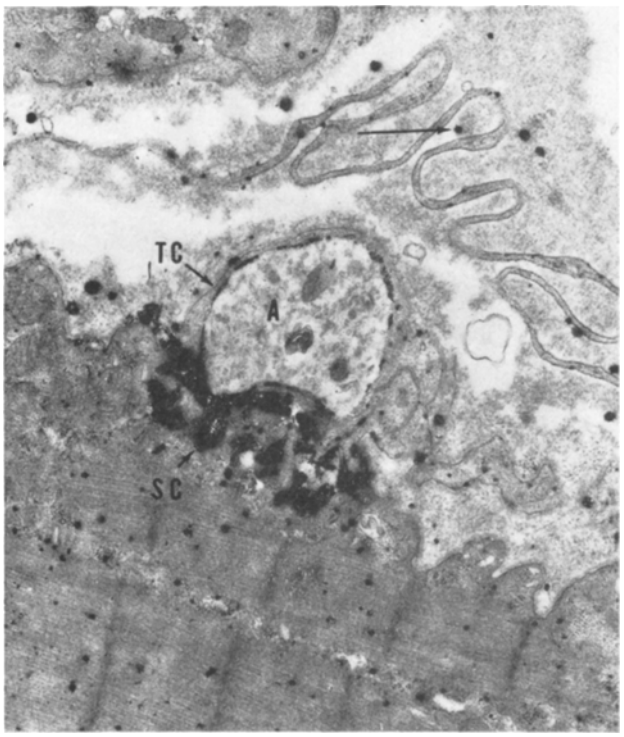

endings exhibit an acetylcholinesterase activity and are therefore likely to be cholinergic. The contraction of the extraocular muscles by adrenergic agents[7,8] still awaits an explanation.

Zusammenfassung. Elektronenmikroskopisch wird gezeigt, dass die aus nichtmyelinhaltigen Nervenfasern stammenden kleinen motorischen Endplatten in den Augenmuskeln der Ratte Azetylcholinesterase-Aktivität haben.

H. Teräväinen

Department of Anatomy, University of Helsinki, Helsinki 17 (Finland), 2 December 1968.

[7] I. S. Sanghvi, Invest. Ophthal. *6*, 269 (1967).
[8] K. E. Eakins and R. L. Katz, Invest. Ophthal. *6*, 253 (1967).

AChE activity in the small myoneural junction arising from unmyelinated nerves in the extraocular muscles of the rat. The reaction totally fills the irregular synaptic clefts (SC) and activity is also seen between the teloglial cell (TC) and the axon terminal (A). The scattered precipitate (arrow) is regarded as an artifact (see text). × 17,000.

Cervical Sympathectomy and the Origin of Small Nerve Endings in the Extraocular Muscles of the Rat

Two structurally different types of myoneural junctions are present in the extraocular muscle fibres. One of the endings comes from myelinated nerves and has the structure of an ordinary myoneural junction of skeletal striated muscle fibres[1,2]. The structure of the second type of myoneural junction is different. It is very small (even less than 1 μ), is derived from unmyelinated nerves, and possesses a fine structure resembling that of the cholinergic excitatory synapse[2]. The location of the neurones supplying the unmyelinated nerves to these 'multiple endings'[2] is unknown and it is not even known by which anatomical pathway their axons reach the extraocular muscles.

A lesion of the superior cervical ganglion causes partial paralysis of the levator palpebrae superioris muscle (e.g. Davson[3]). Stimulation of this ganglion can sometimes also cause contraction in the extraocular muscles[4], which have been reported to have nerve endings from unmyelinated nerves of the sympathetic perivascular plexus[5,6]. Therefore, we decided to study the fine structure of multiple endings in the extraocular muscle fibres after experimental removal of the superior cervical ganglion.

Methods. Adult Sprague-Dawley laboratory rats were used in the experiments. The superior cervical ganglion was removed either uni- or bilaterally under ether anaesthesia. The rectus superior, lateralis and medialis muscles were prepared 12 h, 2, 4 and 17 days after the operation after decapitation of the animals under ether anaesthesia. The muscles were fixed at 4 °C with 2.5% glutaraldehyde in phosphate buffer at pH 7.2[7] and post-fixed with 1% OsO_4 in the phosphate buffer. After dehydration with graded series of ethyl alcohol, Epon 812[8] was used for embedding. The sections were counterstained with lead citrate[9].

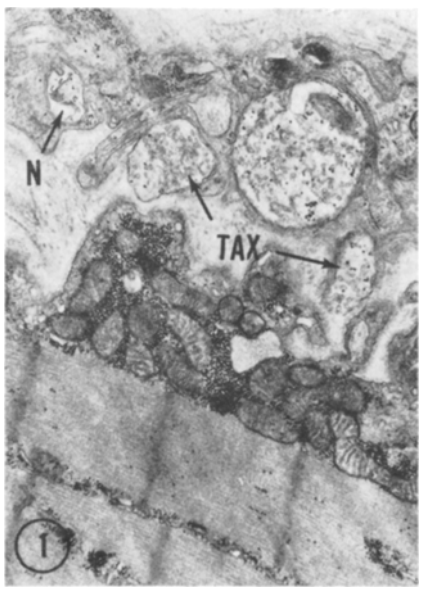

Fig. 1. Myoneural junction derived from unmyelinated nerves (N) in the unoperated rat. 2 axon terminals (TAX) apposed to the muscle plasma membrane and 2 vesiculated axon processes are seen. × 15,000.

Results. Typical small axon terminals[2] from unmyelinated nerves were seen to be apposed to the plasma membrane of slow-type muscle fibres (Figure 1) in the unoperated animals.

The fine structure of the multiple endings in the operated group was identical with that of the controls after both uni- and bilateral (Figure 2) cervical ganglionectomy, even 17 days after the operation.

The possibility that the multiple endings from unmyelinated nerves were derived from the superior cervical ganglion is thus excluded. The possibilities that the neurones of origin are located in the ciliary ganglion or arrive with the third or fifth cervical nerves are now under investigation.

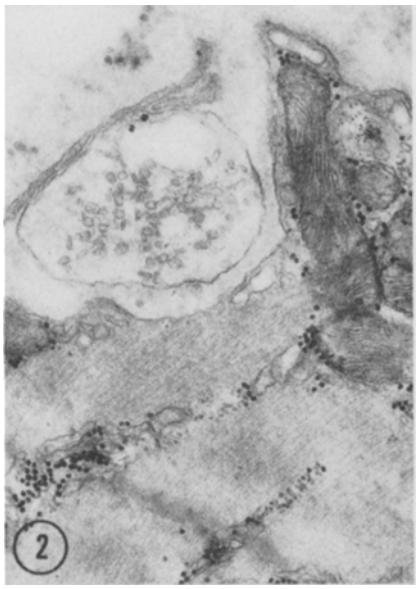

Fig. 2. Myoneural junction from unmyelinated nerves 17 days after bilateral removal of superior cervical ganglion. No degenerative changes. × 30,000.

Zusammenfassung. Die Ultrastruktur der aus nichtmyelinhaltigen Nervenfasern stammenden kleinen motorischen Endplatten in den Augenmuskeln der Ratte wurde untersucht. Strukturelle Veränderungen waren nach Entfernen des Ganglion cervicale superior nicht festzustellen.

H. TERÄVÄINEN and L. RAUANHEIMO

Department of Anatomy, University of Helsinki, Helsinki 17 (Finland), 2 December 1968.

1 M. v. DÜRING, Z. Zellforsch. mikrosk. Anat. *81*, 74 (1967).
2 H. TERÄVÄINEN, Z. Zellforsch. mikrosk. Anat. *90*, 372 (1968).
3 H. DAVSON, in *The Eye* (Academic Press, New York 1962), vol. 3.
4 G. A. BRECHER and W. G. MITCHELL, Am. J. Ophthal. *4*, 144 (1957).
5 J. R. WOLTER, in *Proc. 7th Int. Congr. Ophthal.* (Univ. Press, Toronto 1954), vol. 3, p. 1865.
6 J. R. WOLTER, Acta neuroveg. *5*, 257 (1953).
7 D. D. SABATINI, K. BENSCH and R. J. BARRNETT, J. Cell Biol. *17*, 19 (1963).
8 J. H. LUFT, J. biophys. biochem. Cytol. *9*, 409 (1961).
9 E. S. REYNOLDS, J. Cell Biol. *17*, 208 (1963).

Identification of a Catecholamine in the Skin of the Toad, *Xenopus laevis*, and the Relation to the Physiological Melanophore Reaction

In 1960 DAVEY[1] published a hypothesis about the activity of MSH – in vitro – in relation to the physiological melanophore reaction. He postulated an indolalkylamine stored in the skin of *Xenopus laevis*, which caused dispersion of the melanophores initiated by MSH.

In order to investigate the storage of a biogenic amine in the skin of *X. laevis*, which is released during the dispersion reaction of the melanophores, an analysis was made of skin of light and dark adapted animals by the method developed by FALCK et al.[2].

Therefore toads were adapted during various periods to a white or black background. The toads were killed by plunging them into liquid nitrogen and pieces of skin were cut off in the frozen state and immediately lyophylized. Dried pieces of skin were treated with gaseous formaldehyde, embedded and sectioned for fluorescence microscopy.

The tissue sections were examined with activation filter UG1, maximum of transmission at 365 nm, and barrier filter 41, transmission from 410 nm (filter notations according to Schott). Just below the epidermis a strip of green fluoresceing material was found. Compared with animals which were adapted for 3–6 days to a white background (Figure A), a decrease in the green fluorescence and a narrowing of the strip was observed in animals which were adapted for the same period to a black background (Figure B).

Using the sodium borohydride reduction method of CORRODI et al.[3], the fluorescence could definitely be ascribed to biogenic amines. The colour of the fluorescent material indicated a catecholamine rather than an indolalkylamine.

In order to investigate the nature of this catecholamine, a chemical analysis of a skin extract was made. Using the fluorimetric method of BERTLER et al.[4], a distinction can be made between DOPA, noradrenaline, adrenaline and dopamine. An extract was made of about 800 mg dorsal skin of a light adapted toad. The catecholamines were separated on a column with negatively charged groups (Dowex 50W X-8 200–400 mesh) by elution with different solvents. With this method each

1 K. G. DAVEY, Can. J. Zool. *38*, 715 (1960).
2 B. FALCK, N.-Å. HILLARP, G. THIEME and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962).
3 H. CORRODI, N.-Å. HILLARP and G. JOHNSON, J. Histochem. Cytochem. *12*, 382 (1964).
4 A. BERTLER, A. CARLSSON and E. ROSENGREN, Acta physiol. scand. *44*, 273 (1958).