Short communication

A Device for the Recording of Isolated Muscle Cell Contractions Using Silicone Tensometer

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The generation of force represents one important parameter to be measured in studying muscle cell processes. Technically this is an exceptionally demanding task, mainly when recording is to be made from a single cell or its part, since forces thus generated are below 1 mN. Commercially available mechanoelectrical transducers with a sufficiently high sensitivity are commonly used for similar measurements. Moving anode valve type RCA 5734 has most frequently been used for these purposes (Hodgkin and Horowicz 1959; Zachar et al. 1964; a.o.). Disadvantages of this method include a relatively high heating current required, which can electrically interfere with other measurements, and a higher operation temperature with a resulting drift. Piezoelectrical converters, also having a high sensitivity, are another type of device used for similar experiments (Gilai and Kirch 1978; Close 1981); however, their high resistance presents additional problems of shielding. Special transducers working on the optical principle have also been developed (Helam and Podolsky 1969; Fabiato and Fabiato 1976).

We developed a new method of isometric measurement of muscle cell contraction; the method is highly sensitive and easy to build up. It used Czechoslovak made silicone tensometer AP-120-6-12.

Construction of the Mechanical Part

A silicone tensometer is used as the force sensing element. The silicone tensometer is a non-hysteretic mechanoelectrical transducer with a high effectivity of conversion of mechanical deformation into electrical resistance. Its characteristics have a long-term stability. The tensometer is a small plate ($6 \times 0.6 \times 0.1$ mm) with wiring on its shorter sides.

The contraction force of the muscle cell has to be converted into deformation of the silicone plate in a sufficiently sensitive way, and the silicone tensometer has to be protected at the same time from being damaged by occasional shocks during

the fixation of muscle fibres. An arresting mechanism has proved unsuitable since it would increase the weight of the arm transmitting the contraction force to the tensometer, thus interfering with the measurement. The simplest solution to the problem, meeting at the same time all the above requirements, has been the insertion of the tensometer to the interrupted part of the sensing arm.

The mechanical part of the device consists of a micromanipulator enabling to adjust the sensing part to a position suitable for the measurement; a holder and a sensing arm with a tensometer. We shall describe here the construction of the sensing arm only, since the other parts are standard and adjusted to actual layout of the experiment. Major requirements the sensing arm should meet, include a high rigidity to avoid deformation, and a low weight because of the dynamic properties.

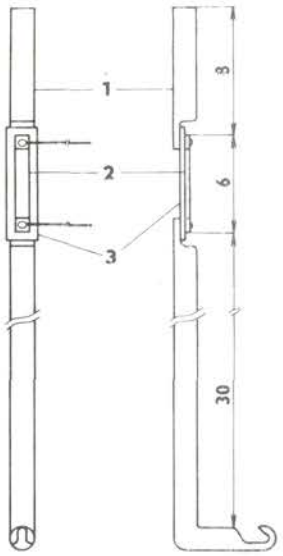

Fig. 1. Construction of the sensing arm with a tensometer (1 — steek tube made of a syringe needle, 2 — tensometer, 3 — glass fibre bundle).

The arm represents a level converting the contraction force into tensometer deformation. The length of the arm thus determines the resulting sensitivity. Fig. 1 shows the construction of the arm. The arm has been made of a thin stainless steel tube (external diameter 0.5 mm). A syringe needle may be used for this purpose as it meets the requirements of mechanical characteristics and is biologically inert. The needle can be bent to obtain the shape required. External diameter, thickness and length should all be chosen according to the experimental conditions. (Fig. 2). A small hook is grinded into one end of the needle, serving to hang up the tendon of the muscle cell. The other end of the needle is fixed in a holder. The tensometer

is inserted at a distance of approx. 8 mm from the fixed end of the arm, with its longitudinal axis parallell to that of the arm. To protect the tensometer from shocks it is attached to a thin bundle of glass fibres. Such a thin flat bundle can be obtained from the commonly available laminate fabric. Two-component epoxide adhesive was used to attach the tensometer to the bundle, and to attach the pad with the tensometer to the grinded ends of the interrupted arm. During the procedure attention should be payed that the epoxide adhesive is hard enough to obtain a sufficient rigidity of the arm as a whole, to maintain the sensitivity of the tensometer. This design also provides electrical insulation of the tensometer from the metallic part of the arm and thus from the preparation measured.

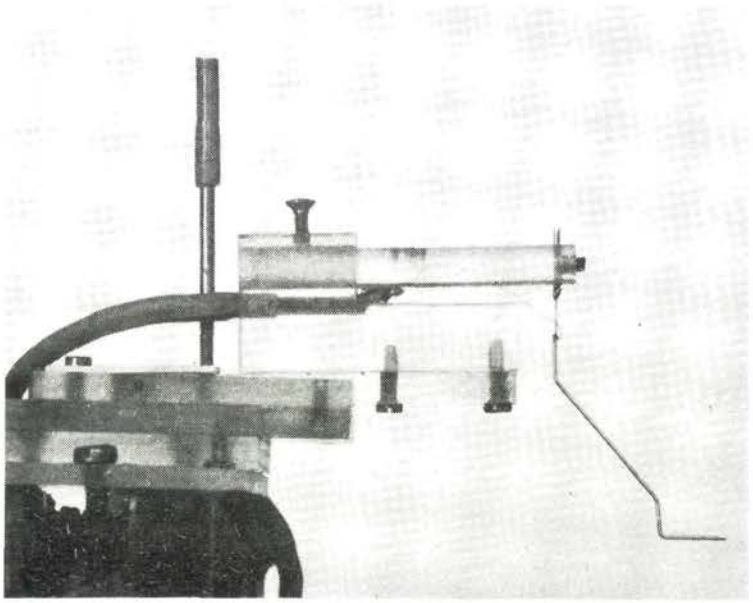

Fig. 2. View of the sensing arm used to record contractions of muscle fibre segments.

Electrical circuit

Fig. 3 shows the electrical circuit of the tensometer. The tensometer used (AP-120-6-12, produced and distributed by Okresní podnik služeb, Gottwaldov, Czechoslovakia) has the following characteristics, important for the connection used:

Nominal resistance $R_{0,25}$	120 Ω
Non-linearity in the deformation range $\pm 1 \times 10^{-3}$	± 0.8 %
Thermal coefficient of resistance for 20—30 °C	± 0.08 %/°C.

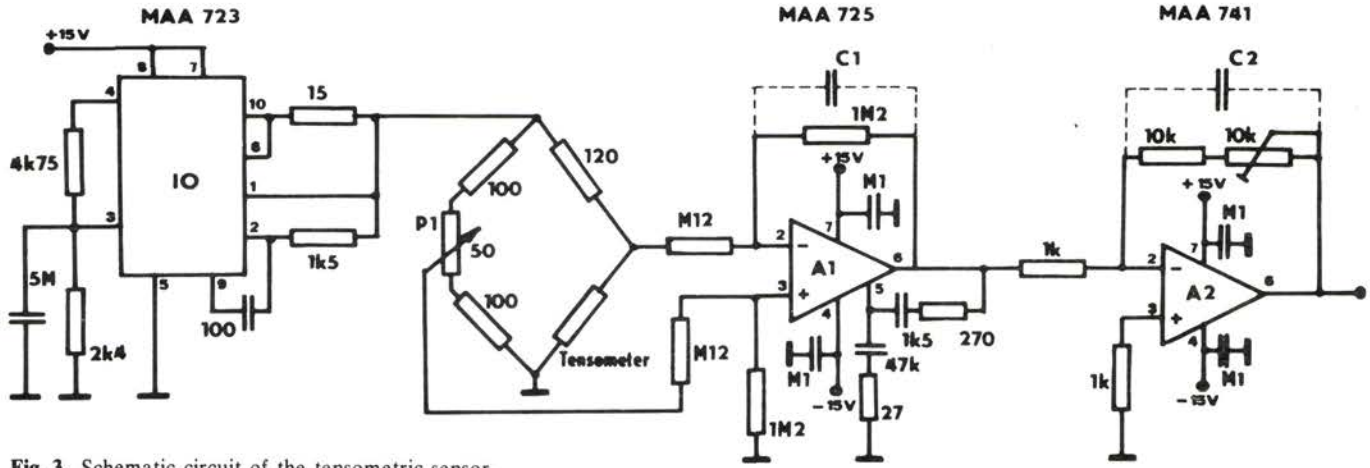

Fig. 3. Schematic circuit of the tensometric sensor.

The tensometer is inserted in one branch of the Wheatston bridge, the other branches having standard resistance identical with the nominal resistance of the tensometer. Potentiometer P_1 is used to set zero output voltage at the tensometer amplifier at rest or with basic load. The bridge is fed from a source of constant stabilized voltage, consisting of an integrated stabilizer MAA 723 connected according to the recommendations of the manufacturer. At a feeding voltage of 2.4 V to the bridge a resting current of 10 mA will flow through the tensometer. Any deformation of the tensometer will result in changes in its resistance and the current flowing through it will create a corresponding voltage drop. Voltage changes are then recorded by a differential amplifier A_1, from the bridge diagonal. The amplifier consists of an operational amplifier MAA 725 with a 10-fold amplification, and amplifier A_2 with an amplification set for the actuall sensing element. We have set the amplifier to 14-fold amplification to obtain an imput voltage of 1 V for a force of 1 mN. This voltage is then fed in a recorder.

The construction design and its circuit is mainly suitable for dynamic measurements. With respect to its small size, the tensometer AP-120-6-12 allows the recording of dynamic courses of up to 10^5 Hz (as stated by the manufacturer). This range is however partially limited by the arm.

In measuring muscle cell contractions the actual range used will be 10 μN—10 mN. This range corresponds to deformations of $\pm 1 \times 10^{-3}$ with a transmission linearity better than 0.8 %.

Thermal dependence is of no importance for tensometric recording of muscle contractions: it will not interfere with the dynamics and will only be shown as a shift of the output DC voltage level. For the connection described this shift will be 30 mV/°C, i.e. better than the thermal coefficient granted by the manufacturer.

The discrimination ability is limited by noise at the output of the amplifier A_2 (less than 10 mV p—p). The noise can be reduced by filtering the high frequency noise using a low frequency filter at the output, or by limiting the frequency characteristics of amplifiers A_1 and A_2 using capacitances C_1 and C_2 (for $f = 10$ kHz $C_1 = 10$ pF, and $C_2 = 1$ nF) in feedback connection. The discrimination ability will be about 1 mV, corresponding to a force of 1 μN.

Conclusions

The described device has already been used over a longer period to measure isometric contractions of isolated frog and crayfish muscle cell (Lipskaja et al. 1983) and to measure contractions of 0.5 mm long muscle fibre segments (Poledna and Lacinová 1985). Fig. 4 shows a series of records of contractions of voltage clamped fibre segments at various depolarizations and pulse lengths.

As compared to mechanoelectrical transducers of other constructions, the advantages of our device include a high sensitivity (in the order of 1 μN), a wide

range of forces recorded (1 μN to 10 mN), linearity over the entire range, easy construction, low costs, good shielding, long-term calibration stability, and good dynamic characteristics. The device can readily be adjusted to actual experimental conditions and a variety of preparations. The range of forces to be measured and/or dynamic properties of the device may be determined by the selection of the appropriate tensometer bed.

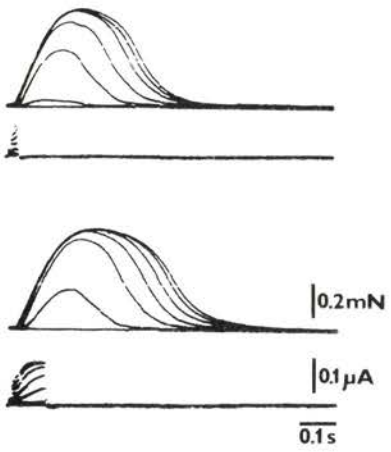

Fig. 4. Records of contractions of a frog muscle fibre segment under voltage clamp for depolarizations from 10 to 70 mV and pulse lengths of 30 and 100 ms. The physiological saline contained 10^{-7} mol/l TTX. Simultaneous recording of ionic currents (top).

The device described herein is widely applicable for various biological experiments and may successfully replace devices used so far.

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Received November 12, 1985/Accepted January 6, 1986