AN ELECTRON MICROSCOPE STUDY OF MYOSIN, ACTIN, AND ACTOMYOSIN

by

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In connection with our ultracentrifuge studies of myosin, actin, and actomyosin, we have also studied some of our solutions in the electron microscope. We have thereby seen some structures which have not been published before, and it therefore seems to us to be of interest to publish some pictures. The micrographs have been taken with the gold shadow method of WILLIAMS AND WYCKOFF[1].

Earlier, JAKUS AND HALL[2] published some pictures of actin and myosin. Their myosin is obviously contaminated with actomyosin. No pictures have been published of pure myosin. PERRY AND REED[3] have studied actin and actomyosin. Furthermore SZENT-GYÖRGYI[4] published some pictures of STAUDINGER AND ROZSA in Bern where, however, the resolving power of the electron microscope was poor.

Myosin

Earlier we have published[5] some pictures from myosin once crystallised according to SZENT-GYÖRGYI[6] showing that these crystals are of the same paracrystalline nature as the crystals of tobacco mosaic virus. One can clearly see how they are composed of very long fibrils. The crystals end in thin points. Where the fibrils terminate the myosin molecules are often irregularly clustered. The crystalline needles can be broken up in threads of uniform thickness by ultrasonic treatment. Myosin precipitated by dialysing a potassium chloride solution forms even needles but much smaller in size (Fig. 1).

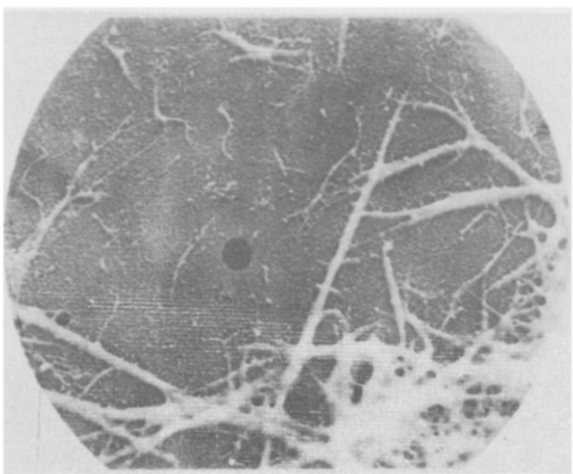

Fig. 1. Myosin crystals obtained through dialysing a potassium chloride solution (8000 ×).

Crystalline myosin is soluble in distilled water. The solutions seem to contain the myosin in a jelly-like state. From the electron micrographs one can see that the jelly is composed of fibrils of myosin which are twisted together (Fig. 2).

In myosin dissolved in 0.3 M KCl, the thread structure has

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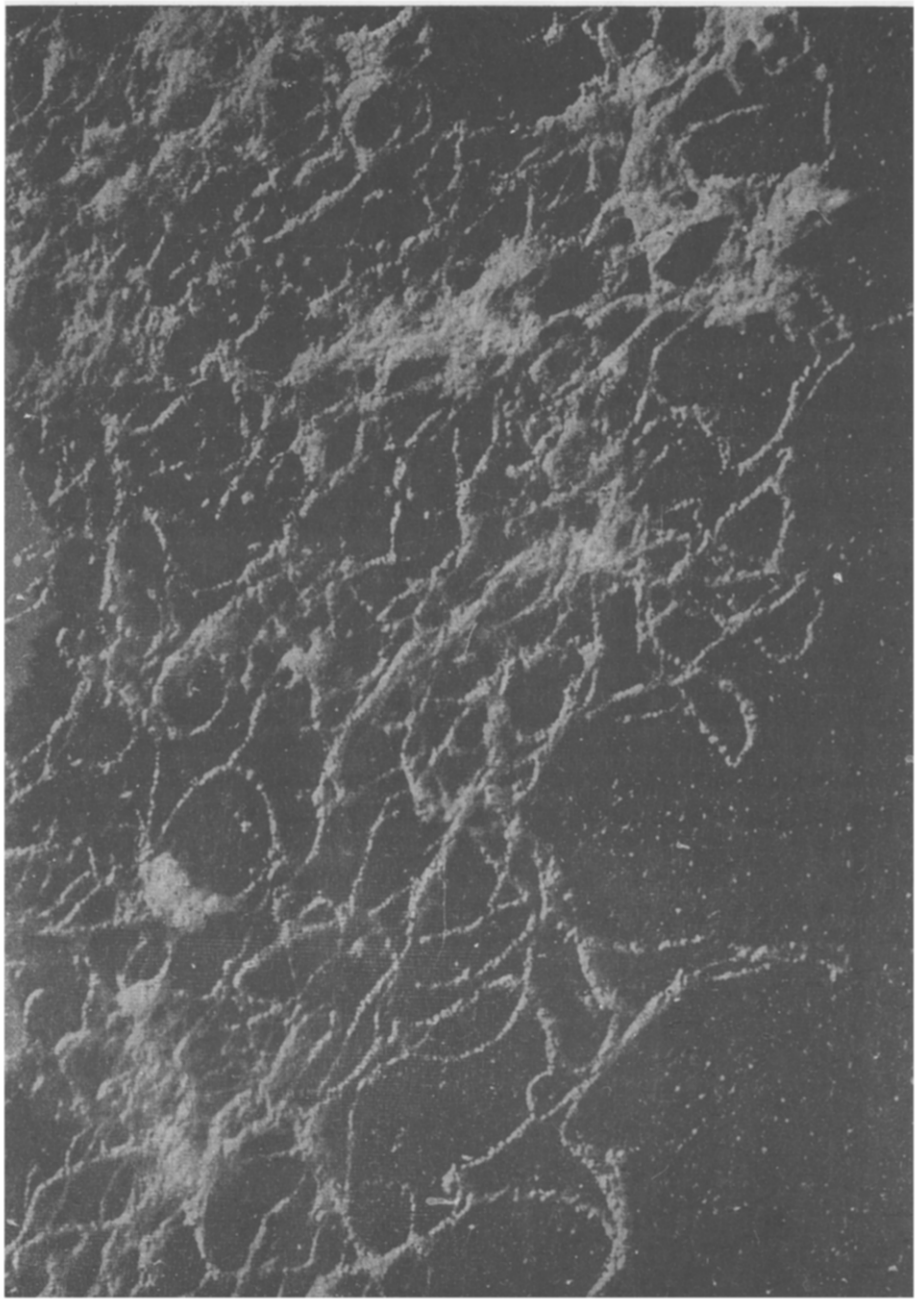

Fig. 2. Crystallised myosin dissolved in pure water (40000 ×).

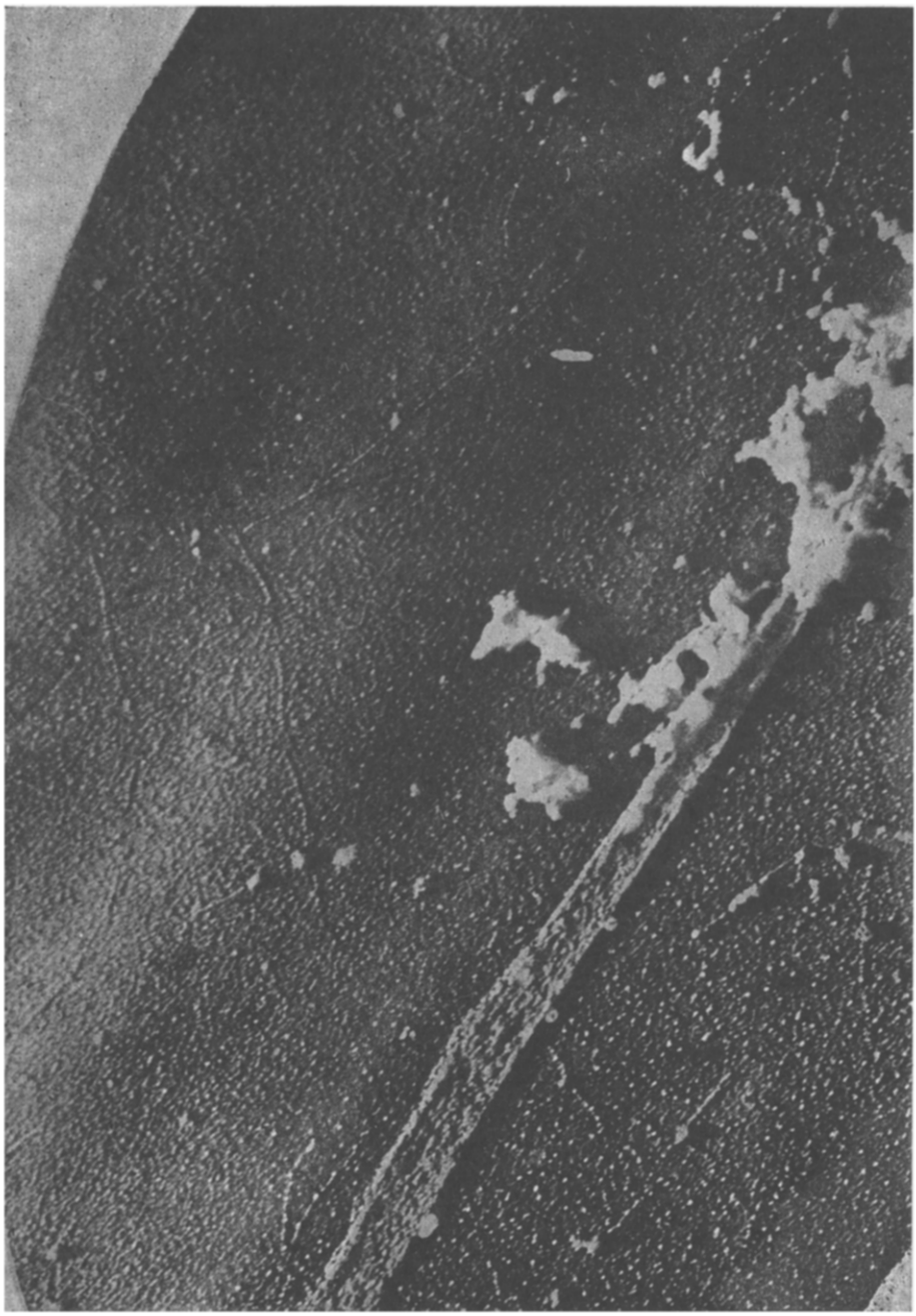

Fig. 4. F-actin polymerized with 0.001 M $MgCl_2$ (40000 ×)

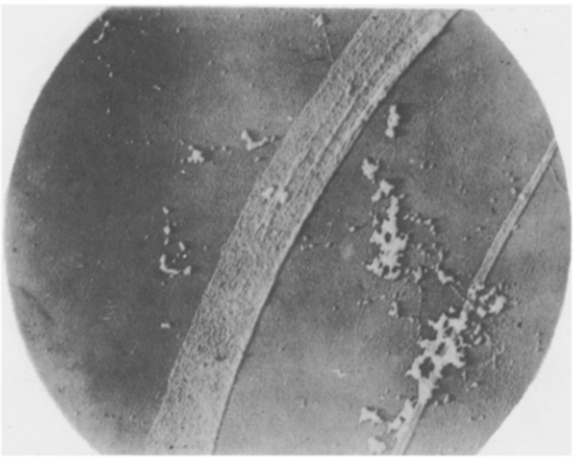

Fig. 5. F-actin polymerized with 0.001 M $MgCl_2$ (8000 ×)

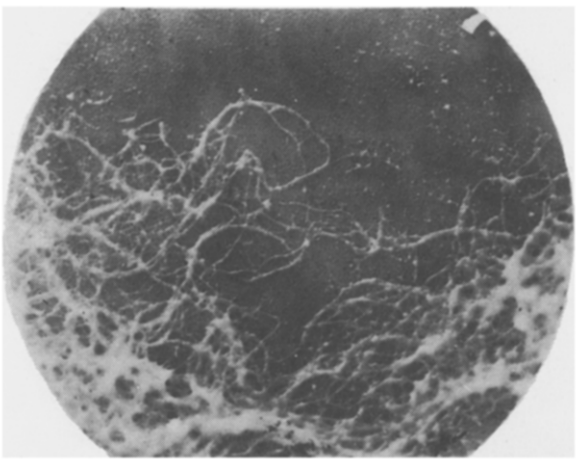

Fig. 6. Actomyosin from 1 part F-actin and 3 parts myosin. p_H 7. "Jelly part" (8000 ×)

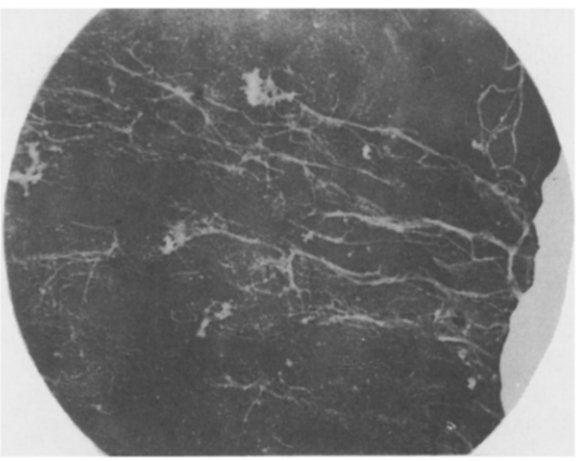

Fig. 7 Actomyosin from 1 part F-actin and 3 parts myosin. p_H 7. "Thread fraction" (8000 ×)

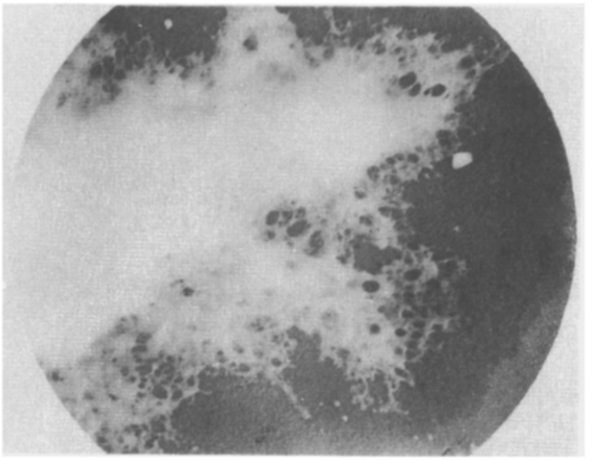

Fig. 8. Actomyosin plus ATP. "Jelly fraction" (8000 ×)

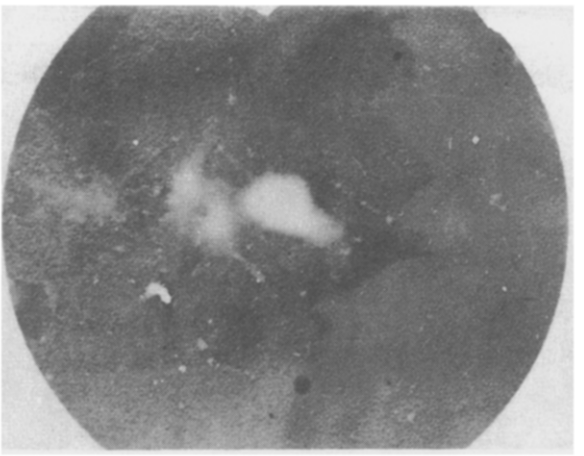

Fig. 9. Actomyosin plus ATP. "Thread fraction" (8000 ×)

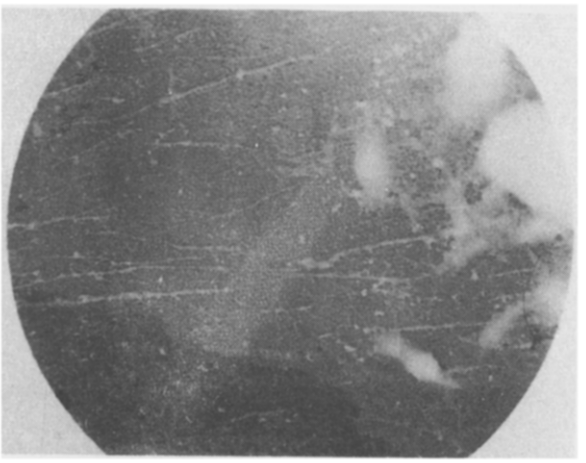

Fig. 10. Actomyosin plus ATP with some uncontracted threads. (8000 ×)

disappeared. Only here and there is possible to see some clusters of myosin. The myosin seems to lay smoothly on the surface even at fairly small concentrations. At very small concentrations this layer is broken but no free molecules are visible.

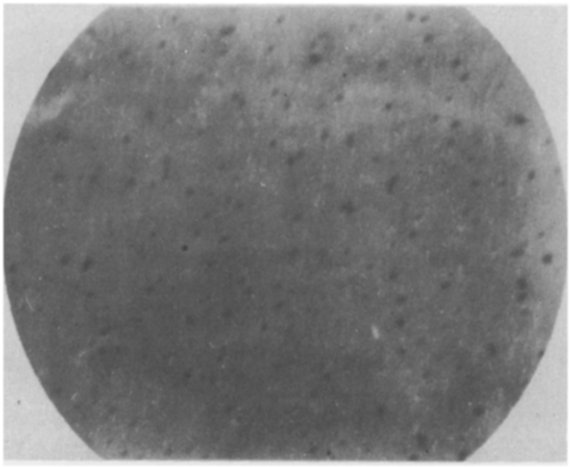

Fig. 3. C-actin from muscle powder which is insufficiently treated with acetone (Unstained 20000 ×)

Actin

The pictures of G-actin, prepared according to STRAUB[7], which PERRY AND REED and SZENT-GYÖRGYI have published, show that the preparation has not been pure. Pictures with clusters of actin are obtained when the actin is insufficiently purified (Fig. 3). Such clusters appear as dark irregular spots on unstained micrograph pictures. When the muscle powder is treated several times with acetone they disappear. Possibly the material which holds the actin molecules together dissolves in acetone.

The polymerized actin (F-actin) is, as JAKUS AND HALL have shown, in a fibroid state. They found that the length of the fibrils was a function of the p_H at which the G-actin was polymerized. They polymerized it with acids. We have polymerized our G-actin in water with 0.001 M $MgCl_2$. It was possible to see that the threads were composed of small particles (Fig. 4, p, 662). The threads of F-actin aggregate very easily. The aggregation generally takes place in a special manner. The threads bind themselves together to form very thin sheets. The unusal ease with which the solutions of F-actin show great double refraction may depend on such structures (Fig. 5, p. 663).

Actomyosin

We have mainly studied the actomyosin obtained by adding an actin solution to a myosin solution in the optimal ratio (1 part actin to 3 parts myosin). Some experiments with the native actomyosin, the so-called B-myosin of SZENT-GYÖRGYI[8], did not show any good results. One could see long free threads, but they were very diffuse because the whole surface was covered with a layer of protein. At very great dilutions, the protein film was broken but at the same time the threads could not be detected. From our experience of the fact that B-myosin is much contaminated with myosin and from our experience of myosin stated above the film here is probably due to myosin.

In the ultracentrifuge their seems to be a difference between B-myosin and synthetic actomyosin. Synthetic actomyosin has more jelly-like properties. In actomyosin one can see one component which sediments very quickly to the bottom and has marked jelly-like properties, and another component which sediments more slowly.

In our actomyosin solutions from synthetic actomyosin, this very fast jelly-like component was the greater. We have separated the two components in the centrifuge and tried to take micrographs of both. We have, however, not seen any principal difference between them. Both are composed of threads but they are much more intermingled

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in the jelly part (Figs 6 and 7). If care is not taken to use actin myosin in the optimal ration, one obtains as did PERRY AND REED, pictures where the actomyosin is obviously covered by a protein film.

Under a certain concentration of salt (0.15 M KCl), the actomyosin contracts with adenosintriphosphate (ATP). In the so-called jelly fraction, the actomyosin contracts to clusters greater than the electron micrograph (Fig. 8). In the other fraction the clusters were much smaller (Fig. 9). In both cases we were not able to detect any structure in the material. In some cases we could see that a smaller part of the threads did not contract in spite of the fact that nearby threads contracted (Fig. 10). That this possibility can occur is shown by the investigations of BANGA AND GUBA (quoted by SZENT-GYÖRGYI[4, 6]). They showed that when the myosin had a small amount of proteins adsorbed the actomyosin did not contract with ATP. We certainly had little of such myosin in our solutions.

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SUMMARY

In general the present work confirms the results of previous investigaters. It shows further that the myosin molecules can form threads when high salt concentrations are not used. In other cases myosin will form a protein film.

Only when enough acetone treatment has been used, is all the G-actin in globular form without the particles being clustered together. The fibrils of F-actin can build up very thin sheets when the polymerization occurs in the presence of a little $MgCl_2$.

Synthetic actomyosin consists of long threads thicker than threads of myosin or F-actin. They are often mingled together. If care is not taken to avoid contamination of too much myosin the latter will lay as a thin film over the actomyosin and make it not easily visible. Native actomyosin (B-myosin) investigated was always poorly visible on account of such a film. Actomyosin threads with ATP contract to jelly clusters in which it is not possible to see any structures. Actomyosin threads which do not contract have also been seen.

RÉSUMÉ

Le présent travail confirme en général les résultats des auteurs antérieurs. Il montre en outre que les molécules de myosine peuvent former des filaments en l'absence de fortes concentrations en sel. Sinon, la myosine forme une couche mince.

Ce n'est qu'après un traitement convenable à l'acétone que toute l'actine G prend l'état globulaire sans que les particules s'agglomèrent. Les fibrilles de l'actine F peuvent constituer des couches très minces si leur polymérisation a lieu en présence d'un peu de $MgCl_2$.

L'actomyosine synthétique est formée par de longs filaments plus épais que ceux de la myosine ou de l'actine F. Ces filaments sont souvent enchevêtrés. Si l'on n'a pas soin de suffisamment éliminer la myosine, cette dernière se dépose en films minces sur l'actomyosine et la rend difficilement visible. C'est pour cette raison que l'actomyosine native (myosine B) n'est jamais bien visible. Les filaments d'actomyosine en présence de ATP se contractent en amas gélatineux dans lesquels il n'est possible de voir aucune structure. On a constaté également l'existence de filaments d'actomyosine qui ne se contractent pas.

ZUSAMMENFASSUNG

Im allgemeinen bestätigt die vorliegende Arbeit die Ergebnisse früherer Untersucher. Sie zeigt weiterhin, dass die Myosinmoleküle Fäden bilden können, wenn keine hohen Salzkonzentrationen benutzt werden. In anderen Fällen bildet Myosin einen Eiweissfilm.

Nur nach einer genügenden Behandlung mit Azeton befindet sich alles G-Aktin in Kugelform, ohne dass die Teilchen zusammengeklumpt sind. Die Fibrillen von F-Aktin können sehr dünne Blätter bilden, wenn die Polymerisation bei Anwesenheit von etwas $MgCl_2$ erfolgt.

Synthetisches Aktomyosin besteht aus langen Fäden, die dicker sind als die Fäden von Myosin

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oder F-Aktin. Sie sind oft miteinander verknäuelt. Wenn nicht dafür gesorgt wird, Verunreinigung durch zuviel Myosin zu vermeiden, legt sich dies als dünner Film über das Aktomyosin und macht es schlecht sichtbar. Natives Aktomyosin (B-Myosin), das untersucht wurde, war infolge eines solchen Films immer schlecht zu sehen. Aktomyosinfäden kontrahieren mit ATP zu gelatinösen Häufchen, in denen keinerlei Struktur zu sehen ist. Aktomyosinfäden, die nicht kontrahieren, wurden auch beobachtet.

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