## Transformation of Chemical into Mechanical Energy by Glycerol-Extracted Fibres of Insect Flight Muscles in the Absence of Nucleosidetriphosphate-Hydrolysis

Crosslinked gels of neutralized polyacryliacid which were incubated in salt solutions displayed reversible isotonic contraction cycles after addition of HCl[1-3] and exhibited a teinochemical effect (*τεινειν* = to stretch, to dilate): When such gels were mechanically stretched while they remained incubated in salt solution, a reversible decrease in pH was observed[4,5]. Thermodynamic analysis led to the general teinochemical principle[4]: 1. A completely quantitative transformation of chemical into mechanical energy is possible with contractile systems provided that the system can be reversibly changed by length changes and by the addition of chemical reagents; 2. such energy transformations are coupled with a teinochemical effect: reagents which induce (isometric) relaxation are absorbed by the contractile system when it is stretched at constant activity of that reagent.

Glycerol-extracted insect flight muscles showed repeatable isometric contraction-relaxation cycles after addition and removal of such ATP-analogues as Mg-pyrophosphate[6] and $\gamma$-imido-ATP (AMPPNP)[6-8] which are not hydrolysed by actomyosin. In this paper it will be shown that it is possible to obtain external work provided that the fibres are stretched in the presence of a plasticizer and released in its absence and that the fibres behave like a teinochemical system.

*Methods.* Fibre bundles (6–8 fibres) of the dorsal longitudinal muscle from *Lethocerus maximus* were stored for up to 8 months in 50% glycerol and glued between 2 glass rods of an highly isometric tension recording apparatus[9,10]. One glass rod was connected to a 5734 RCA-force transducer (compliance $5 \times 10^{-6}$ cm/dyne). The other rod was fixed on a micrometer drive whose position could be monitored by the displacement of a Grass-force transducer FTO3C. Length changes were calibrated with an ocular micrometer and from the reproducibility of stiffness measurements of fibres in rigor, it was concluded that length control was accurate within $\pm$ 1 µm.

Stiffness ($\Delta$ force per fibre and per unit strain) was measured immediately after a release (0.25% $L_0$) completed within 0.5 sec ('immediate stiffness') and 5 to 10 min after that release (static stiffness).

Rigor contractions were generated by the method of WHITE[11]: The bundle was first suspended in an ATP-relaxing solution (15 m*M* Mg-ATP, pH 6.5, pCa > 7) and then immersed in 2 ml of an ATP free rigor solution (20 m*M* Imidazol, 10 m*M* $NaN_3$, 4 m*M* EGTA, 50 m*M* KCl, pH 6.5, 22–26 °C) in order to produce rigor tension. ATP was then washed out from the fibres by several succesive solution changes. Then, the fibres were incubated for up to 20 min in 2 ml of a Mg-AMPPNP solution (5 m*M* in addition to rigor solution) and were then immersed in a freshly made rigor solution, in which they contracted again. After this pretreatment the experiment was continued either by isometric tension experiments when the fibres were immersed into analogue solutions of increasing concentration (0.1 m*M* to 16 m*M*) or by positive (or negative) length-tension cycles (see legend to Figure 3).

*Results.* After depletion of ATP, the fibres contract isometrically and develop their typical rigor characteristics[6,11] (Table). When the fibres are transferred into a 5 m*M* Mg-analogue solution, tension and stiffness fall and reach a steady value 5 min after incubation in that solution (Table). This analogue-induced relaxation can be partially reversed (Figure 1): The fibres contract and become stiffer when they are re-immersed in a rigor solution. The final values are about 75% of the tension and stiffness in the original rigor solution (Table). Such isometric tension cycles can be repeated many times (up to 11 cycles) without any noticable change in the tension amplitude (Figure 1).

The effect of analogue concentration upon static isometric fibre tension is summarized in Figure 2: Increasing Mg-AMPPNP concentrations result in enhanced relaxation of the fibres; however, the relationship between tension and concentration is not a simple hyperbolic one; the Mg-AMPPNP concentrations neces-


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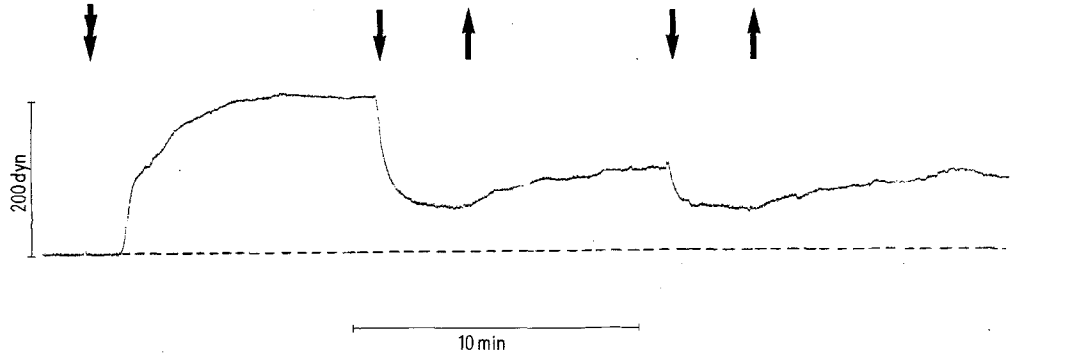


Fig. 1. Contraction cycles without ATP. A bundle of 8 fibres (DLM, *Lethocerus maximus*) suspended in ATP relaxing solution is transferred (at ↓) into ATP free rigor solution in order to produce rigor tension. At↓ partial relaxation is induced by immersing the bundle in a 5 m*M* Mg $\gamma$-imido-ATP solution (AMP-P-N-P). 4 min later tension is steady at 7.5 dyne per fibre. At ↑ the fibres are transferred back into the rigor solution which generates a tension rise up to 15 dyne per fibre. Repeatable contraction-relaxation cycles can be observed after removal (↑) and readdition (↓) of Mg $\gamma$-imido-ATP.

sary to induce 50% and 90% relaxation are 2.5 m$M$ and 16 m$M$ respectively. At comparable concentrations $Mg^{++}$ (added to rigor solution as $MgCl_2$) and Na-AMPPNP have a much smaller isometric relaxing effect than Mg-AMPPNP (Figure 2).

A positive work cycle was obtained when (after the pretreatment described above) the fibre was released by 0.5% $L_0$ in rigor solution, restretched in relaxing solution containing the analogue (5 m$M$) and induced to contract

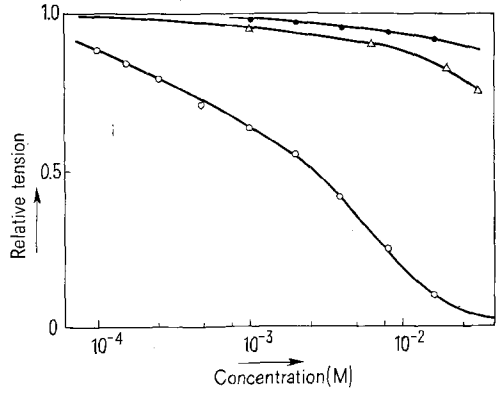


Fig. 2. The effect of Mg-AMPPNP concentration upon tension of glycerinated DLM fibres of *Lethocerus maximus*. Rigor tension was generated by the method of WHITE. Subsequent immersion of the bundle (6 fibres) into a Mg-AMPPNP solution (5 m$M$) induces relaxation which is partially reversed after a reincubation in rigor solution (compare Figure 1). After this pretreatment the fibres were immersed in solutions with increasing Mg-AMPPNP (○), Na-AMPPNP (□) or $MgCl_2$ (△) concentrations. Note that the Mg complex of AMPPNP has a stronger relaxation effect than its components.

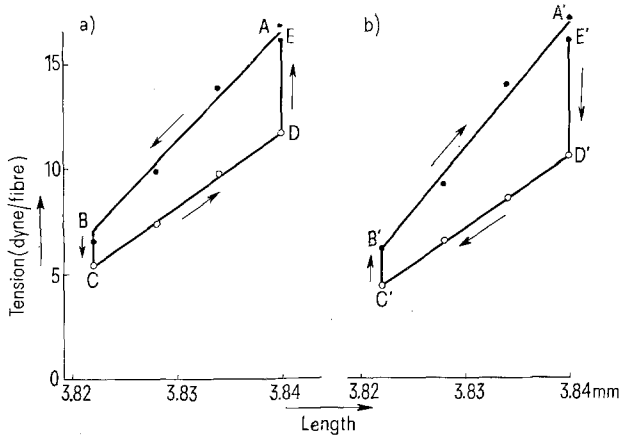


Fig. 3. Reversible work cycles with DLM fibres (*Lethocerus maximus*) After pretreatment which involved (see Figure 1) generation of rigor tension, analogue induced relaxation and partial regeneration of rigor tension by washing out the analogue, a bundle (8 fibres, 23 °C) was subjected to a positive and a negative work cycle. A positive work cycle starts at A when the fibres are immersed in rigor solution. While remaining in that solution the bundle is released stepwise (A–B) and steady tensions (△) recorded 5 min after those releases. At B, Mg-AMPPNP added to rigor solution induces partial relaxation until 5 min later tension is steady at C. The bundle, while it remains immersed in the analogue solution, is stretched stepwise to $L_0$ (C–D) and tensions (□) are recorded 5 min after those stretches. At D the fibres were immersed into rigor solution to induce isometric contraction. At the end of this positive work cycle, tension (E', ▽) did not differ notiecably from the tension at the start (A) of the cycle. A negative work cycle (b) was performed by reversing the steps E'-D'-C'-B'-A' of the positive cycle. Tension at the end of the negative cycle (A') is virtually the same as in the beginning of the positive cycle (A).

(isometrically) by immersion in rigor solution. Figure 3 shows that fibre tension at the end of a positive cycle (E) was nearly the same as at the beginning (A). During 5 positive cycles the fibres have performed positive work ([16.5 ± 1.6 (S.E.) merg/fibre/cycle], equivalent to the area circumscribed by the cycles). Such cycles could also be performed in the reverse (negative) direction. During 3 negative cycles the same fibres absorb mechanical work (18.3 ± 2.0 merg/fibre/cycle). At the end of the last negative cycle the fibre was in the same state as at the beginning of the first positive cycle. It was not possible, with the same bundle, to get noticeable work performances when a 5 m$M$ $MgCl_2$ solution was used instead of the 5 m$M$ Mg AMPPNP solution. AMPPNP-induced work performances (14.5; 15.3; 16.5; 18.3 merg/fibre/cycle) were essentially reproducable for the four fibres tested.

Application of the teinochemical principle to glycerol-extracted fibres. AMPPNP is not split by actomyosin[12]; even the maximum splitting rate compatible with YOUNT's estimates[12] (less than 2% in 16 h under conditions in which ATP is 90% cleaved in 10 min) would supply to the fibres less than 2% of the free energy necessary to produce the observed mechanical work (Figure 3). Hence, the evidence that the same state (same tension at same length and concentration) of the fibres is obtained after positive and after negative work cycles demonstrates that the chemical reactions between AMPPNP and crossbridges as well as the mechano-chemical responses to lengthening are reversible. Thus, the prerequisite of the teinochemical principle[4] is fullfilled.

Statement 1.[13] of the teinochemical principle implies then that a completely quantitative transformation of chemical (free) energy into mechanical work and vice versa is possible with glycerol-extracted DLM fibres of *Lethocerus maximus*.

Statement 2. of that principle is the teinochemical effect. It demands that the observed Mg-AMPPNP induced (isometric) relaxation is coupled with an absorption of Mg-AMPPNP by the fibres when they are stretched at constant activity of that analogue. The quantitative aspects of the teinochemical effect were already given by GIBBS[14,15]; they were later experimentally verified for artificial contractile systems[4,5,16] and for a denaturated actomyosin system[17]:

$$\left(\frac{N}{L}\right)_a = -\frac{\partial f}{\partial \log(a)} \frac{1}{2.303\,RT} \qquad (1)$$

where $N$ is the number of mole AMPPNP absorbed by the fibre; L is the fibre length; $(a)$ is the activity of

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[13] This formulation is typical for the 'engine approach' in thermodynamics[28]. The meaning of the statement[4–7] is that under isothermal and reversible conditions the cyclically effected work performances must be compensated by the supply of an equal amount of free energy. In the absence of energy providing reactions (and of contamination processes) free energy is to be supplied from immersion solutions (corresponding to reservoirs of the 'engine') while AMPPNP is transported from the 5 m$M$ solution via the 'engine' to rigor solution. It is the (equivalent) osmotic work released by this dilution process which is quantitatively transformed by the 'engine' into mechanical work[29].

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Tension and stiffness of glycerinated DLM fibres (*Lethocerus maximus*) in rigor solution and Mg-AMPPNP solution

| Solution | Tension (dyne/fibre) | yield point (dyne/fibre) | Immediate stiffness (dyne/fibre) | Static stiffness (dyne/fibre) |
|---|---|---|---|---|
| A rigor[a] | 21.5 | 21.5 | 3600 | 3500 |
| B rigor + 5 m*M* Mg-AMPPNP[b] | 7.3 | 8.0 | 1700 | 1100 |
| C rigor | 15.2 | 18.3 | 2300 | 2200 |

[a] 50 m*M* KCl, 20 m*M* Imidazol, 10 m*M* Na-azide, 4 m*M* EGTA, pH 6.5. [b] aequimolar in $MgCl_2$ and adenyl imidodiphosphate (purchased by Serva, Heidelberg).
The fibres were first suspended at room temperature (22 °C) in a ATP relaxing solution (15 m*M* MgATP, pH 6.5, pCa 7). After immersion in a rigor solution tension was generated (A); tension drops after analogue addition (B) and is partially regenerated after reimmersion in rigor solution (C).

Mg-AMPPNP in the incubation solution; $f$ is the force acting on the fibre; RT is equal to $2.45 \times 10^{-10}$ erg/mole at room temperature.

Neglecting the differences between activities ($a$) and concentrations, it is easy to evaluate $(\partial f/\partial \log a)L = -7$ dyn/fibre from the slope at 5 m*M* of the force – analogue concentration curve (Figure 2). According to equation (1) the amount of the stretch induced AMPPNP absorption $(\partial N/\partial L)_a$ is 120 pmol/cm fibre at constant AMPPNP concentration (5 m*M*), which means that a stretch of 1% $L_o$ should induce an extra AMPPNP absorption of 1.2 pmole per cm fibre length.

A direct, but still lacking, experimental verification of the teinochemical effect [equation (1)] would give reinforced evidence that the described system is fully reversible; but would [if the results fit equation (1)] otherwise give no new information upon the system.

It is interesting to discuss the effect of analogue addition upon actomyosin linkages in connection with evidence obtained by biochemical studies[12], mechanical studies[11] (Table), and optical studies (electron micrographics[16], X-ray diffraction patterns[19,20] and fluorescent polarisation[21]). As will be discussed further elsewhere, such results suggest that analogue binds to crossbridges when it induces relaxation, and that in complete relaxation bridges are released from the arrowhead position on the actin. Free energy (added by increasing the AMPPNP concentration) must be supplied in order to bind the AMPPNP to the crossbridges and in order to release crossbridges from their attachment to actin in the arrowhead conformation[22]. Removal of AMPPNP causes contraction and removes free energy from the contractile system. Hence, it is likely that the contraction and the formation of rigor linkages is the spontaneous process in the crossbridge cycle, while the detachment is the energy requiring step.

After a (reversible) complete isometric contraction – relaxation cycle, no work is performed by the fibres and the free energy supplied for the binding must be exactly equalized by the free energy released due to the removal of the analogue. However, in doing mechanical work as described in this paper, extra free energy is required and is supplied to the contractile system by the stretch induced analogue absorption. It is that extra free energy associated with analogue binding which is quantitatively transformed into mechanical energy during a work cycle: a cyclically effected transformation of chemical into mechanical energy is only possible, if a stretch dependent analogue affinity enables the storage of free binding-energy when the crossbridges are stretched. The calculated 1.2 pmole analogue which are absorbed during a 1% $L_o$ stretch of the fibres corresponds to a stretch induced analogue binding of 20%–40% of the total number of crossbridges[23] present in 1 cm fibre length, which is taken to mean that the affinity of the crossbridges for the analogue is markedly increased by the stretch.

From the evidence suggesting a stretch-dependent affinity of crossbridges to AMPPNP, it is tempting to speculate about one possible role of the hydrolytic products of ATP-splitting[24,25] in actively contracting muscle. If that product behaved analogueously to the AMPPNP with respect to actomyosin binding, a shortening of the crossbridge would decrease the affinity of crossbridges to the products, while a stretch would cause it to increase. The theory of processes[26] implies that the decreased product affinity induces an enhanced rate of product dissociation (the rate limiting step of ATP-splitting).

Hence, the ATP-splitting rate would be enhanced when crossbridges are released, and retarded when they are stretched. Such an effect might account for the extra ATP-splitting observed in actively shortening muscle[27].

*Zusammenfassung.* Glycerin-extrahierte Faserbündel von fibrillären Insektenflugmuskeln (*Lethocerus maximus*) leisten reversible mechanische Arbeit, wenn sie in Rigorlösung entdehnt werden, durch Zugabe von AMPPNP[8] isometrisch relaxieren, in der AMPPNP-Lösung gedehnt werden und durch Entzug von AMPPNP zur isometrischen Kontraktion gebracht werden. Dabei wird die mechanische Arbeit quantitativ aus chemischer Energie (Verdünnungsenergie des Analogs) produziert; dies ist nur möglich, wenn AMPPNP dehnungsinduziert von den Fasern absorbiert wird.

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