preliminary dilution plate counts, high soil populations of *Chrysosporium* spp. were not recovered when nutritive agar contained 5 g/l of neutralized CHA ($\approx 5 \cdot 10^{-2}$ mole/l), whereas *Aspergillus, Fusarium, Penicillium* spp. as well as members of the Mucoraceae, and a grey sterile fungus, were isolated frequently when the CHA concentration was 20 g/l ($= 2 \cdot 10^{-1}$ mole/l). At intermediate concentrations, neutralized CHA eliminated species of *Acremonium, Gliocladium, Myrothecium, Paecilomyces,* and *Trichoderma.* It would also be interesting to know whether different strains of fungal species exhibit marked differences in their sensitivity to the aromatic amines examined in this study.

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Low temperature-induced contracture of depolarized smooth muscle and the effects of calcium and multivalent cations[1]

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Summary. Lowering of temperature caused tension development in the smooth muscle of the guinea-pig vas deferens, which was dependent on extracellular Ca. Mn^{2+} and La^{3+} reversed the effect and induced phasic contraction on rewarming.

It has been reported in skeletal and smooth muscle that rapid cooling caused contracture due to the release of intracellular Ca^{2}[2,3]. The contracture is also observed in depolarized preparations in which a change in membrane potential by cooling can be excluded. However, in the depolarized smooth muscle, the final effect of cooling varies with the type of smooth muscle. In the smooth muscle of *Taenia coli* and longitudinal muscle of the stomach of the guinea-pig, relaxation of depolarized preparations has been reported[4,5]. On the other hand, the smooth muscle of the urinary bladder and circular muscle of the stomach show a contractile response to lowering of temperature[5,6].

We have also observed that the smooth muscle of the guinea-pig vas deferens shows an increase in tension when cooling treatment is applied in the course of the tonic phase of contracture induced by high potassium concentration[1,7]. The present experiments were performed to investigate the effects of Ca and multivalent cations on the cooling-induced contracture of depolarized smooth muscle of the guinea-pig vas deferens.

Vasa deferentia were dissected from the abdomen and longitudinal preparations were made using the prostatic one-third of the vasa. The preparations were mounted in an organ bath filled with modified Tyrode solution of the following composition: NaCl, 137 mM; KCl, 2.7 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM, $NaHCO_3$, 11.9 mM; NaH_2PO_4; 0.4 mM; glucose, 5.6 mM; equilibrated with a gas mixture of 95% O_2 and 5% CO_2. The depolarizing solution (K-Tyrode solution) was made by replacing all NaCl in the solution with KCl. When Mn^{2+} and La^{3+} were used, Tris-buffer was used instead of bicarbonate buffer and the solution was gassed with 100% O_2. The change in pH due to the temperature alteration was not corrected, since a similar change in pH had no significant effect on the tonic contraction. In addition, no obvious difference in the tension development by cooling was observed between bicarbonate buffer and Tris-buffer, which is known to be more affected by the change in temperature than the former.

The developed tension was observed isometrically by a mechano-electronic transducer. The changes in membrane potential and membrane resistance were studied by the double sucrose-gap method[8]. The temperature was controlled by changing the temperature of the water surrounding the organ bath or the tube of test solution in the double sucrose-gap apparatus.

High-K induced contracture of the vas deferens was composed of a transient phasic contraction and a tonic contraction which was sustained for longer than 1 h without obvious change in tension. When the cooling treatment was applied during the course of the tonic phase, an increase in tension was observed; the membrane showed a slight further depolarization and the membrane resistance increased. By rewarming the preparation, the membrane potential and resistance returned to the initial level and the developed tension returned to the level of the tonic contraction. Although tension development by cooling was not observed in normal Tyrode solution (in contrast to other smooth muscles), this may be due to the difference in the speed and/or degree of cooling.

Since the highest tension development was obtained by cooling the preparations from 35 °C to 20 °C, most of the experiments were performed by lowering the temperature to 20 °C. At this temperature, the maximum tension was $179 \pm 10.6\%$ (mean $\pm$ SE, $n = 5$) of the control tonic contraction at 35 °C.

The tonic tension disappeared and no tension development

was induced by cooling when Ca in the solution was removed. Similar results were obtained by the addition of verapamil (5×10^{-6} M) in the presence of Ca ions.

On the other hand, elevation of the Ca concentration caused an increase in cooling-induced tension development. The maximum tension obtained by cooling in the presence of 10 mM Ca was $389 \pm 15.6\%$ ($n = 5$) of that of the tonic contraction observed under normal conditions (2 mM Ca, 35 °C). Although the height of the tonic contraction at 35 °C was increased by elevation of the Ca concentration, the maximum tension in the presence of 10 mM Ca was only $131 \pm 6.1\%$ ($n = 5$) of that of the control (fig. 1). These results indicate that extracellular Ca plays an important role in the initiation of tension development by cooling.

Mn^{2+} and La^{3+} have been shown to block Ca fluxes in smooth muscle[9-11]. We therefore studied the effects of these ions on the cooling-induced contracture. The application of either ion during the course of the tonic phase of high-K induced contracture caused a decrease in tension. This, however, was followed by a re-elevation of tension when the treatment was prolonged. Cooling in the presence of either ion caused a relaxation, in contrast to the contractile effect in the absence of these ions. When the preparations were rewarmed from 15 °C to 35 °C in the presence of either ion, a phasic contraction of similar amplitude to that of the high-K induced contracture appeared (fig. 2). The phasic contraction after rewarming, however, cannot be explained by the change in Ca movement through the cell membrane. These effects of Mn^{2+} and La^{3+} were also observed in preparations which had been soaked in Ca-free solution or in those which had been treated with caffeine in Ca-free solution with the aim of eliminating Ca from the preparations[12].

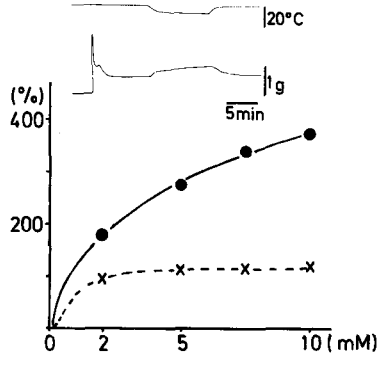

Figure 1. The relationship between calcium concentration and tension induced by cooling. The tension of tonic contraction at 35 °C in the presence of 2 mM Ca was taken as 100% and maximum tensions at 20 °C with various concentrations of Ca were expressed as percentages of this value. ●—●, maximum tension at 20 °C; ×---×, tonic tension at 35 °C. The inserted figure shows the effect of cooling to 20 °C applied during the course of the tonic phase of high K-induced contracture in the presence of 2 mM of Ca.

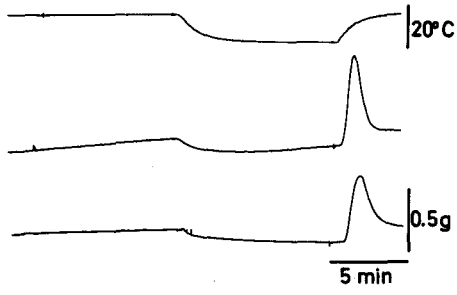

Figure 2. Effects of cooling on the depolarized vas deferens in the presence of lanthanum. This record was taken after the 5th repetition of the cooling treatment in the presence of La^{3+}, so that the basic tension increased. Top, temperature; middle, tension recorded in the presence of La^{3+} and Ca; bottom, tension recorded in the presence of La^{3+} and absence of Ca. Compare with the insert in figure 1.

These results indicate that the tension development on cooling is controled by extracellular Ca. However, since the membrane resistance increased, the tension development might not be due to an increase in Ca influx but rather to a decrease in Ca extrusion through the cell membrane. Reports that the Ca efflux of smooth muscle decreases[13,14] and that Ca content increases[13,15] at low temperature would support this interpretation.

The elevation of basic tension in the presence of Mn^{2+} or La^{3+} may be explained by the release of intracellularly bound Ca under conditions in which the influx and efflux of Ca is inhibited by these ions[9-12]. However, it seems difficult to explain the relaxation by cooling, since this treatment has been thought to cause the release of Ca or inhibition of Ca uptake by the sarcoplasmic reticulum[2]. In addition, the effects of cooling and rewarming could also be observed in the preparation from which Ca had been eliminated. An alternative explanation is that, when it is in a depolarized state, Mn^{2+} or La^{3+} can penetrate the cell membrane[16] and initiate the contractile reaction of muscle protein which is dependent on temperature. The relaxing effect observed on cooling the preparation in the presence of Mn^{2+} or La^{3+} cannot be explained by their inhibitory action on Ca translocations through the plasma membrane of the cells since it was also observed in the absence of extracellular Ca. We have no explanation for this effect at the present time. The phasic contractions observed on rewarming in the presence of Mn^{2+} or La^{3+} might be due to release of intracellularly bound Ca^{9-12}. Alternatively, Mn^{2+} or La^{3+} might penetrate through the depolarized cell membrane and initiate a temperature-dependent activation of the contractile proteins.

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