EXPERIENTIA

Vol. 24 – Fasc. 6 Pag. 529–640 15. 6. 1968

Oscillatory Mechanism in Fibrillar Insect Flight Muscle

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During flapping flight of certain higher insects (flies, bees, wasps, beetles) their indirect (fibrillar) flight muscles contract and relax, i.e. they oscillate according to a myogenic rhythm (PRINGLE[1,2]) many hundred times/sec and in certain small species (midges) even up to a thousand times/sec[1] in order to produce the wing-beat by compressing and decompressing the elastic thorax. The latter acts as a lever for the wings. Although in these cross-striated muscles, the amplitude of the oscillatory contractions is only a few % of the muscle length (about 1–2% in flies and bumble bees, see BOETTIGER[3]) and although the force generated is much smaller than in vertebrate skeletal muscle (about 0.3 kg/cm^2, see MACHIN and PRINGLE[4]) the power output may be 10 times larger (MACHIN and PRINGLE[4], NEVILLE and WEIS-FOGH[5]) and, on a weight basis, it may even be comparable to the power output of an aircraft engine (WEIS-FOGH[6]). Since in muscle the amount of mechanical work controls the extent of the chemical reaction providing the contractile energy, the low metabolic rate of the resting muscle shoots up to as much as 40 cal/g muscle every min (e.g. bee wing muscle, see WEIS-FOGH[6]) at the onset of oscillation.

The contractile machinery for the isobaric and isothermic chemo-mechanical energy transformation are the bundles of myofibrils within muscle cells (muscle fibres); fibrils are essentially chains of sarcomers, about 2.5 μ long, separated by Z-discs and consisting of a double array of thick and thin filaments[7]. The thin actin filaments (about 1 μ long) are attached to the Z-discs and interdigitate with the array of thick myosin filaments into which they slide when the sarcomer shortens in contraction by the sliding filament mechanism (HUXLEY and HANSON[8]).

Like a recent review on insect flight muscle[2], this paper will also be concerned with the question of how the pull on the sliding actin filaments, i.e. the contractile force, might be generated during oscillation (see section 3). So far, the nature of the conversion of chemical energy – released by the splitting of adenosine triphosphate (ATP) – into mechanical work is still mysterious; more is known about the regulating mechanisms operating during oscillation: firstly a calcium release mechanism in the muscle cell, which activates muscle (see the survey of HASSELBACH[9]) and permits oscillation; secondly, a 'built-in oscillator' within the myofibrils which triggers the individual contraction cycles during oscillation according to a myogenic rhythm of high frequency (section 1) and thirdly, an energy release control which automatically increases the rate of chemo-mechanical energy flow when the muscle is doing work by shortening under tension (FENN[10]) and adapts it to power output to which it is proportional (section 2).

(1) *The triggering of contractile cycles*

Myogenic and neurogenic oscillation. Unlike neurogenic oscillation of synchronous (also called 'afibrillar', because myofibrils – though present – are not easily distinguished with the light microscope) flight muscles, during myogenic oscillation of fibrillar, asynchronous flight muscles contraction is not triggered by nerve impulses and by action-potentials in synchrony with the contractile cycles (PRINGLE[11], BOETTIGER and MCCANN[12], ROEDER[13]). It therefore seems unlikely that in fibrillar muscles contractile activity is switched on by an increase, and switched off by a decrease in the sarcoplasmic level of free calcium ions, as it is in neuro-

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[2] J. W. S. PRINGLE, Prog. Biophys. biophys. Chem. *17*, 1 (1967).
[3] E. G. BOETTIGER, in *Recent Advances in Invertebrate Physiology* (Ed. B. T. SCHEER; Univ. of Oregon Press 1957), p. 117.
[4] K. E. MACHIN and J. W. S. PRINGLE, Proc. R. Soc. *B. 151*, 204 (1959).
[5] A. C. NEVILLE and T. WEIS-FOGH, J. exp. Biol. *40*, 111 (1963).
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[7] H. E. HUXLEY and J. HANSON, Abstr. 1st Europ. Electron Microsc. Congr. Stockholm, p. 202 (1957); D. E. ASHHURST, J. Cell Sci. *2*, 435 (1967); E. ZEBE, W. MEINRENKEN and J. C. RÜEGG, Z. Zellforsch. mikrosk. Anat. *87*, 603 (1968).
[8] JEAN HANSON and H. E. HUXLEY, Nature *172*, 530 (1953).
[9] W. HASSELBACH, Prog. Biophys. biophys. Chem. *14*, 167 (1964).
[10] W. O. FENN, J. Physiol. *58*, 175 (1923).
[11] J. W. S. PRINGLE, J. Physiol. *108*, 226 (1949).
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[13] K. D. ROEDER, Biol. Bull. mar. biol. Lab., Woods Hole *100*, 95 (1951).

genic oscillation (SMITH[15]). For free calcium is supposedly released[14] from the stores in the sarcoplasmatic vesicles when they receive a signal transmitted from the depolarized, excited cell membrane via the 'T-system' of the sarcoplasmatic reticulum. (Survey see HASSELBACH[9]; SMITH[15].) An increase in the sarcoplasmatic free calcium level above the resting value (about $10^{-7}M$, see PORTZEHL et al.[16]) per se activates the myofibrillar ATPase and contraction of the myofibrils (CALDWELL[17]) as it does in isolated myofibrils in vitro (A. WEBER et al.[18]), when ATP is supplied as an energy source. The isolated myofibrils suspended in Mg-ATP solutions relax again when the myofibrillar ATPase is inhibited by reducing the free calcium ion concentration to below $10^{-8}M$ by means of chelating agents or, physiologically, by pumping the released calcium back into the vesicles of the sarcoplasmatic reticulum (HASSELBACH[9]). Thus, a series of contraction-relaxation cycles such as they occur in twitches or during neurogenic oscillation of afibrillar flight muscles in lower insects (e.g. moths and butterflies), is only possible so long as the interval between stimuli producing a calcium release from the reticulum is larger than the time required to pump the calcium back into the vesicles of the reticulum. As the frequency of stimulation increases to a critical value, the sarcoplasmic concentration of ionized calcium remains high and the twitches fuse to a maintained contraction, a tetanus. For this reason afibrillar flight muscle cannot perform neurogenic oscillation at a frequency higher than about 100 c/sec, in spite of the fact that their sarcoplasmic reticulum is extremely well developed and has an especially active vesicular Calcium pump (see SMITH[15]).

By contrast, in the so-called fibrillar flight muscles of flies, beetles, bugs, the sarcoplasmic reticulum is poorly developed[15] and accordingly the tetanus fusion frequency is quite low, about 10 c/sec in fly muscle[19] and basalar beetle muscle[4], under *strictly isometric* recording conditions. And yet, these 'slow' muscles are able to oscillate in situ above the tetanus 'frequency barrier' at frequencies of up to several hundred/sec. In the sound-producing cicada, about 4 click movements (sound pulses) are evoked in the fibrillar tymbal muscle by a single motor nerve impulse and stimulation at 50 c/sec would set up a myogenic rhythm at about 320 oscillations/sec, though under strictly isometric conditions the tetanus fusion frequency was 60/sec and a single twitch lasted 0.1 sec (PRINGLE[20]).

The frequency of oscillation is near the resonance frequency of the thorax-wing system and may be doubled simply by cutting the wings. Thus it depends on the mechanical parameters (inertia of the wings, stiffness of thorax) rather than on the rate of stimulation (PRINGLE[4,11]). The latter may be similar to the tetanus fusion frequency but lower than the oscillation frequency provided that it maintains the muscle in a sufficiently activated state[20]. Accordingly, the frequency of oscillation is not directly controlled by the rate of nervous stimulation (it is myogenic, not neurogenic) and the oscillatory cycles are not then in synchrony with action potentials.

The myofibrillar automatism of myogenic oscillation. The myofibrillar origin of myogenic oscillation was demonstrated when JEWELL, PRINGLE and RÜEGG[21], and JEWELL and RÜEGG[22], discovered the oscillatory performance of myofibrillar bundles of flight muscles in physiological salt solutions containing ATP as the only source of energy. They used the indirect flight muscles of huge tropical waterbugs which have a comparatively low wingbeat frequency of about 10 to 20 c/sec. The myofibrillar contractile structures were functionally but not necessarily physically isolated (see ABBOT and CHAPLAIN[23]) from the vesicular calcium pump, the sarcosomes, the excitable membrane and the sarcoplasmatic soluble constituents of the cell by SZENT-GYÖRGYI's glycerol-extraction procedure[22]. Fibres, about 70 μ thick and cut to a length of about 0.5 cm, were glued to a torsionband lever (Figure 1)

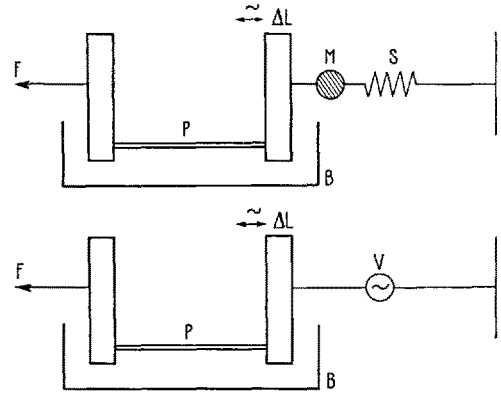

Fig. 1. Experimental set-up for free oscillation (above) and driven oscillation (below). The preparation (P) is immersed into a bath (B) containing ATP salt solution; it changes length sinusoidally (amplitude of oscillation ΔL) when attached to a resonant lever system (torsion band lever) of the elasticity (S) and the mass (M) (above), or when it is sinusoidally stretched by the electromagnetic vibrator (V) which is driven by a sinusoidal wave generator (below). In the free oscillation experiments (Figure 2) L is recorded; in the driven oscillation experiments (Figure 3) both L and tension (force, F) are registered and displayed on the oscilloscope. During insect flight, the elasticity (S) is largely determined by the thorax and the mass (M) by the wings.

[14] E. B. RIDGWAY and C. C. ASHLEY, Biochem. biophys. Res. Commun. *29*, 229 (1967).
[15] D. S. SMITH, Prog. Biophys. biophys. Chem. *16*, 107 (1966).
[16] H. PORTZEHL, P. C. CALDWELL and J. C. RÜEGG, Biochim. biophys. Acta, *79*, 581 (1964).
[17] P. C. CALDWELL, Proc. R. Soc. B. *160*, 512 (1964).
[18] A. WEBER, R. HERZ and I. REISS, Proc. R. Soc. B. *160*, 489 (1964).
[19] E. G. BOETTIGER, Anat. Rec. *111*, 443 (1951).
[20] J. W. S. PRINGLE, J. Physiol. *124*, 269 (1954).
[21] B. R. JEWELL, J. W. S. PRINGLE and J. C. RÜEGG, J. Physiol. *173*, 6P (1964).
[22] B. R. JEWELL and J. C. RÜEGG, Proc. R. Soc. B. *164*, 428 (1966).
[23] R. H. ABBOT and R. A. CHAPLAIN, J. Cell Sci. *1*, 311 (1966).

and immersed into a physiological salt solution of pH 7 containing 5 m*M* ATP as the only energy source. The preparation relaxed in the virtual absence of calcium ions (which were chelated by adding EGTA) but it contracted after addition of Ca^{++} and developed tension associated with increased ATPase activity in proportion to the log of the concentration of calcium ions (between 10^{-8}–10^{-6} *M* Ca), when the lever was stiff (SCHÄDLER[24]). But when the natural frequency of the lever was reduced to about 10–40 c/sec by increasing its compliance, the preparation did not contract isometrically; instead it produced an oscillatory contraction, lasting for as long as the calcium ion concentration was increased (up to 23 h[22]). The amplitude increased with increasing calcium concentration up to 1.5% of the muscle length and the frequency was always near the resonance frequency of the lever system used (Figure 2).

This type of oscillation is analogous to that obtained in surviving isolated longitudinal flight muscles of *Bombus* working under an inertial load (BOETTIGER[3]); but probably different from the auto-oscillations obtained in glycerinated mammalian skeletal muscle fibres by treatment with ATP (GOODALL and LORAND and MOSS[25], see discussion by JEWELL and RÜEGG[22]). It is noteworthy that the contraction and relaxation cycles during oscillation occur with ATP as the only energy source under constant chemical conditions, in particular at a constant, buffered calcium ion concentration, and at a rate which can be varied by varying the resonance frequency of the recording lever system (Table I). Thus, the switching 'off and on' of contractile activity is apparently controlled by the small changes in length occurring during oscillation rather than by changes in calcium ion concentration. Let us suppose (PRINGLE[20,26]), that these length changes are sensed by the myofibrils and that shortening switches the contractile machinery 'off' and lengthening of the muscle-fibre switches the machinery 'on' again, with a delay. Thus the myofibrillar preparation attached to a reso-

Table I. Effect of increasing the natural frequency of the lever system on the oscillatory response of extracted fibres from the dorsolongitudinal muscle of *Lethocerus cordofanus* (data from JEWELL and RÜEGG[22])

Experiment	Stiffness of lever (g/cm)	Natural frequency of: lever with fibre (c/sec)	Response in ATP solution[a] with 2×10^{-7} *M* Ca^{++}	
			Mean tension (dynes/fibre)	Oscillation (c/sec)
1	0.35	4.5	2	5.3
2	0.35	10	2	12
3	0.35	24	3	27
4	400	> 500	6	none

[a] Containing 5 m*M* ATP, 5 m*M* $MgCl_2$, 2 m*M* EGTA-calcium buffer, 10 m*M* histidine buffer, pH 7. Ionic strength adjusted to 0.08 with KCl 20 °C. In experiments 1–3 the natural frequency of the lever was increased by decreasing the equivalent mass of the lever system. In flying *Lethocerus maximus*, the wingbeat frequency is about 25 c/sec and the firing rate of the motor nerves of flight muscles is about 4/sec. Note that on a stiff lever the fibre produces an isometric contraction after immersion into the contraction solution, but it is unable to oscillate.

[24] M. SCHÄDLER, Pflügers Arch. ges. Physiol. *296*, 70 (1967).
[25] M. C. GOODALL, Nature *177*, 1238 (1956). – L. LORAND and C. MOSS, Nature *177*, 1239 (1956).
[26] J. W. S. PRINGLE, in *The Physiology of Insecta* (Ed. M. ROCKSTEIN; Academic Press, New York 1965), vol. 2, p. 283.

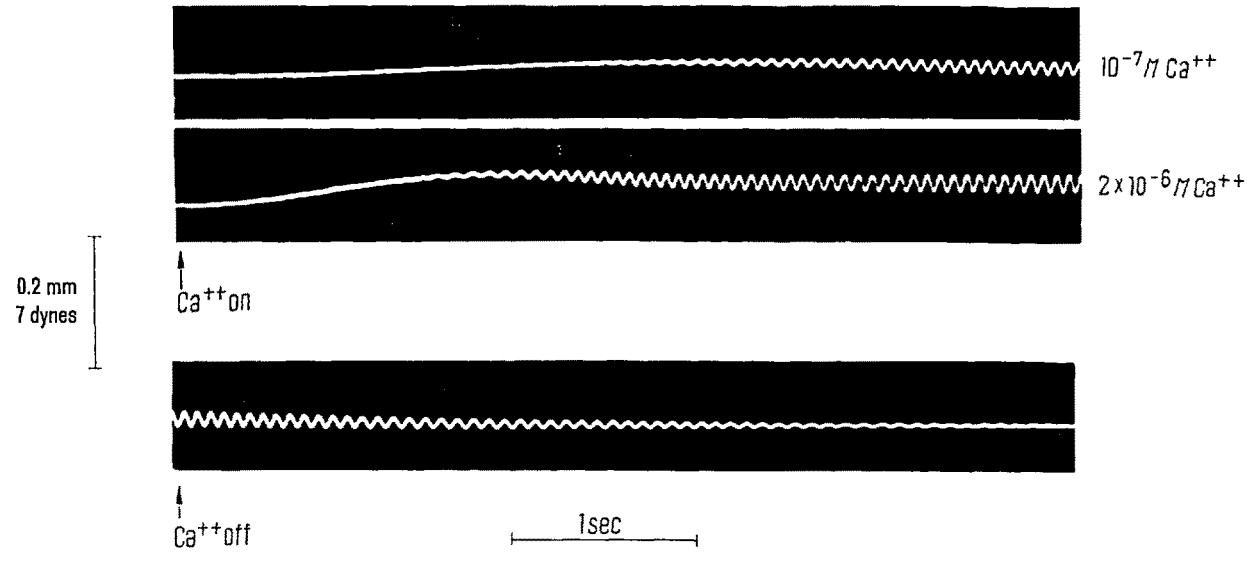

Fig. 2. Auto-oscillation of glycerinated fibrillar muscle (single fibre) attached to a weak torsionband lever in ATP salt solution containing 5 m*M* ATP and 5 m*M* $MgCl_2$. Concentration of free calcium adjusted with EGTA-calcium buffers, pH 7, 20 °C. Note that oscillation requires a steady level of free calcium and that the amplitude but not the frequency of oscillation depends on the concentration of Ca^{++}. Preparation: from dorso-longitudinal muscle of *L. maximus*. Adapted from JEWELL and RÜEGG[22].

nant lever system will contract and shorten, getting deactivated and thus relaxing and lengthening under the pull of the restoring force of the lever system. Then it becomes activated; a counterforce is evoked and it will contract again; this cycle would be exactly timed by the (restoring) movement of the resonant lever system.

As expected from the hypothesis described, there is an increase in contractile tension and ATPase activity after forcible elongation above resting length (RÜEGG and TREGEAR[27], Table II). As in living fibrillar muscle[3], the increase in tension occurs with a delay after an abrupt stretch, while an abrupt release of tension produces a delayed fall in tension (JEWELL and RÜEGG[22]). Because of the delayed activation and deactivation, sinusoidal stretches and releases (at up to 4% L_0 amplitude and at e.g. 2 c/sec driven oscillation) by means of a vibrator (Figure 1) produce (more or less) sinusoidal tension changes lagging behind the length changes (Figure 3)[22,28,29] in extracted fibres, as well as in living flight muscle during auto-oscillation (BOETTIGER[3]) and during sinusoidally imposed length changes (MACHIN and PRINGLE[30]).

Table II. Effect of stretching an extracted fibre bundle on its ATPase activity, the passive resting tension and the superimposed contractile tension. Preparation from the dorso-longitudinal muscle of *L. maximus* (data from [28])

Fibre length (% of resting length)	Passive tension in relaxing solution (dynes/fibre)	Contractile tension (dynes/fibre)	ATPase activity (nmol/min/cm fibre)
100	0	7.5	0.18
102.5	1.7	11	0.2
105	4	13	0.3
107.5	5.5	17	0.45

Medium for measuring ATPase activity and contractile tension contained $3\times10^{-7}M$ Ca^{++} and Na azide to inhibit the sarcosomal ATPase. Relaxing solution contained 4 mM EGTA, but no Ca. Conditions otherwise as in Table I.

[27] J. C. RÜEGG and R. T. TREGEAR, Proc. R. Soc. B. *165*, 497 (1966).
[28] J. C. RÜEGG, Am. Zool. *7*, 457 (1967).
[29] J. C. RÜEGG, Verh. schweiz. naturf. Ges. 184 (1965).
[30] K. MACHIN and J. W. S. PRINGLE, Proc. R. Soc. B. *152*, 311 (1960).

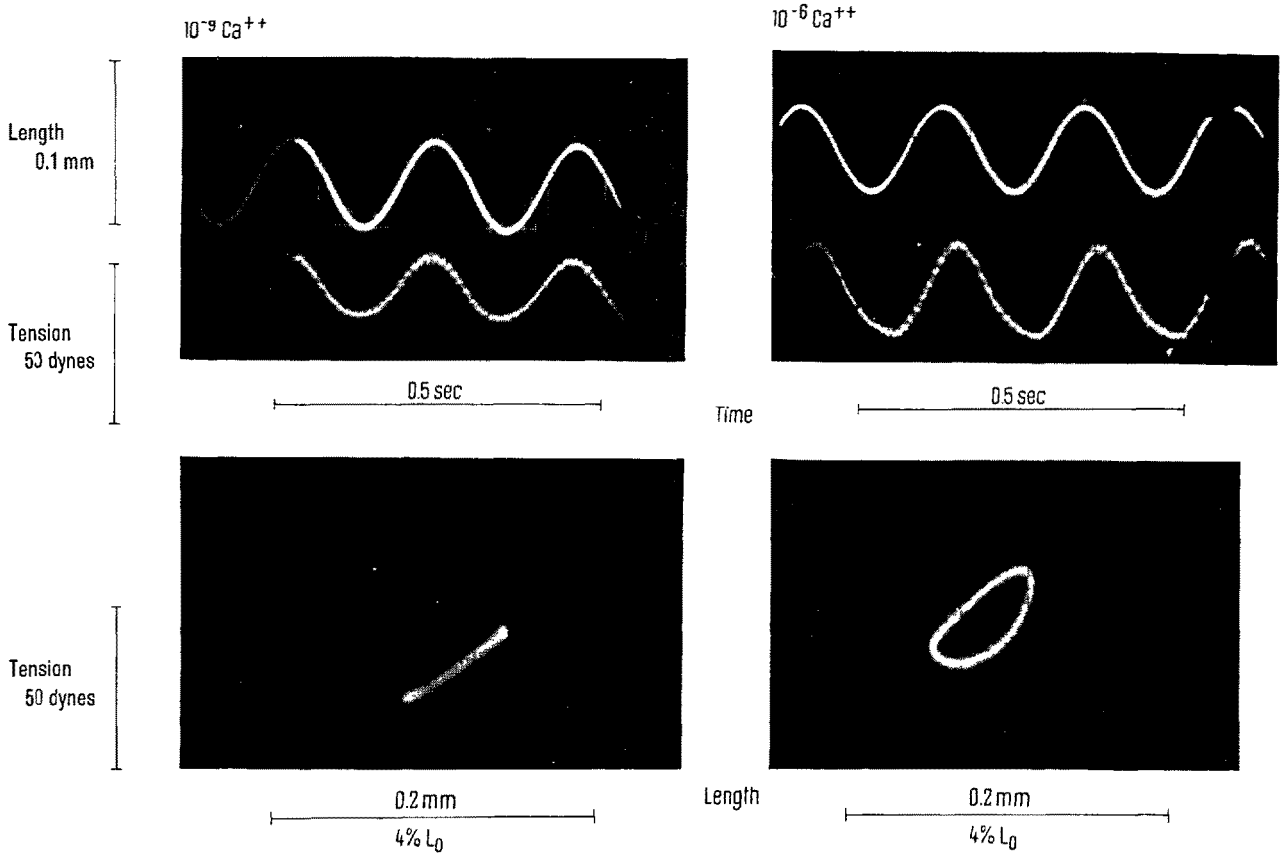

Fig. 3. Oscillation of glycerinated fibrillar muscle (fibre bundle) in ATP salt solution driven by sinusoidal stretching (sinusoidal analysis). Above: Sinusoidal tension changes lag behind the sinusoidal length changes in activating solution ($10^{-6}M$ Ca) but not in relaxing solution (about $10^{-9}M$ Ca^{++}). Below: The tension-length diagram is described in a counter-clockwise fashion, i.e. the tension is greater during shortening than during elongation, indicating that the sinusoidally stretched preparation does work (the area of the loup) in each oscillatory cycle at high concentration (right) but not at low concentration (left) of calcium ions. Composition of ATP salts solution as in Table I. Preparation: dorso-longitudinal muscle of *L. cordofanus* (adapted from RÜEGG[29]).

Oscillatory power. Consequently, in each oscillatory cycle, tension is greater during the shortening half cycle than during the stretch half cycle, and more mechanical energy is released during shortening than is required to stretch the preparation. The difference represents the positive oscillatory work per cycle (up to 1 erg/cm fibre[28]), which is the same as in living beetle muscle (cf. MACHIN and PRINGLE[4]), and is of course indicated by the area enclosed by the tension length diagram (Figure 3). The 'driven oscillation' experiments (employing a vibrator, mimicking an undamped resonant lever system of variable natural frequency) show that the delayed generation of tension by sinusoidal length changes produces oscillatory power which may obviously be large enough to overcome the damping of the torsionband lever system and of the insect thorax-wing system during maintained free oscillation. The generation of delayed tension and extra ATPase activity by stretching and thus oscillation and the production of oscillatory power is only possible if the ATPase activity is sufficiently activated by increasing the level of free calcium above 10^{-8}–$10^{-7}M$ at about 0.1 ionic strength and with Mg ions present. Without Mg, with inosinetriphosphate replacing ATP, or at very low ionic strengths, there is excessive activation already without stretching the preparation. No further delayed activation occurs upon stretch, nor does the preparation then give an oscillatory response after raising the free calcium concentration.

(2) *Mechano-chemical coupling of ATPase activity and power output*

As H. E. HUXLEY[31] pointed out, the 2 most important features of muscle are that the contractile mechanism is able to transform with a high efficiency the energy liberated in the chemical reaction of ATP splitting into mechanical work, and that the amount of mechanical work which is done controls the extent of chemical reaction (ATP splitting) which takes place. The functionally isolated contractile machinery of the 'myofibrillar bundles' (glycerol-extracted fibres) shows both of these properties, when it is driven during oscillation by ATP as the only energy source under controlled chemical conditions. During driven oscillation at about 2 c/sec produced by sinusoidal stretch and release of the fibre, the ATPase activity is greater than during isometric contraction under the same ionic conditions by an amount proportional to the rate of doing work (RÜEGG and TREGEAR[27], RÜEGG[28]). The latter can be increased simply by increasing the frequency of the amplitude of oscillation[27,28]. The linear relationship between extra ATPase activity and power output suggests an efficiency of about 3000 cal work/mole of ATP split. But this linearity is somewhat surprising in view of the fact that at high ATPase activity the ratio of ATP:ADP, and thus the free energy of the reaction, will be considerably lower than at low ATPase activity. If a waterbug fibre is sinusoidally stretched and released without doing work, for instance at 0.2 c/sec or at 60 c/sec there is no extra ATP-splitting produced during oscillation (STEIGER[32]; see Table III). These findings demonstrate not only the close coupling of power output and extra ATPase activity; they also give evidence that the oscillatory extra ATPase at 2 c/sec is not simply a 'diffusional artifact', for if it were, the artifact would be even more pronounced at high frequencies. Other investigations (MANNHERZ[33]) showed that the rate of ATP diffusion does not rate-limit the ATPase activity of fibres. Slow sinusoidal length changes (at 0.2 c/sec) should increase the ATPase activity above that at mean length if extending the preparation above the mean length were to produce a large stretch activation which is not compensated by the reduction of ATPase activity upon the release below the mean length. Since the net change in average ATPase activity is negligible upon slow oscillation, the large increase observed at about 2 c/sec must be due to a dynamic activation of the rate of energy release which occurs when the muscle is working by shortening under tension, and which is distinct from static stretch activation.

Obviously the increase in metabolic rate of muscle in proportion to the rate of doing work (FENN effect[10]) has its origin in an increase of the splitting rate of energy-rich phosphate compounds (biochemical FENN effect see WILKIE[34], MARÉCHAL[35], DAVIES[36]). But it is

Table III. Coupling of ATPase activity and oscillatory poweroutput

Frequency (c/sec)	Experiment 1		Experiment 2	
	ATPase of 1 cm fibre (nmol/min)	Power of 1 cm fibre (μcal/min)	ATPase of 1 cm fibre (nmol/min)	Power of 1 cm fibre (μcal/min)
0	0.3	0	0.37	0
0.2	–	–	0.36	0
2	0.75	2.4	0.64	0.4
60	0.25	0	–	–

Experiments showing the effect of increasing the frequency of driven oscillation above the frequency optimum (experiment 1) and of decreasing it below the optimum (experiment 2). Preparation: extracted fibre bundles from dorso-longitudinal muscle of *L. maximus*. Amplitude of oscillation 3.6% muscle lengths (1) or 2.5% (2). Conditions as in Table II. ~ 20 °C. Unpublished data of STEIGER.

[31] H. E. HUXLEY and J. HANSON, in *The Structure and Function of Muscle* (Ed. G. H. BOURNE; Academic Press, New York 1960), vol. 1, p. 183.
[32] G. STEIGER, Pflüger's Arch. ges. Physiol., in press.
[33] H. J. MANNHERZ, Pflüger's Arch. Ges. Physiol. *291*, 94 (1966).
[34] D. R. WILKIE, J. Physiol. *195*, 157 (1968).
[35] G. MARÉCHAL, *Le métabolisme de la phosphorylcréatine et de l'adénosine triphosphate durant la contraction musculaire* (Arcia, Bruxelles 1964).
[36] R. E. DAVIES, Proc. R. Soc. B. *160*, 480 (1964).

not known how and where in the cell the work-load is sensed by the contracting muscle and how it adapts the rate of chemo-mechanical energy flow to the rate of doing work. The 'automatic' release of calcium ions into the sarcoplasm under work-load has been implied in various suggestions (see A. V. HILL[37], MARÉCHAL[35]). But probably a more direct mechanism is involved: In a sliding filament contractile system, the rate of sliding ought to be controlled by the rate at which energy is released to the contractile system and vice versa (cf. A. V. HILL[38], A. F. HUXLEY[39]). If crosslinks between actin and myosin filaments operate cyclically, as assumed in most present theories of muscle contraction (A. F. HUXLEY[39], H. E. HUXLEY[31], DAVIES[40], PRINGLE[2]), the rate of ATP-splitting by the actin activated myosin ATPase (the actomyosin ATPase) depends on the probability of actin myosin interaction and thus, among other things, on the rate at which sites on actin filaments are sliding past and presented to enzymatic sites on the myosin filaments.

Thus, the ATPase activity increases when the muscle is shortening under tension because of a direct coupling of ATPase activity and power output in the sliding filament system of the myofibril. Such a mechanochemical coupling was postulated by many workers and has now been demonstrated in the functional isolated myofibrillar contractile structure of insect flight muscle supplied with ATP as the only energy source[27–29]. Mechanical ATPase activation is probably the enzymatic basis for the tremendous increase[6] in metabolic rate (FENN effect) observed in insect fibrillar muscles during flight.

(3) *Generation of oscillatory tension and chemomechanical energy transformation*

Tension is probably generated by means of crossbridges or side-pieces of the myosin filaments which interact with actin filaments when ATP is enzymatically split at the crossbridge site (A. F. HUXLEY[39], H. E. HUXLEY[31], PERRY and COTTERIL[41]). Possible mechanism of the generation of tension by the crossbridges in an oscillatory work cycle may perhaps best be visualized with the help of a model experiment (Figure 4). When extracted fibres of insect flight muscle were suspended in Calcium free, ATP containing relaxing solution, they were relaxed and extensible because of the plasticizing action of ATP which is known to dissociate actomyosin into actin and myosin in vitro (see WEBER and PORTZEHL[42]). Correspondingly, the crossbridges pointing at right angles to the direction of actin filaments were not connected to these filaments (REEDY, HOLMES and TREGEAR[43]). This mechanical and structural state persisted when the ATP was replaced by its analogue MTP (6-morpholino 9/2'3'-*O*-isoproyliden β-ribofuranosilpurin-5-triphosphate) which is barely attacked by the myofibrillar ATPase (WHITE, quoted by PRINGLE[2]). When the plasticizing agent was washed out with salt solutions, the crossbridges attached to the actin filaments and pulled these filaments by about 150 Å (about 1.5% of the half sarcomer length) towards the middle of the sarcomer by means of an angular stroke, so that they now point at an angle of approximately 45° towards the centre of the sarcomer, giving an 'arrowhead' appearance to the thin filaments (REEDY, HOLMES and TREGEAR[43]). The structural change just described is associated with rigor contraction, i.e. with a great decrease in extensibility and with the shortening of the contractile structure by about 1.5–2%. This stretches the series elastic components and develops about 10 dynes or $\sim$ 0.1 ergs mechanical work/cm fibre (WHITE, quoted by PRINGLE[2]). Since the mechanical work involved in the stretching of the series elastic components is supposedly produced without splitting of energy-rich phosphates,

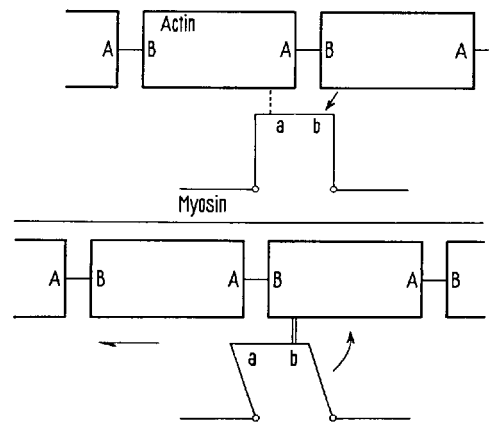

Fig. 4. Hypothetical scheme of contractile mechanism (modified from PERRY and COTTERILL[41], taking account of angular crossbridge movement. (See [43]; recent experiments[47] show that the crossbridge movement is probably more complicated than shown in this simplified scheme.) Above: relaxed state; actin filament (greatly simplified) and myosin filament are not connected mechanically but may interact enzymatically at the 'a' sites of the myosin sidepieces and the 'A' sites. Below: rigor state; the myosin filaments combine mechanically at the 'b' sites of the sidepieces with the 'B' sites of the actin filaments. The sidepiece (crossbridge, H-meromyosin) then bends, so as to form the arrowhead configuration pointing[43] towards the centre of the sarcomer (at left hand side). The driving reaction pushing the actin filament (about 150 A) towards the left (centre sarcomer) and thus generating force is supposedly the spontaneous 'arrowhead' formation between crossbridge and actin. This may occur after the ATP plasticizer is locally removed by the enzymatic site 'a' activated with 'A'. Repetitive cycles of crossbridge action may lead to oscillation, to maintained tension, or to shortening (see text).

[37] A. V. HILL, Proc. R. Soc. B. *160*, 516 (1964).
[38] A. V. HILL, Proc. R. Soc. B. *126*, 136 (1938).
[39] A. F. HUXLEY, Prog. Biophys. biophys. Chem. *7*, 255 (1957).
[40] R. E. DAVIES, Nature *199*, 1068 (1963).
[41] S. V. PERRY and X. COTTERILL, Nature *206*, 161 (1964).
[42] H. H. WEBER and H. PORTZEHL, Prog. Biophys. biophys. Chem. *4*, 60 (1954).
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merely by removing the plasticizer (MTP), it would appear that the primary reaction involved in the release of energy required for the angular (force generating) crossbridge stroke stems from the chemical interaction of crossbridges (essentially the heads of myosin molecules or H-meromyosin[44]) with actin filaments. Indeed, if isolated heads of myosin molecules (H-meromyosin) are mixed with isolated actin filaments in the absence, but not in the presence of ATP-plasticizer, they spontaneously attach to the filaments and then produce an angular stroke so that they now form the characteristic arrowhead structure, first described by HUXLEY[44] and later discovered in longitudinal sections of insect flight muscle fixed in rigor (REEDY[45]).

Since the combination of myosin crossbridges with actin, as well as the force generating crossbridge movement, takes place spontaneously with the equilibrium greatly in favour of the combined state ('arrowhead' state), energy must be provided to break the links when ATP or an ATP-analogue are bound to the myosin sites at the crossbridges. In fact, addition of ATP or of its analogue MTP to the fibres in rigor restores the initial relaxed mechanical and structural state and thus completes the work-cycle of the crossbridge. The cycle starts anew when ATP is washed out or enzymatically split at the enzymatic site of crossbridges (see PERRY and COTTERILL[41]) in contraction. A repetitive cyclic action of this type involving at least 1 ATP molecule split/crossbridge cycle requires that ATP splitting occurs (because of activation with actin) just before myosin crossbridges and actin combine, but is inhibited after actomyosin formation in order to allow the breaking of the links by newly diffusing ATP combining with myosin. (Figure 4; see PERRY and COTTERILL[41].) Such a repetitive oscillatory crossbridge action may take place in synchrony during oscillation of insect fibrillar muscle (PRINGLE[2], but see TREGEAR[46]) or asynchronously during tetanic contraction of frog muscle when crossbridges are known to beat continuously (HUXLEY and BROWN[47]). Work-cycles of crossbridges are about 3×10^{-13} ergs in a tetanus (DAVIES[40]) and about 2×10^{-13} ergs in an oszillatory cycle of glycerinated water bug muscles (crossbridge work obtained by dividing the fibre work in reference [28] by the number of ATP molecules split, assuming 1 ATP split/crossbridge action). The size of the work/crossbridge cycle is thus similar to that obtained in rigor contraction (i.e. about 10^{-13} ergs) on the assumption that here all the crossbridges (about 1.5 pmoles/cm fibre according to CHAPLAIN and TREGEAR[48]; but see [45]), generate tension. Thus, it is possible that the mechanical work and force is generated in the same way in all 3 cases, i.e. by the spontaneous 'arrow head' formation of myosin crossbridges attaching to actin when the ATP plasticizer is removed.

Delayed generation of tension after stretch. The oscillatory mechanism is not understood unless we find out why an increase in length (e.g. by stretch) is followed by a delayed tension development and a proportional activation of the ATPase activity. Contractile tension (GORDON et al.[49]) and rate of ATP splitting (WARD et al.[50]) depends on actinmyosin interaction and the degree of overlap of thick and thin filaments; thus it is proportional to the number of actin myosin links in operation. It seems conceivable, therefore, that stretching the extracted insect muscle fibres affects the myosin filament which are connected to the Z-line ([51,52] but cf. ASHURST[53]) and increases the number of contractile actin myosin linkages which are in operation, perhaps by increasing the calcium affinity of contractile structures (CHAPLAIN[54]) or by increasing the production of ADP (ABBOT, quoted by PRINGLE[2]). However, the calcium ion concentration required for half maximal activation (about $10^{-7}M$ at pH 7, see VOM BROCKE[55], MARUYAMA et al.[56], SCHÄDLER[24]) and thus presumably also the calcium affinity, is the same in stretched and unstretched preparations[24]. The latter can be further activated by stretch even in a solution containing a saturating concentration of free calcium (about $10^{-5}M$), and at various ADP concentrations. It is tempting to speculate that activation by stretch is due to the removal or displacement of an inhibition, analogous to that removed by MEINRENKEN[57] when he extracted from glycerinated insect fibres certain protein inhibitor factors including EBASHI factor[58] with pH 8 *Tris* buffer. For, after extraction, the contractile tension induced with Mg-ATP at saturating calcium concentration is often increased; it is even greater than in stretch activated preparations and comparable to the tension in the 'high-tension' state obtained with ITP (ZEBE et al.[51]), at low ATP concentration (JEWELL and RÜEGG[22]), at very low ionic strength (RÜEGG and STUMPF[59]) or without added Magnesium ions (ZIEGER[60]). But any further activation by stretch is then impossible.

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(4) *Conclusions*

In conclusion, insect fibrillar muscle oscillates by means of a myofibrillar automatism with ATP as the source of energy and with calcium and magnesium ions as necessary activators because any increase in length of myofibrils produces a delayed rise in tension and any decrease in length produces a delayed fall. Future work will have to show what relation (if any) exists between somewhat similar oscillatory phenomena of skeletal muscle described by ARMSTRONG et al.[61] and the oscillation of insect fibrillar muscle. Oscillations of tension have also been described in heart muscle[62] and smooth muscle[63] but these are almost certainly unrelated to myogenic oscillation since, unlike insect flight muscles, the vertebrate muscles do show fluctuations of the membrane potential in synchrony with the mechanical events. The mechanism of stretch activation and of the ATPase activation in relation to power output, as well as the nature of the highly efficient energy transfer from the ATP molecules to mechanical work, is still a mystery. But recently considerable progress has been achieved in studying the thermodynamics of contracting glycerinated skeletal muscle fibres (WEBER and PORTZEHL[42]) and of living striated muscle (e.g. MARÉCHAL[35], DAVIES[36], WILKIE[34]). Further research on the molecular level seems rather promising now that the efficient chemo-mechanical energy conversion and the mechano-chemical energy coupling can be studied directly on the working (oscillating) 'isolated' contractile machinery in vitro and under controlled chemical and mechanical conditions with ATP as the only source of energy, which is liberated by the ATPase of actin activated myosin[64].

Zusammenfassung. Während des Insektenfluges oszillieren die fibrillären Muskeln dank einem myofibrillären Automatismus. Oszillation der Myofibrillen ist in ATP-Salzlösungen selbst nach Isolierung der kontraktilen Strukturen möglich. Dies erlaubt In-vitro-Untersuchungen der mechano-chemischen Energiekoppelung der Muskelkontraktion.

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[64] Acknowledgment. The author is grateful to his colleagues in the laboratory for allowing him to quote much of their unpublished work and to Prof. J. W. S. PRINGLE and his group in Oxford for many stimulating discussions.

SPECIALIA

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The Synthesis of (±)-Maackiain

(−)-Maackiain (I) was isolated from *Maackia amurensis* Rupr. et Maxim. var. Buergeri (Maxim.) C. K. Schneid.[1], from *Andira inermis* (Wright) H. B. K.[2], and from *Swartzia Madagascariensis* Desv.[3]. By methylation I was converted into (−)-pterocarpin (II) which had been isolated from *Pterocarpus santalinus* L.[4]. Lately, (±)-maackiain I was obtained from *Sophora japonica* L.[5], and from *Dalbergia spruceana*[6].

In previous papers[7,8], the authors have reported the total synthesis of (±)-pterocarpin (II) via the methyl ether (III) of medicagol (IV), which was obtained by the procedure of WANZLICK's benzofurano-3′, 2′: 3, 4-coumarin synthesis[9]. In this paper we describe the synthesis of (±)-I from benzyl ether (V) of IV according to the modified procedure reported earlier[8].

In a manner similar to the experiment described earlier[7,10], the benzyl ether V (m.p. 251–253°, IR 1742 (α-pyrone), 1033, 940 cm^{-1} ($O-CH_2-O$) (Nujol), UV

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