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.

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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

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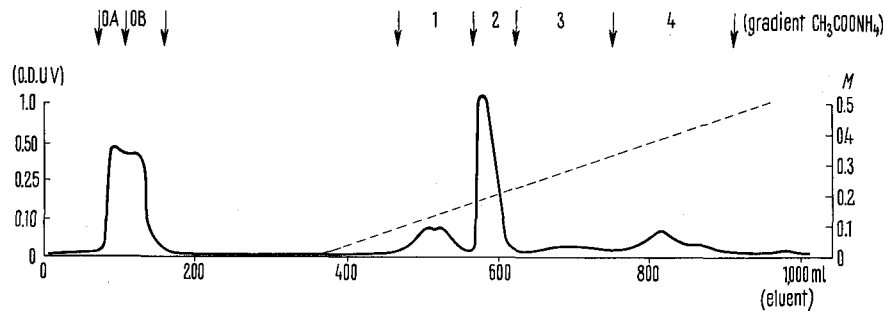

Ion exchange of deproteinized homogenate on DEAE-cellulose, equilibrated with buffer acetic acid-ammonium acetate 0.005 M; pH = 5. Column of 2 × 30 (h) cm; 40 ml of ultrafiltrate diluted to 80 ml with distilled water ($\simeq$ 400 O.D. UV); flow rate 30 ml/h, fractions of 5 ml; linear gradient with ammonium acetate 0.5 M. In ordinate: UV-absorption.

Fraction	Compound administered (μg)	No. of parabiotic rats	Weight of ovaries mg % g body-weight $\pm$ S.E.	Inhibition % (P)
Controls	–	10	103.8 $\pm$ 3.3	–
OA	127	10	84.4 $\pm$ 5.5	19
OB	642	11	82.3 $\pm$ 3.3	21 ($0.001 < P < 0.01$)
1	286	9	98.1 $\pm$ 1.7	5
2	123	10	70.8 $\pm$ 9.1	32 ($0.01 < P < 0.02$)
3	106	10	101.9 $\pm$ 4.7	2
4	131	10	87.4 $\pm$ 3.1	16

Pituitary-inhibiting activity of the fractions obtained by chromatography on DEAE-cellulose, in the celomatic parabiosis test, according to SHIPLEY[7]

were lacking. In all the fractions there were spots with absorption at 254 nm, probably of nucleosidic (OA, OB) and nucleotidic (1, 2, 3 and 4) nature. Since it is eluted in ammonium acetate between 0.19 and 0.22 M, fraction 2 might be rich in dinucleotides.

Dry-weight and phosphorus contents (according to GOODWIN[6]) are: OA, 10.9 mg, 42 μg of phosphorus; OB, 55 mg, 36 μg; 1, 24 mg, 92 μg; 2, 10 mg, 1,875 μg; 3, 9 mg, 938 μg; 4, 11.2 mg, 33 μg.

Inhibition-test has been carried out on the chromatographically isolated fractions, presumably corresponding to enrichment in nucleosides and peptides (OA, OB), mono-(1), di-(2), tri-(3) and tetra-nucleotides (4).

Inhibitory activity, evaluated by celomatic parabiosis method, using immature Sprague Dawley female rats and adult castrate females, according to SHIPLEY[7], seems to be concentrated chiefly in fraction OB and 2 (Table).

In a further group of experiments, the isolated fractions were administrated to the parabionts, at a total dosage of 250 μg of dry substance per pair. Under these conditions, fraction OB showed the strongest activity (–40%), while the activity of fraction 2 practically disappeared, whereas, at a lower dosage, this fraction showed a strong activity.

Since in the 2 groups of experiments there is a remarkable difference in concentration (123–250 μg for fraction 2; 642–250 μg for fraction OB), it seems reasonable to suppose that, in the first case, a direct trophic activity on the target-organ (ovaries) may nullify the indirect inhibition of the trophinic activity at pituitary level. This phenomenon is frequently observed in the regulative action of the pituitary feed-back mechanisms.

It seems more difficult to explain the reason for the inhibitory effect, which seems to increase in the OB fraction, when the concentration is lowered to a third part. We cannot exclude an unmasking of the direct pituitary action when the general trophic activity of the nucleosidic compounds is lowered. A general trophic activity of those substances is already known.

In order to exclude a possible estrogenic activity in the fractions we have tested each fraction with the RUBIN test[8]. All results are entirely negative. The estrogenic total activity in any fraction seems to be lower than 0.03 μg (as estrone) per 250 μg of tested product.

In regard to the androgenic contents, we refer to our previous observation[5] and recall that the concentration of these steroids is very low, and in any case lower than the threshold of the activity on the pituitary gland in the test we use. While the nucleotidic contents of the extracts seems to be essential for biological activity, we cannot exclude that some peptidic molecules may also act as a necessary complement (as a 'tactic catalyst'?). Furthermore, some more recent data, which we have obtained, seem to stress the biological meaning of the late compounds.

Riassunto. L'attività ipofiso-inibitrice, presente negli estratti idrosolubili di strutture gametiche, è apparsa concentrabile in frazioni cromatograficamente caratterizzabili, di probabile natura dinucleotidica. Una minor attività si è riscontrata anche a livello di frazioni peptidiche e nucleosidiche.

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