Tonic Properties of the *Flexor carpi radialis* Muscle of the Male Frog (*Rana temporaria*)

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Summary. The mechanical activity of the isolated *flexor carpi radialis* muscle (FCRM) of the mature male frog (*Rana temporaria*) was compared to that of the sartorius muscle. Muscles were stimulated directly by massive shocks at 20°C and isometric tension was recorded during single twitch, twitch series, long tetani and long tetanus series. Some groups of muscles were poisoned with IAA-N₃ or FDNB. The FCRM showed a high resistance to fatigue during a tetanus series and a "fatigue contracture" during a twitch series. The rate of the tetanic tension rise was smaller by five to six times than in the sartorius muscle (0 to 50% Pₒ) and the twitch/tetanus ratio was always very small. During continuous activity and after the blocking of recovery metabolic pathways, FCRM used its energetic stores two to three times slower than the sartorius muscle. These stores did not seem to be more important.

The results indicate that the FCRM is not a "tonic" muscle but, quite possibly, is composed of several types of fibre like many other frog muscles. However, some characteristics of mechanical response cannot be explained by the common classification into two types of fibre.

Key words: Male Frog — *Flexor carpi radialis* Muscle — Tonic Properties — Fatigue.

In the mating-season, mature male frogs (*Rana temporaria*) clasp females for several days. The behaviour of muscles associated with clasping-reflex has already been investigated by other authors who have registered mechanical activity (Wachholder, 1930; Melichna et al., 1972; Thibert and Nicolet, 1972) or electrical activity (László and Tigy, 1959). László and Tigy claimed that these muscles can sustain large tension over prolonged periods of time by a myosin crystallization; in other words to explain the tonic phenomenon, these authors suggest a "catch" hypothesis, in which metabolic cost should be very small for maintenance of tension.

In the present work we have investigated the mechanical response of the isolated FCRM which plays a prominent part in the clasping-act; then, by comparing it with the response of the sartorius muscle, we have
attempted an indirect estimation of the energy cost of sustained activity, after poisoning with iodoaeetic acid (IAA) or 1-fluoro-2,4-dinitrobenzene (FDNB).

Methods

Preparation. Experiments were carried out during two periods (January—February and November—December, 1973) on *sartorius* and *flexor carpi radialis* muscles of mature male frogs (*Rana temporaria*), obtained from the same supplier in the surroundings of Besançon (France). After excision, the muscles were stored for 2 hrs or overnight, at 6°C, in Ringer solution (NaCl 113 mM; KCl 2.0 mM; CaCl₂ 1.8 mM; Phosphate buffer 2.0 mM; pH measured: 7.1). Some muscles were poisoned with 0.4 mM IAA for 40 or 60 min and maintained in N₂ for 10 min. Other muscles were poisoned with 0.82 mM FDNB for 30 min. Unpoisoned muscles were stimulated in Ringer gassed with O₂ and poisoned muscles in desoxygenated Ringer gassed with N₂ (P₀₂ was lower than 2 mm Hg).

The *Sartorius* muscles were dissected out, their standard length *l₀* measured, still attached to the pelvic bone. After storage they were mounted vertically in a through between two plate electrodes by connecting the distal tendon to the chain of the strain-gauge and by clamping the pelvic bone.

The *FCRM*, thick and triangular in male frog, is attached along the *Crista medialis* of the *humerus* and the distal tendon is attached to the *Centrale* of the *Carpus* (Ecker and Wiederscheim, 1896). Dissection and mounting were not easy and for technical reasons only the left muscle was used. The skin was removed from the forelimb, and, in order to standardize the degree of stretching, the muscle length was measured on the two free sides when the angle between the *ulna* and the *humerus* was 110°—120°. The muscle was dissected out attached to the *humerus*. The distal tendon was tied to the chain of the strain-gauge. The mounting apparatus allowed to fasten the *humerus* firmly by clamping the *caput humeri* and *eminentia capitata*. Axial rotary motion was avoided by a check on the *Crista ventralis*. The angle between the *humerus* and the chain was about 15°. The apparatus did not allow changes of fibre length. Indeed, because of muscle asymmetry, a change Δ*l* in muscle length brought about in fibres a relative change Δ*l*/*l* higher on one side than on the other. Hence, we could not investigate the length-isometric tetanic relation and, in our experiments, *FCRM* were stretched at the *in situ* length. Moreover, tetanic tensions of *FCRM* are given in kg/g; an approximative and comparative value in kg/cm² can be obtained by multiplying tension in kg/g by the coefficient 1.7, which was roughly the length of middle muscular fibres in our animal batches, (in cm).

Stimulation. Massive stimulation in Ringer solution, with rectangular pulses, was used. The muscles were stimulated transversely by a pair of platinum plate electrodes (20 mm long, 20 mm large and 18 mm apart), across which we could apply up to 30 V. However, the used voltage across the bath was always inferior to 15 V, or current density was lower than about 200 mA/cm². The experiments required that the stimulator should be able to deliver stimuli of alternating polarity in order to minimize polarisation effects at the electrodes. To that effect we devised and built an electronic switch similar in design to those previously described (Ross and Brust, 1965; Okong'o *et al.*, 1970). The stimuli were provided by combination of a classical stimulator with a pulse amplifier, the power supply of which should have a low output impedance. When a stimulus was delivered, the primary pulse caused a fast relay to rock, which reversed the connections of stimulation electrodes. Maximum frequency of stimulation was 150 shocks/sec.