and the myosin heads. Tropomysin does not bind to myosin in the absence of actin (*15*), however; hence there is no reason to expect tropomyosin to interact directly with myosin heads in the presence of actin.

In conclusion, evidence that is incompatible with a simple steric blocking model of muscle regulation has been presented. It appears that the mechanism of inhibition of the Mg-ATPase during relaxation of skeletal muscle (and perhaps other actomyosin contractile systems as well) cannot be accurately portrayed merely as the movement of tropomyosin from a contraction and rigor position to a relaxation position in which it physically blocks the myosin from interacting with F-actin by covering F-actin's binding site for myosin. Consideration must also be given to the compelling evidence that changes do occur in the F-actin moiety.

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16. I thank Dr. D. R. Kominz who made the initial observations that prompted this study and who provided me with encouragement and laboratory space. I also thank Dr. S. Mulhern for preparing the NEM-HMM and Dr. E. Eisenberg for supplying most of the other proteins and for helpful discussions during the course of this work.

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Fertilization of Sea Urchins Needs Magnesium Ions in Seawater

Abstract. *When sea urchin eggs were inseminated in seawater free of magnesium, the fertilization rate was very low. Spermatozoa that had been treated with egg jelly to induce the acrosome reaction also failed to fertilize eggs in seawater free of magnesium. These results indicate that magnesium is indispensable for some process or processes at fertilization, such as membrane fusion or sperm penetration.*

Seawater contains calcium and magnesium as the main divalent cations. It has been shown that calcium ions are required for inducing the acrosome reaction, that is, the formation of a process from the apical part of the spermatozoon on contact with egg jelly in marine invertebrates, and is thus indispensable for their fertilization (*1*). Little attention has been paid, however, to the significance of magnesium ions in fertilization. Below, we present evidence for the necessity of magnesium ions for fertilization in sea urchins.

Gametes of the sea urchins *Hemicentrotus pulcherrimus, Strongylocentrotus intermedius*, and *Anthocidaris crassispina* were used as materials.

Eggs of *H. pulcherrimus* were washed four times with artificial seawater free of calcium and magnesium (Ca, Mg-free ASW) (*2*). The eggs were then transferred to artificial seawater (ASW) (*2*) containing different amounts of calcium and magnesium. Insemination was made by mixing each of such egg suspensions (50 ml) with a sperm suspension diluted with Ca, Mg-free ASW (0.1 ml), to give a final dilution of 1 : 50,000 of the original semen. After insemination, the suspensions of egg and sperm were stirred for 15 minutes. The spermatozoa were then killed according to either the method of Rothschild (*3*) or that of Hagström (*4*), with some modifications. The eggs were transferred to normal seawater and the percentage of formation of fertilization membrane was counted. Several hours later, the percentage of dividing cells was also counted. The two sets of values coincided well with each other.

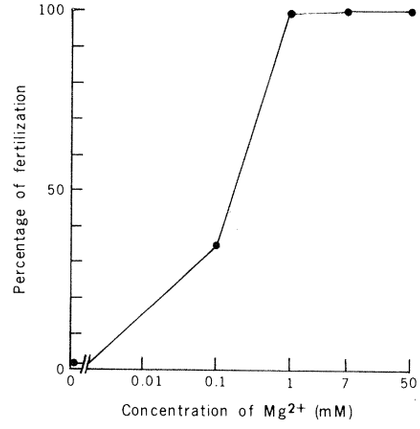

Fig. 1. Fertilization rate of *H. pulcherrimus* as a function of magnesium ion concentration in the presence of 10 m*M* calcium. The jelly layer of the eggs was removed by acidic seawater (*p*H 5) before washing with Ca, Mg-free ASW.

As shown in Fig. 1, the fertilization rate decreased markedly as the magnesium concentration of the ASW was reduced, even though 10 m*M* calcium chloride was included. When the eggs were suspended in ASW containing 10 m*M* calcium chloride and 50 m*M* magnesium chloride, all of them were fertilized. Similar results were obtained with *A. crassispina*.

When the concentration of inseminating spermatozoa was increased considerably (up to a final dilution of 1 : 5000), the fertilization percentage reached almost 100 percent in magnesium-free ASW that contained 10 m*M* calcium chloride. However, if the spermatozoa were washed once with Ca, Mg-free ASW prior to insemination, the fertilization rate became very low (less than 9 percent). In normal ASW, the washed spermatozoa could fertilize almost 100 percent of the eggs. These data are interpreted as suggesting that the high fertilization rates obtained with high sperm concentrations were due to magnesium ions either contained in the seminal plasma or associated with the sperm surface.

These results indicate that magnesium ions are also required, in addition to calcium ions, for fertilization. However, Loeb (*5*) reported that sea urchin eggs were fertilized in magnesium-free ASW that contained calcium, in contrast to our

results. It may be that the washing of the eggs and the dilution of semen in Loeb's experiments were not sufficient to remove most of the magnesium ions.

We also checked the effect of removal of magnesium ions on sperm motility. Semen was diluted to 1 : 50,000 with ASW containing various concentrations of calcium and magnesium. The swimming speed was estimated by measuring the tracks made by the spermatozoa on dark-field micrographs. The results revealed that there is no significant difference in speed among the different sperm suspensions. In other words, the reduction in the fertilization rate observed above is not attributable to any adverse effects of the absence of magnesium ions on sperm motility.

Takahashi and Sugiyama (*6*) showed that, when sea urchin spermatozoa were treated with dissolved egg jelly, their acrosome reaction was induced and these reacted spermatozoa could fertilize the eggs even in the virtual absence of calcium if insemination was carried out within 30 seconds after the treatment with egg jelly. Their results indicated that calcium ions in seawater are not necessary for the processes following the acrosome reaction. Therefore, as the next step, we examined whether or not magnesium ions are needed for the subsequent processes at fertilization.

For this purpose, eggs of *S. intermedius* were washed and suspended as described above. Spermatozoa were treated with dissolved egg jelly to induce the acrosome reaction as described by Dan *et al.* (*7*). Insemination was accomplished by mixing the egg suspensions with the jelly-treated sperm suspension within 30 seconds after the spermatozoa were exposed to the jelly solution. The fertilization rate was determined as described above.

The results revealed that, in the calcium-free ASW that contained 50 m*M* magnesium chloride, the fertilization rate was 100 percent, whereas when the magnesium chloride concentration was reduced to less than 10 m*M*, almost no fertilization occurred (Fig. 2). The same was true in *H. pulcherrimus* and *A. crassispina*.

It appears to be paradoxical that more magnesium ions were needed to cause fertilization with the acrosome-reacted spermatozoa treated with jelly than with the normal spermatozoa, because the treated spermatozoa conducted only a part of the whole fertilization process. In this respect, we found that the spermatozoa treated with jelly showed higher fertilizability when incubated prior to insemination in calcium-free ASW that contained 50 m*M* magnesium chloride, as compared with those incubated in calcium-free ASW that contained 1 m*M* magnesium chloride. The result implies that the presence of a high concentration of magnesium ions is favorable to preserving the structure and function of the reacted acrosome. In fact, the reacted spermatozoa lose their fertilizability rapidly, and do not seem to be equivalent to the nonreacted ones.

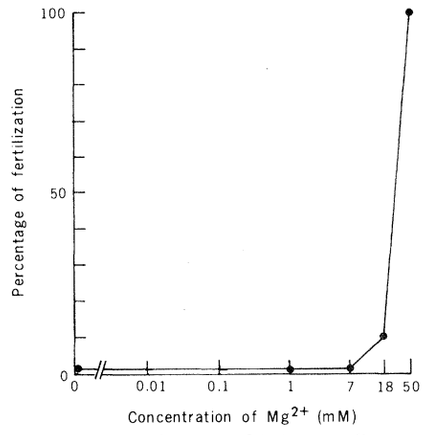

Fig. 2. Fertilization rate of *S. intermedius* as a function of magnesium ion concentration in the absence of calcium. The spermatozoa had been treated with dissolved egg jelly to induce acrosome reaction immediately before insemination.

In addition, we found that treatment with butyric acid of unfertilized eggs, washed four times with Ca, Mg-free ASW, caused the formation of fertilization membrane in the absence of calcium or magnesium ions, or both. Therefore, it is conceivable that the membrane formation itself does not require these divalent cations, the exogenous ones at least. This result is consistent with those obtained by Steinhardt and Epel (*8*) with the artificial activator A23187.

From these results we suggest that magnesium ions in seawater are indispensable for fertilization of sea urchins, probably at some step or steps between the acrosome reaction and the formation of fertilization membrane, such as fusion between sperm acrosomal membrane and egg plasma membrane or penetration of the spermatozoon into the egg, including membrane lysin and the like.

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Bone Cells in Culture: Morphologic Transformation by Hormones

Abstract. *Hormones and purine nucleosides and nucleotides induced cultured bone cells to transform transiently from a spherical to a stellate shape. Cytochalasin B also induced the transformation. The change was blocked by colchicine and vinblastine, but not by lumicolchicine or cycloheximide. This morphologic transformation may provide a dynamic model of hormone action and bone cell modulation in vitro.*

We report here that bone cells in culture undergo spontaneous and hormone-induced transient morphologic transformation from spherical to stellate shape.

Bone cells were isolated from fetal Holtzman rat calvariae (21 days gestation) and cultured in Falcon tissue culture flasks (3×10^5 cells added to each flask; growth area, 25 cm^2) by modification of the method of Peck *et al.* (*1*) and were observed intermittently for 12-hour periods through an inverted phase-contrast microscope. The bone cells in culture reached monolayer confluence in 7 to 10 days and appeared spherical in shape.

A low level of transient morphologic transformation accompanied cell maturation in culture. As they reached their 7th day in monolayer, cells became stellate in sporadic areas of some flasks and formed circumscribed, lacelike networks