also incubated for $1^1/_2$ h in the same incubation medium and were examined under the light microscope. DFP (Diisopropyl fluorophosphate) and 62 C 47 [1, 5-*bis*(4-trimethyl ammonium phenyl)pentan 3-one iodid] at concentrations of $10^{-5}M$ completely abolished the reaction of AChE. No inhibitors of nonspecific cholinesterase were added to the incubation medium as the latter enzyme is not present in the perikarya of rat sensory ganglia[1,2,10].

Light microscope observations showed a network-like pattern of the reaction product mainly in the small neurones (Figure 1). Similarly, the electron microscope revealed a positive reaction mainly in the small neurones. The reaction product was deposited in the cysternae, vesicles and vacuoles of the Golgi apparatus, and at the plasma membrane (Figures 2, 3). The electron micrographs indicated that preservation of the tissue is adequate. No reaction product was seen in the nuclei, mitochondria or endoplasmic reticulum.

The present data are partially in agreement with other reports on the activity of AChE in the central nervous system and sympathetic ganglia[4–7]. In these neurones, localization of the reaction product was observed in the Golgi apparatus and plasma membrane as well as in the rough endoplasmic reticulum. However, in the present studies no positive reaction was observed in the rough endoplasmic reticulum of the neurones. It is not possible at present to explain the absence of activity of AChE in the endoplasmic reticulum.

The present results may add information to the assumption that some of the neurones of the sensory ganglia are cholinergic[3,11,12].

*Resumen.* Utilizando el microscopio electrónico se demostró actividad enzymática de la acetilcolinesterasa en el aparato de Golgi y a nivel de la membrana plasmática de las neuronas del ganglio del trigémino en ratas.

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## Electron Microscopic Localization of Acetylcholinesterase in Small Multiple Endings in the Extraocular Muscles of the Rat

There are 2 different types of myoneural junctions in the extraocular muscles of different animals (for earlier literature see e.g. TERÄVÄINEN[1]). One of the junctions is formed from a myelinated nerve and has a structure comparable with a normal motor end plate of striated skeletal muscle fibres in both the light microscope and the electron microscope. The other type of myoneural junction differs strikingly from the motor end plate, since it is derived from a non-myelinated nerve and terminates in many small endings.

Since the light microscopic distribution of cholinesterase activity was closely correlated with the electron microscopic structure of the junction, it was suggested that these 'multiple endings' are cholinergic excitatory synapses to extraocular muscle fibres[1]. Because the distribution of cholinesterase had been studied only by light microscopy, it was decided to examine the acetylcholinesterase (AChE) activity of these multiple endings at the electron microscope level.

*Methods.* Adult Sprague-Dawley rats were killed under ether anaesthesia by decapitation, and the rectus medialis muscle was immediately removed and fixed for 20–40 min at 4 °C with 2.5% solution of glutaraldehyde buffered with phosphate at pH 7.2[2]. The muscles were cut into small pieces of about 0.5 mm thick with a razor blade and washed at 4 °C with $0.25M$ sucrose for about 12 h.

The presence of acetylcholinesterase (EC. 3.1.1.7) was demonstrated using acetylthiocholine iodide (Fluka AG, Buchs) as substrate together with $10^{-5}M$ tetra-isopropylpyrophosphoramide (iso-OMPA; L. Light & Co. Ltd., Colnbrook) in the incubation solution to exclude activity due to other cholinesterases (EC. 3.1.1.8). Control studies were made with both *iso*-OMPA and $10^{-5}M$ 284C51 (Burroughs and Wellcome, London) in the incubation solution. The ferro-ferricyanide method[3] was used at 4 °C and pH 6.0, with an incubation time of 60–180 min. The specimens were then washed for 30 min in phosphate buffer, dehydrated with graded series of ethyl alcohol and embedded in Epon 812[4]. The sections were afterstained with lead citrate[5].

*Results and discussion.* AChE activity was observed in the small multiple endings in the rectus medialis muscle. The reaction was localized between the synaptic membranes. With longer incubation times, the end product of the reaction totally filled the synaptic cleft and activity was also observed between the membranes of the axon terminal and the teloglial cell (Figure). The synaptic vesicles were negative. Some scattered granules with a different form from the precipitation granule of the reaction product were observed throughout the sections. They were unrelated to particular structures and regarded as artifacts.

The ferro-ferricyanide method has previously been used with some success to localize cholinesterase activity in the motor end plate, where the enzyme activity was intense and the incubation time shorter[6]. In the present work, the method did not appear to be very suitable for electron microscopic histochemistry if the activity to be demonstrated was only moderate, as was the case in these small junctions[1]. Therefore the exact location of AChE in respect of the synaptic membrane of these small junctions, remains to be shown. However, the present work demonstrates beyond any doubt that these

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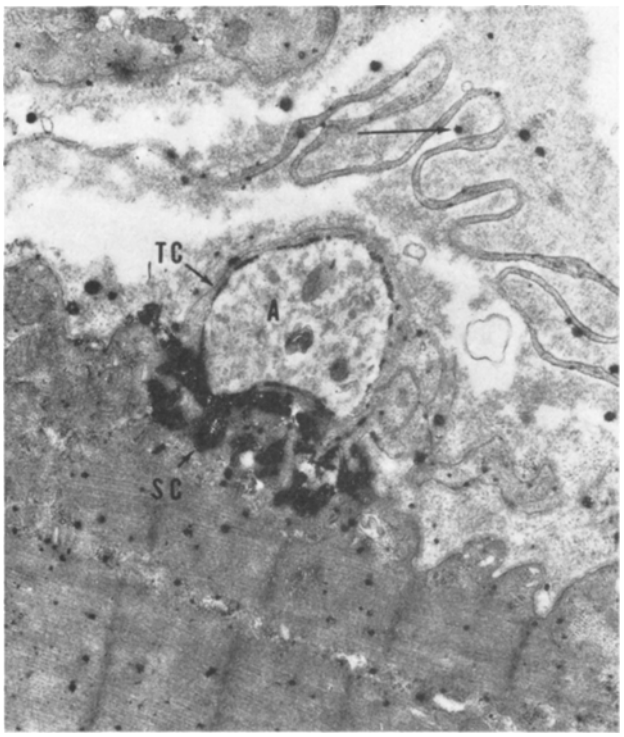


endings exhibit an acetylcholinesterase activity and are therefore likely to be cholinergic. The contraction of the extraocular muscles by adrenergic agents[7,8] still awaits an explanation.

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

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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].

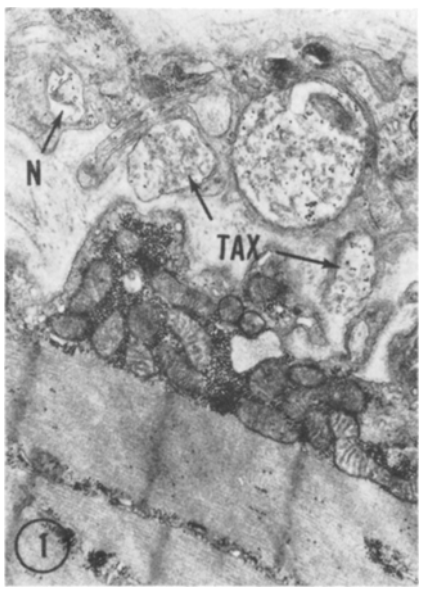


Fig. 1. Myoneural junction derived from unmyelinated nerves (N) in the unoperated rat. 2 axon terminals (TAX) apposed to the muscle plasma membrane and 2 vesiculated axon processes are seen. × 15,000.