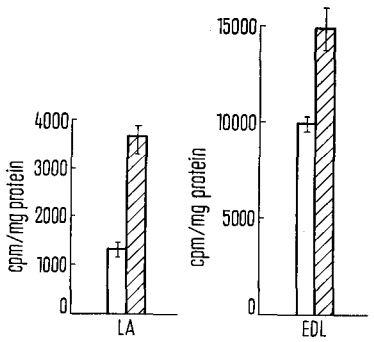
regulative metabolic reaction not dependent on the nervous system to stretch of the myofibrils might be assumed, as the increase of oxygen consumption occurs also in a curarized muscle. However, it must be remembered that a freely immersed muscle coils up and may even go into contracture, and thus the surface area exposed to the radioactive medium may also decrease. It will be necessary to study further the mechanism by which stretch increases rate of metabolism of proteins and to differentiate metabolic and mechanical factors. Generally unstretched muscles are used in routine incorporation experiments and therefore this factor should be considered in this type of experiments.

We may conclude that the use of stretched muscle in incorporation studies offers advantages and maintains also more natural conditions for muscle function. As stretch affects the rate of incorporation, it appears necessary to state the degree of stretch applied to a muscle in incorporation studies into muscle.



Incorporation of ^{14}C-leucine into the proteins of the levator ani muscle (LA) and the extensor digitorum longus muscle (EDL). The muscles were incubated for 2 h while the muscles were either stretched (shaded columns) or not stretched (white columns).

Zusammenfassung. Gegenüber nichtgedehnten Muskeln weisen gedehnte Skelettmuskeln in vitro eine beträchtlich erhöhte Rate von Aminsäureninkorporation in das Muskelprotein auf.

M. BUREŠOVÁ, E. GUTMANN and M. KLICPERA

Institute of Physiology, Czechoslovak Academy of Sciences, Praha 4 (Czechoslovakia), 5 September 1968.

A Relation Between Positive Phase Shift and Elastic Modulus Enhancement of Smooth Muscle

Muscle is a special kind of visco-elastic material capable of synthesizing energy during the contractile process. The mechanical behavior of relaxed smooth muscle can be matched by a three-parameter model composed of a Maxwell element in parallel with a spring[1]. All 3 parameters, essentially 2 tensile moduli to quantify energy-conserving characteristics and 1 viscosity to quantify energy-dissipation, have been measured[2,3]. These 3 parameters are known to increase with the level of contractile tone[3,4] so that they may also be a measure of the energy-synthesizing characteristics.

If a sinusoidal strain is imposed on in vitro smooth muscle[4,5], and on some insect flight muscles[6], the induced stress is generally out of phase with the strain and at high frequencies the phase angle (angular difference between stress and strain waves) is negative as would be expected for an ordinary viscoelastic body. However, at low frequencies there is a positive phase angle[4-6] which appears to represent a net production of mechanical energy by the muscle. The present study shows that the same low frequency oscillatory strains also induce a net increase in the contractile tone of smooth muscles, resulting in levels of tensile moduli which equal and even exceed those produced by drugs or electrical stimulation. The purpose of this report is to describe this new phenomenon and to give the conditions for producing it.

Experimental procedure. Rectangular specimens with a central slit were removed from the urinary bladder, pulmonary artery, and large veins of anesthetized (40 mg/kg sodium pentobarbital in the heart) rabbits, cats, and dogs. All these tissues contain numerous smooth muscle bundles. Specimens were supported horizontally in a fluid medium by 2 hooks slipped through the central slit. The medium was NaCl solution made isotonic with Ringer's solution by adding KCl (0–0.08 N) and/or $CaCl_2$ (0–0.08 N) and/or EDTA (0–0.08 N), and *Tris* buffer to keep pH at 5 levels between 5.5–8.5. Temperature was maintained at 0°, 15°, 25°, or 37 °C and recorded on an Offner Dynograph as previously described[4].

One supporting hook was attached to a vibration exciter which strained the specimen parallel to the central slit at frequencies (ω) from 0.01–50 Hz. Movement of the exciter was monitored with a fiberoptic displacement transducer (response 1100 Hz) whose output was registered as strain on the Dynograph and gave the X input of a storage tube oscilloscope. The other supporting hook was attached to a stress transducer (response 350 Hz) whose output was also registered on the Dynograph and served as the Y input to the oscilloscope. Stress-strain loops led to computation of absolute dynamic modulus ($|E|$) and phase shift ($\tan \phi$)[4,5].

Specimens were stretched by a step function and permitted to stress-relax to a steady mean tension. If the tension rose as evidence of recontraction, 1 or 2 oscillations at $\omega = 0.2$ Hz were superimposed every 2 min on the rising tension to permit concurrent measurement of $|E|$ and $\tan \phi$. When there was no evidence of recontraction, oscillatory strains of less than 1% were then superimposed

[1] J. T. APTER, Bull. math. Biophys. *26*, 367 (1964).
[2] J. T. APTER, Circulation Res. *19*, 104 (1966); J. T. APTER, M. RABINOWITZ and D. H. CUMMINGS, Circulation Res. *19*, 111 (1966); J. T. APTER, Circulation Res. *19*, 115 (1966).
[3] J. T. APTER, Circulation Res. *21*, 901 (1967).
[4] J. T. APTER and E. MARQUEZ, Circulation Res. *22*, 393 (1968).
[5] J. T. APTER and E. MARQUEZ, Biorheology, in press.
[6] J. C. RÜEGG, Experientia *24*, 529 (1968).

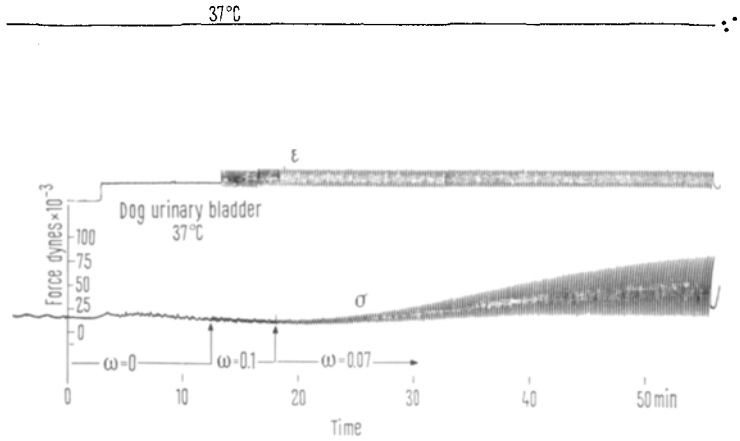


Fig. 1. Time course of stress (σ) changes registered when dog urinary bladder was strained in various ways (ε). Note absence of recontraction after a step-function stretch, but the presence of spontaneous oscillations. An oscillating strain at 0.1 Hz produced an oscillating stress associated with decreasing mean tension and decreasing amplitude of stress but an oscillating strain at 0.07 Hz (ω^*) was immediately accompanied by a gradual rise in amplitude of the sinusoidal stress and of mean tension.

on the steady tension at a variety of frequencies. Stress-strain loops showed that some low (< 1 Hz) frequencies were accompanied not only by a phase lead (positive phase angle), but also by a net increase in mean tension and in absolute dynamic modulus, $|E|$, or modulus enhancement. Frequencies above a critical value, ω^*, were not effective in producing phase lead nor modulus enhancement but the lower limit of effective frequencies must be lower than 0.01 Hz, the lowest available in these tests. The phenomenon was characterized under varying conditions by the ω^* and by τ^* which is the time to double $|E|$ at ω^*. Measurements of the time (τ) to double $|E|$ at lower frequencies (ω) were also recorded. Activated specimens were stored in their fluid media at 4 °C for testing on subsequent days.

The system showed no positive phase shift nor modulus enhancement at any available forcing frequency with specimens of rubber, elastin, collagen, silk, or nonviable smooth muscle (removed, for example from animals anesthetized with 40 mg sodium pentobarbital i.v.).

Results. Figure 1 shows the time course of the stress response to a sinusoidal ($\omega = 0.07$ Hz) strain of dog urinary bladder in Ca-free, K-free Ringer's solution containing EDTA. Detailed examination of the data showed that strain led stress so that there was a positive phase angle in contrast to the usual negative phase angle of ordinary visco-elastic materials. Mean tension, elastic modulus ($|E|$), and the phase gain ($\tan\phi$) began to increase immediately (Figure 2); indeed, after the first oscillation. Mean tension doubled in 15 min; $|E|$ doubled in 10 min; $\tan\phi$ doubled in 5 min. It is interesting that the mean tension and $|E|$ changed at differing rates although both are evidence of modulus enhancement. This is perhaps due to the non-linear relationship between stress and strain for smooth muscle. All manifestations of increase in visco-elastic properties continued for at least 120 min. Once begun, the modulus enhancement continued even if the activating oscillation were stopped, but at a slower rate (longer τ). During storage for several days at 4 °C the muscle relaxed and was capable of reactivation, although with some decrease in responsiveness as quantified by a reduced ω^* and longer τ^*. Activated muscle remained viable for 2 or more days while ordinary smooth muscle was viable for no longer than 2 days.

The highest frequency (ω^*) at which modulus enhancement occurred was a function of the source of the tissue, of the ions present, of the temperature, the mean tension, and the time (in vitro time) elapsed since the specimen was isolated from the living animal. The ω^* was highest

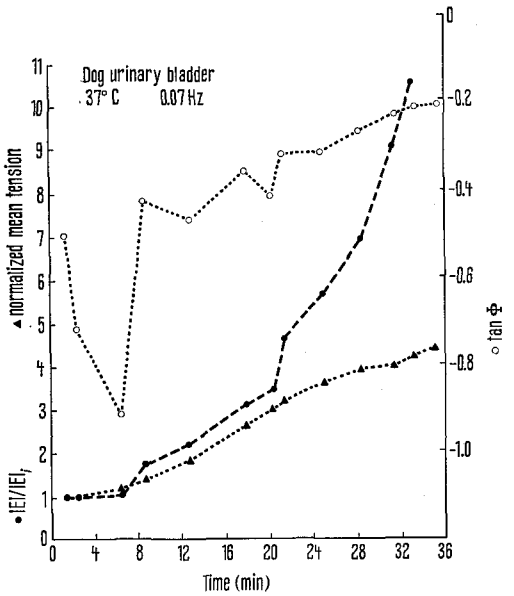


Fig. 2. Data taken from stress-strain loops obtained during the experiment depicted in Figure 1. Time course of change in absolute dynamic modulus, $|E|$, normalized with respect to its initial value $|E|_i$, of change in similarly normalized mean tension, and of the phase lead (positive phase shift is plotted as negative $\tan\phi$). Note the marked increase in phase lead occurring after a few min of oscillation. It was a regular finding and was associated with the most marked elastic modulus enhancement.

for the first 4 h after isolation and fell noticeably thereafter. The shortest time (τ) to double $|E|$ was regularly associated with high ω: in fact, there was a nearly constant product of ω and τ, so that

$$\omega \times \tau \approx k \qquad (1)$$

where k is a constant and $\omega \leq \omega^*$. Therefore it appeared to require approximately the same number of oscillations to double $|E|$, whatever the strain oscillation frequency. The value for k was nearly constant for a given smooth muscle type even though ω^* became lower as in vitro time increased. At low temperatures ω^* was lower, but, again, τ^* was higher, maintaining relationship (1) in general.

Higher mean tensions were associated with higher ω^* and shorter τ^*. If Ca^{++} was absent, the self-amplification still occurred so long as K^+ was also absent, but not if K^+ was present. Values for ω^* were higher and τ^* shorter if Ca^{++} normality was greater than K^+. In high Ca^{++} solutions or in the absence of both Ca^{++} and K^+, the initial step-function stretch was followed by stress relaxation and then by a slow recontraction process, all within 1 min. Such specimens also had a high ω^*, and it is logical to suppose that the step-function was serving simply as a half-oscillation initiating the self-amplification. However, τ (time to double $|E|$) was considerably longer after a step-function-induced phenomenon than with steady oscillation at ω^*. Arterial smooth muscle had a lower ω^* than does urinary bladder but rabbit arterial ω^* was highest.

Discussion. The positive phase shift indicating that muscles produce energy has also been found in insect flight muscles oscillated at their natural frequency[6]. However, the modulus enhancement found here on smooth muscles and, indeed, a logical consequence of energy production has not been reported previously. The smooth muscle showed a maximal positive phase shift with its associated modulus enhancement when the frequency of the forcing was the same as the frequency of spontaneous oscillations (Figure 1). Although myofibrillar contractile structures isolated from insect flight muscle can oscillate spontaneously only in the presence of Ca^{++}, smooth muscle oscillates and its modulus is enhanced even in the absence of this ion. This finding suggests that stretch and release of the smooth muscle cell membrane itself may occur during mechanical oscillation of the specimens and take part in producing the modulus enhancement.

This phenomenon of modulus enhancement may prove useful in investigating the excitation-contraction coupling mechanism of muscle as are similar phenomena in flight muscle[7] and 'catch' in mammalian smooth muscle[8] and for developing a mathematical formulation for muscular contraction without resorting to such empirical analogs as HILL's contractile element[9]. It may also prove useful as a simple and rapid means to test the viability of muscular tissues in organs preserved for transplantation[10].

Values for ω^* and τ^* for dog urinary bladder in a bath containing $[Cl^-] = 0.159N$; $[Na^+] = 0.158M$; [EDTA] = $0.001M$; *Tris* buffer = $0.005M$ at 37 °C pH 7.0, and mean tension $< 2.7 \times 10^4$ dynes cm^{-1}

Specimen No.	ω^* (Hz)	τ^* (min)	k (Hz-min)
1	0.03	19	0.57
1	0.04	25	1.00
1	0.05	19	0.95
1	0.06	25	1.50
1	0.07	14	0.98
1	0.08	9.5	0.765
1	0.05	19	0.95
7	0.05	27	1.35
17	0.05	26	1.30
19	0.05	19	0.95
27	0.05	29	1.45
32	0.05	19	0.95
12	0.1	12	1.2
43	0.1	14	1.4
84	0.1	10	1.0
99	0.1	15	1.5
111	0.1	9	0.9
112	0.1	12	1.2

Zusammenfassung. Bei glatter Muskulatur, sowie bei quergestreifter Insektenflugmuskulatur, war eine positive Phasenverschiebung zwischen oszillatorisch verformender Dehnung und dem Zug nachweisbar, die ein Zeichen für Energieproduktion ist. Wenn die positive Phasenverschiebung auftrat, fand sich oft auch eine Erhöhung des Elastizitätsmoduls und sogar eine verlängerte In-vitro-Überlebenszeit der glatten Muskulatur.

JULIA T. APTER and W. GRAESSLEY

Section of Mathematical Biology, Presbyterian-St. Luke's Hospital Chicago (Illinois 60612, USA) and Department of Chemical Engineering, Northwestern University, Evanston (Illinois, USA), 2 October 1968.

[7] J. W. S. PRINGLE, Prog. Biophys. *17*, 3 (1967).
[8] W. H. JOHNSON, J. S. KOHN and A. C. SZENT-GYÖRGYI, Science *130*, 160 (1959).
[9] A. V. HILL, Nature *166*, 415 (1950).
[10] Supported by U.S.P.H.S. Grant No. GM-14659-02.

Biochemical Study on the Pituitary Inhibition of Gonadal Origin

We had previously succeeded in showing[1-5] that the pituitary-inhibiting activity from the gametogenic structures, depauperates from sexual steroids, seems to act chiefly on follicle stimulating hormone (FSH). Such activity seemed to be thermolabile (at $\geq$ 90 °C for 10 min) and destroyed by the DNAse II; it seemed to persist practically unaltered even in extracts deprived of any detectable effect of androgenic or estrogenic type.

Owing to the high nucleotide content of the raw material and the activity of the nucleases, we decided preliminarily to direct our research towards the identification of substances of this type, as possible responsable for the pituitary inhibition.

The bovine nemasperm homogenate was ultrafiltrated through an SM 12136 membrane (Membranfilter, Göttingen), porosity < 0.005 µ, and fractionated on DEAE-cellulose (Figure). The fractions were grouped as OA and OB, 1, 2, 3 and 4. Chromatographies on a thin layer of cellulose G (solvent system: *n*-butanol, acetone, acetic acid, ammonia water 5%, water, 9:3:2:2:4) showed ninhydrin-positive substances in fractions OA, OB, 1 and 2; in fractions 3 and 4 ninhydrin-positive compounds

[1] G. FACHINI, C. TOFFOLI, A. GAUDIANO, M. MARABELLI, M. POLIZZI and G. MANGILI, Nature *199*, 195 (1963).
[2] G. FACHINI and G. GIANFRANCESCHI, Experientia *20*, 404 (1964).
[3] G. FACHINI and C. CIACCOLINI, Endokrinologie *50*, 79 (1966).
[4] G. FACHINI, Endokrinologie *50*, 83 (1966).
[5] G. FACHINI, Annls Endocr. *27*, 679 (1966).