

although openings of the secretory vacuoles or sacs into the perivascular spaces could not be observed. The second characteristic finding in the perivascular spaces is the occasional presence of fusiform striated structures, which are found exclusively in an intimate association with the perivascular basement membrane near the SCO basal processes. These structures are oriented parallel to the perivascular basement membrane and composed of numerous fine filaments about 80 Å in diameter showing cross striations with a repeating periodicity of about 1200 Å (Figure 2). Similar striated structures have been hitherto disclosed in the perivascular space of the SCO of some mammals<sup>11-13</sup> and are postulated to be atypical collagenous elements. The significance of these striated structures is still not completely elucidated, but according to some authors<sup>11,12</sup> it is assumed that they might play an important role in metabolic interaction between the SCO and the capillaries.

The capillary endothelial cell contains abundant, probably pinocytotic, vesicles and well-developed organelles such as the Golgi apparatus and the rough endoplasmic reticulum. In its cytoplasm also homogeneously dense granules about 2000 Å in diameter enclosed by the limiting membrane were often seen. However, it has not been determined whether or not they are absorbed secretory substances of the SCO cells. No fenestration of the endothelial cells was noticed.

The aspects of the perivascular spaces described above may serve as morphological evidence for the existence of the possible basal secretion in the gecko<sup>14</sup>.

**Zusammenfassung.** Im Verlauf von elektronenmikroskopischen Untersuchungen am Subkommissuralorgan (SKO) von *Gecko japonicus* wurden zwischen den SKO-Zellen und den Kapillaren breite perivaskuläre Spalträume beobachtet, die mit flockigen Substanzen gefüllt sind und mitunter quergestreifte Strukturen aufweisen. Die funktionelle Bedeutung dieser perivaskulären Spalträume wurde im Hinblick auf die basale Sekretion des SKO diskutiert.

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Department of Anatomy, School of Medicine, University, Kurume (Japan), 24 December 1968.

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<sup>12</sup> R. WETZSTEIN, A. SCHWINK and P. STANKA, *Z. Zellforsch.* 67, 493 (1963).

<sup>13</sup> N. X. PAPACHARALAMPOUS, A. SCHWINK and R. WETZSTEIN, *Z. Zellforsch.* 90, 202 (1968).

<sup>14</sup> This paper is dedicated to Professor Dr. E. TONUTTI, Director of the Department of Clinical Morphology, University Ulm (Germany), on the occasion of his 60th birthday.

## Lack of Effect of Ciliarectomy on the Fine Structure of the Small Multiple Endings in the Extraocular Muscles of the Rat

The extraocular muscles of the rat are innervated in part by ordinary myoneural junctions arising from myelinated nerves and in part by multiple small junctions from unmyelinated nerves<sup>1</sup>. These 'small multiple endings' exhibit acetylcholinesterase activity, as has been observed with both the light<sup>1</sup> microscope and the electron<sup>2</sup> microscope, and their fine structure is similar to that of a typical cholinergic excitatory synapse<sup>1</sup>. Neither the site of the nerve cell bodies connected with these unmyelinated nerves nor their physiological function is known (for references see TERÄVÄINEN<sup>1</sup>). Because extirpation of the ganglion ciliare is reported to result in progressive atrophy of the extraocular muscles<sup>3</sup>, and electrical stimulation of the ciliary ganglion is stated to cause contraction of the extraocular muscles<sup>4</sup>, we decided to study the fine structure of the small multiple endings after removal of the ganglion ciliare.

The ganglion ciliare of adult Sprague-Dawley rats was either removed or electrocoagulated under ether anaesthesia. The rectus superior and lateralis muscles were prepared in ether anaesthesia for 24 h, 2, 4, 6, 9, and 21 days after the operation and fixed immediately at 4°C and pH 7.2 for 2½ h with 2.5% glutaraldehyde<sup>5</sup> buffered with phosphate. The contralateral unoperated side served as a control. After postfixation with 1% osmium tetroxide in the phosphate buffer, the specimens were dehydrated in graded series of ethyl alcohol and embedded in Epon 812<sup>6</sup>. The sections were counterstained with lead citrate<sup>7</sup>.

Small terminals of unmyelinated axons were apposed to the electrondense postsynaptic membrane of the muscle fibres on the unoperated control side (Figure 1), as earlier described<sup>1</sup>. We did not observe degenerative

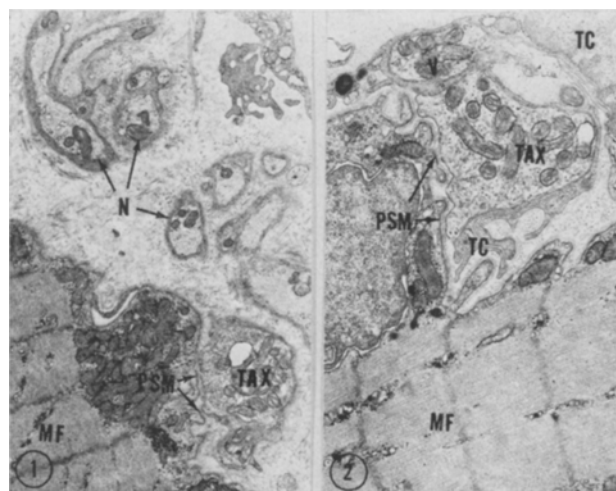


Fig. 1. Electron micrograph of a small myoneural junction in the extraocular muscle of the unoperated side. One axon terminal (TAX), filled with vesicles and mitochondria, is seen to be apposed to the electron-dense postsynaptic membrane (PSM) of the muscle fibre (MF). The section also passes through the unmyelinated nerves (N) from which the terminal is derived. The structure of the muscle fibre is of the slow type, with small mitochondria and a relatively weakly developed sarcoplasmic reticulum incompletely separating the muscle fibrils.  $\times 10,000$ .

Fig. 2. Small myoneural junction 21 days after removal of the ganglion ciliare. No degenerative changes are present (compare with Figure 1).  $\times 15,000$ .

changes in the fine structure of these multiple endings or in the extraocular muscle fibres even 21 days after the operation (Figure 2).

These results demonstrate that in the rat the neurones of the ciliary ganglion do not innervate the extraocular muscle fibres. The contraction of the extraocular muscles observed by some observers after electrical stimulation of the ciliary ganglion<sup>4</sup> must have been due to other mechanisms, possibly concomitant excitation of the oculomotor nerve close to the ganglion. No atrophy of the extraocular muscles was observed such as has been reported earlier after removal of the ganglion ciliare<sup>3</sup>. A follow-up period of 21 days should be long enough for such atrophy to occur, if the endings were connected with cells of fibres in the ganglion ciliare. It is concluded that the small multiple endings originate outside the ciliary ganglion.

*Zusammenfassung.* Die motorischen Endplatten der nicht myelinhaltigen Nervenfasern in den Augenmuskeln

der Ratte wurden elektronenmikroskopisch untersucht. Strukturelle Veränderungen treten nach Entfernen des Ganglion ciliare nicht auf.

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## Localization of Acetylcholinesterase Activity in Myotendinous and Myomalous Junctions of the Striated Skeletal Muscles of the Rat

The attachment of a striated skeletal muscle fibre in a tendon has received special attention since COUTEAUX<sup>1</sup> demonstrated histochemically that increased acetylcholinesterase (AChE) activity was present in this region of the muscles of the frog and mouse. This differentiated part of the muscle fibre is called the myotendinous junction (MTJ). Locally increased AChE activity seen in very thorough light microscope studies of the MTJ has since been reported in other animals, such as the rat<sup>2</sup>, the goat<sup>3</sup>, birds<sup>4</sup>, the cat and dog<sup>5</sup>, and man<sup>6</sup>. Electron microscope studies have changed the older theories of the structure of the MTJ (e.g.<sup>7</sup>), showing that the myofibrils are separated from the tendon by the sarcolemma and that no other special structures, such as nerves, are present<sup>5, 8, 9</sup>.

In mammals, the fibres of the skeletal muscle usually run from end to end of the muscle. However, in the human sartorius and gracilis muscles, muscle fibres have been described which do not continue to the tendon but join another muscle fibre<sup>10</sup>. These fibres have been thought always to have a tendon-like connective tissue component connecting the muscle fibres<sup>10</sup>, and accordingly it has been held that true fibre-to-fibre junctions like the intercalated disc of the heart muscle do not exist in striated skeletal muscles. The present report describes such a myomalous junction.

*Materials and methods.* The diaphragm and the rectus superior, medialis and lateralis of the extraocular muscles of adult Sprague-Dawley rats were used in the experiments. The muscles were removed under ether anaesthesia and fixed in toto for light microscopy at 4°C with 3.5% formal-calcium for 4–12 h. The method used for light microscopic localization of cholinesterases was based on the GÖMÖRI<sup>11</sup> modification of the KOELLE<sup>12</sup> thiocholine technique with minor modifications (TERÄVÄINEN<sup>13</sup>). Tetra-isopropylpyrophosphoramidate (iso-OMPA; L. Light & Co. Ltd., Colnbrook) and 1:5-bis-(4-allyl dimethylammoniumphenyl)pentan-3-one diiodide (284C51; Burroughs and Wellcome, London) were used to discriminate between other cholinesterases (E.C. 3.1.1.8) and acetyl-

cholinesterase (E.C. 3.1.1.7). Acetylthiocholine iodide (Fluka AG., Buchs) was used as a substrate for AChE and butyrylthiocholine iodide (Fluka AG., Buchs) for ns. ChE (for inhibitors see e.g. TERÄVÄINEN<sup>13</sup>).

The pieces of muscle were immersed for 20–60 min in 3% glutaraldehyde buffered to pH 7.2 with phosphate, rinsed for 2–3 h in the phosphate buffer and incubated according to the method of KARNOVSKY<sup>14</sup> for about 1 h to enable the distribution of AChE to be studied electron microscopically. The muscles were then sectioned longitudinally at approximately 200–300  $\mu$  with a razor blade and the area required was separated, using injection needles as knives, under an ordinary light microscope. The separations were postfixed with 1% osmium tetroxide in the phosphate buffer. After dehydration in a graded ethyl alcohol series, the tissue blocks were embedded in Epon 812<sup>15</sup>, sectioned and stained with lead citrate stain<sup>16</sup>.

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