An Isolated Human Voluntary Muscle Preparation.* (22048)

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The use of isolated mammalian and amphibian nerve-muscle preparations for the study of drug and ion interactions at the motor end-plate is well known. The object of this communication is to describe a method of utilizing isolated human muscle obtained from surgical operations for similar study. Fasciculi from various muscle groups have been tried, but so far the human intercostal, obtained at thoracotomies, has been used most often because of its small discrete fasciculi, short fibers, availability, and accessibility.

Method. After the muscle specimen was removed, a suitable fasciculus was dissected out and placed in a vial containing Krebs' solution, which had been saturated with 95% O_2 and 5% CO_2. The vial was closed and initially packed in chipped ice during the time it was transported from the hospital to the laboratory. Subsequently it was ascertained that chilling of the muscle was unnecessary, and this practice has been discontinued. Each fasciculus was about 0.5-1.0 mm thick and 10-15 mm long. It was suspended in a bath of Krebs' solution between 2 platinum electrodes as previously described(1). Tracings were usually about 20-30 mm in height. Because the nerve supply to the muscle cannot ordinarily be isolated, an attempt was made to stimulate the intramuscular nerves without at the same time stimulating the muscle fibres themselves by using a square wave of very short duration. The square wave stimulator was triggered by one of a pair of rocking mercury switches; the other mercury switch was used to stimulate the muscle fibres directly as before(1), and the switches were rocked to provide alternate direct and indirect stimuli at 10-second intervals.

The electrodes used to stimulate the intramuscular nerves were placed about 5 mm apart on either side of the muscle rather than longitudinally since Rushton(2) has shown that transverse current preferentially affects intramuscular nerves. To the same end 10%, rather than 5%, carbon dioxide in oxygen was used, since evidence has been presented(3) that conditions which lower the pH *in vitro* reduce the chronaxie of nerve relative to that of muscle in the human subject. In our experience the use of 10% carbon dioxide did favor the stimulation of intramuscular nerves selectively. It has been determined that a square-wave pulse of 0.01-0.07 millisecond duration and 5-10 volts (as measured across the electrode by an oscilloscope) will stimulate only the nerve endings in this preparation, and the contractions elicited by the indirect stimuli are equal to or slightly less than those from direct stimuli. Furthermore, contractions from indirect stimuli are blocked completely by 1-1.5 μg/ml of decamethonium bromide or 2.5-3.0 μg/ml of d-tubocurarine chloride.

There was some evidence that the action of C10 on this muscle was biphasic as previously reported for the rabbit lumbrical(1).

Summary. A preparation of human voluntary muscle suitable for studies on neuromuscular transmission *in vitro* is described and some of the actions of curare and of decamethonium on it are recorded.

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1. Jenden, D. J., Kamijo, K., and Taylor, D. B., *J. Pharm. Exp. Therap.*, 1954, v111, 229.

2. Rushton, W. A. H., *J. Physiol.*, 1930, v70, 317.

3. Casselli, C., and Pinelli, P., *Arch. Fisiol.*, 1953, v531, 94.

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