humidity, with wide ranges of temperature conditions and diverse types of breeding sites.

Analysis of zymograms of various gene-enzyme systems in 6 species (table) shows that only 23% of all the structural gene loci are polymorphic in *A. aquasalis*, compared to 46–58% in other species (crit. 1). Similarly an average individual of this species is likely to have only 8.1% of its genome in heterozygous condition, whereas in other species it is 2–3 times higher. Again *A. aquasalis* possesses fewer of its individuals (8.4%), as heterozygotes, considering all loci studied, than other species (11–17%). From these measures of genetic variability in different anopheline species, we can conclude that our results support the 'selectionist' view of significance of genetic (allozymic) variability.

1 This work was supported by a grant from CNPq – Brasil.
2 J.L. Hubby and R.C. Lewontin, Genetics *54*, 577 (1966).
3 R.C. Lewontin, Columbia Univ. Press, New York 1974.
4 M. Kimura, Cold Spring Harb. Symp. Quant. Biol. *20*, 33 (1955).
5 M. Kimura, Thesis, University of Wisconsin, Madison 1956.
6 M. Kimura and J.F. Crow, Genetics *49*, 725 (1964).
7 F.J. Ayala, M.L. Tracey, L.G. Barr, J.F. Mac Donald and S. Perez-Salas, Genetics *77*, 343 (1974).
8 R. Milkman, Genetc. Res. *25*, 229 (1975).
9 R.P. Wagner and R.K. Selander, A. Rev. Ent. *19*, 117 (1974).
10 M. Nei, Molecular Population Genetics and Evolution. North-Holland, Amsterdam 1975.
11 W.H. Li, Genetics *90*, 349 (1978).
12 M. Kimura, Genet. Res. *11*, 247 (1968).
13 J.G. Scandalios in: Isozymes, vol.4, p.1. Ed. C.L. Markert. Academic Press, New York 1975.

Frequency-domain study of the mechanical response of living striated muscle

G.H. Rossmanith, J. Unsworth and R.D. Bell[1]

School of Mathematics and Physics, Macquarie University, North Ryde (New South Wales 2113, Australia), 18 April 1979

Summary. Small-amplitude sinusoidal displacements, in the frequency range 4–100 Hz, were applied to intact whole frog sartorius muscle whilst in a state of tetanus. At low frequencies the muscle was observed to do oscillatory work, while at higher frequencies it tended towards elastic behaviour. Frequency-response plots obtained were compared with those from other muscle preparations. Results were interpreted in terms of mechano-chemical transduction properties of muscle.

Dynamic stiffness and phase values over a range of frequencies have been obtained for a variety of muscle preparations: intact and glycerinated insect fibrillar muscle[2,3]; glycerinated frog sartorius muscle[4]; chemically stimulated frog semitendinosus fibres[5], and intact, whole, frog sartorius muscle[6].

Intact insect fibrillar muscle displayed a minimum in the dynamic stiffness response, while in the phase response both minimum and maximum features were evident[2]. The phase response was negative in the minimum region and positive in the maximum region. Negative phase values imply that the muscle is doing oscillatory work[2]. The same features were observed in glycerinated insect fibrillar muscle. It was concluded that the stiffness and phase responses were not a feature of excitation and contraction coupling but an intrinsic property of the contractile mechanism[3]. Negative values for the minimum-phase region were taken to be a characteristic feature of the oscillatory behaviour of insect fibrillar muscle.

Glycerinated frog fibres displayed the same negative phase region[4], but the frequency range was not extended far enough to define the maximum phase feature. However this emphasized that the capacity for oscillatory work was not peculiar to insect fibrillar muscle. Studies performed on chemically stimulated frog semitendinosus fibres over the frequency range 0.25–133 Hz displayed a minimum in the stiffness response and the minimum-maximum features in the phase response[5].

All the above systems could maintain a state of tetanic contraction for many minutes. Living muscle, stimulated electrically, cannot endure such long periods of tetanic contraction. Halpern and Alpert[6] used a broad-band length pertubation signal which enabled them to obtain dynamic stiffness and phase values over a wide range of frequencies from each 1.3-sec tetanic stimulation. Their results, however, departed from the pattern emerging from the above investigations. They characterized the dynamic stiffness trend by a 2-plateau shape, and only observed positive values for phase. Their study led them to model their response by a first-order transfer function with a finite delay, in contrast to transfer functions of orders 2 and 3 which were necessary for insect fibrillar muscle[7] and chemically stimulated frog fibres[5].

Our investigation was directed at establishing whether dynamic stiffness and phase trends, as observed in insect fibrillar muscle and chemically stimulated frog fibres can be observed in living frog muscle. We used small-amplitude sinusoidal length changes, as were used by all other workers, except Halpern and Alpert[6]. Although requiring more tetanic stimulations than the wide-band signal to define stiffness and phase trends, sinusoidal length changes had the advantage of much greater frequency-domain power for the same time-domain amplitude[8].

Methods. Whole sartorius muscles from the tree climbing frog *(Litoria caerulea)* were dissected and mounted horizontally in a small rectangular glass bath containing oxygenated Ringers solution (NaCl 115 mM, KCl 2.5 mM, $CaCl_2$ 1.8 mM, Na_2HPO_4 2.15 mM, NaH_2PO_4 0.85 mM, pH 6.9). The temperature of the bathing solution was measured using a thermocouple and a Keithley 160 multimeter and controlled by means of a Tauchlora cooling system.

Typical values for muscle weight and length were 32 mg and 2.3 cm. The tendon end of the muscle was firmly secured to the length driver, and the pelvic end to the force gauge. By means of a Spectra Physics 4 mW He-Ne laser, and displaying the diffraction fringes on a translucent screen, the sarcomere length was adjusted to rest length L_o, considered to correspond to an average of 2.2 μm along the muscle.

The muscles were supramaximally stimulated using a single pair of bright platinum wire electrodes. Duration of stimulation was kept to 2 sec and the muscle allowed 2 min rest between tetani.

The frequency of the sinusoidal length displacements was controlled by a digital computer (Hewlett-Packard 2100S) via a voltage from the digital to analog (D/A) interface

applied to the V_{CG} input of an Interstate Electronics Corporation F34 function generator. A Toyo Baldwin vibrator, which was stiffened using a metal diaphragm giving it a resonant frequency of 280 Hz and a compliance of 2 μm/g, was used to apply mechanical displacements of constant amplitude and not exceeding 0.1% L_o to the muscle. The displacements were monitored using a Hewlett-Packard linear variable displacement transformer which has an upper 3-db frequency of 350 Hz. The force gauge was constructed from 4 Kyowa silicon semi-conductor resistance elements (KSP-2-E4) mounted in a Wheatstone bridge network and bonded to a nickel-steel cantilever. The sensitivity was 1.3 mV/g, the static compliance 10 μm/g and the resonant frequency 290 Hz. The signal corresponding to the force was a.c. coupled to a Tektronix strain gauge amplifier and after amplification passed to the analog to digital (A/D) interface, as was the displacement signal. The force and displacement signals were frequency analysed on the Hewlett-Packard 2100S digital computer. Dynamic stiffness was taken as the ratio of the amplitude of oscillatory component of force to the amplitude of oscillatory component of length. At the conclusion of an experiment the data was corrected for frequency characteristics of the transducers and amplifiers. These correction characteristics were obtained from a calibration run where the muscle was replaced by a length of waxed cotton loaded to approximately the tetanus force level. The corrected data was displayed on a Hewlett-Packard 7210A digital plotter. Such a plot is shown in figure 1.

Results and discussion. Figure 1 shows a typical frequency response obtained from tetanized frog sartorius muscle. This response differs from that which was reported by Halpern and Alpert[6] and aligns itself with the trends seen in insect fibrillar muscle[2,3,7] and other frog skeletal muscle preparations[5]. The similarity to insect fibrillar muscle is very striking as seen when comparing the Nyquist-plot form of the data (figure 1b) with the plots reported by Machin and Pringle[2] and Jewell and Rüegg[3]. By analogy with calculations performed on frequency responses obtained from insect fibrillar muscle[7], frog sartorius dynamics also reflect a transfer function which is at least of order two. It can be shown that under sinusoidal steady-state conditions, the work W done by the muscle over one cycle of the sinusoid is given by

$$W = -2\pi \cdot y_{rms} \cdot x_{rms} \cdot \sin\theta ,$$

where y_{rms} and x_{rms} are the root mean square values of force and displacement sinusoids, and θ is the phase angle. The expression for maximum energy E transferred between muscle and the experimental rig is given by

$$E = y_{rms} \cdot x_{rms} \cdot \cos\theta .$$

This energy is stored in the muscle during 1 quarter cycle of the sinusoid and returned during the next quarter cycle. W and E are plotted against frequency in figure 2. The data used was the same as gave rise to the frequency response

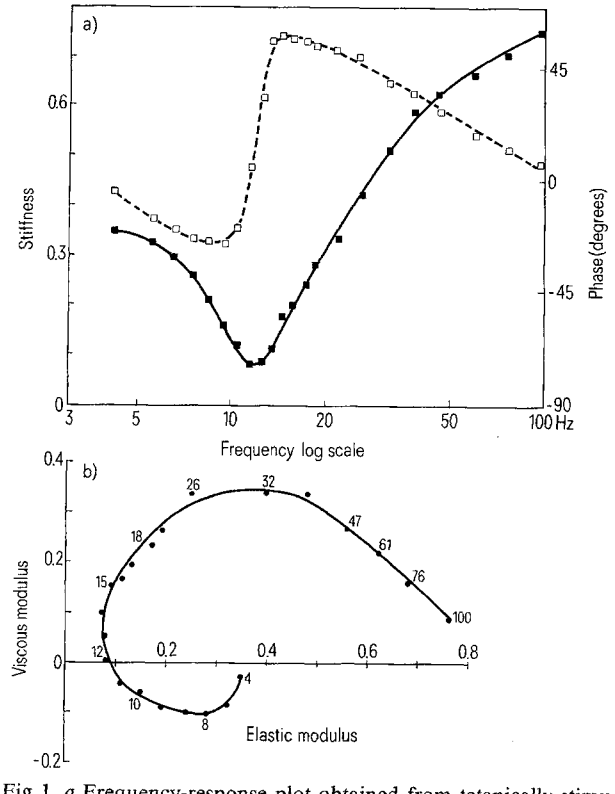

Fig. 1. *a* Frequency-response plot obtained from tetanically stimulated frog sartorius muscle maintained at 14 °C. Phase and dynamic stiffness values are indicated by the symbols □ and ■ respectively. *b* A Nyquist-plot of the data shown in (a). Dynamic stiffness and modulus values are given in arbitary units.

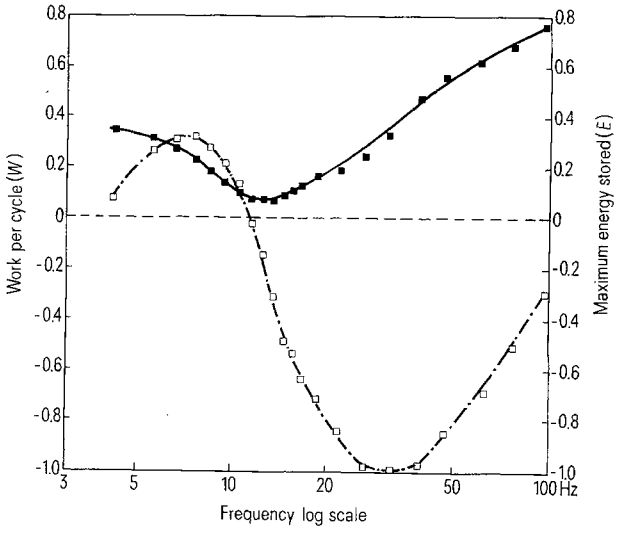

Fig. 2. Plot of maximum energy stored or exchanged (E) by the muscle, and work per cycle (W) done by the muscle, as a function of frequency of applied length oscillation. Positive values for W indicate that for this range of frequencies the muscle is doing net work on the external system. Values of W and E are indicated by the symbols □ and ■ respectively. The data for this plot is the same as gave rise to the frequency-response plot shown in figure 1.

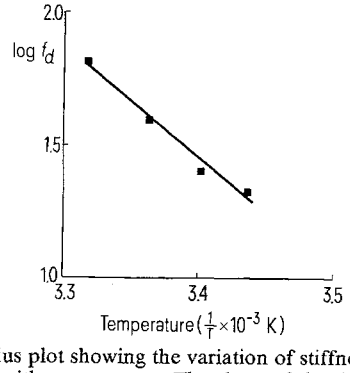

Fig. 3. Arrhenius plot showing the variation of stiffness - minimum frequency, f_d, with temperature. The slope of the line indicates an activation energy of 18 kcal/mole and a Q_{10} of 3.

plot shown in figure 1. This energetics plot reveals that for a range of frequencies net work per cycle is being done by the muscle (i.e. W positive) and reaches a maximum. As frequency is increased, W begins to decrease and becomes zero approximately at the frequency where dynamic stiffness is a minimum (figure 1a). This frequency is denoted by f_d. At this frequency, E also reaches a minimum. Beyond f_d, viscous effects dominate and W becomes negative. In the vicinity of f_d therefore, a change occurs in the performance of the muscle, with net work being done before f_d, while viscous effects dominate at frequencies beyond f_d. As frequency is increased further, W goes through a minimum and then begins to increase again. This is paralleled by a steady increase in E. Therefore at higher frequencies another change appears in muscle behaviour - from one where viscous effects are at their peak to behaviour which becomes more like that of an elastic device. This tendency towards elastic behaviour at higher frequencies is in agreement with tension transient studies performed on living frog fibres[10].

Implications of these studies to the mechanical response of tetanized muscle when subjected to sinusoidal length changes was highlighted by a study performed by Steiger and Rüegg[11]. They found that activated insect fibrillar muscle, when driven with small-amplitude sinusoidal perturbations, not only performed oscillatory work (i.e. W positive) but also displayed what was termed an 'oscillation-induced extra ATPase'. This oscillation-induced extra ATPase (termed the biochemical equivalent of the Fenn effect) varied with frequency of oscillation in a similar manner to the work curve. No extra ATPase was induced for frequencies greater than the frequency where dynamic stiffness was minimum (i.e. f_d). They concluded that work per cycle and ATPase activity reflect a frequency dependence of actin-myosin interaction rate in a sliding filament system. For the limited frequency range investigated by Steiger and Rüegg[11] the oscillatory work curve obtained by them agreed with the work curve shown in figure 2.

When the temperature of the bathing solution was changed we observed the stiffness minimum, and hence the frequency where extra induced ATPase activity ceased, to move. Figure 3 shows this temperature dependence for the range 18–28 °C. The Arrhenius plot revealed an activation energy of the process reflected by the parameter f_d, to be 18 kcal/mole. This figure is in close agreement with values quoted for the activation energy of ATPase activity of myosin[12] which was proposed as the rate limiting step of the cross-bridge cycle.

To further investigate this temperature dependence a more efficient procedure for obtaining frequency responses must be employed. Reports on this are in preparation.

1 The authors wish to thank the Australian Research Grants Commission for provision of the laser, and L.I. Patterson for construction of the mechanical apparatus.
2 K.E. Machin and J.W.S. Pringle, Proc. R. Soc. Lond. B *152*, 311 (1960).
3 B.R. Jewell and J.C. Rüegg, Proc. R. Soc. B *164*, 428 (1966).
4 P. Heinl, Pflügers Arch. *333*, 213 (1972).
5 M. Kawai, P.W. Brandt and M. Orentlicher, Biophys. J. *18*, 161 (1977).
6 W. Halpern and N.R. Alpert, J. appl. Physiol. *31*, 913 (1971).
7 R.H. Abbott, Cold Spring Harb. Symp. quant. Biol. *37*, 647 (1973).
8 G.H. Rossmanith, thesis, Macquarie University, 1977.
9 M.E. Van Valkenberg, Prentice-Hall, Englewood Cliffs, N.J., 1964.
10 L.E. Ford, A.F. Huxley and R.M. Simmons, J. Physiol. *269*, 441 (1977).
11 G.J. Steiger and J.C. Rüegg, Pflügers Arch. *307*, 1 (1969).
12 M. Bárány, J. gen. Physiol. *213*, 455 (1967).

Induction of zygospores at high temperatures in the thermophilic species *Mucor miehei* with aspartic acid and phenylalanine

B.S. Mehrotra[1] and B.M. Mehrotra

Botany Department, Allahabad University, Allahabad (India), 6 March 1979

Summary. The importance of amino acids in the induction of zygospores in mucors has been realized for the first time. It was found that in the thermophilic species *Mucor miehei* zygospores at high temperatures (40–50 °C) can be induced by addition of phenylalanine and aspartic acid.

During our studies of thermophilic fungi it was observed that the homothallic and thermophilic species *Mucor miehei* Cooney and Emerson produces zygospores at 35 °C but fails to produce them at 50 ± 1 °C. Earlier, Lasure and Ingle[2] had also observed that this otherwise homothallic species failed to produce zygospores and a pigment at higher temperatures (45 °C). It was thought that such fungi are possibly able to produce certain essential amino acids for the induction of zygospores at low temperatures but fail to do so at high temperatures. This expectation has been justified by subsequent studies. They are briefly reported here.

Our own isolate of *M. miehei*, obtained from soil, was grown in flasks with a medium containing: dextrose 40 g; KH_2PO_4 0.5 g; $MgSO_4.7\ H_2O$ 0.25 g; thiamine chloride 0.5 mg; asparagine 2 g, and 1000 ml distilled water. For each experiment 1 set of 9 replicates was incubated at 35 °C and another set at 50 °C. At the end of the 4th, 8th and 12th day of incubation a set of 3 flasks were taken out from each of the 2 incubators and the contents of each set was separately passed through a filter. The mycelial extract of each set was obtained in ethanol and then analysed chromatographically for its amino acid content following Consdon et al.[3]. Phenol, saturated with ammonia solution, and a mixture of n-butanol-acetic acid and water (4:1:5) were used as 1st and 2nd running solvents, respectively. Ninhydrin at a concentration of 0.1% (w/v) in n-butanol was sprayed to locate the amino acid and amide spots. The spots were developed on heating the chromatograms for 20 min at 80 °C. 13 amino acids were detected (table 1) in the colony grown at 35 °C but only 8 were found in the mycelium growing at 50 °C. The 5 amino acids missing at 50 °C were aspartic acid, γ-amino n-butyric acid, asparagine, leucine and phenylalanine. From this it was concluded that 1 or more of these 5 amino acids are possibly essential for the initiation of sexual activity in the fungus and failure of the fungus to synthesize them at high temperature is responsible for the absence of zygospores at that temperature. This conclusion was substantiated by the experiments that followed.