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

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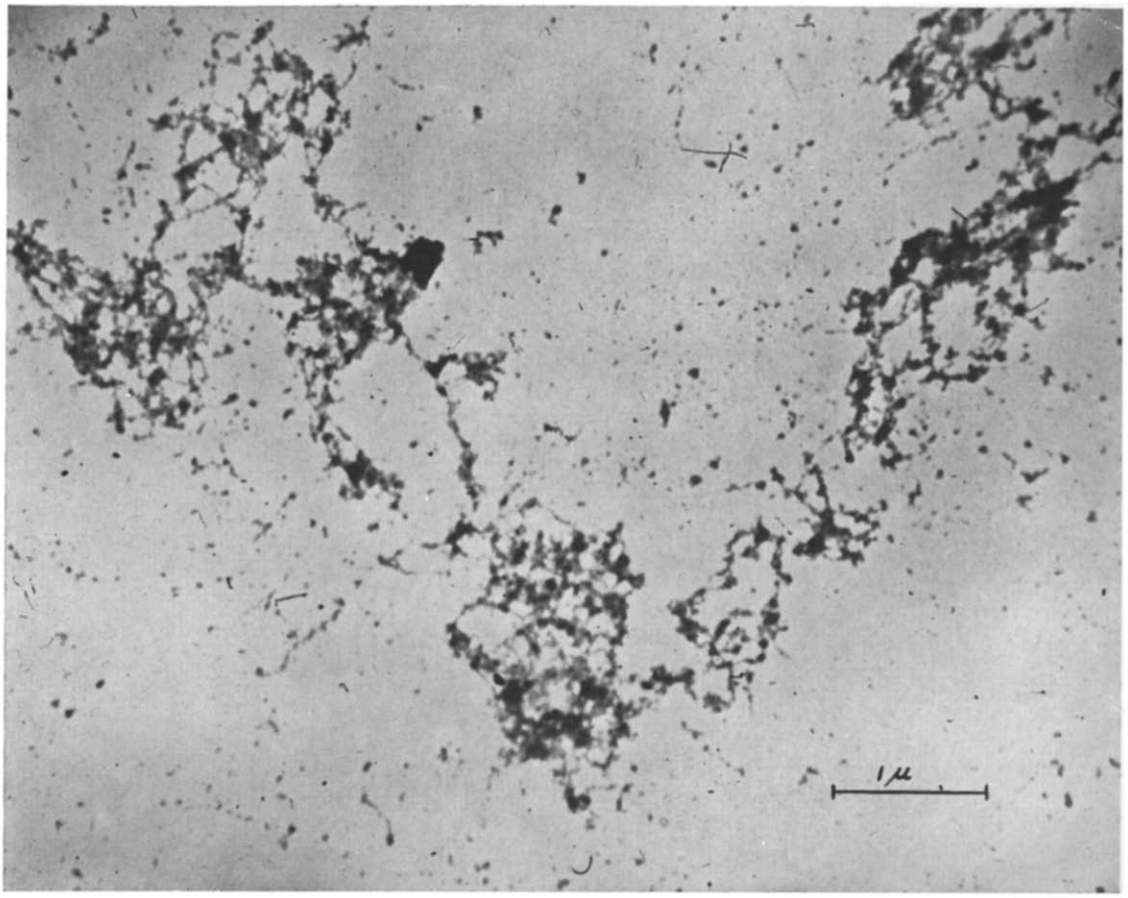

Fig. 1. F-actin from G-actin treated with 0.1 M KCl and dialysed. pH 7

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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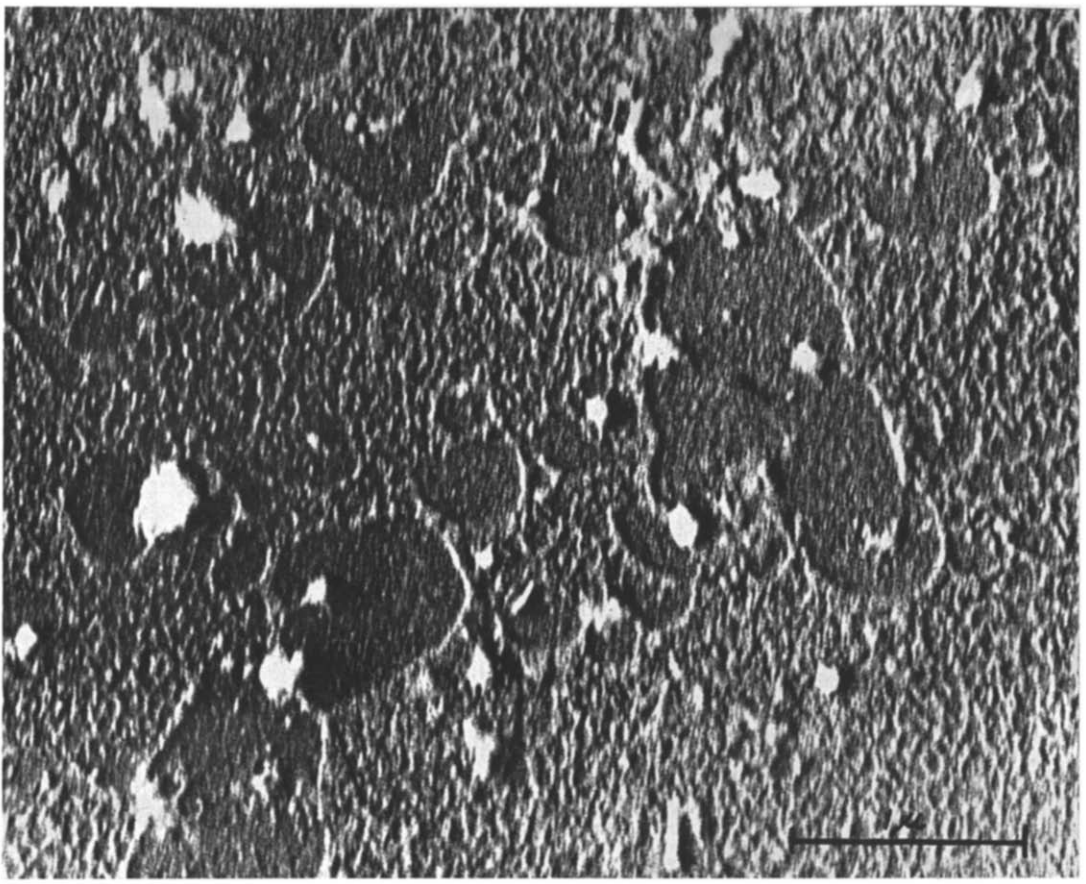

Fig. 2. F-actin from G-actin treated with 0.1 M KCl, pH 7, gold-shadowed

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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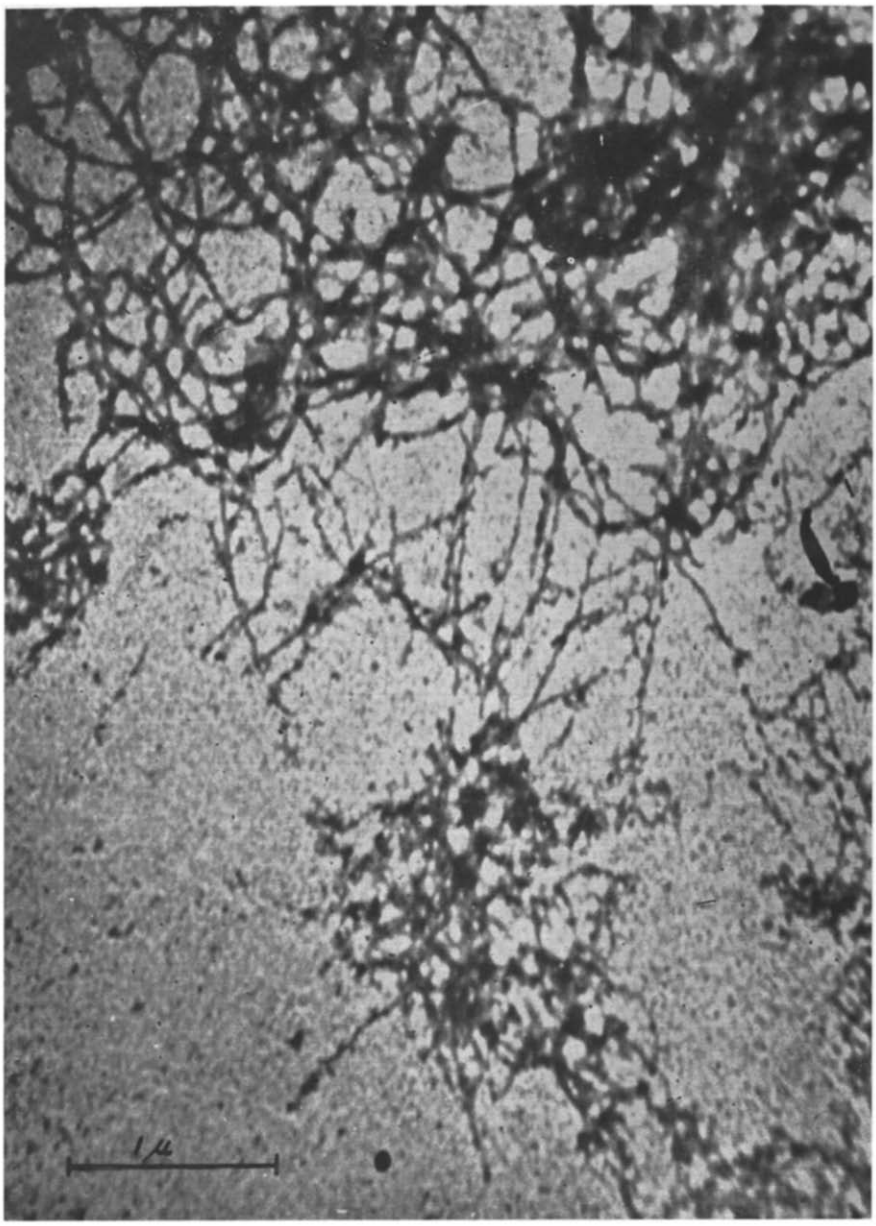

Fig. 3. F-actin from G-actin treated with 0.1 M KCl, pH 7

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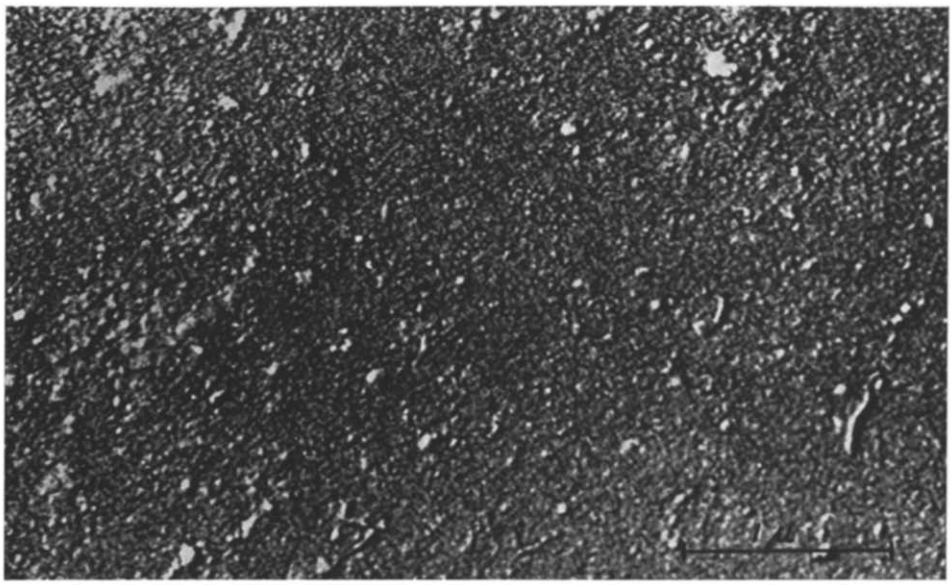

Fig. 4. G-actin, dialysed, pH 7, gold-shadowed

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

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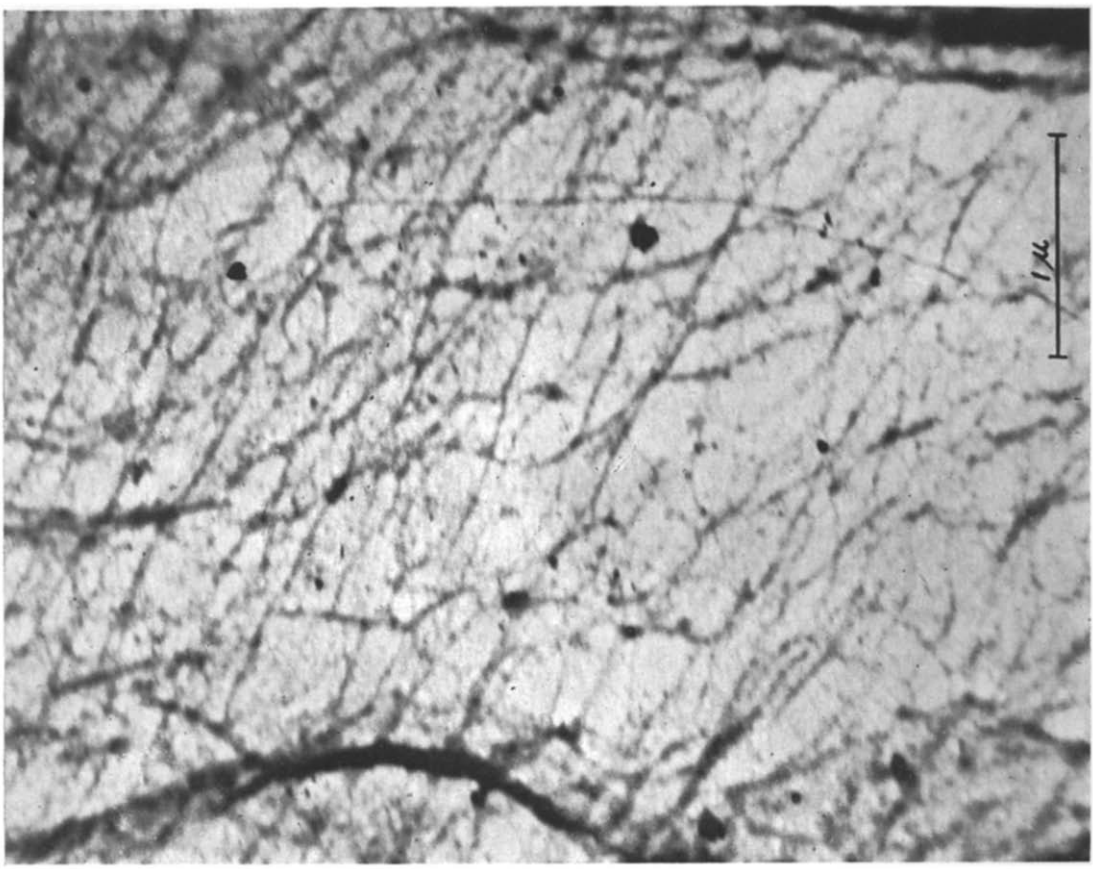

Fig. 5. Actomyosin from 2.5 parts F-actin and 8.4 parts myosin (actin iso-electrically precipitated), pH 7

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

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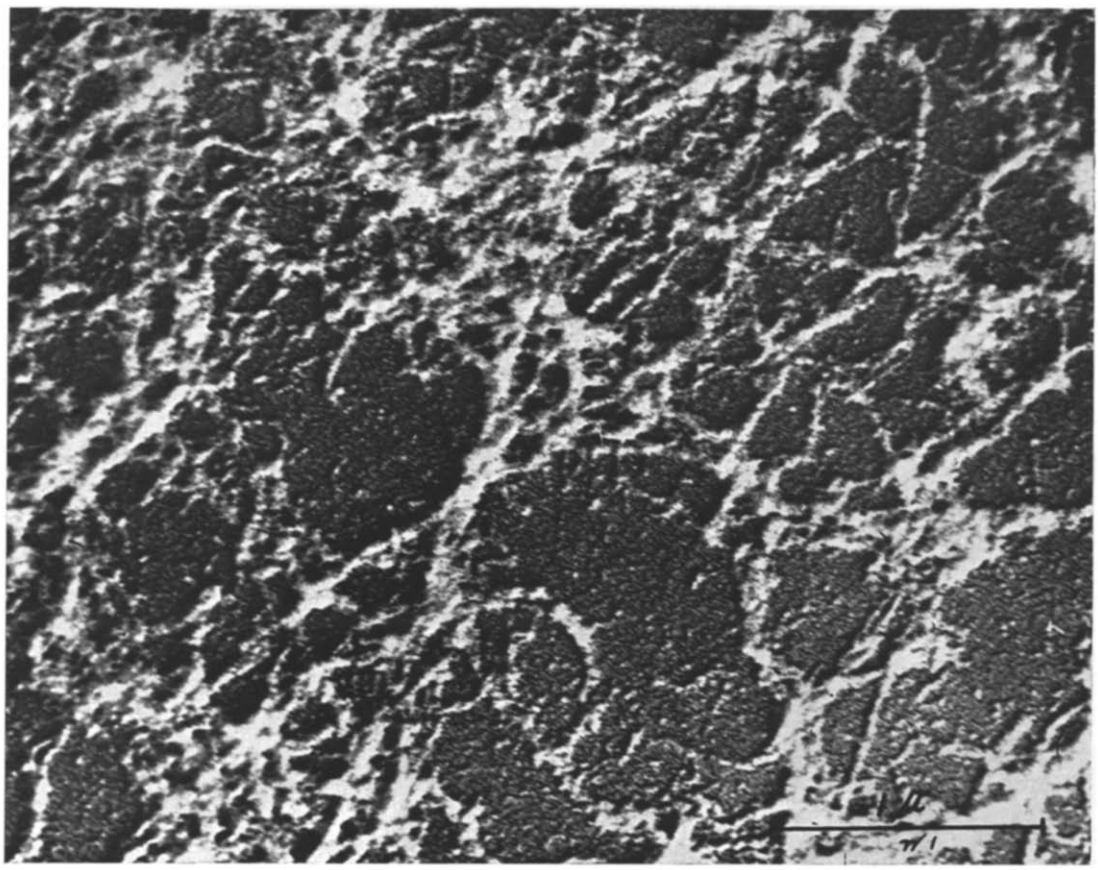

Fig. 6. Actomyosin from 3 parts F-actin and 8 parts myosin, pH 7, gold-shadowed

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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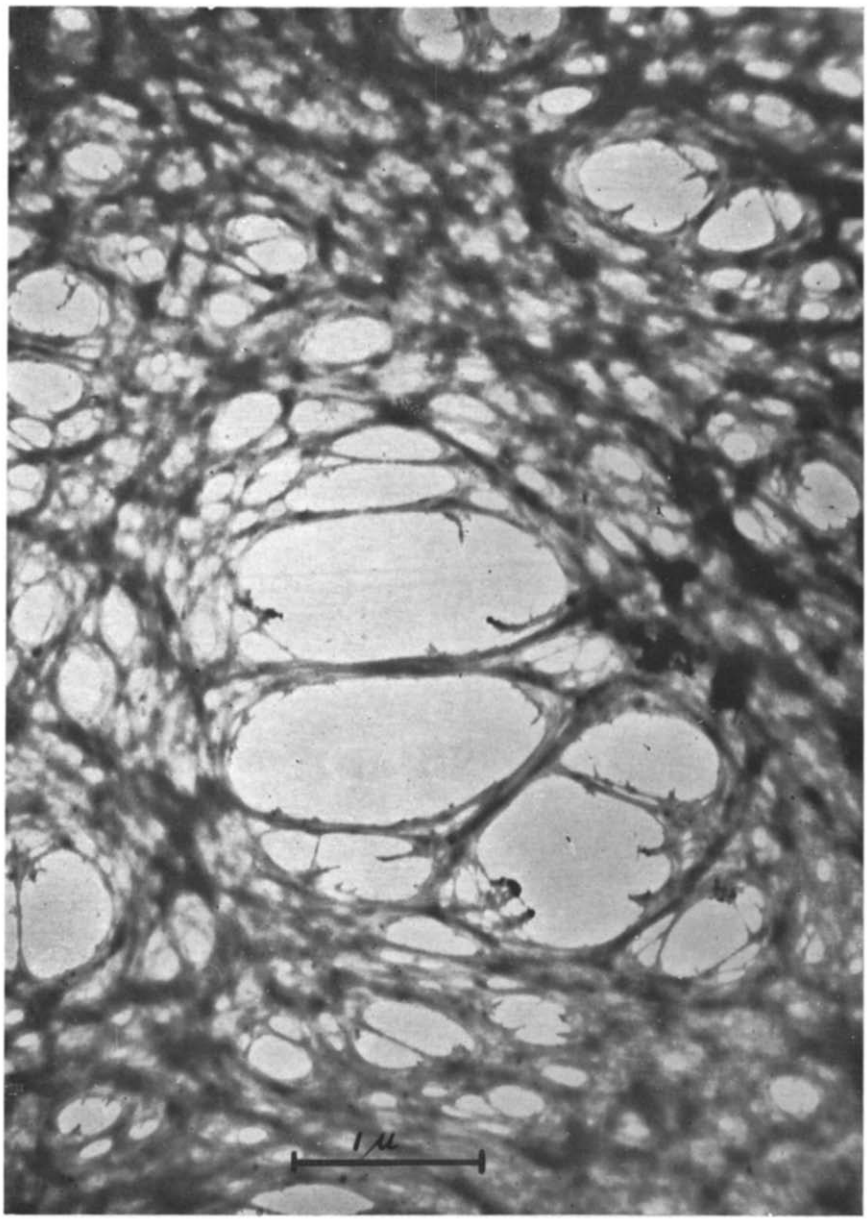

Fig. 7. Actomyosin from 2.5 parts F-actin and 8.4 parts myosin, pH 7

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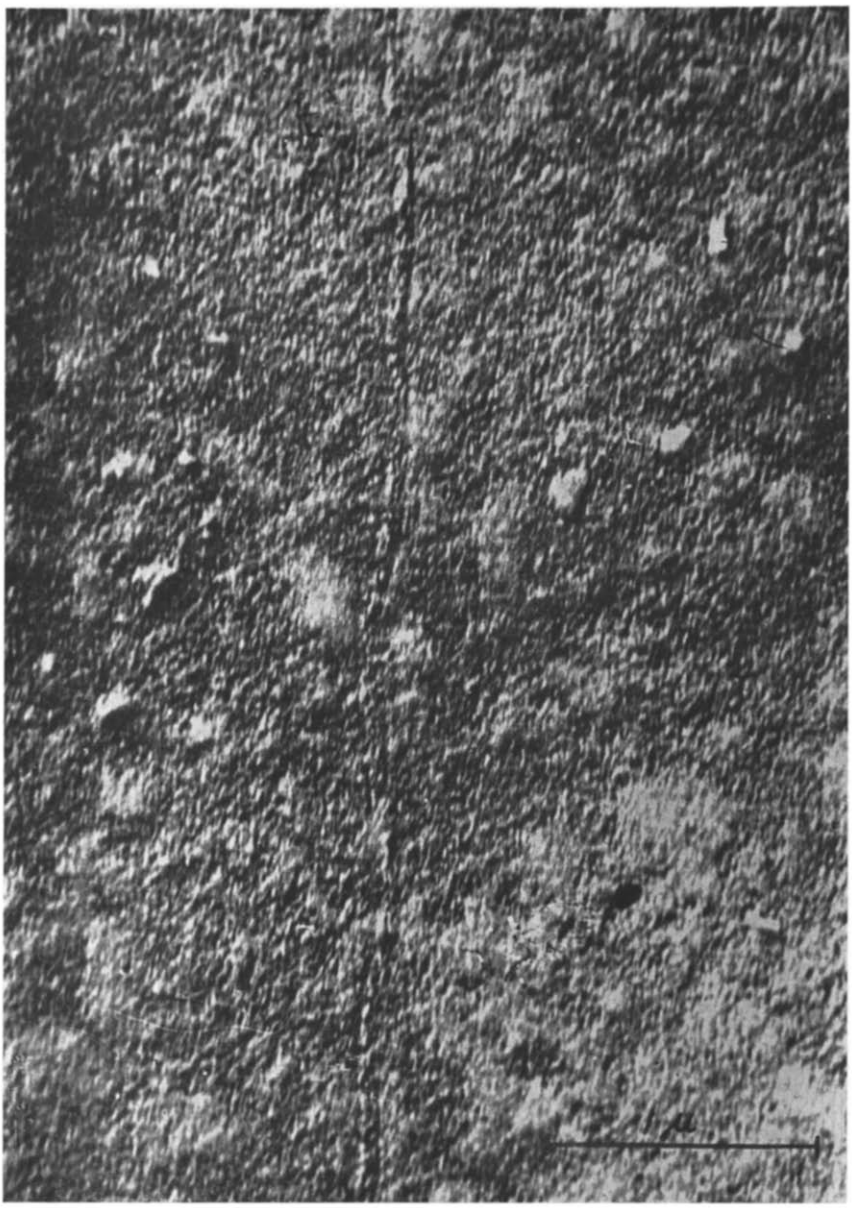

Fig. 8. Actomyosin plus ATP. Same actomyosin as used for Fig. 6 plus 0.0013 M ATP, p_H 7, gold-shadowed

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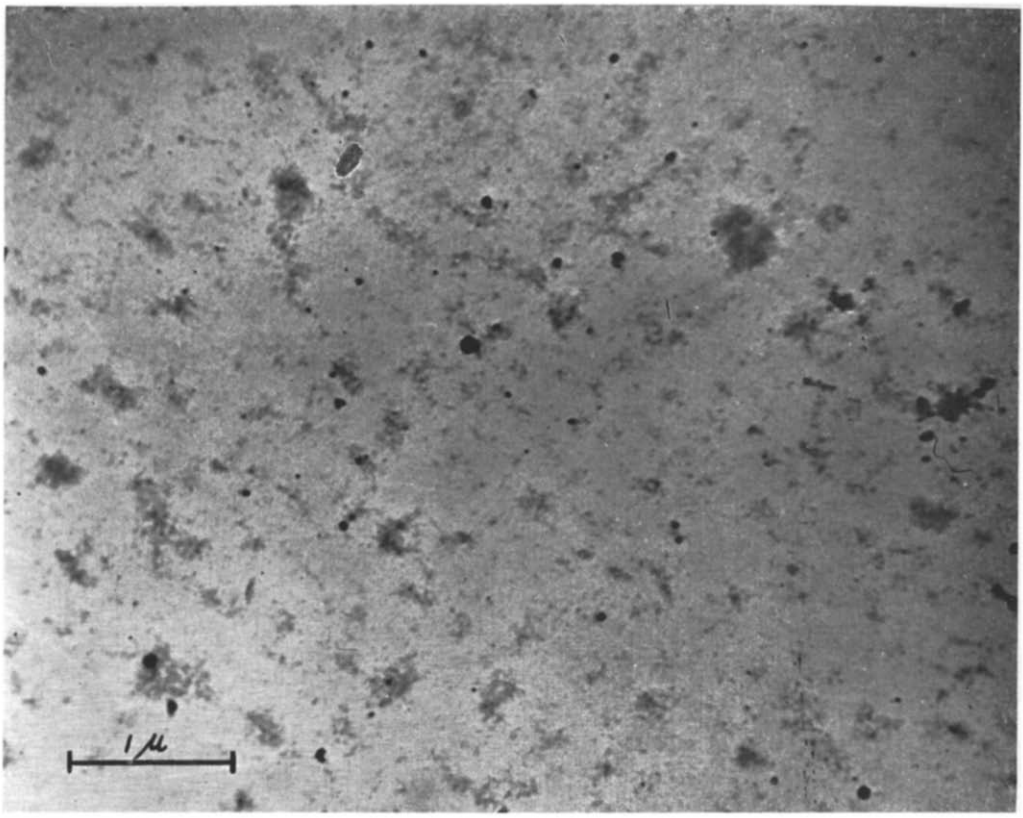

Fig. 9. Actomyosin plus ATP. Same actomyosin as used for Figs. 5 and 7 plus 0.0013 M ATP, pH 7

II. X-RAYS

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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

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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

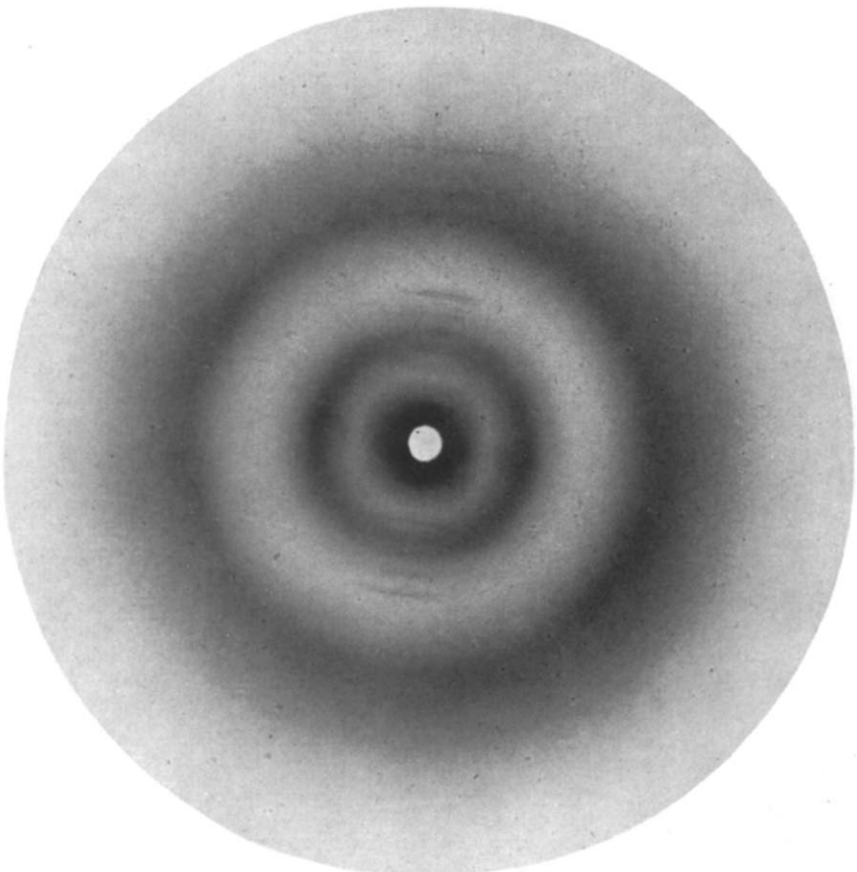

Fig. 10. X-ray fibre photograph given by F-actin film photographed with the beam parallel to the surface of the film. (CuKα rays; collimator 5 cm × 0.25 mm; original film-to-specimen distance 4 cm)

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-

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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'.

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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.

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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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