Organ Culture of Rat Skeletal Muscle Subjected to Intermittent Activity

In spite of the fact that the method for survival of conveniently large organs or their parts has been known for almost 10 years (TROWELL[1]), organ cultures of skeletal muscles have been used only exceptionally (MILEDI and TROWELL[2], HARRIS and MILEDI[3]).

The aim of this work was to obtain a model of an organ culture, enabling, in the course of cultivation, a physiological functional load analogous to the function of the organ in vivo.

A rat peroneus brevis muscle (mean weight 91 ± 13 mg, length 22 ± 2 mm, maximum diameter 2.8 ± 1.5 mm, $n = 10$) was maintained in a modified medium 199 (SLONIM et al.[4]) with the addition of 4 g glucose, 50,000 U PNC and 10 mg STM/l of the complete medium. Since the solution was oxygenated by means of a mixture of 95% oxygen and 5% carbon dioxide, the concentration of HCO_3^- was increased to 44 *M* equiv. for the stabilization of the pH value at 7.4. The temperature was maintained at 20 °C. At higher temperatures symptoms of fatigue were more marked, although judging from the size of the mechanical response the optimum temperature was 29 °C.

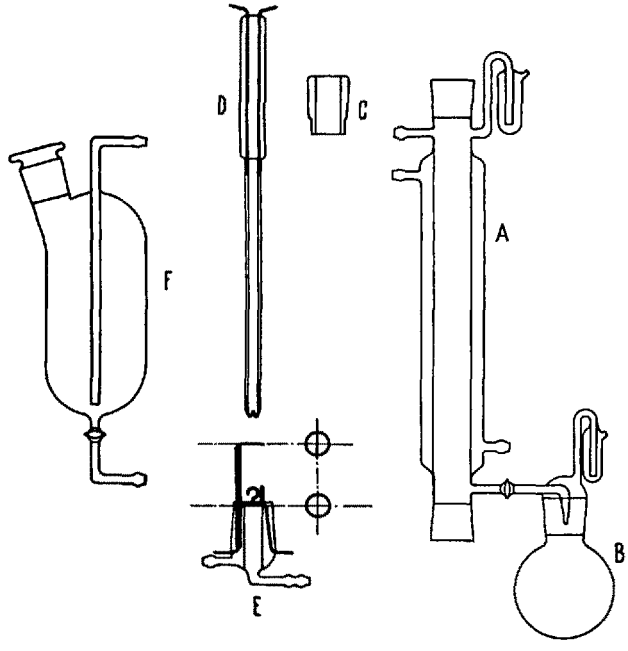
Direct massive stimulation with bipolar (LILLY et al.[5]) rectangular pulses was employed. The preparation was stimulated once in 5 min by a 5-sec train of 1 msec pulses at a frequency of 35 c/sec. The frequency of 1 tetanic contraction in the course of 5 min was selected because it had been ascertained in preliminary experiments that shorter intervals between individual contractions result in a smaller mechanical response. The pulse frequency in train 35 c/sec yields the greatest amplitude of the mechanical response of this muscle. Platinum electrodes give rise to an electric field in the medium parallel to the muscles fibres. Such an arrangement allows a maximum response to be attained at a lower stimulation voltage.

The mechanical reaction of the muscle preparation was recorded by means of a resistance extensometric device (a silicone tube 1 mm in diameter filled with mercury) and the muscle length and its changes were registered on a servocompensation recorder. The starting mechanical tension was 5 g.

The preparation was kept in a glass apparatus, which is shown in the Figure, consisting of a reservoir of the medium F connected by its upper part through a silicone tube with chamber A. The lower part of the reservoir is connected with the part E containing the electrodes, the fixing hoocklet for the preparation and a central tube with a jet for the gas inlet. The double-walled chamber A containing circulating water maintains the temperature of the medium as well as of the device. The apparatus, when ready for use, is connected with the surroundings by means of 2 fermentation stoppers filled with an antiseptic, and by an inlet tube for gas on the part E, which is connected to the gas cylinder by means of a sterile cotton filter. The whole apparatus is sterilized before use in an autoclave. The muscle preparation is extended between the glass hook in part E and the platinum tip of the extensometric device. The starting mechanical tension is preset by inserting the holder D. The level of the medium in chamber A can be regulated by means of the reservoir level. The medium flows through chamber A and is collected in vessel B. Rate of flow of the medium (4 ml/h) is controlled by the difference in levels of the fermentation plugs and by the tap in part A.

Using the above arrangement, the muscles in the last 10 experiments, out of a series of 52, survived for 1 week maintaining their mechanical activity. The longest survival time attained was 192 h. Up to 4 days, the contraction amplitude remained unchanged, or even increased. After the fourth day it slowly decreased. The survival time with preserved mechanical activity depended on the slope of this decrease. Preparations from the last 10 experiments were evaluated histologically. In none of the cases was there a necrotic focus or alterations indicating any anoxic damage to be found. The amount of glycogen remained the same throughout the whole muscle cross-section and it was somewhat higher than in non-cultivated contralateral controls. In 3 instances necrosis was established in the surface layers down to a depth of 3–4 fibres. This was probably caused by too intensive flow of the medium (TROWELL[6]).

The method described above facilitates long-term biochemical and pharmacological investigations on the isolated working muscle and makes a study of early post-denervation changes in vitro possible.



Zusammenfassung. Es wird eine Methode angegeben, um die wichtigen biochemischen und pharmakologischen Untersuchungen an Muskulatur (Rattenmuskel) bei Erhaltung der mechanischen Aktivität über 8 Tage durchzuführen.

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