each showing the unit-membrane structure. Between them lies an intermediate osmiophilic layer (inset to Fig. 10). In the cytoplasm on each side of the desmosome there is always an accumulation of microtubules bunched close to the desmosomes; they seem to be attached by a filamentous layer to the desmosome membranes on both sides. As in Fig. 10 microtubules may run from one desmosome to the other. The microtubules are arranged in bundles, in the plane of the flat glial process, and are thus seen together in longitudinal (Fig. 10), oblique (Figs. 6, 8), or transverse section (Figs. 7, 11), but never arranged at random within one process.

The glial layers formed by the sheets of cells and processes enclose the synaptic region of the lamina, broken only where retinula fibres and ganglion cell axons enter the synaptic region, usually in bundles (Figs. 1, 12). Here the glial processes attach themselves closely to the nerve fibres, and accompany them towards the cartridges. Glial cell processes, however, do not enter the cartridges, and do not invaginate into nerve cell bodies of axons except in the special circumstances of degeneration.

DISCUSSION

As deduced from morphological observations the 3 types of glial cells in the lamina all seem to serve 3 basic functions. They presumably play a part in nutrition of nerve cells, fibres and synapses, including perhaps the regulation of ions; they must act as a resistive barrier to the flow of ionic currents; and finally the numerous membranes and fibres presumably support the nerve elements. Certainly arthropod glial sheets are difficult to penetrate with micro-electrodes.

The basis of the mechanical support is established by the dark cells with their thick non-cellular layer which is similar to the basement lamina. These layers connect together the light and the dark cells. It is from these glial support areas that numerous long processes of the light cells spread out to ensheath the ganglion cells. This seems to be the only source of sheaths for the ganglion cells, since the dark cells have never been observed in contact with nerve cells. The third glial type, the sheet cells with their processes, form a compact investing layer. They do not penetrate deeply between the nerve cells, though they may make contact with nerve cell bodies lying near to their own layer of nerve fibre bundles which enter through them on the way to the synaptic region.

Histochemical study of these glial structures would be helpful in answering numerous questions. The extracellular amorphous material between glial cells in insects (Smith & Treherne, 1963) has been identified by Pipa (1961) as an acid mucopolysaccharide which may hold cations, for example, potassium, in an extracellular store. Where the ramifications of this extracellular basement lamina material meet them the processes of the light cells contain a finely divided substance which adheres to the basement lamina material. This is only one of a number of signs of an exchange of material along the glial cell processes.

The numerous thin processes of the spindle-shaped cells form the two thick glial layers, 15–25 μ deep, which shut off the synaptic region. Some of the same processes form a thin sheath around the nerve fibres as they enter the synaptic region. The remarkably high number of vesicles in the sheet processes bordering the lacunar system and spreading right down to the cartridges is to be compared with pinocytotic vesicles in endothelial cell cytoplasm of vertebrates. Since we have not so far made experiments with markers such as ferritin, we cannot prove that these vesicles are a sign of pinocytosis. However, their occasional communication with the cisterns, and the fact that some sections indicate that they apparently bud off from the cisterns, favour this assumption.

Without more direct evidence we can only infer that the glial processes play an important role in the supply of nutrients and ions to the synapses. Waterman & Wiersma

(1963) and Horridge & Sandeman (1964) found a high sensitivity to loss of the blood supply in the optic lobe of Crustacea. Five minutes after injury of the ophthalmic blood vessel the afferent optic signals can no longer be recorded. The optic lamina contains no large vessels; at the nearest they lie outside the lamina between the basement membrane of the retina and the ganglion cell layer. So there must be some efficient morphological system which takes the place of blood vessels to the cartridges. Here we believe lies the significance of the network of extracellular cisterns between the processes of the sheet cells, directly comparable with the glial lacunar system in insects (Wigglesworth, 1960). The desmosomes between the glial processes dissect the system to smaller lacunae, though it presumably remains continuous. If the vesicles represent pinocytosis, and if they flow down towards the synaptic region, along the lines marked out by the bundles of microtubules, then the flow line to the synaptic region is complete. On this theory the tubules would be regarded as an agent causing the flow, which would explain why they are attached to the membranes. For plant cells a similar theory has been proposed by Ledbetter & Porter (1964).

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ABBREVIATIONS

ag	Golgi apparatus	*gs*	glial sheet cell
cl	cartridge layer of lamina	*gsp*	glial sheet cell process
cs	cement substance	*gv*	glial cell vesicle
db	dense body	*lg*	light glial cell
dg	dark glial cell	*lgp*	light glial cell process
dp	dark cell process	*m*	mitochondrion
ds	desmosome	*mt*	microtubule
gc	ganglion cell layer	*n*	nucleus
gl	glial layer	*rf*	retinula fibre

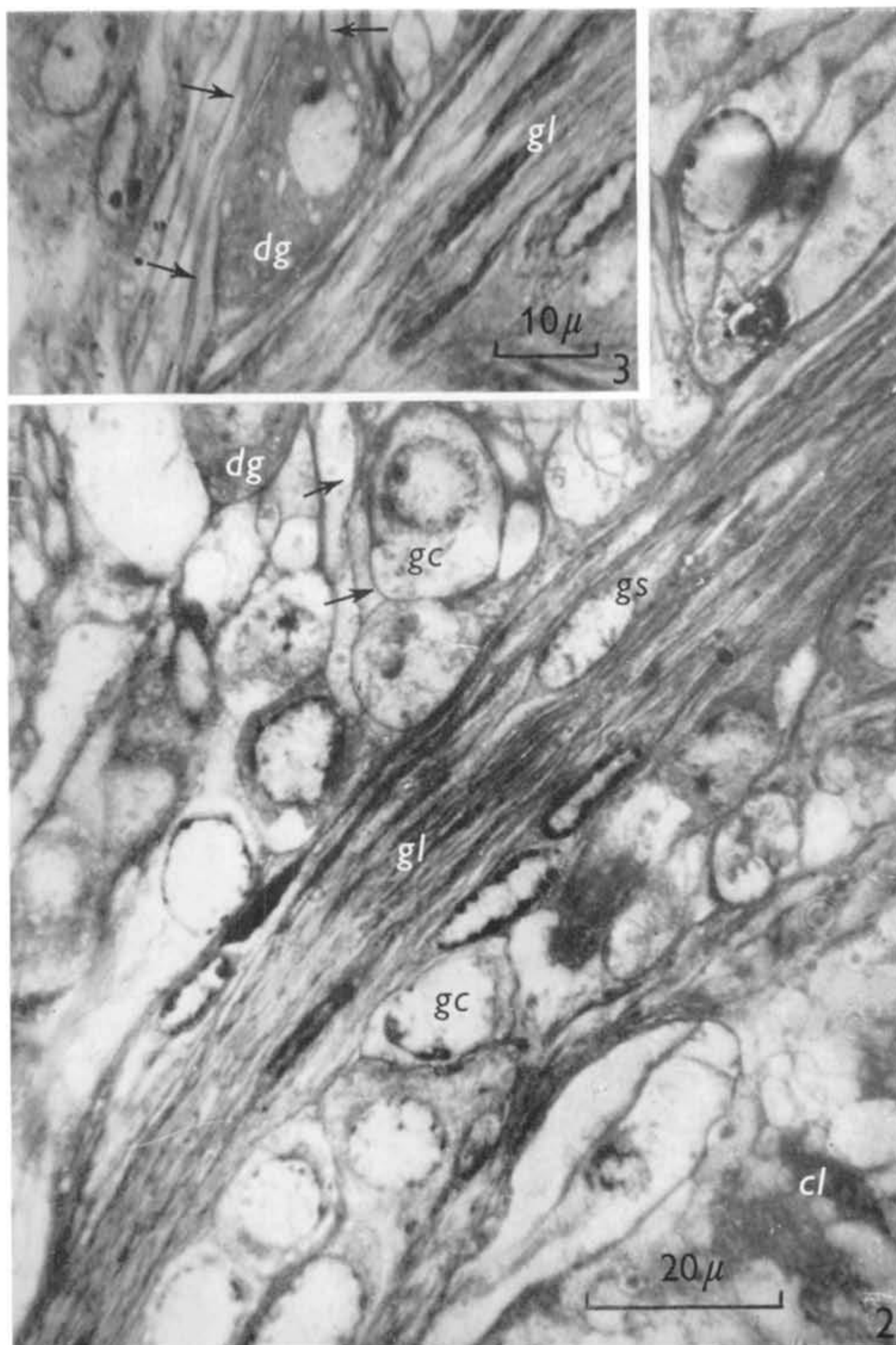

Fig. 2. Light micrograph of the peripheral glial layer of the lamina from an Araldite section stained with toluidine blue. Across the centre of the picture the thick multiple glial layer (*gl*), composed of glial sheet cells (*gs*) and their processes, separates two groups of ganglion cells (*gc*) from each other and isolates the cartridge region of the lamina (*cl*). Thick processes of light glial cells surround both the ganglion cell bodies (*gc* and arrows) and the dark glial cell (*dg*).

Fig. 3. Light micrograph of a dark glial cell surrounded by processes of glial cells bordering the glial layer (*gl*). Arrows show light glial processes.

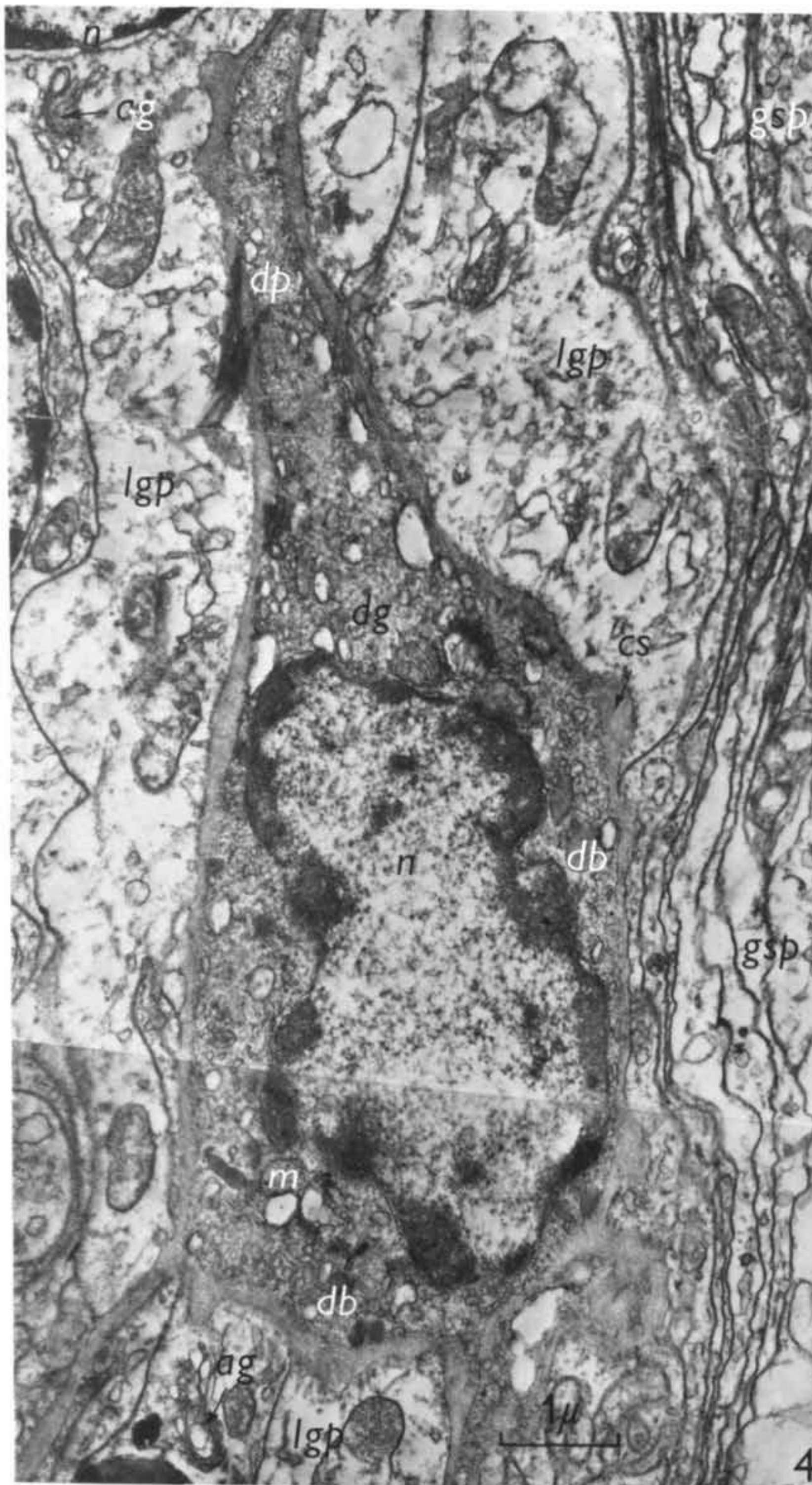

Fig. 4. Electron micrograph of the dark cell (*dg*) shown in Fig. 3. The cytoplasm is filled with dense vesicles and membranes, dense bodies (*db*) and small mitochondria (*m*). A cell process (*dp*) and the cell body are surrounded by light glial cell processes (*lgp*). The light cells contain a poorly developed Golgi apparatus (*ag*). Between the dark cell and the processes of the light cell the intercellular gap is filled with a dark cement substance (*cs*). On the right are a few sheet cell processes (*gsp*).

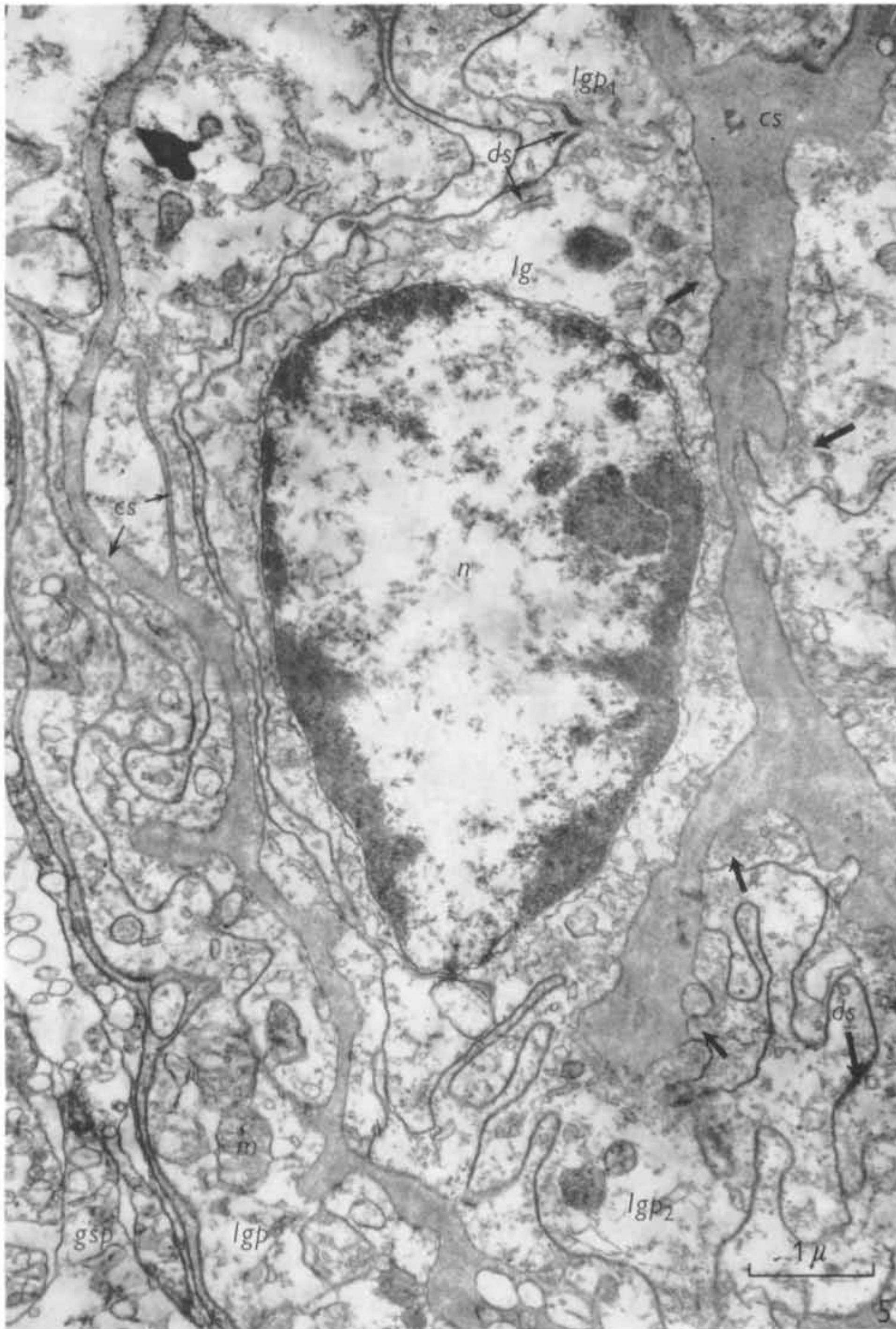

Fig. 5. Light glial cell (*lg*) from which sinuous processes emerge at top and bottom (lgp_1 and lgp_2). In this area several light cell processes interdigitate and form deep foldings with desmosomes between (*ds*). Extracellular cement substance (*cs*) ramifies between some of the lamellae. An aggregate of dense material is accumulated within the cell processes at some places where they border the cement substance (arrows).

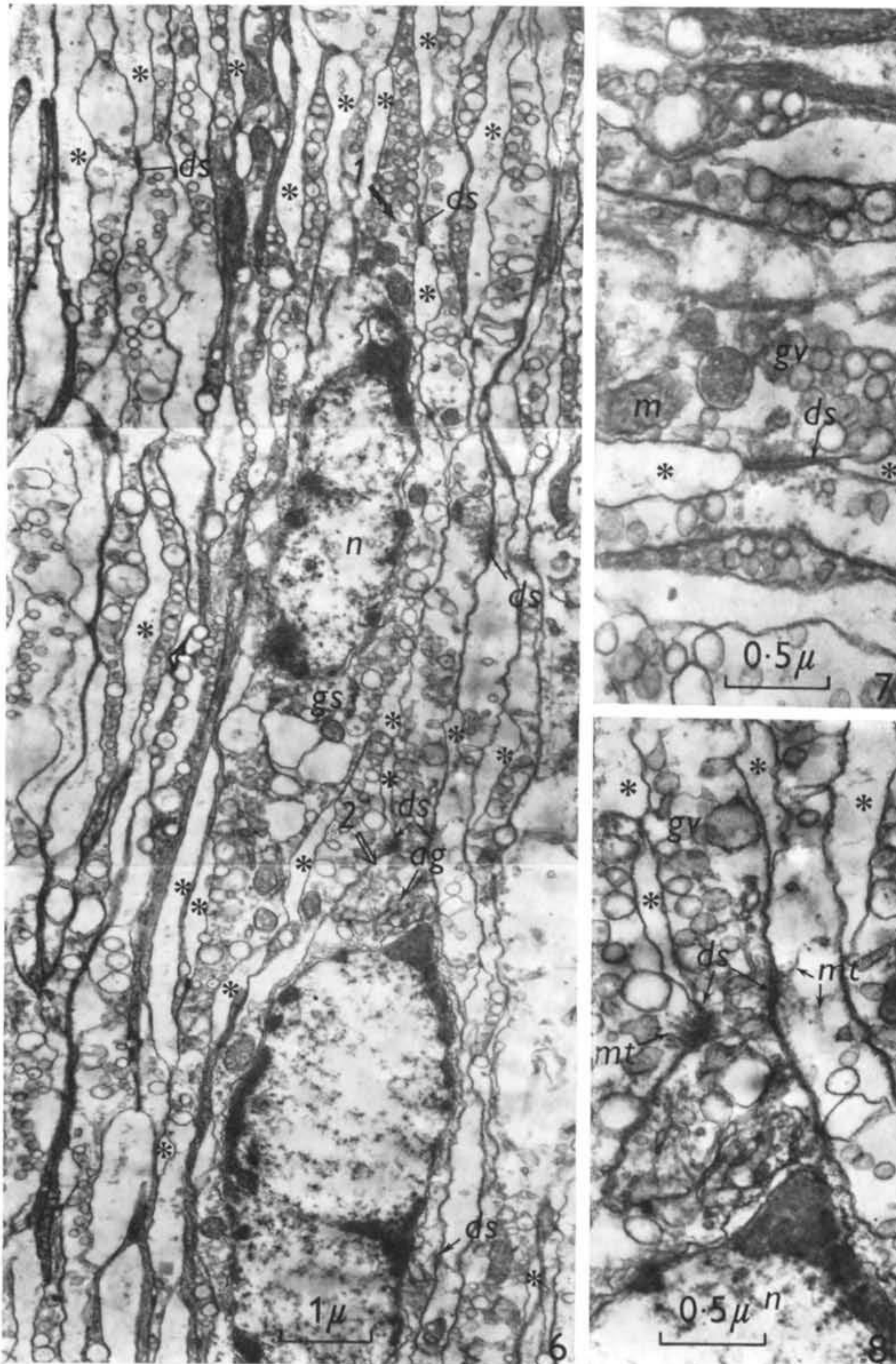

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Fig. 6. Part of the glial layer of Fig. 2, containing 2 glial sheet cells (*gs*), each with spindle-shaped nucleus, small Golgi apparatus (*ag*), long processes (arrows 1 and 2) and large vesicles. These cells are surrounded by frothy processes of other sheet cells. Desmosomes (*ds*), between processes, delimit extracellular cisternae which are marked by asterisks. The desmosome near arrow 1 at upper right is enlarged in Fig. 7, and that near arrow 2 in Fig. 8.

Fig. 7. Desmosomes (*ds*) between 2 sheet cell processes which contain large vesicles (*gv*) and mitochondria (*m*). Microtubules in cross-section lie adjacent to the thickened desmosome membrane which pinches off the 2 extracellular spaces shown by asterisks.

Fig. 8. Contacts (*ds*) between 2 bordering sheet processes, with microtubules (*mt*) in oblique section close to the desmosomes.

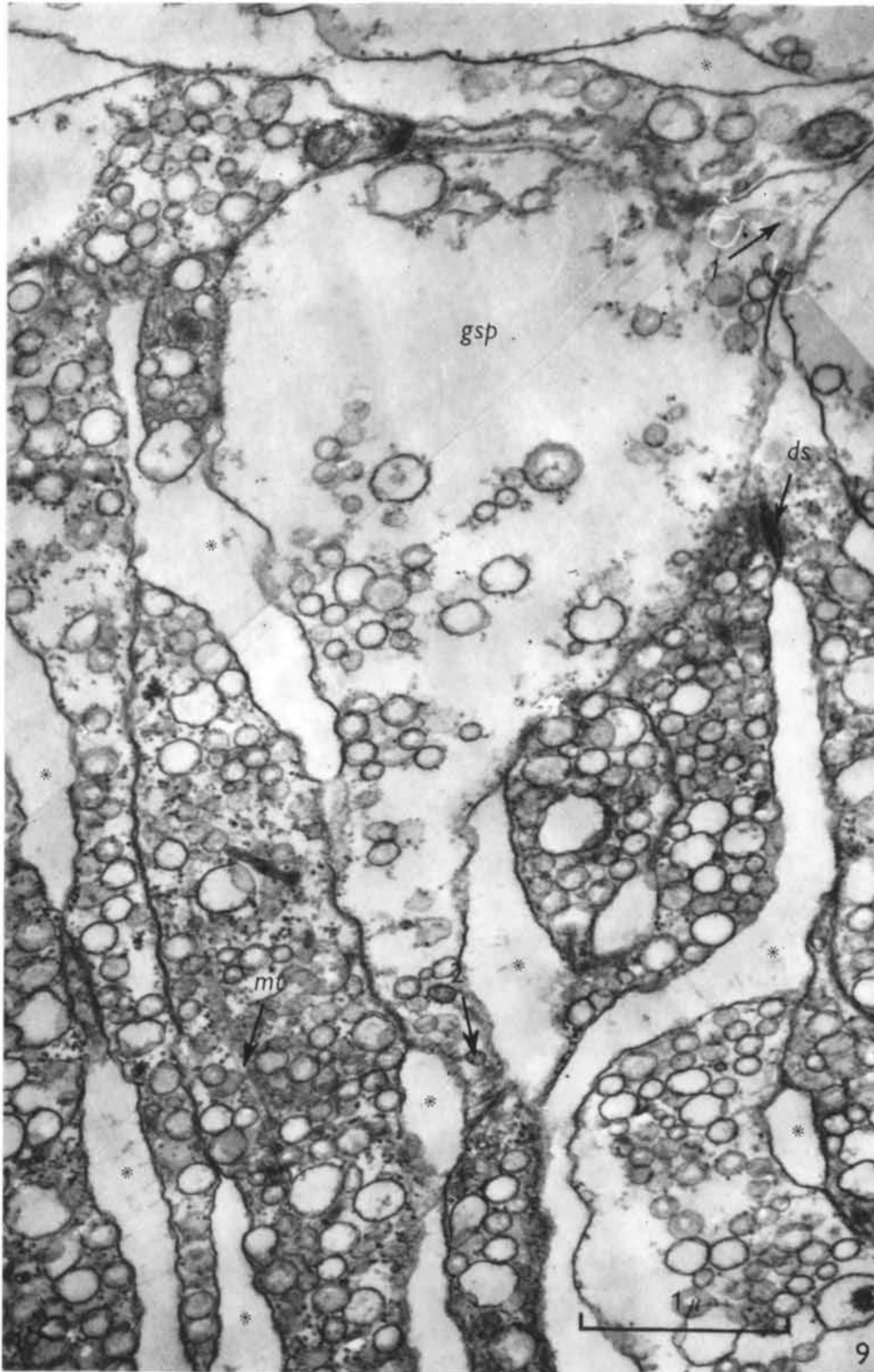

Fig. 9. Features of sheet-cell processes. The majority of the flat sheet-like processes are cut transversely and appear thin, but in the centre the one (*gsp*) which continues at 1 and 2 is expanded where it is twisted and cut transversely. There are typical microtubules (*mt*) and desmosomes (*ds*); asterisks mark extracellular cisternae.

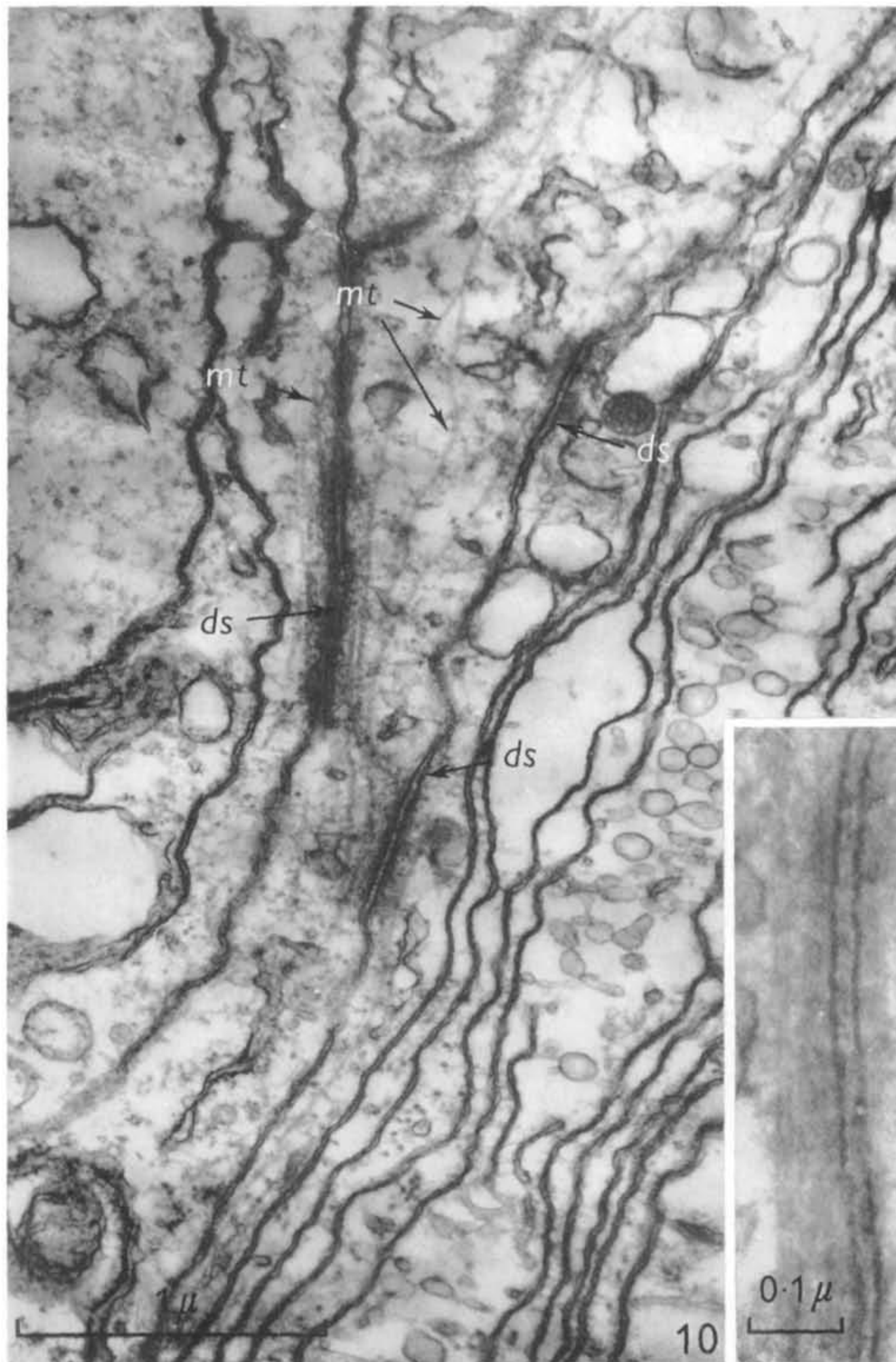

Fig. 10. Desmosomes (*ds*) between sheet-cell processes. The adhesion of microtubules (*mt*) to the desmosomes is especially characteristic; the same microtubules run from one desmosome to another. Microtubules are attached to the thickened desmosome membranes by microfilaments. In the inset the structure of the desmosome is shown as two symmetrical unit membranes with an additional osmiophilic layer along the central line of the intercellular gap.

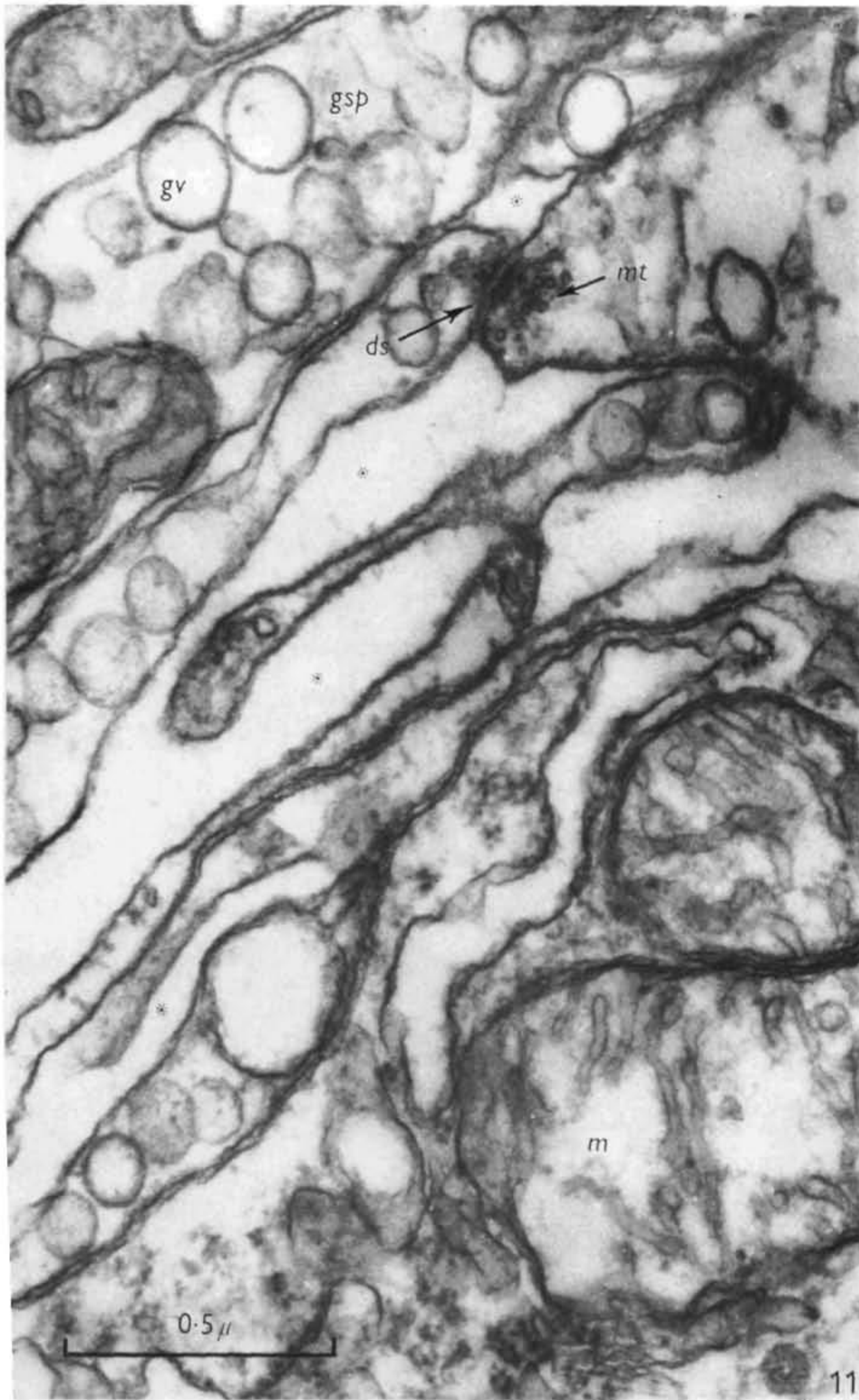

Fig. 11. Desmosome (*ds*) with microtubules (*mt*) in cross-section and so appearing as vesicles. In the processes (*gsp*) are large vesicles (*gv*) and mitochondria (*m*). Asterisks denote extracellular spaces.

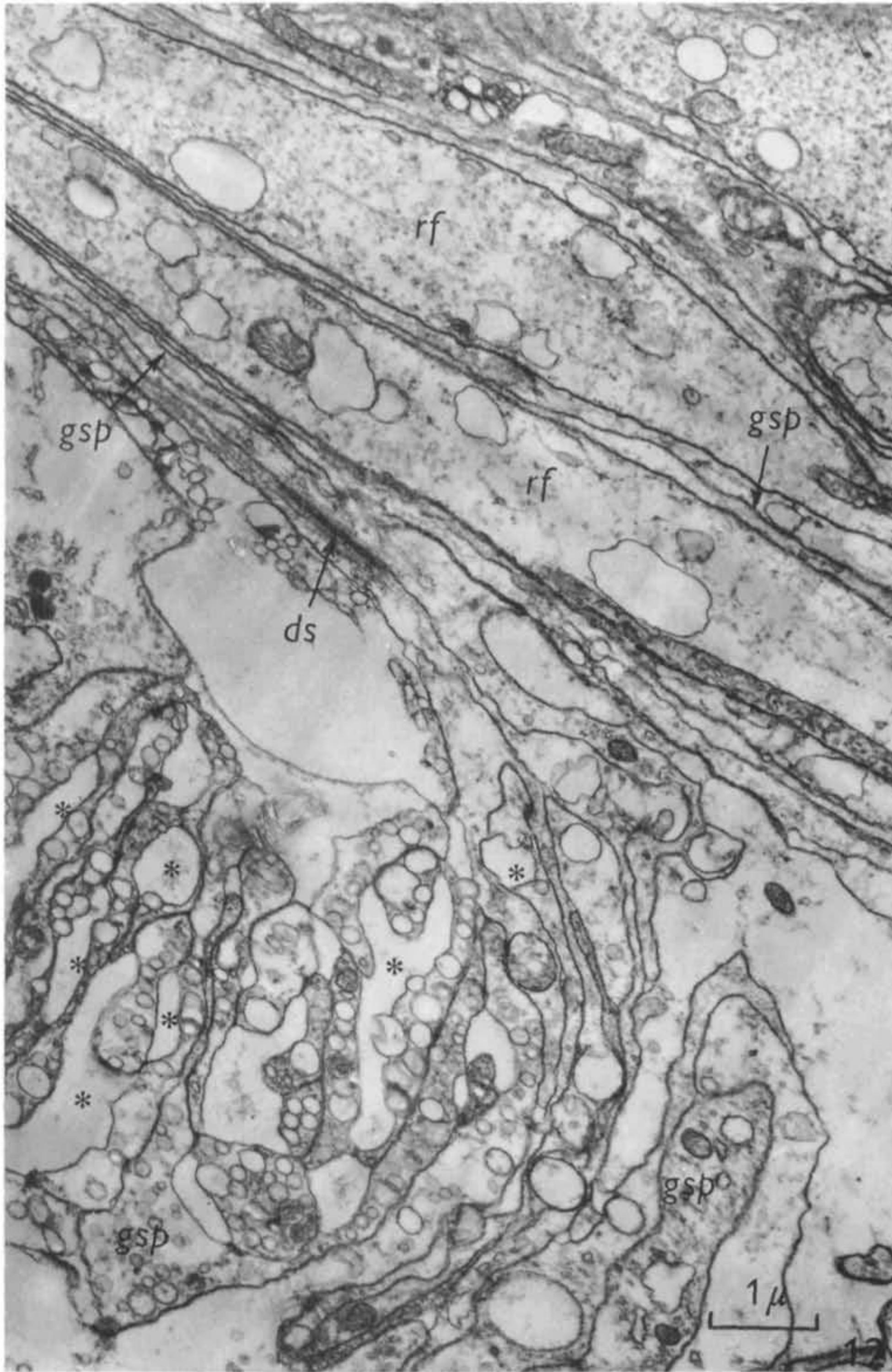

Fig. 12. Micrograph showing the processes of sheet cells (*gsp*) of the glial layer turning to accompany retinula fibres (*rf*) as these enter the lamina. As before, there are desmosomes (*ds*) between glial processes; the extracellular cisternae, which are noticeably not in the vicinity of the retinula fibres, are marked by asterisks.