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## ELECTRON MICROSCOPY OF ACTOMYOSIN

by

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The macroscopic appearance of the contractile muscle proteins depends on pH, ionic strength, and ATP content. It is possible to show that the electron microscopic appearance of these proteins depends also on the pH and on the presence or absence of ATP if in the buffered system the salt concentration is kept at such a low level that the effect of drying is minimized.

No pH effect was found with F-actin under the conditions of these experiments. Actin solutions reveal indefinitely long, freely distributed, uniform threads at all pH values between 5.5 and 8.5. Myosin and actomyosin have a very similar appearance in the pH range of 5.5 to about 7: fibrous aggregates at pH 5.5 and aggregates embedded in an amorphous film at pH 7. Between pH 7.3 and 8 myosin appears as an amorphous film in contrast to actomyosin which reveals actin-like threads embedded in an amorphous matrix.

In the presence of ATP and Mg, actomyosin forms a contracted plug at low pH <sup>1</sup>. This plug consists of fibrous aggregates, in some respects similar to myosin or ATP-free actomyosin at the corresponding pH. However, a difference is evident in that many, relatively short, actin-like threads can be seen fraying out at the end of the fibrous aggregates. It seems likely that they are the remnants of a continuous network within the intact plug which became disrupted during preparation.

In the presence of ATP and Mg, actomyosin forms a clear solution at around neutral pH <sup>1</sup>. The electron micrographs show a continuous amorphous protein film like that observed in myosin solutions at a somewhat higher pH. In addition, indefinitely long, actin-like threads are uniformly distributed throughout the fields resembling F-actin prepared alone. This is in contrast to ATP-free actomyosin at the same pH where shorter threads are concentrated in the cloud-like areas of amorphous myosin. After the ATP has been completely hydrolyzed, the actomyosin forms a fine, non-contractile precipitate. Its microscopic appearance now coincides with that of ATP-free actomyosin.

At alkaline pH actomyosin forms a firm, transparent gel after much of the ATP has been hydrolyzed. Microscopically a network is seen which consists of strands made up of loosely associated, indefinitely long, actin-like threads embedded in an amorphous matrix. It appears that the contracted plug and the gel are similar in having a framework of actin-like threads, but differ in that the myosin-like matrix forms a fibrous aggregate around this network at the acid reaction, and forms a characteristically amorphous matrix around the network at high pH.

It was not possible to characterize actomyosin units as such morphologically. The structures in general consist of actin-like threads embedded in a myosin-like matrix which corresponds to the myosin alone at the respective pH. The presence of ATP accentuates the appearance of actin-like threads. The pictures obtained of the clear solution in the presence of ATP and Mg at near neutral pH and physiological salt concentration appear to demonstrate the dissociation of actomyosin.

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