Force Generation in Glycerinated Insect-Flight Muscles without ATP

The elementary contractile process in muscle involves presumably the cyclic attachment and detachment of cross-bridges between actin- and myosinfilaments[1,2]. Recently, HUXLEY and SIMMONS[3] proposed a force generation mechanism according to which tension is generated by means of a 'spontaneous' rotation of attached cross-bridges. After completion of the rotational movement (power stroke of the bridge), ATP is required in order to break the actin-myosin linkages rather than for immediate force generation[3]. Recent results[4] further suggest that each ATP-molecule combined with myosin is immediately hydrolysed – thus suggesting that ADP rather than ATP is bound to the cross-bridge in the detached state. If this concept is correct, it should be possible to replace ADP by pyrophosphate and to induce in glycerinated fibres a tension increase by removing it. Pyrophosphate is known to be bound to the cross-bridges[5,6] and to dissociate actin from myosin[7] (however unlike ATP or tripoly-phosphate) without being split by the myosin ATPase. In the experiments with pyrophosphate, it was necessary to 'prime' the fibres by a preceding 'rigor contraction'[8,9] which must be followed by a partial relaxation induced by Mg-pyrophosphate.

Methods. Four solutions (pH = 6.5) with basic composition (rigor solution) 20 m*M* Histidin, 10 m*M* Na-azide, 4 m*M* EGTA and 50 m*M* KCl and specific composition as listed in the Table were used.

Fibre bundles of the dorsal longitudinal muscle (DLM) of *Lethocerus maximus* were stored for up to 6 months in 50% glycerol and mounted on an highly isometric tension recording apparatus[10,11]. The rigor contraction was induced by the method of WHITE[9], which involves first suspension in ATP-relaxing solution and then immersion into (2 ml) ATP-free rigor solution, in which cross-bridges are known to become attached in the arrow head (angled) configuration[12,13]. We also ensured that any ATP left within the fibre was thoroughly washed out by several successive solution changes before continuing the experiment with the following sequence: [PP relaxing solution → rigor solution]$_n$ → ATP-relaxing solution where n was up to 14! Subsequently the preparation was again immersed into relaxing solution and rigor solution for checking its condition. Only fibres which fully relaxed in all ATP-relaxing solutions were used for evaluation.

The static stiffness (Δ force per unit change of strain and per fibre) was determined 5 to 20 min after a sudden length decrease (release -0.25% L_o).

Results. The fibres developed, after depletion from ATP by an immersion into rigor solution, a typical rigor tension[9] (in the mean 22.7 dyne/fibre) under isometric conditions. This tension diminishes to about 50% within 2 h.

When the fibres are brought from rigor solutions into the PP-relaxing solution, a fall of tension is observed; after 3–5 min incubation time tension becomes practically steady at 30% (6.8 dyne/fibre) of maximal rigor tension. This relaxation can be partially reversed (Figure): The fibres contract again when they are transferred again into a (freshly made up) rigor solution, finally reaching a value of 46% (10.3 dyne/fibre) of maximal tension in rigor, and they relaxe again with MgPP. These cycles can be repeated many times (up to 14 cycles). The extent of contraction within these cycles did not depend on the duration (range 3–30 min) of incubation in PP-relaxing solution. Similar reversible tension changes were observed with AMP-PNP[14] instead of MgPP and also between 1 m*M* MgPP/9 m*M* NaPP as relaxing solution and 10 m*M* NaPP as contracting solution. But addition of inorganic phosphate (3 m*M*) to all solutions diminished the contraction responses described which were nearly abolished at pH 7. Pyrophosphate without Mg did not cause an appreciable relaxation. We did not succeed in obtaining similiar contraction cycles with glycerinated rabbit psoas.

The tension increase produced by washing out the plasticizer pyrophosphate is also associated with a doubling of stiffness, while pyrophosphate induced relaxation is associated with a decrease in stiffness. Note also the near proportionality between stiffness and tension (Table); the latter can be abolished by a 'quick release' of less than 0.6% L_o.

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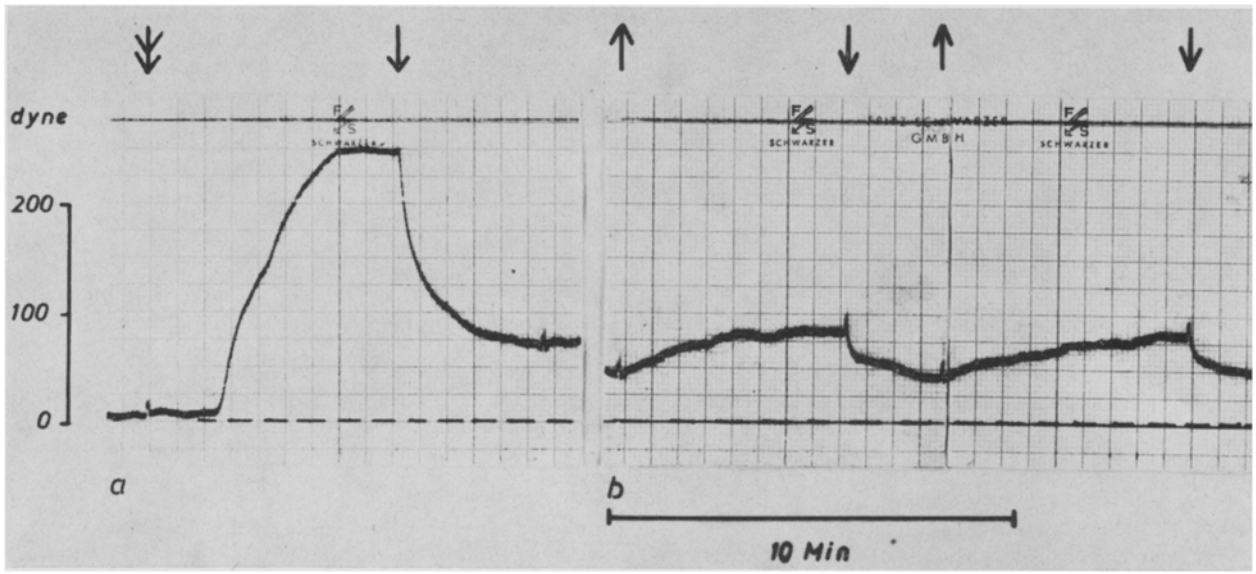
Tension and stiffness of glycerinated fibres of dorsal longitudinal muscles from *Lethocerus maximus* in rigor-, pyrophosphate- and tripolyphosphate-solutions (22 °C, pH = 6.5)

Composition of relaxing solution	In relaxing solution		After transfer to rigor solution	
	Resting tension (dyne/fibre)	Stiffness (dyne/fibre)	Tension increase[a] (dyne/fibre ± S.E.)	Stiffness (dyne/fibre)
10 m*M* MgATP[b]	0	250	+22.7 ± 1.3 (10)	3800
0.5 m*M* $MgCl_2$, 5 m*M* NaPP[c]	6.8	1100	+ 3.5 ± 0.2 (38)	1800
1 m*M* $MgCl_2$, 1 m*M* NaPPP[c]	7.7	1200	+ 2.8 ± 0.2 (11)	1800

[a] Number of experiments in parenthesis. [b] In addition to 20 m*M* Histidine, 10 m*M* Na-azide, 4 m*M* EGTA, 30 m*M* KCl. [c] In addition to basic solution (see text), Na-pyrophosphate (PP) (A.R. Merck), Na-tripolyphosphate (PPP) recrystallized.



Contraction cycles without ATP. a) A bundle of 8 fibres (DLM) suspended in ATP relaxing solution is transferred (at ↕) into ATP-free rigor solution (pH = 6.5, 22 °C) in order to produce rigor contraction. At ↓ (incomplete) relaxation is induced by immersing the preparation into PP-relaxing solution containing 0.5 m*M* Mg-pyrophosphate in place of ATP. b) 20 min later the fibres exhibit a resting tension of about 7 dynes (cf. Table, column 2). Note that repeatable contraction-relaxation cycles can be induced by removal (↑) and readdition (↓) of Mg-pyrophosphate.

Conclusions. The experiments just described show that it is possible to generate stiffness and a force comparable to the active tension (up to 5 dynes per fibre) merely by removing a plasticizer (pyrophosphate), i.e. without the presence or splitting of a nucleoside triphosphate. Since this effect can be reversed, it may be concluded that force is generated because of the spontaneous formation of actin-myosin linkages after removal of the actin-myosin dissociating agent, and that energy (supplied by the binding of pyrophosphate) is required to break the linkage and induce relaxation. These experiments must, of course, be considered in conjunction with morphological data[15] suggesting that myosin cross-bridge orientation does undergo a reversible change after addition of pyrophosphate to and removal from ATP-free rigor solution. It will now be investigated whether the energy transformations caused by pyrophosphate can be used to obtain mechanical work-cycles, and whether it is reversible in the sense that chemical energy (free energy of dilution) can be transformed into mechanical energy and vice versa: such processes might be indeed analogous to those occurring in contracting polyacrilic acid gels[16, 17].

Zusammenfassung. Durch Zugabe und durch Auswaschen von Mg-Pyrophosphat (0,5 m*M*) oder Mg-Tripoly-Phosphat (1 m*M*) konnten (in Abwesenheit von ATP) in glyzerinextrahierten Fasern von fibrillären Insektenmuskeln (*Lethocerus maximus*) reversible Kontraktionszyklen und Änderungen des Dehnungswiderstandes bewirkt werden.

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Electron Microscope and Optical Diffraction Studies on Glycerol-Extracted Insect Flight Muscle Fibres Relaxed by Pyrophosphate[1]

During the contraction-relaxation cycle of muscle, ATP has two functions: a plasticizing function (dissociation of actomyosin into actin and myosin), and the provision of energy by splitting. Results of Taylor et al.[2] suggest that the energy liberated by ATP splitting is used to bring back the detached myosin heads (= cross bridges) into the relaxed, rectangular position[3]. If this suggestion is correct, the angled cross bridges of a muscle in rigor should detach from the actin filaments without a change of conformation by the action of pyrophosphate (PP) which is known to imitate only the plasticizing action of ATP[4].

To test this hypothesis, the rigor state of glycerinated fibres of the dorsolongitudinal flight muscles of *Lethocerus spec.*[5] was used as a starting condition[6]. To be sure that no ATP was left in the fibre, the rigor solution (50 m*M* KCl, 20 m*M* histidine, pH 6.5, 10 m*M* NaN_3) was changed several times. When the fibres were transferred to the