depending on the amount of food intake. This influences in a crucial way the still undifferentiated hypothalamic centers and stabilizes the threshold to peripheral feedback stimuli at a different level. Accordingly, at a prepubertal age (between 10 and 16 years of age) a diet relatively poor in calories but otherwise balanced in all its components might be *one* determinant factor in programming the species-specific life span of humans and prolong it to its maximal expectancy. This kind of intervention will not prevent ageing, but it might well postpone the onset of some of its most typical degenerative phenomena (artheriosclerosis, hypertension, increased genesis of tumors, a.o.) which most probably relate to an imbalanced hormonal status.

Potentials in mammalian skeletal muscle from collagenase-treated tissue

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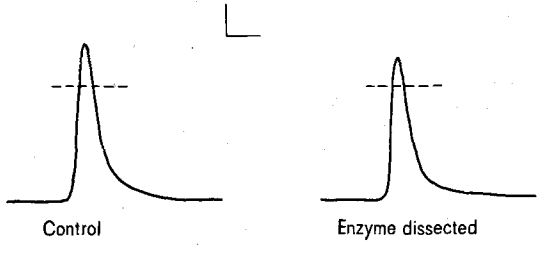
Summary. Transmembrane potentials were recorded from skeletal muscle fibres dissected with the aid of collagenase perfusion. Collagenase treatment had little or no effect on the action or resting potentials.

Because dissection of single skeletal muscle fibres or small bundles of fibres often is difficult due to tough connective tissue, we have looked for a technique that would reduce this component without cellular injury. Collagenases have been shown to digest primarily intercellular proteins and have allowed the isolation of intact smooth muscle[1] and cardiac cells[2]. We have used collagenases to prepare skeletal muscle for homogenization[3], thus we decided to explore the possibilities of enzyme-assisted dissection of mammalian skeletal muscle fibres and bundles. Preliminary experiments demonstrated that simple submersion of the tissue in an enzyme-containing solution was inadequate for disruption. Therefore, a hind-limb perfusion technique similar to the liver perfusion protocol employed in the isolation of liver cells[4] was adopted. The integrity of preparations subjected to this technique was assessed by examining the transmembrane action potential.

Electrical potentials from rat semi-tendinosus muscle

	Normal*	Enzyme-treated*	p
Resting potential	-68.9 ± 1.0 mv (15)	-66.6 ± 1.0 mv (23)	0.09
Overshoot	$+20.4 \pm 2.4$ mv (15)	$+17.6 \pm 1.2$ mv (16)	0.33
Action potential	89.4 ± 2.5 mv (15)	84.1 ± 1.9 mv (16)	0.10

* Indicates that 3 animals were used and numbers in parentheses indicate the number of penetrations recorded. Electrical potentials are the means $\pm$ SE of all the penetrations recorded.



Tracings of action potential recorded from control and enzyme dissected muscle. The dashed line in each represents the isopotential line. Vertical bar is 20 mV. Horizontal bar is 2 msec.

Materials and methods. Sprague-Dawley rats 200–300 g were anaesthetized with ether and a small cannula was introduced into the right femoral artery. After the cannula was tied in place, room temperature (22 °C $\pm$ 2 °C), calcium-free Hank's solution was perfused through the limb by means of a peristaltic pump until the veins were cleared of blood. Then, the femoral vein was severed and the perfusate allowed to escape for a 5-min-period. Subsequently, collagenase (Type I, Sigma Co.) in complete Hank's solution (containing calcium) that had been filtered through Gelman 0.45 µm filtres was perfused, at room temperature, for 5 min. Thereafter, normal Hank's solution without enzyme was perfused for 10 min to remove the collagenase and digestion products. Dissection and removal of small bundles of fibres from the semitendinosus muscle was performed in Hank's solution. Control preparations were dissected from nonperfused, ether anaesthetized animals and placed directly into complete, room temperature, Hank's solution.

For electrical recording, control and test muscle bundle preparations were transferred to a horizontal muscle chamber containing oxygenated Hank's solution. Transmembrane potentials were measured with 3 M KCl filled glass microelectrodes having 5 to 20 MΩ resistance and connected through calomel half cells to a Transidyne MPA-6 preamplifier with stray capacitance neutralization. Stimulation was accomplished with suprathreshold square wave pulses of 0.2 to 0.4 msec duration applied through platinum electrodes. A floating microelectrode arrangement was utilized to minimize mechanical artifacts. Signals were displayed on a Tektronic Type 503 oscilloscope equipped for Polaroid photography.

Results. When perfused with enzyme-containing solution, hind-limb muscles gradually swelled to nearly twice their initial diameter. Under direct microscopic view, it was apparent that muscle fibre bundles were well dispersed and that they responded to electrical stimulation with vigorous contractions. Subsequently, minimal mechanical dissection was necessary to obtain small bundles of muscle fibres that were less readily obtained from un-

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treated preparations. However, because some of the supporting structure had been removed by enzyme treatment, these bundles were easily damaged.

Results of the microelectrode studies are presented in the table and the figure. Although a slight reduction was noted in the resting potential with the enzymetreated muscles, the action potential overshoot of the isoelectric line and the action potential amplitude were not significantly ($p < 0.05$) different from the untreated preparations.

Discussion. Proteolytic enzymes such as trypsin have been shown to have deleterious effects on skeletal muscle transmembrane potentials[5]. Collagenases are more specific in cleaving primarily intercellular proteins and have been used to recover viable cells from a variety of tissues[1,2,6]. In the experiments reported here, we applied collagenase to rat skeletal muscle using a hind-limp perfusion technique to facilitate enzyme distribution and subsequent fibre bundle dispersion. The results indicate that collagenase pretreatment has only a slight, or negligible, effect on the cellular resting potential and action potential magnitudes. Action potential durations were observed in some preparations to become prolonged. However, this occurred only when the action potential and resting potential amplitudes began to decay. We feel this technique offers a simple adjunct to mammalian skeletal muscle dissection, and thus may be particularly useful in studies of electrophysical and thermomechanical phenomena.

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The effect of dehydration on the neurohypophyseal blood flow in rats with hereditary diabetes insipidus[1]

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Summary. Neurohypophyseal blood flow increases in water-deprived rats. This increase is independent of vasopressin release, since it occurs even in rats with hereditary defect of hypothalamic vasopressin synthesis.

Using ^{125}I-antipyrin, Lichardus et al.[4] found an increase of neurohypophyseal blood flow in rats after water deprivation, and they concluded that it accompanied the release of vasopressin (VP). Earlier it was reported that any stimulation of neurohypophyseal hormones release causes a vasodilatation in posterior pituitary[5]. Furthermore, water deprivation causes the release, from the neurohypophysis, of both oxytocin and VP[6]. An attempt is presented to ascertain whether neurohypophyseal blood flow increases after water deprivation even in rats which are unable to synthetize VP, i.e. in homozygous Brattleboro rats with hereditary diabetes insipidus[7].

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Body weight, adenohypophyseal and neurohypophyseal weight, flow fraction of cardiac output and ^{86}Rb/g-uptake in control (C) and water deprived (WD) Wistar, heterozygous (non-DI) and homozygous (DI) Brattleboro male rats

	Wistar		Non-DI		DI	
	C	WD	C	WD	C	WD
Body weight (g)	204.4 ± 5.56 (9)	187.5[a] ± 2.50 (8)	247.1 ± 7.47 (7)	216.3[a] ± 6.25 (8)	190.7 ± 7.35 (7)	160.6[a] ± 5.21 (8)
Adenohypophysis						
Weight (mg/100 g b.wt)	–	–	1.98 ± 0.061	1.93 ± 0.071	2.05 ± 0.061	2.29[c] ± 0.108
Flow fraction of cardiac output ($\% \times 10^{-3}$)	–	–	2.49 ± 0.257	2.31 ± 0.206	2.74 ± 0.229	3.04[c] ± 0.175
^{86}Rb-uptake (%/g)	–	–	0.62 ± 0.056	0.60 ± 0.046	0.67 ± 0.048	0.67 ± 0.046
Neurohypophysis						
Weight (mg/100 g b.wt)	0.52 ± 0.026	0.55 ± 0.027	0.43[b] ± 0.027	0.48 ± 0.022	0.68[bc] ± 0.034	0.81[abc] ± 0.016
Flow fraction of cardiac output ($\% \times 10^{-3}$)	4.09 ± 0.268	5.04[a] ± 0.352	3.90 ± 0.350	4.71 ± 0.212	4.92[bc] ± 0.256	7.53[abc] ± 0.657
^{86}Rb-uptake (%/g)	3.94 ± 0.243	4.58[a] ± 0.167	4.55 ± 0.354	4.90 ± 0.210	3.67 ± 0.229	4.68[a] ± 0.390

Data are given as mean ± SEM ( ) indicates number of animals.
[a] significantly different from control animals ($p<0.05$); [b] significantly different from Wistar rats ($p<0.05$); [c] significantly different from non-DI rats ($p<0.05$).