

Histochemical Nature of the Motor Endplate

On the motor endplate of the skeletal muscle fibre, cholinesterase activity has been investigated in detail by many authors, but other enzymes or materials in the endplate have rarely been reported. We attempted to demonstrate histochemically the various enzymes or materials by the device of double staining with cholinesterase stain or by the comparison with cholinesterase stain in the serial sections.

Rat skeletal muscles and human biopsy or autopsy specimens were rapidly frozen by plunging into acetone dry ice mixture, and cut in 10–20 μ thick by cryostat at -25°C . The histochemically demonstrated enzymes and their methods were as follows^{1,2}: succinic dehydrogenase (SDH): NACHLAS et al.; cytochrome oxidase (CO): BURSTONE; lactic dehydrogenase (LDH): BRODY and ENGEL; adenosine triphosphatase (ATPase): PADYKULA and HERMAN, or WACHSTEIN and MEISEL; phosphorylase (PhR): TAKEUCHI; alkaline phosphatase (Al-P): GOMORI; acid phosphatase (Ac-P): GOMORI; cholinesterase (ChE): KOELLE. PAS reaction, sudan black B stain, Janus green B stain, modified trichrome stain³, hematoxylin stain⁴, and lead stain⁵ were also applied. Double staining with ChE was performed and it was successful for SDH, LDH, CO, PAS, hematoxylin stain by applying before ChE stain and for sudan black B stain after ChE stain.

Normal rat muscle endplate: SDH and CO activity was demonstrated moderately in the endplate of both type I and II fibres in the shape of triangular cap in cross section (Figure 1). Janus green B was also stained in the same manner. Such SDH and CO activity may be that of the mitochondria in both terminal axon and subneural portion of the muscle fibre. PhR activity was poor and PAS reaction positive with no diminution by digestion. It is considered that mitochondrial aerobic metabolism is actively present in the endplate portion of muscle fibre. Positive sudan black stain, positive PAS reaction after digestion, affinity to hematoxylin and red staining by modified trichrome stain were confirmed and these findings show presence of the lipoprotein and mucopolysaccharide in the endplate structure as suggested by electron-microscopic study. ATPase (mitochondrial), LDH (fast-moving), and Ac-P were also positive in the endplate. Lead stain by SAVAY and CSILLIK⁶ was positive and it showed the same figure as demonstrated by ChE stain. This lead affinity is thought to be related to the -SH group of ChE protein.

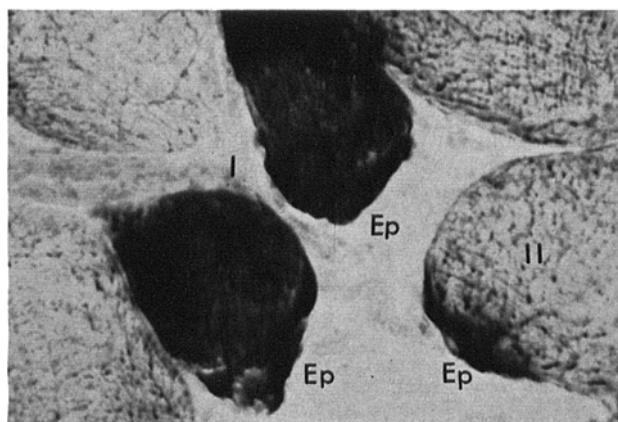


Fig. 1. Succinic dehydrogenase activity in the endplates of type I and II fibres. Ep, endplate. $\times 600$.

Biopsied or autopsied human muscle endplates: endplates of muscles of neuromuscular diseases showed various activities of SDH. Neuropathy, such as amyotrophic lateral sclerosis, spinal muscular atrophy and KUGELBERG-WELANDER syndrome showed weak activity in the atrophied muscle endplates. In myopathy, progressive muscular dystrophy showed normal activity and myasthenia gravis moderate activity in the elongated endplate. Trapezius muscle of ocular myopathy showed the atrophied endplates with weak SDH activity and defect of SDH activity in the subsynaptic portion of muscle fibres (Figure 2), which muscle showed neurogenic pattern by EMG and histology. This is a similar finding to the defect of mitochondria under subsynaptic portion of muscle demonstrated by electron microscopy in GARCIN's case of recurrent ocular motor disturbance⁶, and neurogenic change in muscle was also demonstrated in his case. Interpretation of this finding needs further investigation⁷.

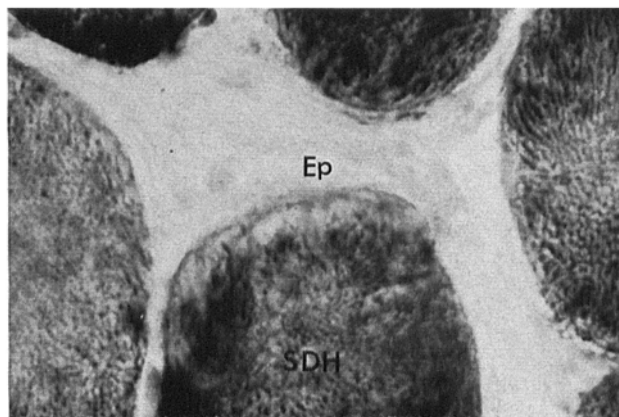


Fig. 2. Cholinesterase activity of the atrophied endplate and decreased succinic dehydrogenase (SDH) activity of both endplate (Ep) and subsynaptic portion of the fibre. Trapezius muscle of the ocular myopathy. Double stain of succinic dehydrogenase and cholinesterase. $\times 600$.

Zusammenfassung. Die Enzyme und Produkte der motorischen Endplatte in der Skelettmuskulatur werden nach der Doppelfärbung mit Cholinesterase untersucht. Dabei erweist sich die Succinodehydrogenase in der Endplatte als stark aktiviert. In der Endplatte der neurogenen, atrophischen Muskelfasern ist sie jedoch vermindert.

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16th February 1967.*

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⁶ R. GARCIN, M. FARDEAU and Mme. GODET-GUILLAIN, *Brain* 88, 739 (1965).

⁷ Acknowledgment: I wish to thank Prof. Dr. K. NAKAO for his guidance in this investigation.