Table II. Differential influence of La^{3+} on end-plate activation and desensitization produced by 0.14 m*M* CARB

La^{3+} (m*M*)	Maximum change in EMR (%)	Half-time of EMR recovery (sec)	Number of fibres
–	39.4 ± 3.0[a]	35.0 ± 5.5[a]	5
0.01	60.6 ± 2.3	75.6 ± 28.2	6,5[b]
1.0	58.0 ± 2.1	14.0 ± 1.9	6

[a] Values are mean ± S.E.; [b] The maximum change in EMR is from 6 fibres and the half-time of EMR recovery is from 5 fibres. The impalement was lost in 1 fibre before desensitization was complete. The Table shows a wide range of standard errors for the half-time of EMR recovery. However the variances were found to be equal by an F-test. With the exception that this may reflect an association of a smaller variance with a smaller mean, no clear explanation of the discrepancy between variances can be offered[19].

that the end-plate response to 0.27 m*M* CARB of muscles equilibrated in 0.1 m*M* DTE decreased with time but appeared to plateau at approximately $1^1/_2$–2 h. Therefore, all subsequent experiments were conducted on muscles kept in 0.1 m*M* DTE for approximately 2 h prior to the determination of CARB activation and desensitization. The CARB used for microperfusion was always dissolved in the same solution which bathed the muscle. Hence, DTE was present in the CARB microperfusate when DTE was present in the bathing solution.

The control or resting EMR of the DTE treated fibres (0.167 ± 0.077 megohms, mean ± S.E.) was not significantly different ($p > 0.05$, *t*-test) from the control EMR of the untreated fibres (0.170 ± 0.138 megohms, mean ± S.E.). These values are lower than those reported for polarized muscle fibres[16]. This difference is attributed to the persistent membrane depolarization produced by potassium.

The inhibition of CARB activation by DTE is evident from the Figure. Record A illustrates the time course of the change in EMR induced by continuous microperfusion of 0.27 m*M* CARB. Record B was obtained from another fibre following 0.1 m*M* DTE treatment. In both instances the EMR recovered after an initial decline indicating PJM desensitization during the sustained CARB perfusion. Although CARB activation was reduced by DTE exposure, the rate of PJM desensitization was significantly greater. The results obtained from numerous fibres are summarized in Table I.

In the presence of 0.01 m*M* La^{3+} the amount of inhibition produced by 0.1 m*M* DTE was lessened. In these experiments the La^{3+} was microperfused along with CARB (no preparations were pretreated with La^{3+} prior to CARB application). Previous studies[11] indicate that no significant change in EMR (recorded from the PJM) results from the perfusion of 0.01 m*M* La^{3+} in the absence of CARB. It was assumed that similar conditions obtained in the present study. Although La^{3+} increased the amount of PJM activation in the DTE treated muscles, the rate of PJM desensitization was not changed (Figure, Table I). These data are consistent with the view that the cholinergic receptor is a protein containing a disulfide bond[1–7] and may explain the progressive loss of response seen in earlier studies with chick muscle[6] and electroplax cells[2].

Earlier studies have shown that La^{3+} not only increases end-plate activation but also accelerates the rate of PJM desensitization produced by CARB[11,17]. However, these two effects of La^{3+} are differentially concentration dependent. In the depolarized preparation, 0.01 m*M* La^{3+} enhances receptor activation[11] without significantly increasing the rate of desensitization induced by CARB (0.054–21.6 m*M*)[18]. The desensitizing action of La^{3+} becomes apparent only at higher concentrations. For example, in the presence of 0.01 m*M* La^{3+} PJM activation with 0.14 m*M* CARB was approximately doubled and desensitization slowed, but not significantly ($p > 0.05$, *t*-test), whereas with 1.0 m*M* La^{3+} both the extent of activation and the rate of desensitization were increased (Table II). These observations suggest that receptor activation and desensitization can be separated pharmacologically and perhaps that La^{3+} has two distinct sites of action[20].

Zusammenfassung. Der Einfluss von Dithioerythritol als Reduktionsmittel und von Lanthan auf die durch Carbamylcholin induzierten Phänomene am Endplattenrezeptor in mit Kalium depolarisierten Muskelfasern wurde unteruscht. Dithioreythritol hemmte die Endplattenrezeptoraktivierung und beschleunigte die Desensibilisierungsgeschwindigkeit während 0.01 m*M* Lanthan die Endplattenaktivierung verstärkte, ohne aber einen Einfluss auf die Geschwindigkeit der Desensibilisierung mit oder ohne Dithioerythritol zu haben. 1.0 m*M* Lanthan hingegen erhöhte sowohl die Geschwindgkeit der Desensibilisierung als auch diejenige der Aktivierung bei Abwesenheit von Dithioerythritol, was den Schluss auf zwei verschiedene Wirkungsorte von Lanthan zulässt.

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The Influence of Magnesium and Calcium Ions on the Force Produced in Rigor Muscle

Removal of ATP from striated muscle fibres induces (under isometric conditions) a rigor contraction[1]. In the subsequent rigor state isometric tension is maintained and the crossbridges (which comprise part of the myosin filament) are fixed to the actin filament in the 'arrowhead' position[2]. In this position the crossbridge heads are at an angle of 45° to the actin filament (Figure 1). It is concluded from recent results that the crossbridges are elastic

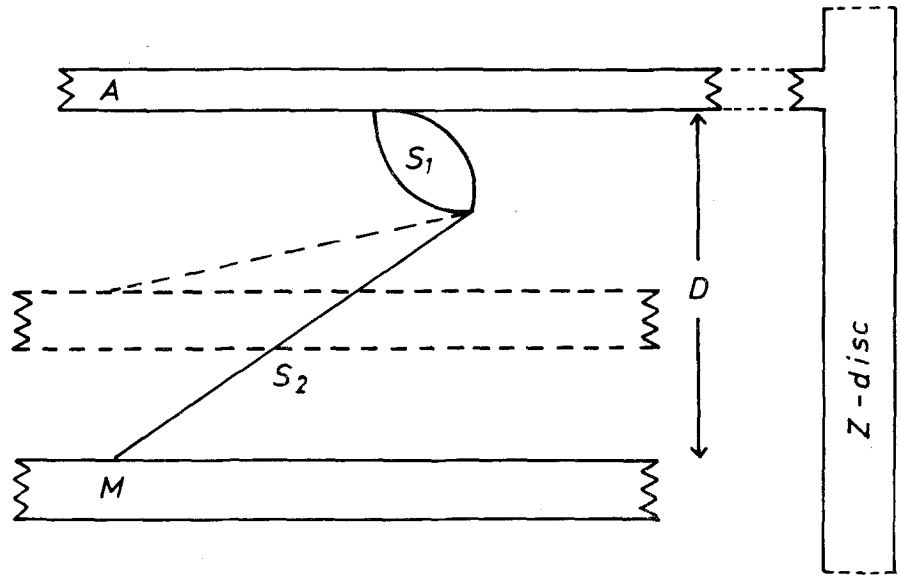

Fig. 1. Diagramm showing a crossbridge fixed to the actin filament in rigor in the 'arrowhead' position (modified from HUXLEY[9]). The relative force between actin and myosin filaments produced by the elastic structure of the crossbridge may decrease by decreasing the interfilamentar distance D. Note the different length of the crossbridge – neck (S_2) at 2 different interfilamentar distances (continous and dotted line). M, myosin filament (backbone); A, actin filament; Z, Z-disc; S_1, (HMM S_1) crossbridge head; S_2 (HMM S_2) crossbridge neck; D, distance between actin and myosin filaments.

structures[3]. Therefore, in rigor, the elastic force developed by the crossbridges and also the fibre tension should depend on the spacing of actin and myosin filaments. As this spacing in rigor is varied by the pH, ionic strength and the concentration of divalent cations (Mg, Ca, Ni, Mn)[4,5] we have investigated whether these influences affect the isometric rigor tension.

Methods. Skeletal muscles [leg muscle of the cayman (*Caiman crocodilus*) and dorsal longitudinal flight muscle (DLM) from waterbugs (*Lethocerus maximus*)] immediately after preparation, were transferred into a 50/50/v/v glycerol-water mixture (20 m*M* imidazole, 10 m*M* Na–azide, pH 7.0) and stored for up to 4 weeks at –16 °C. (Na-azide was given to inhibit the mitochondrial ATPase[6]).

Fibre bundles (length 2–3 mm, diameter 150–400 µm) were dissected from the glycerol extracted muscle. The experiments were carried out on an apparatus providing highly isometric conditions[7]. The fibre bundles were glued (celluloid dissolved in acetone) at one end to a glass rod and at the other to a glass rod connected to a 5734 RCA-force transducer (compliance approximately 5×10^{-6} cm/dyne). The isometric tension was recorded on a 'Schwar-

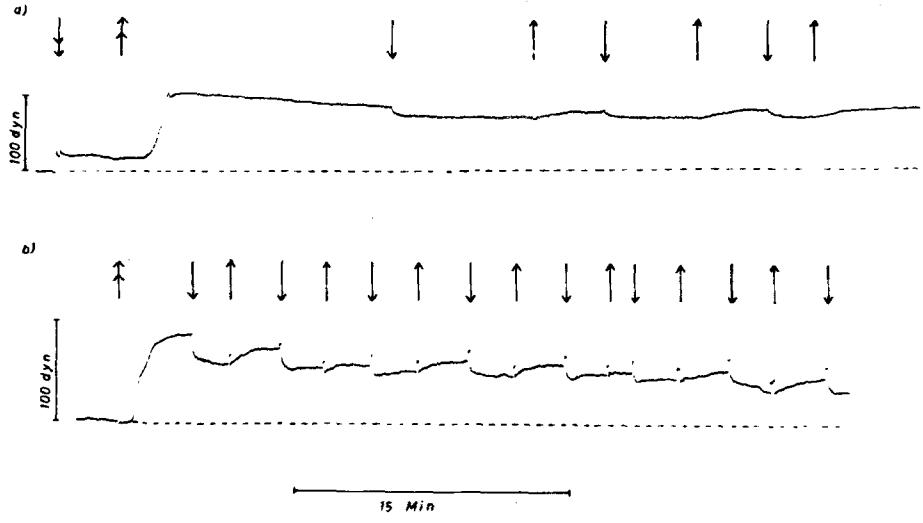

Fig. 2. Magnesium induced tension decrease in rigor, a) of a fibre bundle (diameter ca. 375 µm) from skeleta muscle (leg muscle, *Caiman crocodilus*) and b) a fibre bundle (5 fibres) from insect fibrillar flight muscle (DLM, *Lethocerus maximus*). The fibre bundles were suspended in Mg-ATP-relaxing solution and slightly stretched (↓). After immersion in ATP-free standard rigor solution (↕) isometric tension is produced. Immersion in 30 m*M* Mg-rigor solution (↓) induces a tension fall. Control experiment: After reimmersion in standard rigor solution (↑) tension increases to the original tension level. Cycles like this could be repeated many times.

zer-Direktschreiber'. The ATP-relaxing solution contained (in mM): ATP, 17.5; $MgCl_2$, 17.5; imidazole, 10; EGTA, 4; Na-azide, 4. The (ATP-free) standard rigor solution contained (in mM): KCl, 100; imidazole, 10; EGTA, 4; Na-azide, 4. The other rigor solutions contained (in mM): KCl, 10; $MgCl_2$, 30 (or $CaCl_2$, $BaCl_2$, $SrCl_2$, K_2SO_4 or $KHPO_4$); imidarole, 10; EGTA, 4; Na-Azide, 4. Rigor solutions with lower concentrations of divalent ions (5–30 mM) were obtained by mixing these solutions in the appropriate relation. The pH was adjusted at room temperature to 6.5 by addition of HCl or NaOH. The experiments were carried out at room temperature (ca. 20 °C).

Results. In order to produce rigor tension skeletal muscle fibre bundles relaxed in the ATP-relaxing solution were incubated in the ATP-free standard rigor solution[1]. Herein the isometric tension rose (Figure 2a) to about 0.9 kg/cm^2 (standard deviation 0.3 kg/cm^2, $n = 10$). The following immersion in a Mg-containing rigor solution produced a tension fall to about 85% of the tension level in standard rigor solution (Figure 2a, Table). The 100% tension level was reached again after reimmersion in standard rigor solution. Such tension cycles could be repeated up to 20 times without any notable decrease in the extent. In experiments with glycerol-extracted rabbit psoas fibres (unpublished experiments) and DLM-fibres (Figure 2b) similar findings were obtained. Rigor tension was also produced by transferring the fibres from ATP-relaxing solution directly into Mg-containing rigor solution. The subsequent immersion in standard rigor solution induced a further tension increase of about 15% to the 100% level followed by a tension decrease in Mg-rigor solution.

The tension decreased by raising the Mg^{++} concentration from 5–30 mM. Reversible tension cycles were also induced by other divalent cations (Table): at a concentration of 30 mM the tension fall relative to the 100% level, was similar in Mg-, Ca-, Ba- and Sr-containing rigor solutions. The isometric rigor tension was reversibly decreased by lowering the pH of the standard rigor solution (Table). The reverse effect was obtained in rigor solutions containing SO_4 and by raising the pH (Table), whereas rigor solutions with PO_4^{--} did not seem to affect the isometric tension.

Discussion. Provided that the crossbridges do not rotate on the actin filament while in rigor[8] the elastic force between actin and myosin filaments produced by the crossbridges, and therefore the rigor tension, should increase with wider spacing of actin and myosin filaments.

We assume that this consequence of the crossbridge models (Figure 1)[3,9] is verified by the present experiments, as raising the pH not only increases the rigor tension, but also the interfilamentar distances, to about the same extent of 10%[4,5], whereas the reverse effect on isometric tension and spacing of actin and myosin filaments is induced by divalent cations or lowering the pH. According to ROME[4,5] the variation of the interfilamentar distance might be produced by electrostatic effects between the filaments. Apart from the interpretation discussed, the correlation between rigor tension and myofilamentar distances might also be explained on the basis of the electrostatic model proposed by ELLIOT et al.[10] or KOMINZ's[11] osmotic theory.

Zusammenfassung. Die Rigorspannung glycerinextrahierter Skeletmuskelfasern (*Caiman crocodilus*) und fibrillärer Insektenflugmuskelfasern (DLM) (*L. maximus*) konnte durch Zugabe von zweiwertigen Kationen und pH-Erniedrigung reversibel erniedrigt werden sowie durch pH-Erhöhung und Zugabe von Sulfationen erhöht werden. Da unter ähnlichen Bedingungen im Rigor Veränderungen des Abstandes von Aktin- und Myosinfilamenten nachgewiesen wurden[4,5], wurden die beobachteten Spannungsänderungen im Rigor auf Grund neuerer Crossbridgemodelle[3,9] diskutiert.

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Effect of divalent ions and pH on the isometric rigor tension; [glycerol-extracted fibre bundles from skeletal muscle (*Caiman crocodilus*)]

Solution	pH	Tension[a]	n
10 mM KCl + 30 mM $MgCl_2$	6.5	80.6 ± 10.2	66
10 mM KCl + 30 mM $CaCl_2$	6.5	82.6 ± 11.3	16
10 mM KCl + 30 mM $BaCl_2$	6.5	79.2 ± 15.1	5
10 mM KCl + 30 mM $SrCl_2$	6.5	77.0 ± 13.2	5
10 mM KCl + 30 mM K_2SO_4	6.5	118.2 ± 10.2	10
100 mM KCl	6.0	87.9 ± 5.3	5
100 mM KCl	7.0	112.8 ± 6.3	5

[a] Tensions (mean values and standard deviations from the mean) given in percentage of the values obtained in standard rigor solution (100 mM KCl, pH 6.5, 20 °C). All solutions containend 10 mM imidazole, 4mM Na-azide and 4mM EGTA.

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Overload and Training, Compensatory Hypertrophy and Contraction Characteristics of M. plantaris in Female and Male Rats

In a series of experiments concerning the effect of overload and training on the fast and pennate-fibred M. plantaris (MP)[1–3] we also compared the effect in male and female rats. A recent report on compensatory hypertrophy (CH) of leg muscles in the rat of both sexes[4] produced findings essentially similar to ours, i.e., that there are no differences between the sexes. These authors said: 'This finding is not really surprising, if one considers that