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The interaction of actin and myosin 40 years ago: a commentary by

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on 'An electron microscope and X-ray study of actin'
by W.T. Astbury, S.V. Perry, R. Reed and L.C. Spark
Biochim. Biophys. Acta 1 (1947) 379–392
with Biochim. Biophys. Acta 1 (1947) 506–516 (summary)

In January, 1946 I joined the Department of Biochemistry, Cambridge, and started research on the interaction of actin and myosin in a small basement laboratory with my supervisor, Kenneth Bailey. The immediate post-war period was a seminal time for the development of protein chemistry in Cambridge. Bailey had just discovered tropomyosin, and the other occupants of the laboratory, Fred Sanger and Rodney Porter, were laying the foundations of work that was later to lead to Nobel Prizes [1]. Crystals of myoglobin were being grown in the department and in the Cavendish Laboratory the X-ray crystallographers were turning their attention to proteins.

Actin had been first isolated in 1942 by Straub, working in Szent-Györgyi's laboratory in Szeged, Hungary, but the news of its discovery did not reach Britain until soon after the end of the war. The properties of this remarkable protein were of special interest to Bailey and the Needhams, who were also working in Cambridge. The ability of actin to form with myosin the complex, actomyosin, which in the gel state underwent a dramatic mechano-chemical change, was clearly of profound significance to muscle research. For the first time, a normal cell constituent, ATP, was shown to bring about a physical change in the major protein system of muscle that was also responsible for its enzymic hydrolysis. Clearly, these observations were at the heart of the contractile process. They explained an earlier report by the Needhams [2] in which ATP was shown to produce a fall in the viscosity and flow birefringence of myosin solutions. Although it was not appreciated at the time, it is now clear that these myosin preparations must have contained actomyosin.

After almost 6 years in the army, my biochemistry was somewhat rusty and the original literature describing the isolation of actin and its properties was not generally available. A review of the work carried out by the Szeged group was sent by Szent-Györgyi to Sweden whilst he was in hiding from the Gestapo in Hungary, fearful that the results of his wartime research would be lost to the scientific community. These were subsequently published in the Acta Physiologica Scandinavica [3]. The original papers describing work carried out in the period 1941–43 were in three volumes of the Studies from the Institute of Medical Chemistry, University of Szeged, that had been privately published by Szent-Györgyi and were in limited supply. Fortunately, Bailey had obtained copies of these publications from W.T. Astbury. When scientific contacts had been reestab-

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lished in Europe after the war, Szent-Györgyi had sent copies of the volumes to his old friend Astbury in Leeds. These are now in my possession. Astbury and Bailey had many interests in common, particularly in the field of protein structure. Astbury pioneered the X-ray study of fibrous proteins and had demonstrated that keratin, myosin, epidermin and fibrinogen possessed a characteristic spacing of 5.1 Å along the fibre axis which he ascribed to what he called the α-fold but which is now known to arise from the α-helix. Naturally, he was anxious to see if the fibrous form of this new protein, F-actin, also exhibited a similar X-ray pattern. It was decided that the biochemical studies on the interaction of actin and myosin should be supplemented by a biophysical study in collaboration with the Leeds group.

Early in 1946, preparations of actin were made, probably the first to be produced in Britain, dried down as films on glass plates and taken up to Leeds. The X-ray diffraction diagrams were characterised by moderate angle reflections on or near the meridian. Similar reflections had been observed by Bear [4] in all the types of muscle that he had examined, so it could be concluded that these reflections in the whole muscle pattern were due to actin. The actin diffraction pattern was new, unlike those of the α-proteins but bearing some resemblance to that of feather keratin, although the fibre repeats were different. It was concluded that the detailed construction of the F-actin fibre diagram showed "that the corpuscular units are not strung together in an arbitrary fashion but always in the same way and with atomic precision". This conclusion was subsequently confirmed when the two-helical chain model for the F-actin filament was later deduced by Hanson and Lowy [5].

The electron microscope pictures obtained of actin and its complex with myosin were of indifferent quality compared with those obtained today. It should be pointed out that the first electron microscope study detailing the filamentous structure of the myofibril had appeared only in the previous year [6] and the use of this technique in the study of isolated proteins was in its infancy. The study, however, clearly showed the globular and filamentous forms of actin, the anastomosed network of protein filaments of which actomyosin is composed and the effect of ATP in dissociating this system.

Although the X-ray and electron microscope studies gave an insight into the form of the structures, involved, they gave no indication of the nature of the unique interaction between the two proteins. Study of the actomyosin gel was obviously more relevant to muscle contraction, but because of the obvious difficulties involved with experiments on a heterogeneous system, attention was directed to the sol state, in which the interaction between the proteins could be easily followed by viscosity changes. The interaction was clearly not mainly electrostatic, because the viscometric effects were observed at quite high ionic strengths. A number of hypotheses were examined, particularly the postulate that the interaction was similar to that existing in the enzyme-substrate complex. This would require that actin contained a prosthetic group composed of ATP, or some similar compound containing a pyrophosphate group, which would bind to myosin as a pseudo-substrate but with lower affinity than its normal substrate, ATP. In this way, the dissociation of actomyosin by ATP could be neatly explained. Chromatographic methods for the detection of ATP did not then exist, but its presence could be indicated by the estimation of acid-labile phosphate. Significant levels were detected in actin preparations and, although reduced after exhaustive dialysis, acid-labile phosphate was still present in F-actin preparations. Nevertheless, as the amounts were less than would be expected from the minimum concentrations of ATP required to dissociate actomyosin, this hypothesis was rejected. In retrospect, it is a pity that this observation was not taken further, for clearly we were detecting the bound ADP shown later by Straub and Feuer [7] to be present in all F-actin preparations as a consequence of the hydrolysis of the bound ATP of G-actin on polymerisation. Nevertheless, our conclusions were correct, for the bound nucleotide is not considered to play a role in the interaction of actin and myosin.

The effects of thiol reagents on the interaction indicated that the enzymic activity of myosin was under some conditions closely related to its ability to interact with actin. The inhibition of the Ca^{2+}-activated ATPase of myosin obtained with a range of different types of thiol reagent correlated quite well with the loss in interaction with actin as measured by the viscometric effect. Evidence was also obtained for thiol groups on myosin of different reactivities. In contrast, treatment of actin with similar concentrations of thiol reagents had relatively little effect on its interaction with myosin, despite the fact that actin also possessed free thiol groups in the native form. Apart from the information they provided on the nature of the interaction between actin and myosin, the results of the study of the effects of the thiol reagents on myosin argued strongly for its identity with the ATPase. It may seem strange today that the possibility of myosin's not being an ATPase should be considered, but it should be remembered that all the enzymes studied up to that time had very much lower molecular weights. Against this background the concept of an enzyme of the size of the myosin molecule was not accepted by all. Indeed, at least one paper appeared at the time reporting evidence of non-identity.

The study of the role of the thiol groups suggested that the interaction of actin and ATP occurred either at the same region or at two different closely related

regions of the myosin molecule. The isolation of Subfragment 1 from myosin some years later [8] confirmed that these properties were associated with the part of the molecule now known to represent the head. A variety of techniques such as selective cleavage, cross-linking reagents, specific chemical modification, NMR, etc., have shown that the head of the myosin molecule consists of three domains, probably connected by more flexible regions, that are of special significance for the interactions (see Ref. 9 for review). Although these more recent studies define in much detail the complexity of the interaction in terms of the amino-acid sequences of the two proteins, they confirm earlier studies using less sophisticated techniques that different sites on myosin are involved in the ATPase and actin-binding activities [10,11]. Despite the fact that the sites are separate, the events that occur at them are closely related by a mechanism that we do not yet understand. For example, under conditions when, judged by the viscometric properties, the actoheavymeromyosin complex is presumed to be dissociated, the system was shown to possess high MgATPase activity [12]. Thus, although the strong interaction that is responsible for the high viscosity complex is broken, actin is still able to modify the enzymic activity of the myosin ATPase. Myosin alone does not hydrolyse MgATP at a high rate unless actin is present. To explain this observation, it was proposed [12] that there were two types of interaction between actin and myosin. One would be a relatively strong interaction that is responsible for the high viscosity of actomyosin solutions and is presumably involved in force development in muscle. It was suggested that the other is a weaker type that, unlike the former, exists in the presence of free ATP and is responsible for modifying the enzymic properties of myosin so that it can hydrolyse MgATP at a high rate. This also implied that myosin probably has at least two sites for actin interaction.

Although the original study highlighted the importance of myosin thiol groups in the interaction, it oversimplified the situation. Myosin has quite a high cysteine content and in the original study about half of the total apparent cysteine residues, as judged by direct thiol titration, were modified to obtain almost complete loss of the enzymic activity and actomyosin formation. We now know that the N-terminal head region of myosin contains eight thiol groups. Studies in the 1950s identified two of these groups to be of special significance for the ATPase, namely SH_1 (residue 707) and SH_2 (residue 697). Modification of SH_1 leads to an increase of the Ca^{2+}-ATPase, whereas subsequent modification of SH_2 destroys the enzymic activity completely [13]. Later, careful titration of the thiol groups on heavy meromyosin, which forms a soluble complex with actin that can be studied at low ionic strength, indicated that when SH_2 was modified, and the enzymic activity destroyed, the ability to form the viscous complex with actin was largely preserved [14]. This effect is more readily observed at low ionic strength, explaining why it was not demonstrated in the original studies with actomyosin sols that require higher ionic strength to remain in solution. The subtle relationships between SH_1 and SH_2 are further reflected by the more recent demonstration that these groups can be cross-linked with appropriate reagents. Cross-linking is favoured by the product of enzymic activity, MgADP, which nucleotide in some circumstances can be trapped on the myosin when internal disulphide formation in promoted by DTNB [15,16]. Such studies as these emphasise the close relationship of these two thiol groups with the enzymic site.

The papers that are the subject of this essay were probably the first to describe a detailed study of the interaction between actin and myosin, apart from the original reports of the Szent-Györgyi group. Since that time many thousands of papers describing investigations of the actomyosin system have appeared and the genes of the two proteins have been cloned and sequenced. Clearly, enormous progress in the detailed definition of the system has been made, but unfortunately the results of X-ray diffraction studies are not yet available due to difficulties in preparing the appropriate protein crystals. The advances made over the past 40 years have been largely of detail and definition, with few new concepts emerging. Indeed, it is remarkable that a number of the concepts incorporated in the current hypotheses were developed 20 or 30 years ago from the results of much less sophisticated experiments than those used to confirm them today. The knowledge that we have relates largely to the amino-acid residues involved in the interaction, but we lack information about their three-dimensional form and the molecular dynamics during the contractile event. In consequence, a precise description in molecular terms as to how the force is produced that enables the filaments to slide past one another as yet escapes us. Despite the biochemical definition of the system that existed over 40 years ago, progress in explaining the molecular mechanism of contraction has been somewhat disappointing. Certainly this is so in comparison with the progress achieved in molecular genetics or immunochemistry, subjects that were much less well defined in biochemical terms in the 1940s.

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AN ELECTRON MICROSCOPE AND X-RAY STUDY OF ACTIN

by

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I. ELECTRON MICROSCOPE

by

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Some time ago we commenced an electron microscope and X-ray study[1] with the view of throwing light on the significance of the new muscle protein, actin, discovered by STRAUB[2] and the findings of SZENT-GYÖRGYI and his school on the interrelations of actin and myosin[3]. In the meantime there has appeared an electron microscope study by JAKUS and HALL[4] of actin structures between p_H 7.1 and p_H 4; and whereas these authors transformed globular or G-actin into the active, fibrous condition (F-actin) by lowering the p_H with acetic acid, we had chosen the alternative method of adding 0.1 M KCl to the aqueous extract of G-actin at p_H 7. JAKUS and HALL have shown that G-actin aggregates to form filaments of F-actin which vary in both length and width according to the p_H. Lowering the p_H to 4 results in a considerable decrease in filament length, and the process can be reversed by raising the p_H again with ammonia. JAKUS and HALL also mention electron micrographs of actomyosin, the complex of actin with myosin, but they do not present any of these or any photographs illustrating the effect of adenosine triphosphate (ATP) on actomyosin. Our observations therefore supplement, and in some respects go farther than, those of JAKUS and HALL, and the present seems an appropriate opportunity of giving a preliminary account of them.

EXPERIMENTAL

Aqueous solutions of G-actin were prepared by the method of STRAUB[5], and the activation to F-actin was carried out by adding solid KCl to 0.1 M and allowing to stand for one hour at room temperature. Where necessary to remove the last traces of salt these solutions were dialysed overnight at 4° C, and before electron microscope examination they were tested for activity. For more concentrated solutions of F-actin, such as were used in the actomyosin preparation illustrated in Fig. 5 for example, the aqueous extract of G-actin was precipitated with 0.01 M acetate buffer at p_H 4.7 and the precipitate re-dissolved by adding 0.1 M $NaHCO_3$ to p_H 7.

Myosin was prepared from the back and hindleg muscles of the rabbit, as described by BAILEY[6]; under such conditions of preparation it contains 1 % to 2 % of actin[7].

The examination in the electron microscope of proteins in salt solution is complicated by the presence of the salt, and the problem is to dry a drop of the solution on the supporting film while maintaining the salt concentration at the correct value. Various methods have been tried of overcoming this difficulty[8], and one of the most successful is to form a very thin layer of liquid immediately

References p. 392.

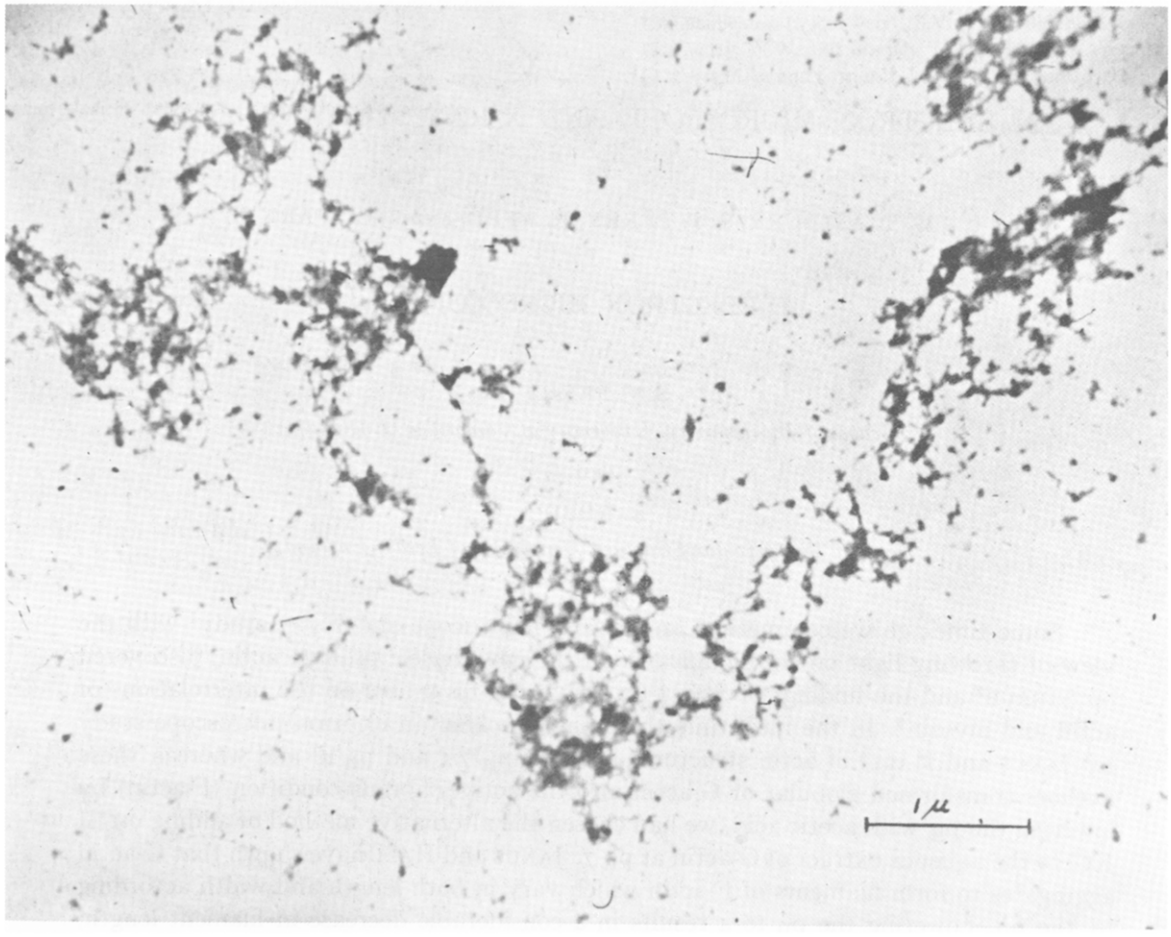

Fig. 1. F-actin from G-actin treated with 0.1 M KCl and dialysed. pH 7

by removing most of the drop with a micro-pipette. Drying then takes place quickly and without aggregation of the protein, and the remaining salt may afterwards be removed by floating the mounted film on the surface of clean distilled water.

The gold-shadowed preparations were made by the method of WILLIAMS and WYCKOFF[9].

In the electron microscope F-actin is seen to consist of fibres which appear to arise from particles, and there is moreover a considerable tendency to aggregate to larger and more robust structures. Fig. 1 shows a preparation obtained by activating G-actin solution with KCl and allowing it to stand for one hour. There is a tracery of fine fibrils against a background of particles. Other samples often showed very little until after metal-shadowing, when close networks of short fibrils were revealed, as illustrated in Fig. 2. In still other cases much longer and more definite fibrous networks were obtained, as in Fig. 3, with a background of smaller fibres and granular bodies. In our experience these different manifestations are due to sensitivity to the conditions of preparation for the electron microscope, but there can be no doubt of the fibrous character of F-actin, and furthermore the fibres appear to form as a result of the joining-together of small corpuscular bodies.

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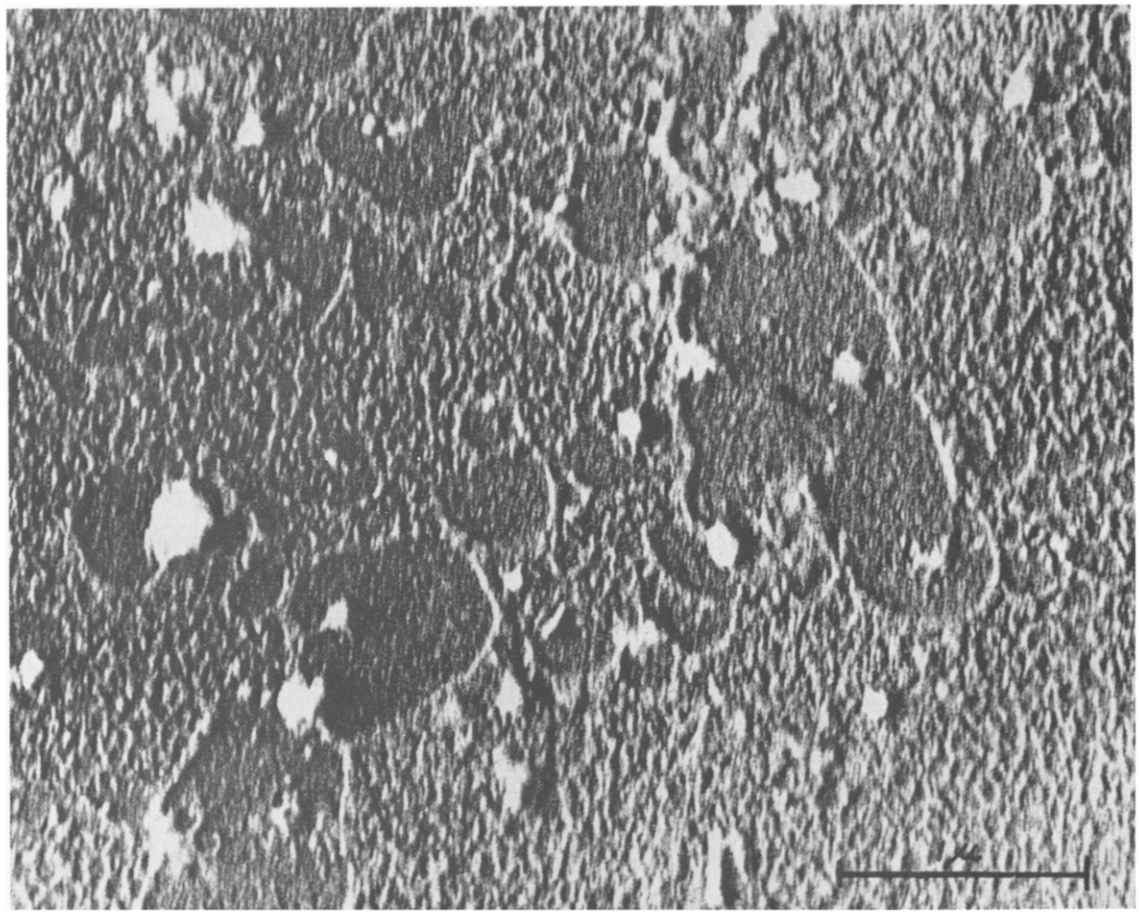

Fig. 2. F-actin from G-actin treated with 0.1 M KCl, p_H 7, gold-shadowed

First attemps to obtain micrographs of G-actin resulted in aggregated forms of the F-actin type, and it was inferred that in the drying-down of even thin liquid layers there was still sufficient salt to cause activation to the F-form. Accordingly, the aqueous solution was then dialysed, whereupon a dense background of corpuscular bodies was obtained invariably. We consider such bodies characteristic of the G-form: they would account for the non-viscous nature of G-actin solutions and would provide the units for building up the fibrous networks of F-actin. Fig. 4 illustrates a preparation of G-actin.

Actomyosin, when prepared by mixing approximately eight parts of myosin with three parts of F-actin, i.e., in the proportions in which they are considered to occur in rabbit skeletal muscle[10], gives a network of anastomosed fibres (Figs. 5, 6, and 7). The high degree of fibre ramification is characteristic and clearly distinguishes actomyosin from F-actin. Myosin itself does not form such networks, and the electron microscope shows the sol to consist of rod-like particles, on the average about 120 A wide and varying greatly in length[11]. There does not appear to be any uniformity of width in the actomyosin filaments; they vary from the fine tracery of Fig. 5 to the texture shown in Fig. 7, which was obtained from a more concentrated dispersion. In general the picture resembles

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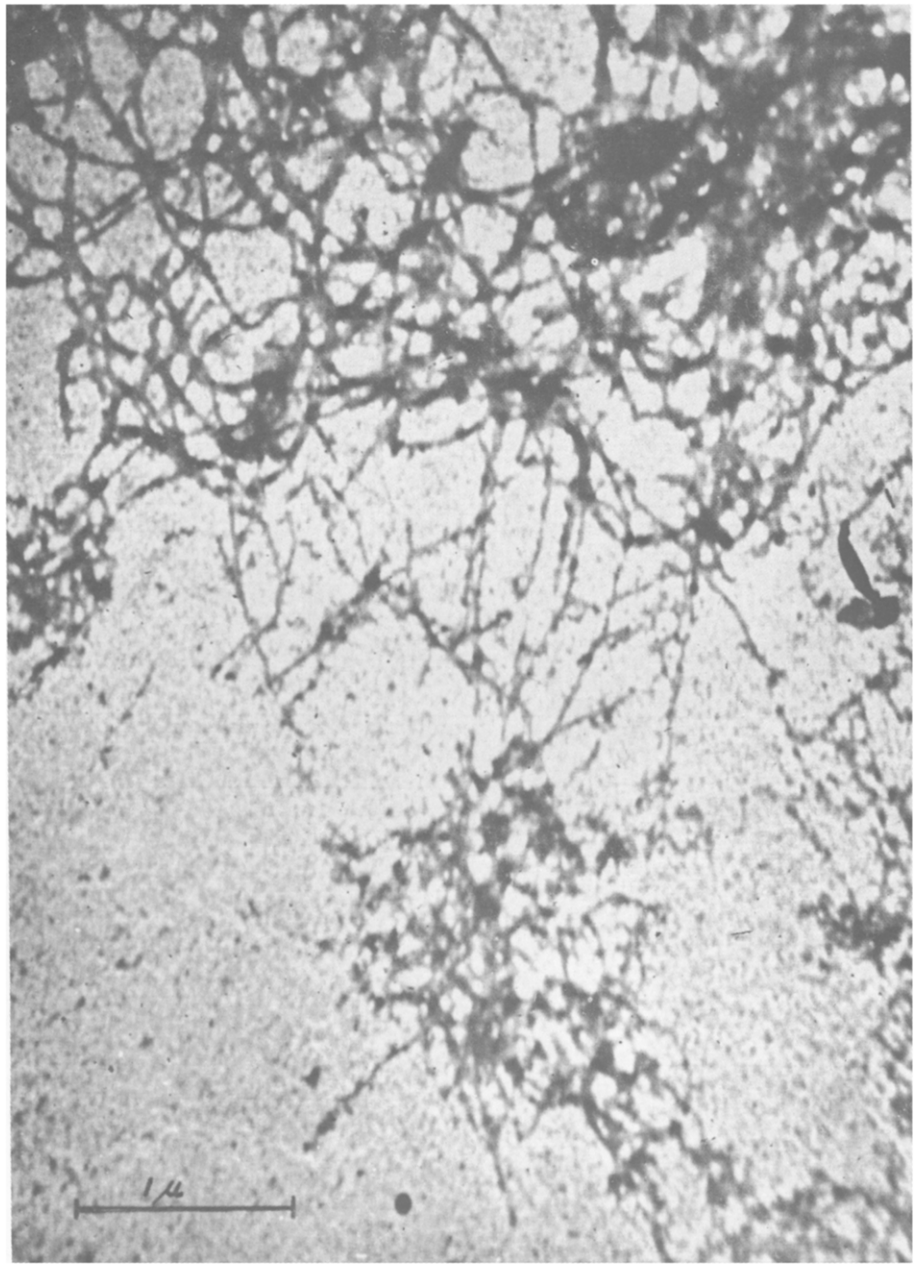

Fig. 3. F-actin from G-actin treated with 0.1 M KCl, p_H 7

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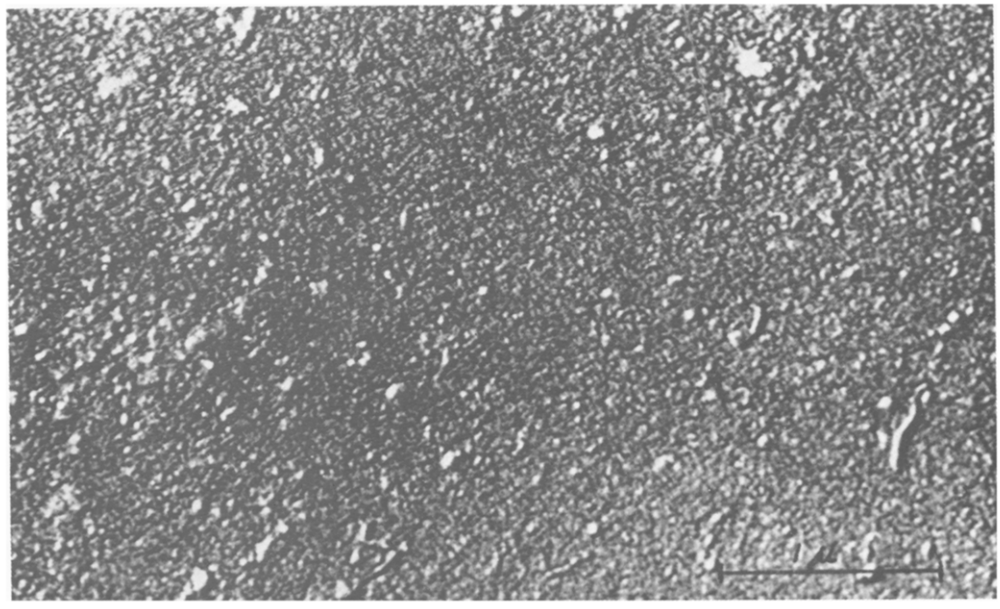

Fig. 4. G-actin, dialysed, pH 7, gold-shadowed

that of a gel and recalls the later stages of the formation of fibrin from fibrinogen [8].

When 0.0013 M ATP is added to actomyosin, the structural changes seen in the electron microscope are paralleled by the observed viscometric effects. The characteristic network of highly anastomosed fibres disappears and leaves fairly evenly distributed clumps of material that are not easy to interpret (Figs. 8 and 9). In the shadowed micrographs the clumps appear featureless, though in some non-shadowed preparations there are indications of an F-actin type of structure consisting of particles and short fibrils. Of the characteristic rod-like particles of myosin there seems to be no trace. Some attempts were made to examine myosin in the presence of ATP, but so far the results have been unsatisfactory. In the main, compared with myosin, the particles seem to have lost their distinctive rod-like appearance, but perhaps this is not unexpected in view of the fact that the myosin preparation used, although precipitated three times, probably still contained 1 % to 2 % of actin; and the dissociation of the actomyosin complex by ATP might conceivably also break down the regularity of the myosin particle structure.

DISCUSSION

The present work therefore confirms in general the findings of JAKUS and HALL, that G-actin is apparently a corpuscular form that is transformed to F-actin by a process of linear aggregation, and both investigations support the indirect conclusions of STRAUB and SZENT-GYÖRGYI in this regard. We appear, however, to have obtained a wider range of F-actin forms than the American workers, probably because we have used the alternative method of activation by the addition of solid KCl. The presence of salt could very well increase the scope of variation in the physical conditions of preparation — in the surface tension relations and in the rate of drying, for example, which in our experience can have considerable influence on the form and texture of protein preparations for the

References p. 392.

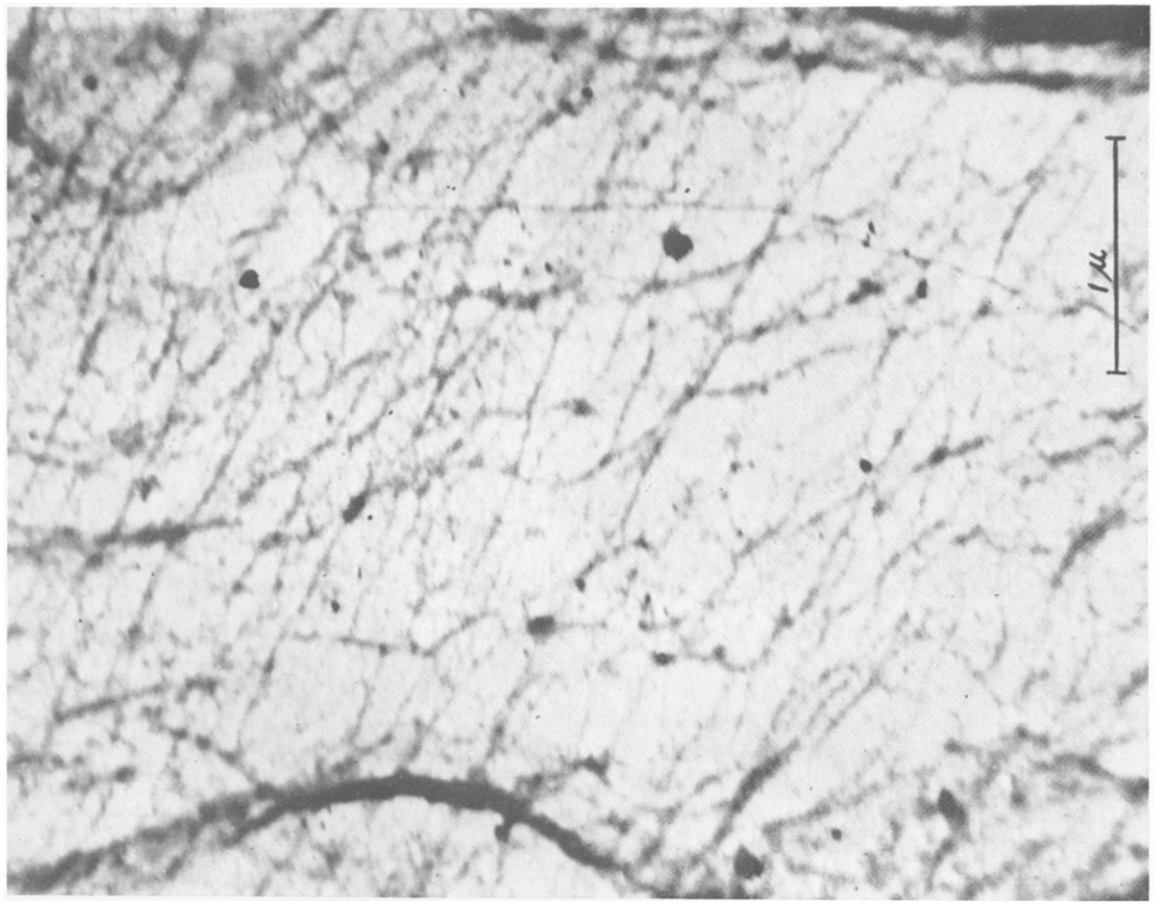

Fig. 5. Actomyosin from 2.5 parts F-actin and 8.4 parts myosin (actin iso-electrically precipitated), PH 7

electron microscope. Our preparation which most resembles the pronounced filamentous form obtained by JAKUS and HALL is illustrated in Fig. 3.

The preliminary studies of actomyosin mentioned by JAKUS and HALL indicate that this complex consists of long slender filaments not unlike those of myosin but significantly thicker than the average actin filaments, but in view of the great difference in viscosity between myosin and actomyosin one might reasonably expect some more striking structural difference. From our own work it appears that actomyosin formed from actin and myosin in physiological proportions surpasses either of its components in the capacity of forming anastomosed networks, and that furthermore the property is lost in the presence of ATP. Such a ramified structure would better account for the high viscosity of actomyosin as compared with myosin. While these observations support SZENT-GYÖRGYI's conclusions that actomyosin threads are more capable of forming fibres than are the two component proteins, it would not be justified at this stage to take this as evidence that the actual contractile fibrils of muscle are composed of actomyosin. The method of preparation of actin involves an alkaline 'loosening' treatment of the muscle tissue followed by drying the residue with acetone, which processes make it not

References p. 392.

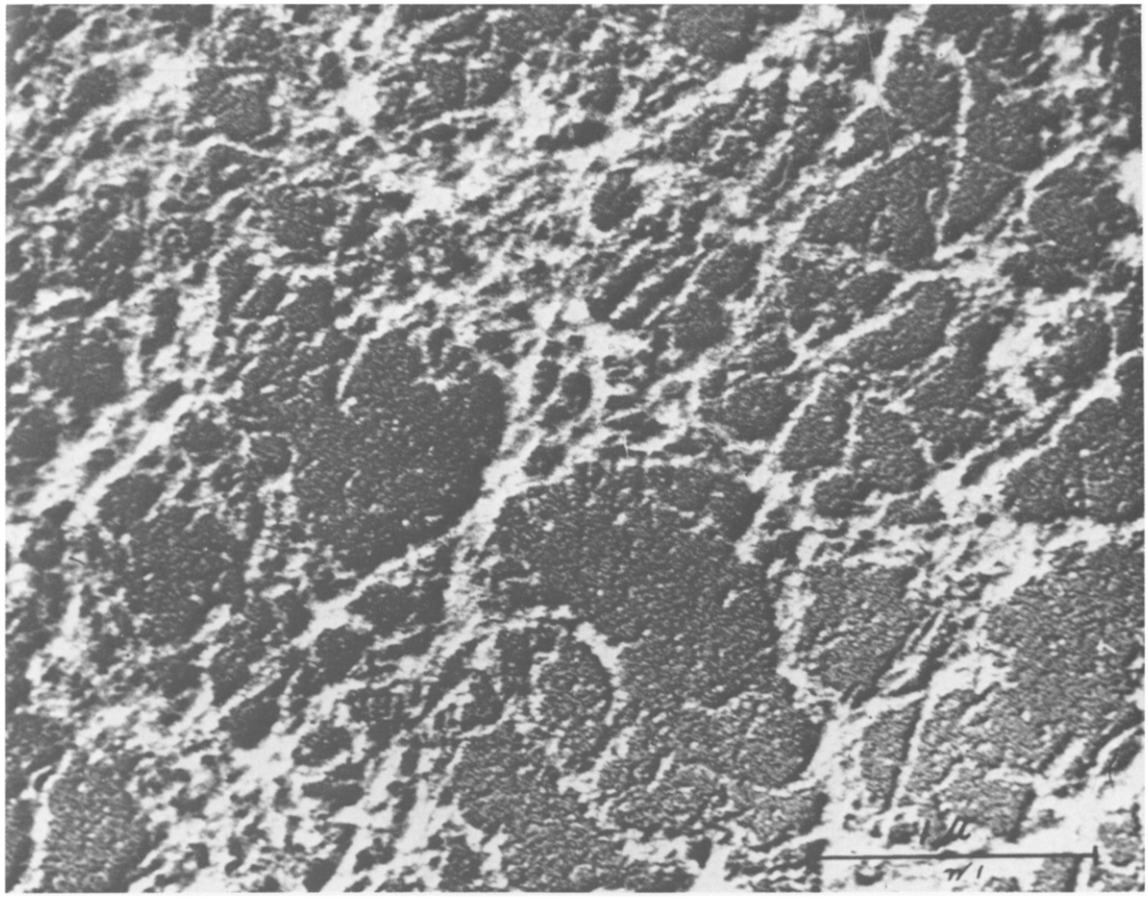

Fig. 6. Actomyosin from 3 parts F-actin and 8 parts myosin, pH 7, gold-shadowed

unreasonable to suppose that in intact tissue the actin may be associated not with myosin but with substances possibly of a lipoid nature.

The electron microscope observations throw no light on the question whether actin in intact tissue exists in the G- or F-form, and also it seems too early to assess the part played by this protein in the contractile process. The fibrous nature of F-actin and its ability to form anastomosed networks with myosin suggest a structural rôle, it is true, but other possibilities are opened up by the ready way in which ATP dissociates actomyosin and by the fact that the sulphydryl groups in myosin essential for its ATP-ase activity must remain intact if it is to interact with actin to form actomyosin[12].

We are indebted to DR. K. BAILEY and PROF. W. T. ASTBURY for their advice and encouragement.

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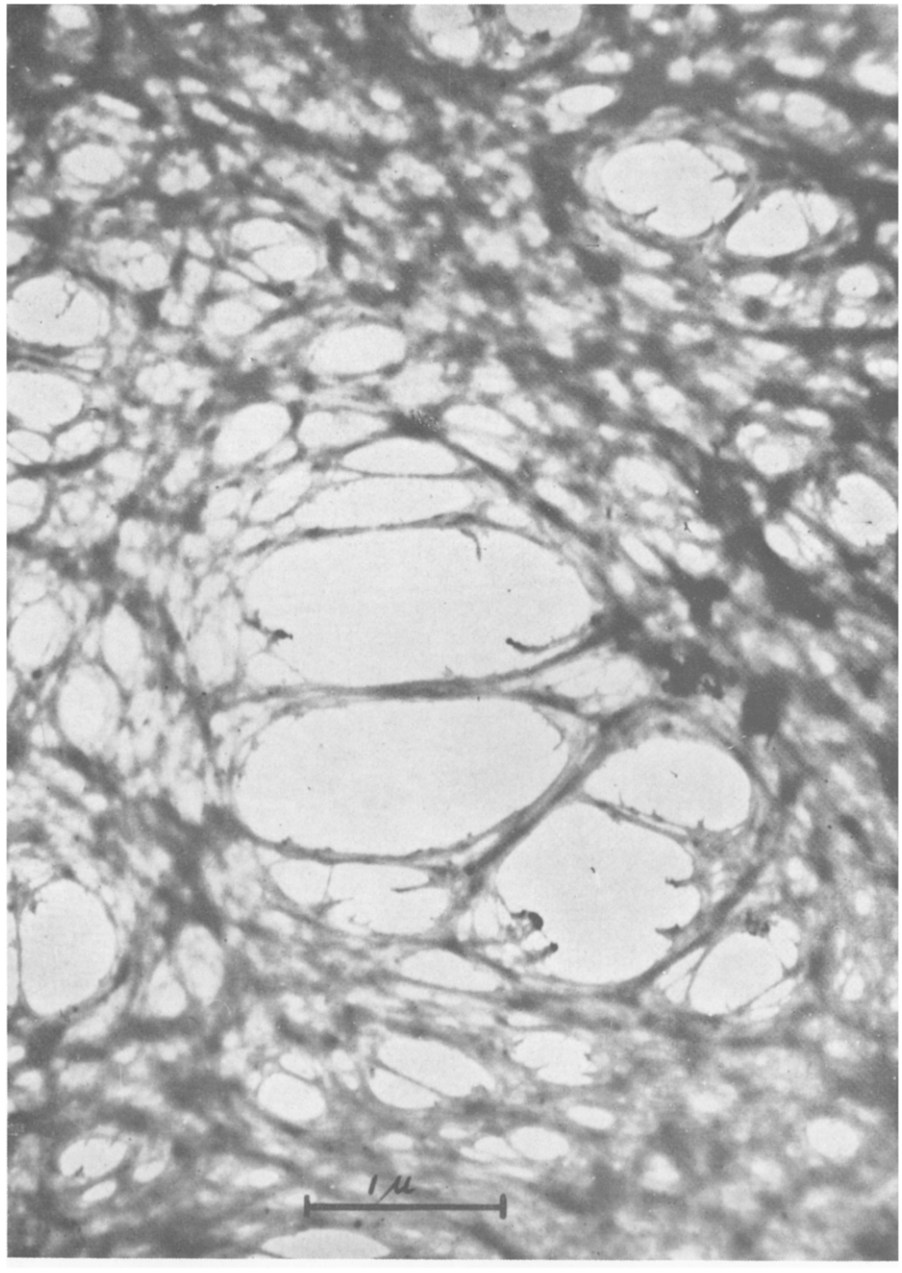

Fig. 7. Actomyosin from 2.5 parts F-actin and 8.4 parts myosin, pH 7

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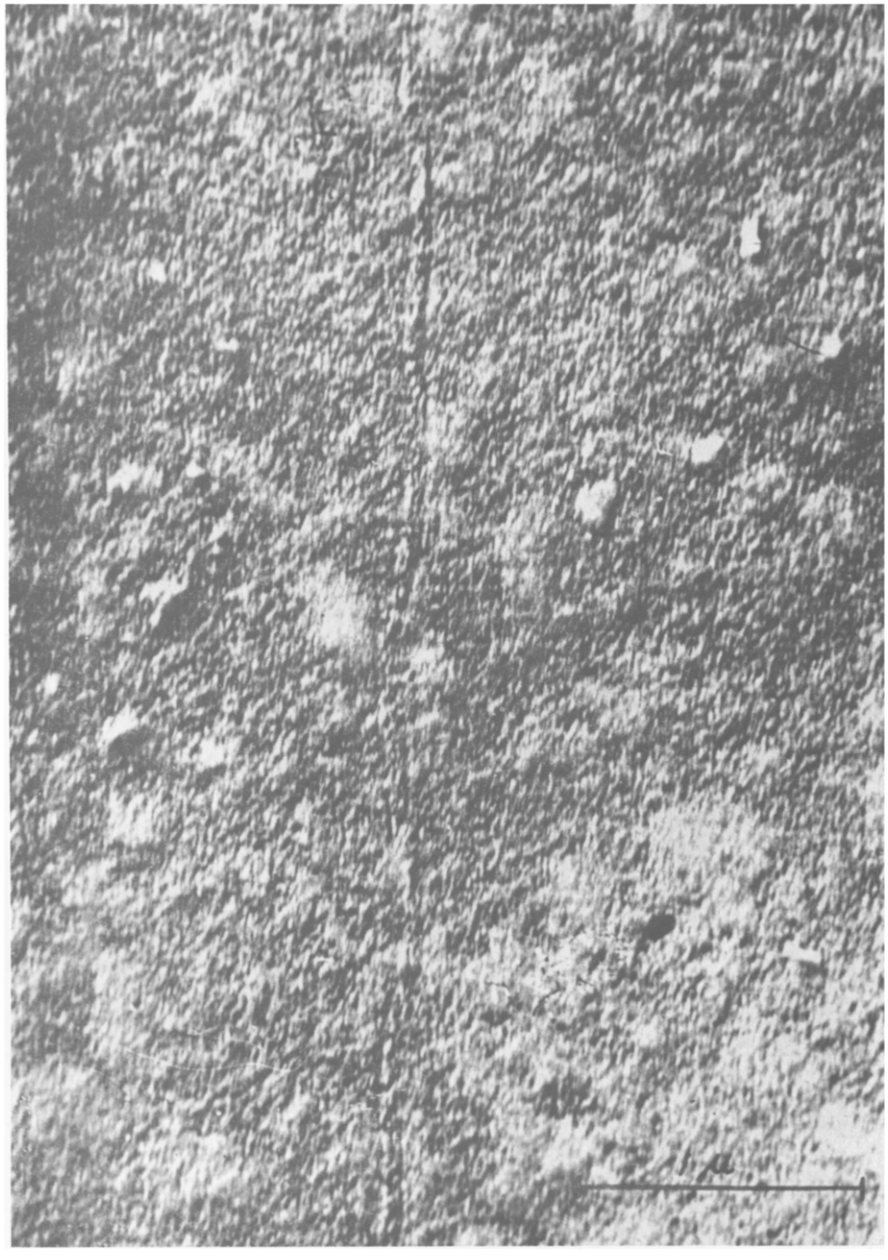

Fig. 8. Actomyosin plus ATP. Same actomyosin as used for Fig. 6 plus 0.0013 M ATP, p_H 7, gold-shadowed

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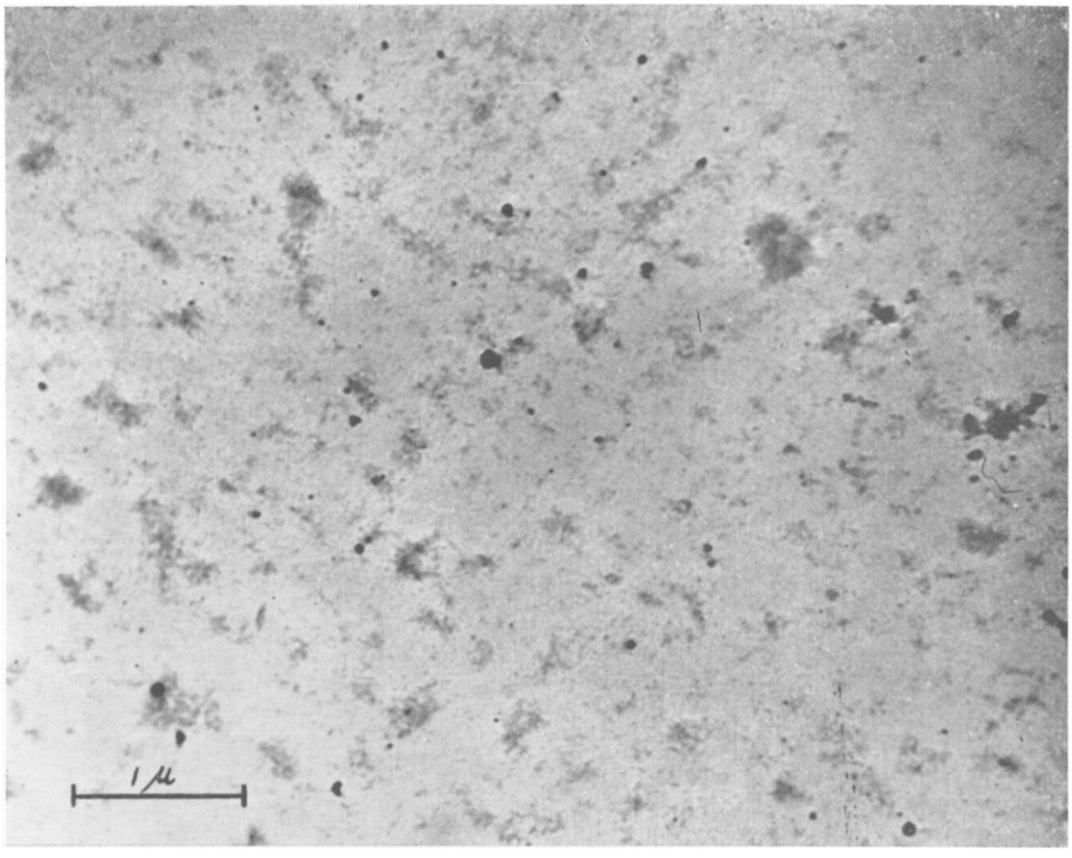

Fig. 9. Actomyosin plus ATP. Same actomyosin as used for Figs. 5 and 7 plus 0.0013 M ATP, pH 7

II. X-RAYS

by

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The actin was examined in the form of thin films, photographed with the X-ray beam parallel to the surface[13]. It was prepared by MR. PERRY by STRAUB's method[5], followed by precipitation at p_H 4.7 by 0.05 M acetate buffer. The precipitated actin was dissolved by neutralisation to p_H 7 with 0.1 M Na_2CO_3, after which it was dialysed overnight at 4° C and tested for activity with respect to actomyosin formation and the ATP effect. The films (about 0.1 mm thick) were made by drying the solution on a glass plate in a current of warm air.

When received they were very brittle and had cracked into small pieces, but it was found possible to build up a block of parallel fragments and to obtain X-ray photographs such as that illustrated in Fig. 10. The very fact that this photograph corresponds so closely to a fibre diagram is, of course, a clear indication that the structures of which the film is composed are elongated: drying a pool of the solution on a glass plate forces

References p. 392.

them to lie down almost parallel to the plate, but in all azimuths, so that when the resulting thin film is photographed with the X-ray beam parallel to its surface the viewpoint is an approximation to that of a fibre photograph. The actin diagram shows about a dozen clear meridional arcs with spacings from about 3.6 A to 27 A (this last being the strongest), some less definite outer reflections, and also certain reflections near the

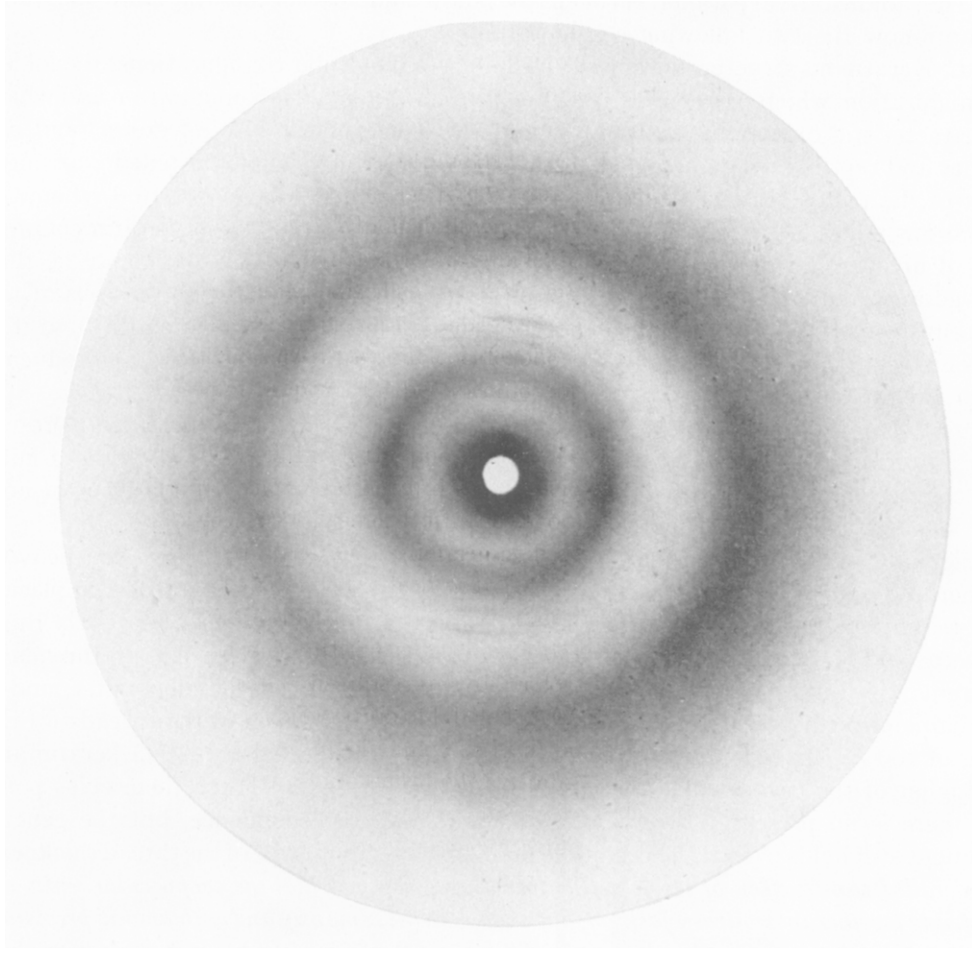

Fig. 10. X-ray fibre photograph given by F-actin film photographed with the beam parallel to the surface of the film. (Cu Kα rays; collimator 5 cm × 0.25 mm; original film-to-specimen distance 4 cm)

centre, i.e., of higher spacings, that are at present imperfectly resolved. From a consideration of all these it follows that the least value of the fibre period is about 54 A, but there is a distinct possibility that it might be twice this, i.e., about 108 A. There are also a number of equatorial reflections and two diffuse rings of spacing about 4½ A and 9 A.

DISCUSSION

Further analysis and details of the actin diagram will be given in a later publication. For the present we wish to point out that it is a new fibre pattern, unlike the α-pattern given by myosin and indeed by all the members of the keratin-myosin-epidermis-

References p. 392.

fibrinogen group, and to draw attention to its general resemblance to the fibre diagram given by feather and reptilian keratin. The fibre period of unstretched seagull quill, for example, is about 95 A, and the strongest meridional reflection is the 4th order of this: the chain-system can, however, be stretched continuously and reversibly by a further 7 % or so[14].

The quantitative parallel between the actin and feather keratin fibre diagrams prompts now the two following considerations:

1. Keratinous structures occur naturally in two molecular configurations: the folded α-configuration, which passes, on stretching, into the extended β-configuration and which is characteristic of mammalian keratin and the whole of the k-m-e-f group; and the feather and reptilian configuration, which is based on a somewhat shortened β-arrangement of the polypeptide chains[15]. Logically therefore, in the developmental scheme of the fibrous proteins, we ought to be able to find somewhere the feather keratin counterpart of myosin and of the other members, too, of the k-m-e-f group It may be that in F-actin we have actually found this counterpart of myosin. Just as the cells of the epidermis sometimes give rise to α-keratin and sometimes to feather keratin[16], so it is conceivable that muscle cells can perform the analogous dual function of producing either myosin or actin.

2. We long ago suggested[17] that the feather pattern, which is quite the best protein fibre pattern known, gave indications of originating in the end-to-end addition of initially corpuscular units ("it is not impossible that we have here an indication of how very long, *but periodic*, polypeptide chains can arise by the degeneration and linking-up of originally globular molecules")*. If this idea and that of the analogy between the feather and actin patterns is well founded, then we are at once in a position to place a very reasonable interpretation on the electron microscope observations, especially those of JAKUS and HALL. The striking micrographs published by the latter of actin threads at different p_H values have the strong appearance of chains of corpuscular units; and if the diameter of these units corresponds to the observed thickness of the threads (of the order of 100 A), that would fit in very well with the fibre period of feather keratin and the higher of the two possible periods of actin. As JAKUS and HALL themselves point out, there is no present certainty of this one-to-one correspondence, but the general argument still holds even if the corpuscle diameter is a submultipe of the thread thickness. *The detailed construction of the F-actin fibre diagram shows that the corpuscular units are not strung together in arbitrary fashion, but always in the same way and with atomic precision.*

Furthermore, we should expect the fibre period of actin to be rather greater than that of feather keratin, because the corpuscular units in actin fibres are not yet joined by covalent linkages such as have been established in the chain system of feather keratin (cf. the proposed 'grid-iron transformation'[18]).

We have also made some preliminary observations on the nature of the actomyosin complex. The actomyosin was prepared by MR. PERRY from 90 parts myosin (BAILEY's method) and 20 parts dialysed actin, and thin films were made as before. They were very different from the actin films, possessing none of their brittleness. On photographing with the X-ray beam parallel to the surface, an α-pattern was obtained similar to that given by the myosin alone[13].

An attempt was also made to obtain a normal β-pattern from actin by moistening it and

* Cf. also the degeneration of crystalline excelsin *in situ* in such a way as to give rise to three fibre patterns symmetrically disposed with regard to the original crystal lattice[18]. In the same paper we proposed a possible mechanism by which the intramolecular rearrangement might come about — the so-called 'grid-iron transformation'.

References p. 392.

squeezing it between pieces of plate glass (a process that works very easily with myosin, for instance); but the photograph became too diffuse to decide whether any β-form was present. When, however, actin film was heated to 60° C for only 20 seconds, it gave a disoriented but otherwise quite normal β-pattern.

SUMMARY

1. Electron microscope studies have been made of F-actin, prepared by activating G-actin by the addition of KCl. It is fibrous and the fibres appear to form by the joining-together of corpuscular units. The results confirm in general the findings of JAKUS and HALL and support the indirect conclusions of STRAUB and SZENT-GYÖRGYI.

2. G-actin is found to consist of corpuscular bodies.

3. Actomyosin, prepared from actin and myosin in physiological proportions, is found to surpass either of its components in the capacity of forming anastomosed fibrous networks, but the property is lost in the presence of ATP.

4. The interpretation of these observations is discussed.

5. An X-ray fibre photograph of F-actin has been obtained. The fibre period is at least 54 A (approx.), but it may possibly be twice this.

6. The analogy between the fibre diagrams of F-actin and feather keratin is pointed out, and it is suggested that in the fundamental scheme of the keratin-myosin-epidermis-fibrinogen group, F-actin may be related to myosin as feather keratin is to mammalian α-keratin.

7. The F-actin pattern is discussed in relation to previous indications that protein fibres may originate in the end-to-end addition of initially corpuscular units.

8. The detailed construction of the F-actin fibre diagram indicates that the corpuscular units from which the fibres are formed are not strung together in arbitrary fashion, but always in the same way and with atomic precision.

9. Actomyosin is found to give a large-angle X-ray pattern similar to that of myosin.

10. F-actin passes into the normal β-configuration on heating to 60° C.

RÉSUMÉ

1. La F-actine préparée par activation de la G-actine, par addition de KCl, a été étudiée au microscope électronique. C'est une protéine fibreuse, et les fibres sont formées par l'union d'unités globulaires. Ces résultats confirment les observations de Jakus et Hall, et sont d'accord avec les conclusions obtenues indirectement par Straub et Szent-Györgyi.

2. La G-actine est formée de corpuscules globulaires.

3. L'actomyosine, préparée à partir de l'actine et de la myosine, en proportions physiologiques, possède une aptitude supérieure à celle de chacun de ses constituants, à former des réseaux de fibres anastomosées, mais elle perd cette propriété en présence de ATP.

4. Discussion de la signification de ces observations.

5. Photographiée aux rayons X, la fibre de la F-actine montre une période d'au moins 54 Å (environ) ou possiblement du double.

6. L'analogie entre le diagramme de fibre de la F-actine et celui de la kératine des plumes, est soulignée; il est suggéré que dans la structure fondamentale du groupe kératine-myosine-épiderme-fibrinogène, la F-actine puisse présenter vis à vis de la myosine, les mêmes relations que la kératine des plumes vis à vis de l' α-kératine des mammifères.

7. L'architecture de la F-actine est discutée, en s'appuyant sur les indications obtenues précédemment, que les fibres protéiques peuvent avoir leur origine dans l'union bout à bout de corpuscules initialement globulaires.

8. L'analyse fine du diagramme de la fibre de F-actine montre que les corpuscules à partir desquels les fibres sont formées, ne sont pas unis au hasard, mais toujours de la même façon et avec une précision atomique.

9. L'actomyosine donne un diagramme de rayons X à grand angle, analogue à celui de la myosine.

10. La F-actine se transforme en la β-configuration normale par chauffage à 60° C.

ZUSAMMENFASSUNG

1. F-Actin, das bereitet wurde, indem man G-Actin durch Zufügung von KCl aktivierte, wurde mit Hilfe des Elektronenmikroskops studiert. Es ist faserig, und die Fasern scheinen sich durch Zusammenfügen runder Einheiten zu bilden. Die Resultate bestätigen im allgemeinen die Ergebnisse von JAKUS und HALL und stützen die indirekten Folgerungen von STRAUB und SZENT-GYÖRGYI.

2. Es wurde festgestellt, dass G-Actin aus runden Teilchen besteht.

References p. 392.

3. Actomyosin, dass aus Actin und Myosin in physiologischem Verhältnis bereitet wurde, übertrifft, wie festgestellt wurde, seine beiden Komponenten in dem Vermögen zur Bildung von querverbundenen Fasernetzen; diese Eigenschaft geht aber durch Anwesenheit von ATP verloren.

4. Die Interpretation dieser Wahrnehmungen wird diskutiert.

5. Eine Röntgenstrahlfaseraufnahme von F-Aktin wurde erhalten. Die Faserperiode ist mindestens 54 Å (angenähert), kann aber auch doppelt so lang sein.

6. Die Analogie zwischen den Faserdiagrammen von F-Actin und Federkeratin wird dargelegt, und es wird vorgeschlagen, dass in dem Grundschema der Keratin-Myosin-Epidermis-Fibrinogengruppe, F-Aktin sich zu Myosin verhalten könne wie Federkeratin zu Säugetier-α-keratin.

7. Die F-Actinstruktur wird diskutiert unter Bezugnahme auf frühere Anweisungen, dass Eiweissfasern durch die Ende-and-Ende-Zusammenfügung von ursprünglich runden Teilchen entstehen können.

8. Der detaillierte Aufbau des Faserdiagramms von F-Actin zeigt, dass die runden Einheiten, aus denen die Fasern gebildet werden, nicht auf willkürliche Weise aneinandergereiht sind, sondern immer auf gleiche Art und mit atomischer Präzision.

9. Actomyosin gibt, wie gefunden wurde, bei grossem Winkel ein dem Myosin ähnliches Röntgenbild.

10. F-Actin geht bei Erwärmen auf 60° in die normale β-Konfiguration über.

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THE ROLE OF SULPHYDRYL GROUPS IN THE INTERACTION OF MYOSIN AND ACTIN

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Much interest has recently been centred upon the work of the Szeged school under SZENT-GYÖRGYI concerning the interaction of myosin and a new protein, actin, first isolated by STRAUB[1]. Actin appears to be a fibrillar component which is extracted from minced muscle either in the form of an actin-myosin complex, or in its free form from muscle mince which, after appropriate treatment, is dried in acetone and then extracted with water. Of the nature and composition of actin, little is known. When first extracted, the aqueous solution is considered to contain corpuscular molecules which aggregate to a fibrous form on addition of salt (STRAUB[2]). This form of actin (F-actin) interacts with myosin in 0.5 *M*-KCl to give a system of greatly enhanced viscosity with all the properties of a weak gel. The addition of adenosinetriphosphate (ATP) ($\geqslant$ 0.00002 *M*) produces a sudden liquefaction and the viscosity falls to the additive effect of that of the two components. When, however, the actomyosin is precipitated as a filament in solutions of low ionic strength, the effect of ATP is to produce an *isodimensional* shrinkage or synaeresis. Although both effects will be discussed, only the interaction occurring at higher salt concentrations has been studied here. These phenomena are described in the detailed reports 'Studies from the Institute of Medical Chemistry University Szeged, S. KARGER: Basle and New York, 1941-2, 1942, 1943, Volumes 1, 2 and 3', and in several reviews (SZENT-GYÖRGYI[3, 4, 5]).

Electron microscope studies (PERRY and REED[6]; see also JAKUS and HALL[7]) have shown, as indeed could be predicted, that the actomyosin effect is one in which the discrete filaments of myosin anastomose freely with those of actin to give an interlocking network rather reminiscent of the fibrinogen-fibrin transformation (REED[8]). Why the two proteins interact in this way is of great interest, although the Hungarian workers have not essayed an explanation. In an attempt to solve the problem, we first explored the possibility that the interaction was of electrostatic type, and probably one between an acidic partner (myosin) and a more basic one (actin). On this view the role of ATP was to compete for the more basic protein. This line of attack was quite unsuccessful, as indeed the results of GUBA[9] had indicated they might be; he showed that the actin-myosin-ATP interrelations occurred at salt concentrations ($\mu = 2$) which might be expected to reduce very greatly the interaction of one protein with another. We were led therefore to the idea that the interaction was a specific one, rather like that between enzyme and substrate, and involving a special chemical grouping, although for this idea there was no precedent. It was fruitful, however, in the finding that the reactive SH groups of myosin are essential for the interaction: the same groups in fact which are

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References p. 516.

also necessary for ATP-ase activity. This correlation of an enzyme and colloid reaction partially explains the action of ATP in preventing the formation of actomyosin. A preliminary account of some of the results has already appeared (BAILEY and PERRY[10]).

SUMMARY

1. The interaction of actin and myosin in 0.5 *M*-KCl is not of normal electrostatic type but depends upon the presence of SH groups in the myosin partner.

2. The SH groups concerned are also connected with the adenosinetriphosphatase activity of myosin. Their oxidation by reagents such as iodosobenzoate, hydrogen peroxide, iodine, substitution by chloromercuribenzoate, or alkylation by iodoacetamide lead to a diminution of enzyme activity and of actomyosin-forming ability, the two properties declining at the same rate.

3. The groups concerned are mainly, but not entirely, those which give the nitroprusside test and are accessible to oxidants.

4. Whether a specific chemical grouping is necessary in the actin partner is not known, though several possibilities have been explored.

5. The findings elucidate why adenosinetriphosphate, which is the substrate for the enzyme reaction, so profoundly modifies the colloid reaction. It is suggested that enzymes utilizing adenosinetriphosphate are always of SH character.

6. The results are discussed in relation to the earlier work of SZENT-GYÖRGYI and the Szeged school.