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When was actin first extracted from muscle?

Recently Vladimir Matveev of the Institute of Cytology, Russian Academy of Sciences, St Petersburg, who is responsible for the web page on the history of cell biology for the International Federation of Cell Biology, circulated an email asking the following question. 'Who is the discoverer of actin, in fact Straub or Halliburton?' Although I had been aware of this problem some years ago when I acquired Halliburton's book published in 1904, this query has stimulated me to attempt to reassess the whole question of when actin was first extracted from muscle.

It is well known that Straub (1942) isolated the protein actin that combines with myosin to form the complex actomyosin. The stimulus for its discovery was the attempt to explain the difference in properties between the two myosin preparations obtained by Banga and Szent-Gyorgyi (1941/42) when minced rabbit skeletal muscle was extracted with Edsall's salt solution (0.6 M KC1, 0.01 M Na_2CO_3, 0.03 M $NaHCO_3$). Myosin A was prepared by extraction for 20 min in the cold before centrifuging to remove the insoluble residue. Myosin was obtained if the extract was left overnight in the cold or 6 h at room temperature before centrifuging off the muscle residue. The two protein preparations differed in that myosin had a much higher relative viscosity than myosin A, which on addition of ATP was reduced to a similar value to that of myosin A. In contrast the viscosity of myosin A was only slightly reduced by the addition of ATP. History does not tell us whether the long extraction time was by intent or the result of an accident, nevertheless the observation had a profound effect on the development of muscle science and indeed cell biology in general.

Banga and Szent-Gyorgyi described the fall in viscosity produced by ATP as the 'activity' of the myosin preparations and considered that the longer term extraction 'activated' the myosin. The seminal observation for muscle biochemistry was the demonstration that fibres made by precipitating myosin at low ionic strength underwent contraction on the addition of ATP (Szent-Gyorgyi 1941/42a). Thus it was shown for the first time that a protein system isolated from muscle responded on addition of ATP, an important product of muscle enzymic activity, with a mechanical change very similar to that occurring in the intact tissue.

Straub was a medical student who at the end of his first year's examination had previously assisted Szent-Gyorgyi in his work on the yellow enzyme and the C4 acid cycle at Szeged, Hungary. He was invited to join the group working on the muscle proteins but agreed to do so somewhat reluctantly as his yellow enzyme work was progressing well. By application of an unconventional method of protein preparation involving the selective denaturation of the myosin by treating muscle with organic solvent, Straub was able prepare actin in substantial amounts in a relatively pure form by extracting the dehydrated muscle residue with water. His original method is substantially the same as that used today to prepare actin. On addition of this protein myosin A was converted into the 'activated' myosin form described by Banga and Szent-Gyorgyi. Therefore the protein was named actin and the complex formed with myosin, actomyosin (Szent-Gyorgyi, 1941/42b; Straub, 1942).

This work was carried out at the Institute of Medical Chemistry at the University of Szeged, Hungary, during World War II. Due to the hostilities in progress it was not possible for Szent-Gyorgyi to describe his results in Western scientific journals so he had the work published in English privately in Hungary in three special numbers of Studies from the Institute of Medical Chemistry University Szeged (1941-1943). Knowledge of the actomyosin work became more readily available to the West in 1945 when it was published as a supplement to the Acta Physiologica Scandinavica (Szent-Gyorgyi, 1945).

At approximately the same time Joseph and Dorothy Needham and colleagues in Cambridge, unaware of the actomyosin work going on in Szeged, were studying the properties of myosin. They had confirmed Engelhardt and Ljubimova's report of the ATPase activity of myosin as indeed had other workers (Szent-Gyorgyi and Banga, 1941; Bailey, 1942; Needham, 1942). Stimulated by the earlier work on myosin of v. Muralt and Edsall (1930) demonstrating the anisometric character of the myosin molecule they were struck by the fact that some of their myosin preparations obtained by salt extraction of rabbit skeletal muscle exhibited strong flow birefringence and marked viscosity (Needham *et al.,* 1941). They noted that not all of their normal myosin preparations had these properties but if the rabbits were starved for 24 h before slaughter the myosin invariably was strongly birefringent. In these cases it can be presumed that the glycogen level was depleted and that glycolysis and ATP production were very low or absent during extraction. These conditions would favour actomyosin extraction as did the conditions for myosin preparation. On addition of ATP to the myosin birefringence and viscosity dropped but returned to the original value on standing, the time taken depending on the temperature. After the birefringence and viscosity was restored to its original value the effect could be repeated by further addition of ATP. This evidence and the fact that ATP was uniquely effective in producing the reversible effect indicates clearly that the Needhams and collaborators were extracting actin with their myosin and their preparation was in fact an actomyosin. It of interest that Szent-Gyorgyi heard of the Needhams' (1941) work from a reprint he received from Verzar in which it was

quoted, but he was unable to access the paper at the time (see footnote to Szent-Gyorgyi, 1941/42b).

Astbury (1950), who was the first to use the term molecular biology applied in its broadest sense and not as it is commonly used today, had at that time proposed that muscle contraction was essentially due to the molecular contraction of protein chains (Astbury and Bell, 1938). In consequence the Needham group believed the birefringence changes they observed reflected a change in length of the contractile molecule, myosin, when it split its substrate, ATP. This interpretation was completely compatible with the earlier demonstration by v. Muralt (1932) that during a single isometric twitch the birefringence of muscle decreases but returns to its original value on relaxation. At that time the Needham group did not have evidence for the presence of an additional protein in the contractile system but with hindsight they were clearly extracting actin complexed to myosin. In the light of their findings they suggested that muscle contraction is essentially an enzyme substrate combination (Needham, 1942).

In 1968, Fink raised the question whether actin may have been isolated from muscle very much earlier, in 1887, by Halliburton working in London. He came to this conclusion from Halliburton's paper 'On muscle plasma' (70 pages long!) in which it was shown that myosin extracts 'coagulated' when another extract 'muscle ferment' was added to them. This work was further described in a series of lectures published in book form (Halliburton, 1904).

Kuhne (1859) showed that muscle plasma obtained as press juice from frog skeletal muscle on standing at room temperature formed a clot which was composed of a protein he called myosin. He was not able to show this effect with the muscle of warm-blooded animals but Halliburton succeeded by making his extractions in the cold. Halliburton showed that salt solutions were particularly effective in extracting muscle plasma but unlike the press juice extract the salt extracts did not coagulate spontaneously. On diluting the original extract in 10% NaCl (1.7 M) to 0.42 M coagulation and 'contraction' of the clot occurred in a few hours at room temperature and more rapidly at 37°C. This clot formation was thought to represent the changes occurring in the muscle protein in situ during contraction or in rigor mortis. The coagulated form of the protein was considered to be myosin and Halliburton suggested that the original plasma protein as extracted was a precursor that he called myosinogen, which was converted to myosin by a ferment, myosin ferment. It was believed at the time that the clot formation obtained with muscle plasma was analogous to the clotting of blood where fibrinogen was converted to fibrin by fibrin ferment. Halliburton made a preparation from muscle by exactly the same method as that used by Schmidt to prepare fibrin ferment from blood which accelerated clot formation in blood plasma Muscle was chopped up and kept under absolute alcohol for 3-10 months, dried and powdered. Myosin ferment was an aqueous extract of this powder. When muscle plasma was diluted with ferment it brought about coagulation much more rapidly than dilution with water. Halliburton proposed that muscle contained a ferment that converted the myosinogen in the plasma to myosin. It is of interest that in his book he appears to be slightly less positive about myosin ferment. He concludes 'I have already alluded to the possible existence of a myosin ferment concerned in muscle coagulation. I will only add that if it does exist it is not identical to fibrin ferment (Halliburton 1904).

How do we interpret Halliburton's finding in the light of modern knowledge of the muscle proteins? There is little doubt that his extract probably contained actin, for the alcohol-treated muscle residue is very like that used by Straub for the extraction of actin. He showed the myosin ferment contained protein that could be precipitated out by ammonium sulphate and which retained its ferment activity. As the whole tissue rather than washed muscle was treated with alcohol, the dried residue no doubt contained soluble glycolytic enzymes, some substrates and possibly ATP, all of which could be extracted in the ferment. Of more concern for the interpretation of Halliburton's results is the nature of the clotting and coagulation processes he describes. There is no doubt that his muscle plasma contained myosin as we know and describe it today. He reports that myosin is precipitated if the plasma extract is diluted 10-20 times with water but states the precipitate obtained on dilution differs in appearance to the clots he obtains from muscle plasma. The clots he describes occurred at 0.42 M NaCl at which ionic strength myosin is quite soluble at pH 7. He does report that the extracts became more acid and it is possible the pH is dropping on standing at room temperature and he is observing isoelectric precipitation. Traces of glycolytic enzymes and their substrates present in the original extract and or the myosin ferment would produce lactic acid. Halliburton appears to be aware of the possibility of lactic acid reducing the pH of his extracts. Actin present in the ferment would form actomyosin which is less soluble than myosin itself and probably more readily precipitated under acid conditions.

The information required to fully assess Halliburton's contribution to the actomyosin story is to know whether he was observing the superprecipitation and synaeresis effect produced by low concentrations of ATP on actomyosin precipitates. This effect is unique and when observed can be distinguished from normal protein precipitation for the contracted protein particles are much more dehydrated and settle rapidly. It is unlikely that any free ATP was present in the diluted muscle plasma consisting of myosin, the enzyme that breaks it down. Clearly the clot produced from the water-diluted plasma is myosin, with perhaps a small amount of actin present. It is possible that low concentrations of ATP were present in the myosin ferment as explained above. If that is the case any actomyosin produced by the addition of actin in the ferment would superprecipitate at low ionic strength. This does not appear to have happened for

Halliburton does not comment on any difference between the clot observed on dilution of muscle plasma with water compared to that seen when muscle ferment is used. The ferment merely increased the rate of clot formation. A possible explanation is that the ferment speeded up lactic acid formation and isoelectric precipitation.

In conclusion: (1) It is very likely that Halliburton was the first to make an extract containing actin from muscle, his myosin ferment. Addition of this to myosin would produce an actomyosin. ATP had not been discovered in 1887 but there is no convincing evidence that his myosin ferment was exhibiting an effect on the actomyosin produced which could be ascribed to ATP. (2) Both Szent-Gyorgyi and Banga and the Needham group independently extracted actomyosin from muscle round about 1941 and showed that ATP produced reversible physical effects on the solution. (3). The major credit must go to Straub for the discovery of the second protein component of the contractile system, actin. The influence of Szent-Gyorgyi on this discovery is clear from the glowing tribute paid in Straub's memorial article on the charismatic role of his mentor in the work of the group at Szeged (Straub, 1987).

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