CELL SURFACES AND FIBRE RELATIONSHIPS IN SYMPATHETIC GANGLION CULTURES: A SCANNING ELECTRON-MICROSCOPIC STUDY

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SUMMARY

Sympathetic ganglia from newborn rats and guinea-pigs were grown in modified Rose chambers and examined with scanning electron microscopy after 5-7 days. The cell types seen were macrophages, neurons, glial cells and connective tissue cells. They presented a wide range of surface morphologies and 3-dimensional configurations, from spheroid with an irregular surface to flattened with a smooth surface. The arrangement of the nerve fibres and cells in the outgrowth was essentially 2-layered with connective tissue cells nearest the substrate and nerve fibres, glial cells and macrophages lying over them. The relationships of sympathetic nerve fibres to the different cell types were also investigated. In all cases nerve fibres closely followed the cellular surface contours although the nature of the relationships varied. Fine finger-like cytoplasmic projections were sometimes seen from connective tissue cells and macrophages. The possible role of these structures in adhesion and motility is discussed.

INTRODUCTION

Many studies have been carried out on the morphology of nervous tissues in culture (see Murray, 1965, for review; Chamley, Mark, Campbell & Burnstock, 1972b; Chamley, Mark & Burnstock, 1972a). However, only a few reports have appeared on the 3-dimensional structure of cultured nerve fibres and their accessory cells as seen with scanning electron microscopy. Boyde, James, Tresman & Willis (1968) reported the appearance of nerve fibres and their accessory cells in spinal cord cultures; Lodin, Faltin, Booher, Hartman & Sensenbrenner (1973) described fibre formation and myelination in dissociated dorsal root ganglia; Wilson, Schenkel & Fry (1971) and Shimada & Fischman (1972) observed the junctions formed in vitro between skeletal muscle and spinal cord. Scanning electron microscopy of sympathetic nerve fibres in association with isolated smooth muscle cells has been reported (Chamley, Campbell & Burnstock, 1973), but no 3-dimensional study of sympathetic nerve cell bodies and their accessory cells has been made. This is undertaken in the present report, together with a study of sympathetic nerve-accessory cell relationships.
MATERIALS AND METHODS

Paravertebral sympathetic ganglia from 5-day-old Sprague-Dawley rats and newborn guinea-pigs were cultured for 5-7 days on collagen-coated glass coverslips in modified Rose chambers (Rose, 1954). The nutrient medium consisted of Medium 199 (Salk, Younger & Ward, 1954) to which 5 mg/ml glucose, 0.05 units/ml insulin, 100 units/ml penicillin and 1 unit/ml nerve growth factor (Burroughs-Wellcome, England) were added. For a more detailed description of the culture techniques see Chamley et al. (1972b).

Individual cells were selected for study and photographed using phase-contrast microscopy (Zeiss Standard RA microscope, Zeiss Ikon camera). The cultures were then dismantled, the cultures washed with Hanks' Balanced Salt Solution (Hanks & Wallace, 1949) and fixed in 5% glutaraldehyde in 0.2 M cacodylate or 0.2 M phosphate buffer for 2 h at pH 7.4. This was followed by dehydration through a graded series of ethanol and immersion in several changes of absolute acetone. The cultures were left overnight in a mixture of equal volumes of acetone and Araldite and then placed into pure Araldite for 2 h at room temperature.

After a further 0.5-0.75 h at 60 °C, the culture surfaces were washed free of Araldite with acetone and returned to the 60 °C oven for 24-36 h to complete polymerization (Alexander, Ritchie & Maloney, 1971; Cleveland & Schneider, 1969). The specimens were mounted on aluminium stubs, coated with 30 nm of gold using a rotary coating device (Dynavac High Vacuum Coating Unit, Model CE 12/14) and viewed in a Cambridge S4 Stereoscan scanning electron microscope operated at 20 kV.

Photographs were taken using a Linhof 70-mm camera back with Ilford FP4 Panchromatic Film.

RESULTS

General observations

The strip of dialysing cellophane placed over the sympathetic ganglion explants flattened their upper surface and prevented any observations of the 3-dimensional structure of constituent cells. However, around the edge of the explant, where there was only a single layer of cells, structures were not compressed by the cellophane and could be readily observed.

Within 5-7 days of culture, many cells had migrated from the explanted ganglion tissue together with regenerating nerve fibres. The outgrowth region was arranged in 2 layers with connective tissue cells near to the collagen substrate and nerve cells, nerve fibres, associated glial and Schwann cells and macrophages overlying them. The different cell types showed a range of surface morphology and degree of flattening from smooth and extremely thin to bulbous and thick. Each cell type and the nature of its relationship with nerve fibres will be considered separately.

Neurons

Observations were made on the neurons both at the edge of the explant (Fig. 1c) and those which had migrated into the outgrowth region (Fig. 1a, b). In both positions they were the largest and thickest of all the cells present with the possible exception of the macrophages. Their upper surface was irregular and frequently showed large bulbous protrusions (Fig. 1b, arrows). Extensive nerve fibre meshworks from centrally placed neurons grew over the neurons at the edge of the explant (Fig. 1c) and, together with the processes of these peripherally placed cells, continued into the outgrowth. The neurons at the edge of the explant were usually round in shape and had fairly limited
contact with the substrate (Fig. 1c) while those in the outgrowth were more flattened at their bases, sometimes showing thin flanges of cytoplasm which extended out from the cell body to attach to the collagen substrate (Fig. 1b). In both cases, several processes could usually be seen emerging from the cell body (Fig. 1b, c).

In the outgrowth region, processes from neurons formed extensive meshworks of fine fibres and bundles. Some of the fine fibres were composed of several individual axons (Fig. 2a, b). Components of these bundles frequently separated and then coalesced with neighbouring bundles (Fig. 2a, b, arrows). Where two fibre bundles crossed, the smaller one usually passed over the larger, closely following the surface indentations. The tips of single fibres and fibre bundles were expanded and flattened to form growth cones containing numerous fine filopodia (Fig. 2c, arrows). On some occasions, fine finger-like extensions were also seen emerging from the preterminal regions of fine fibres (Fig. 2c, short arrows). Individual axons frequently exhibited small swellings (Fig. 2d) corresponding in dimension and distribution to the vesicle-containing varicosities of adrenergic nerve fibres seen in vivo (Burnstock & Iwayama, 1971) and in vitro (Chamley et al. 1972b).

**Glial cells**

Schwann cells were generally bipolar and closely associated with nerve fibres. Their processes extended along the axons (Fig. 2b), frequently wrapping around them in a helical fashion. The nuclear region of Schwann cells was thick with an irregular surface of longitudinal ridges and depressions (Fig. 3, arrow). This region had only limited contact with the substrate. On some occasions, other fibres were seen passing over the Schwann cells and closely following the surface indentations. However, these fibres were not seen to deviate from a straight course.

Other glial cells, which were generally tripolar or quadripolar, were frequently found where several nerve fibres crossed. The cell body was flattened against the substrate and the upper surface was fairly smooth. In contrast to the Schwann cells, nerve fibres which passed over the cell body formed a network on its surface (Fig. 4a, b, arrows). At least one nerve fibre on each cell exhibited an enlarged area in contact with the cell surface (Fig. 4b, short arrow).

**Connective tissue cells**

These were the most flattened of all the cell types in the cultures (Fig. 4a, b). The cell surface was smooth although small microprojections extended from the lateral borders and sometimes from the upper surface. The nucleus with its nucleoli was usually visible as it bulged against the upper cell surface. During mitosis, the cells were rounded up and raised from the substrate, their upper surface appearing irregular with numerous fine cytoplasmic projections connecting them to the substrate.

Fibres which grew over the connective tissue cells followed the contours of the cell surface although they did not otherwise deviate from a straight course across the cells.
Macrophages

Macrophages assumed a variety of forms, from round to elongated with one or more thick processes. Although they were thick, their lower surface was flattened against the substrate. Thin flanges and fine finger-like projections of cytoplasm extended from their lateral edges. The upper surface was irregular with large bulbous protrusions (Fig. 3).

In general, macrophages were not associated with nerve fibres. However, when fibres did lie over these cells, they either followed every indentation on the cell surface or created deep depressions in the thick cells.

Discussion

The outgrowth of nerve fibres and cells from explanted sympathetic ganglion tissue presented an essential two-layered appearance similar to that described by Boyde et al. (1968) in chick embryo spinal cord cultures. Flattened connective tissue cells were in contact with the collagen substrate and over them lay nerve fibres and glial cells. The scanning electron microscope revealed that nerves which appeared to be single fibres under phase-contrast microscopy often consisted of several fibres. This is consistent with transmission electron-microscopic studies in vivo (Pick, 1970) and in vitro (Chamley et al. 1972b).

Sympathetic neurons were observed in the outgrowth and at the edge of the explant. In both positions, these cells were thick with an irregular surface of ridges and depressions. It is unlikely that these surface structures are artifacts due to collapse of the cells around organelles since in our experimental procedure the tissue water was replaced with plastic. In contrast, Boyde et al. (1968) used the air-drying technique which is known to produce a number of artifacts due to collapse of the tissues (see Boyde, Weiss & Veselý, 1972). Thus, the one neuron identified in the outgrowth by these authors had an obvious nucleus and a single nucleolus. The use of different methods may also account for the discrepancy in the texture of the glial cell surfaces, which were smooth in our cultures and irregular and pitted in the study of Boyde et al. (1968). An alternative explanation is that glial cells in sympathetic ganglia are markedly different from those in spinal cord.

Fine finger-like cytoplasmic projections were observed along the lateral borders, and occasionally on the upper surfaces, of some connective tissue cells, and along the lateral borders of some macrophages. However, they did not occur on any of the other cell types. Comparison of the behaviour of the different cells provides some insight into the possible role of these structures. Macrophages and connective tissue cells are extremely motile and do not usually aggregate, although connective tissue cells do sometimes show marginal overlapping (Boyde, Grainger & James, 1969; Hodges, 1970). Neurons, Schwann and glial cells are relatively sedentary and, in the outgrowth region, are usually separated from each other. Thus, it is possible that the density of projections on a particular cell can be correlated with the degree of motility of the cell and its ability to establish cellular contacts. Studies of other cells which show similar
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structures provide support for this suggestion (Pugh-Humphreys & Sinclair, 1970; Sträuli, Lindenmann & Haemmerli, 1971; Hodges & Muir, 1972). Epithelial cells, which grow in confluent sheets, have an even distribution of microprojections (Pugh-Humphreys & Sinclair, 1970) while tumour cells, which form 3-dimensional arrays, are covered with them (Pugh-Humphreys & Sinclair, 1970; Sträuli et al. 1971; Hodges & Muir, 1972). Furthermore, during division, when locomotion and cellular contacts are decreased, the number of microvilli is greatly reduced.

Establishment of contacts with the non-cellular substrate also appears to be mediated via microprojections. For example, the projections on the upper surface of normal and malignant cells disappear during mitosis when adhesion to the substrate is minimal, although some microprojections are still present around the periphery (Boyde et al. 1972; Hodges & Muir, 1972; present observations), presumably to prevent the cell from losing all contact with the substrate. De Bault & Millard (1972) also report general loss of surface microvilli and undulating membranes on neural and non-neural cells as the cells become detached from their substrate following treatment with 6-hydroxydopamine. These results are in contrast to those of Follett & O'Neill (1969) and Follett & Goldman (1970) who reported the presence of microprojections on the upper surface of BHK fibroblasts during division.

For any one cell type, the density of microprojections is subject to the environmental conditions (Hodges & Muir, 1972) and hence may reflect a particular physiological state of the cell. More specifically, a decrease in their number may represent a decrease in the rate of some basic synthetic processes, such as DNA replication and protein synthesis. The loss of microprojections during mitosis when there is no DNA replication (Brown, 1972) supports this view. Furthermore, the number of BHK fibroblasts bearing microprojections increases with population density but decreases at confluency (O'Neill & Follett, 1970) when cellular activity and motility are decreased.

Cell thickness was noticeably variable amongst the cell types present in the cultures. This may be due to differences in the degree of elasticity of cell membranes, so that some cells maintain a spheroid morphology while others flatten when given a suitable attachment site. Variation in cell thickness was associated with differences in morphology of the upper cell surface; the flatter the cell the smoother its surface and vice versa. This relationship is not surprising when one considers that an active cell must have a large surface area for the uptake of precursors and the release of products. Therefore, for a thick cell such as a neuron to have a surface area to volume ratio comparable to, or greater than, that of a flat fibroblast, it would need to have a deeply folded cell membrane.

Nerve fibres showed a variety of relationships with the different cells present but in all cases closely followed the surface contours. For the connective tissue cells and macrophages, the fibres did not deviate from a straight course as they passed over the cells. In this respect, the relationship was no more specific than that of a fibre following the surface contours presented by other axon bundles. In contrast, sympathetic nerve fibres did deviate from their course to form meshworks over isolated smooth muscle cells (Chamley et al. 1973). In no instances were fibres seen to be covered by parts of
connective tissue cells as reported for chick embryo spinal cord cultures (Boyde et al. 1968). Instead it appeared that fibres grew over the connective tissue cells in their path. Bipolar Schwann cells, on the other hand, did show a more particular relationship with the fibre bundles. They were usually seen lying along bundles with their processes winding themselves between and around the constituent axons. This observation is consistent with transmission electron-microscopic studies (Pick, 1970) in which Schwann cells provide cytoplasmic covers for axon bundles. Similar findings were reported by Boyde et al. (1968) for the glial cells in spinal cord cultures although in that case the arrangement may have represented the onset of myelination.

The tripolar and quadripolar glial cells displayed an extremely complex interaction with the nerve fibres. Their processes appeared to follow the axon bundles while their cell bodies were covered with meshworks of fibres, one of which generally exhibited a locally enlarged region on the cell surface. This increased contact between the fibres and the glial cells may reflect a more specialized association than that with connective tissue cells or Schwann cells. For example, certain central nervous system neuroglia have been suggested to be involved in the metabolic support of axons (Fernando & Blunt, 1970) and in the regulation of sodium and potassium in nerve excitation (Dorfman & Ho, 1970). Alternatively, some of the cells may represent the type I neurons of Chamley et al. (1972a) and the dilated area of fibre may be of a synaptic nature.

The authors wish to acknowledge the support of the Australian Research Grants Committee, the National Heart Foundation of Australia and the Life Insurance Medical Research Fund of Australia and New Zealand, and the skilful technical assistance of Mr John Nailon.

REFERENCES


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(Received 15 August 1973)
Fig. 1. Neurons.

A. Phase-contrast micrograph of a bipolar neuron which has migrated into the outgrowth. From newborn rat sympathetic ganglion, 11 days in culture. × 1700.

B. Scanning electron micrograph of the same cell as in A. Note that the cell body is thick with large, bulbous protrusions (arrows) on the upper surface. The base of the cell is flattened against the substrate and a thin flange of cytoplasm (a) extends out for attachment. A nerve fibre bundle (b) adheres closely to the surfaces of 2 glial cells (c, d) which lie close to the neuron. × 1700.

C. Scanning electron micrograph of neurons at the edge of a ganglion explant. Extensive nerve fibre meshworks from centrally placed neurons grow over their upper surfaces and, together with the processes of these peripherally placed cells, continue into the outgrowth. From newborn rat sympathetic ganglion, 6 days in culture. × 1200.
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Fig. 2. A, B. Scanning electron micrograph of sympathetic nerve fibres showing that both small and large bundles are composed of several individual axons, which may separate and coalesce with neighbouring bundles (arrows). The nuclear region (i) and one process of a Schwann cell can be seen amongst the fibres of a bundle.

A, newborn guinea-pig sympathetic ganglion, 5 days in culture. \(\times 6480\).

B, newborn rat sympathetic ganglion, 11 days in culture. \(\times 1700\).

c. Two fibre bundles with growth cones containing numerous filopodia (arrows). Fine finger-like extensions (short arrows) protrude from the preterminal regions. Newborn guinea-pig sympathetic ganglion, 5 days in culture. \(\times 1700\).

d. Individual axons exhibiting small swellings which correspond in dimension and distribution to the vesicle-containing varicosities of adrenergic nerve fibres. Newborn guinea-pig sympathetic ganglion, 5 days in culture. \(\times 1140\).
Fig. 3. Scanning electron micrograph of a Schwann cell (s) lying along a fibre bundle (b) and a macrophage beside it. Note that the nuclear region of the Schwann cell is thick with an irregular surface of longitudinal ridges and depressions (arrow). The macrophage exhibits an irregular bulbous upper surface with fine finger-like projections of cytoplasm extending from its lateral edges (f). Newborn rat sympathetic ganglion, 6 days in culture. x 3540.
Fig. 4A. Phase-contrast micrograph of a quadripolar glial cell (g). Note the nerve fibre meshwork on the cell surface (arrows). c, connective tissue cell. Newborn rat sympathetic ganglion, 6 days in culture. × 1060.

Fig. 4B. Scanning electron micrograph of the same cell. Note that the cell body is flattened against the substrate and the smooth upper surface is overlaid by a meshwork of fibres (arrows). One of these fibres on the cell surface has an enlarged area (short arrow). c, connective tissue cell. × 1060.
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