less sensitive to Ch concentration over the range tested. In denervated muscle, the Ch response is transient, as it is in K-Ringer, but with a faster time course. Its magnitude increases with increasing Ch concentration (Figure 2). Denervation increased the sensitivity of the muscle membrane to choline. It seems likely that this increased sensitivity is ultimately to be explained in terms of changes in the potassium handling machinery in the membrane, in view of the following related phenomena. NICHOLLS[7] demonstrated a fall in total membrane conductance after denervation of muscle, and HARRIS and NICHOLLS[8] found an approximately 20% decrease in potassium uptake of muscle after denervation. HUBBARD[9] has more recently shown a fall in membrane potassium conductance after denervation. Then, in terms of the cholinergic receptor system suggested by PORTELA et al.[3], it is attractive to suppose that denervation somehow affects the molecular conformation of this receptor system present in the muscle membrane, so that its ability to combine with choline, the effect on the potassium handling mechanism of the resulting complex, and the changes of its bioelectric transducer action produced by substituting cesium for potassium are all altered.

Further work to clarify the nature of the striking changes in choline sensitivity in intact muscles from denervated frogs is in progress[10].

Résumé. Dans un muscle non dénervé, la choline provoque une dépolarisation permanente en présence de «Cs-Ringer» et transitoire en présence de «K-Ringer». La dénervation augmente la sensibilité de la membrane musculaire à la choline. Mais dans ce cas, le «Cs-Ringer» donne une réponse transitoire à la choline, semblable à celle obtenue avec le «K-Ringer».

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Effect of Tension Upon Rate of Incorporation of Amino Acids into Proteins of Cross-Striated Muscle

In experimental studies concerning rate of incorporation of amino acids into proteins, generally freely suspended unstretched muscles are used during incubation in the radioactive medium. However, it is well known that heat production[1,2] and oxygen consumption increase in a stretched muscle[3]. Isolated muscles survive longer under the influence of stretch[4] and muscles in organ cultures develop in good condition only when stretched[5]. Thus, stretch applied to the muscle fibre may also be an important factor influencing the rate of incorporation of amino acids into proteins. We have therefore compared rate of incorporation of ^{14}C-leucine into the proteins of 2 muscles, i.e. the levator ani muscle (LA) and the extensor digitorum longus muscle (EDL) of rats using stretched and unstretched preparations. Both muscles are very thin, and therefore well suited for incorporation studies[6,7].

Material and methods. Experiments were performed on (a) the LA muscle of 4-week-old rats and (b) the EDL muscle of-7-days old rats. The muscles were removed with the tendinous insertions; ligatures were applied to both tendinous ends of the muscle with a weight attached to one of the tendons. The resting tension of the muscle giving maximal twitch tension output was determined and the corresponding weight (e.g. 0.2 g for the LA and 0.1 g for the EDL muscle) were used in the experiments. The upper ligature was fixed at the upper circumference of the incubation flask and the muscle, thus vertically stretched, was completely immersed in the incubation medium (Krebs-Ringer bicarbonate buffer, pH 7.4). L-leucine-U-^{14}C was used (0.1 μC/ml, specific activity 32.8 mC/m*M* in the case of EDL muscle and 0.2 μC/ml, specific activity 85.3 mC/m*M* in the case of LA muscle). The muscles were incubated for 2 h (LA) and 90 min (EDL) under continuous shaking at 37 °C. At the end of the experiment, the muscles were homogenized and the proteins precipitated with 5% TCA solution. After removing the nucleic acids and lipids, the precipitated proteins were dried with ether, dissolved in folic acid and number of impulses/min were determined. Number of impulses are referred to mg of noncollageneous proteins determined by the Conway method. In the control experiments, the same muscles immersed freely into the incubation medium were used.

Results and discussion. The Figure shows that incorporation of ^{14}C-leucine into proteins in stretched muscles (LA and EDL) is considerably higher than into the proteins of muscles freely incubated. Expressed in percentage, the incorporation into the stretched muscle increases by 174% in the case of LA muscle and by 50% in the case of EDL muscle. The mechanism of this increase of incorporation is not yet clear. It is possible that the increased oxygen consumption in a stretched muscle might be an important factor. The oxygen consumption in a stretched muscle may increase 2–4 times and is connected with increased ATP hydrolysis and creatine phosphate break-down. With release of stretch, oxygen consumption of the muscle decreases[3]. An auto-

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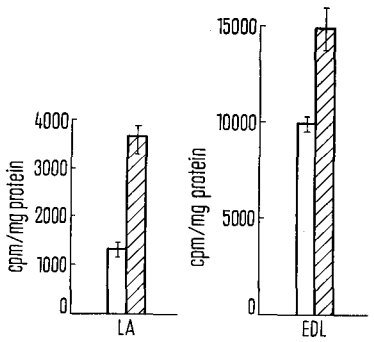
regulative metabolic reaction not dependent on the nervous system to stretch of the myofibrils might be assumed, as the increase of oxygen consumption occurs also in a curarized muscle. However, it must be remembered that a freely immersed muscle coils up and may even go into contracture, and thus the 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.

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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

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