ULTRACENTRIFUGAL ANALYSIS OF CRYSTALLISED MYOSIN

by

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As we have shown previously, very variable values for the molecular weight of myosin are found in the literature, depending on the fact that the solutions used so far were not sufficiently well-defined but that determinations were made on mixtures of myosin and actomyosin. The most reliable measurement should be that which BERGGOLD[1] has carried out on a myosin which WEBER has obviously separated from actomyosin. BERGGOLD gives a value of $1.5 \cdot 10^6$, but further details of this experiment are required to form an opinion of it.

In the experiments described below we used a myosin prepared in crystallised form according to SZENT-GYÖRGYI[2]. In order to obtain an idea of the character of these crystals they were investigated with the aid of the electron microscope.

The crystals dissolved in pure water, but were insoluble in 0.02 M KCl and in this latter solvent they could be studied.

Figs 1–3 are gold shadowed[3] photographs of such crystals. Here the crystals have an appearance which resembles that expected for anisotropic liquid crystals. One could see quite clearly how the ribbon-like structures were composed of long narrow threads. The myosin particles, even when they do not precipitate as crystals, have an obvious tendency to combine into long threads. Under certain conditions one can even see the same type of anisotropic liquid structure with fibrous actin. These structures were completely or partly broken up when the crystal suspensions were treated with super-sound. Thus these structures are similar to those which pertain to tobacco mosaic virus crystals.

The crystallised myosin obtained in this way cannot be absolutely pure since impurities are easily included in such crystals, but it appears from certain other investigations which we made that these impurities do not affect the results of the ultracentrifugal investigations to an essential degree.

ULTRACENTRIFUGAL INVESTIGATIONS OF CRYSTALLISED MYOSIN

Ultracentrifugal analysis has been carried out on both once and twice crystallised myosin. For the theory and technique of the ultracentrifuge we refer to SVEDBERG AND PEDERSEN, *The Ultracentrifuge*, Oxford 1940.

SEDIMENTATION CONSTANT OF MYOSIN

Veronal buffer

The first investigations were made in veronal buffer with 0.46 M KCl and 0.14 M K-veronal-acetate buffer at p_H 6.8.

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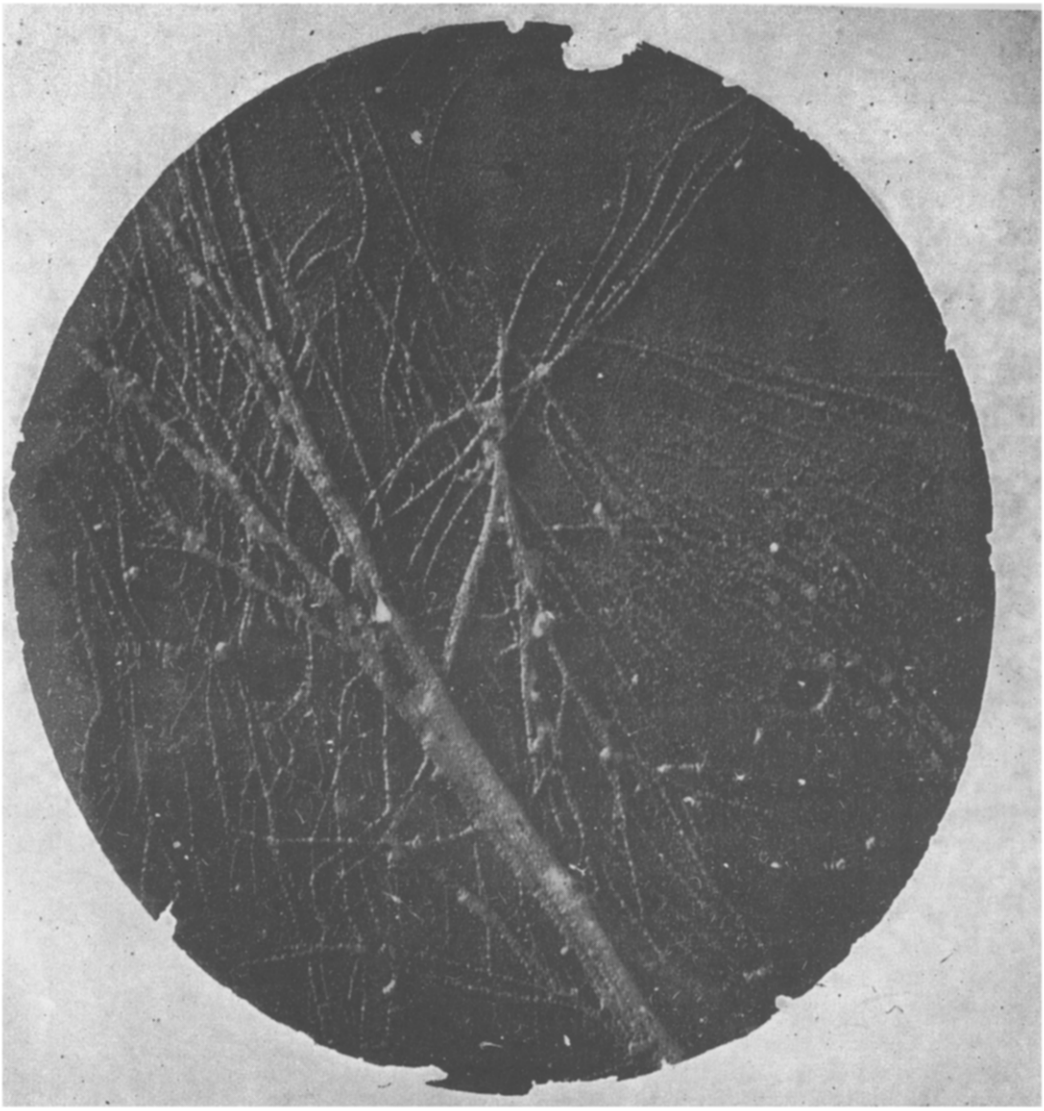

Fig. 1. Electron micrograph of crystallised myosin prepared with gold by the shadow-casting technique. Magnification 26000 ×.

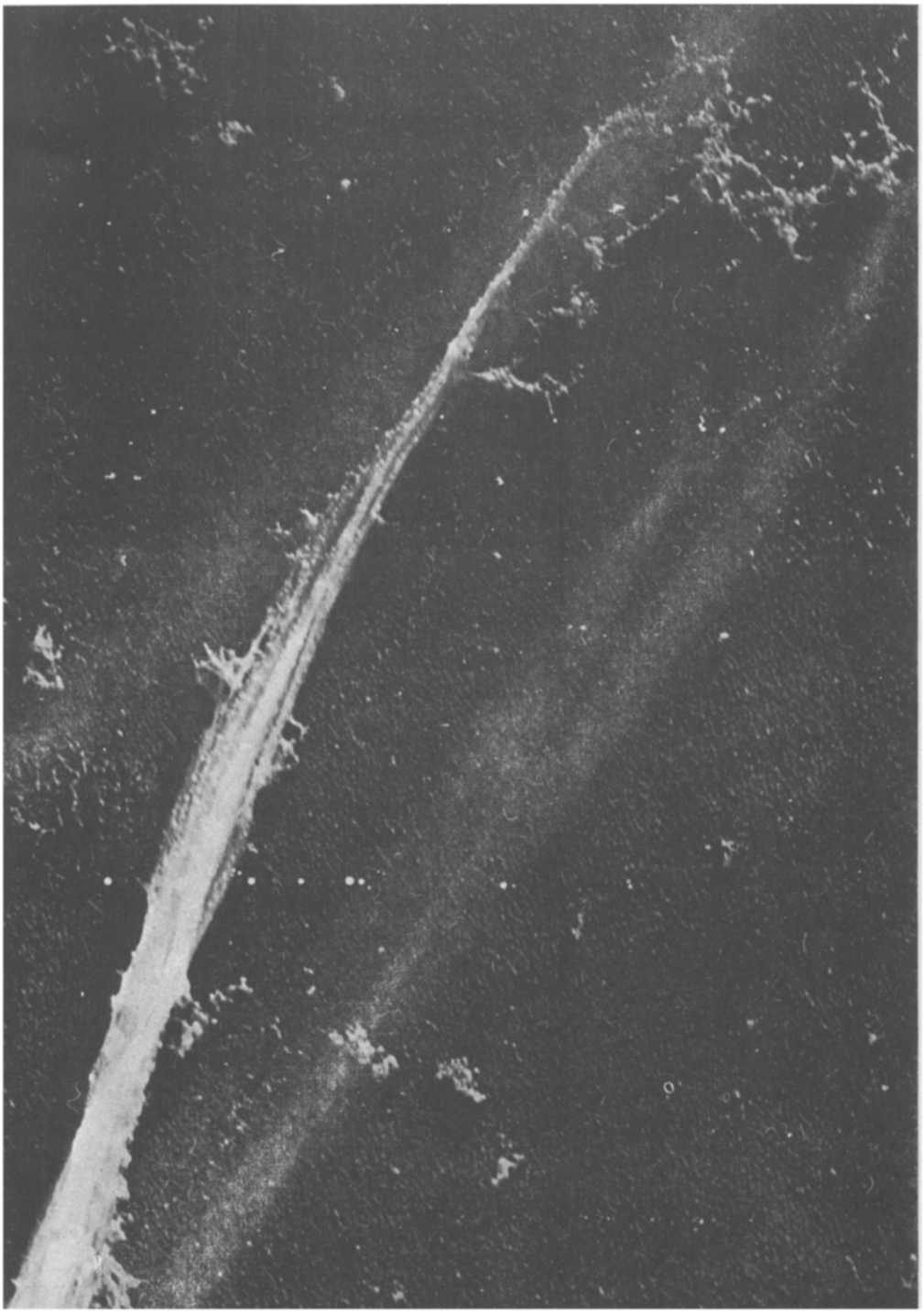

Fig. 2. Electron micrograph of a crystal needle of myosin prepared with gold by the shadow-casting technique. Magnification 40000 ×.

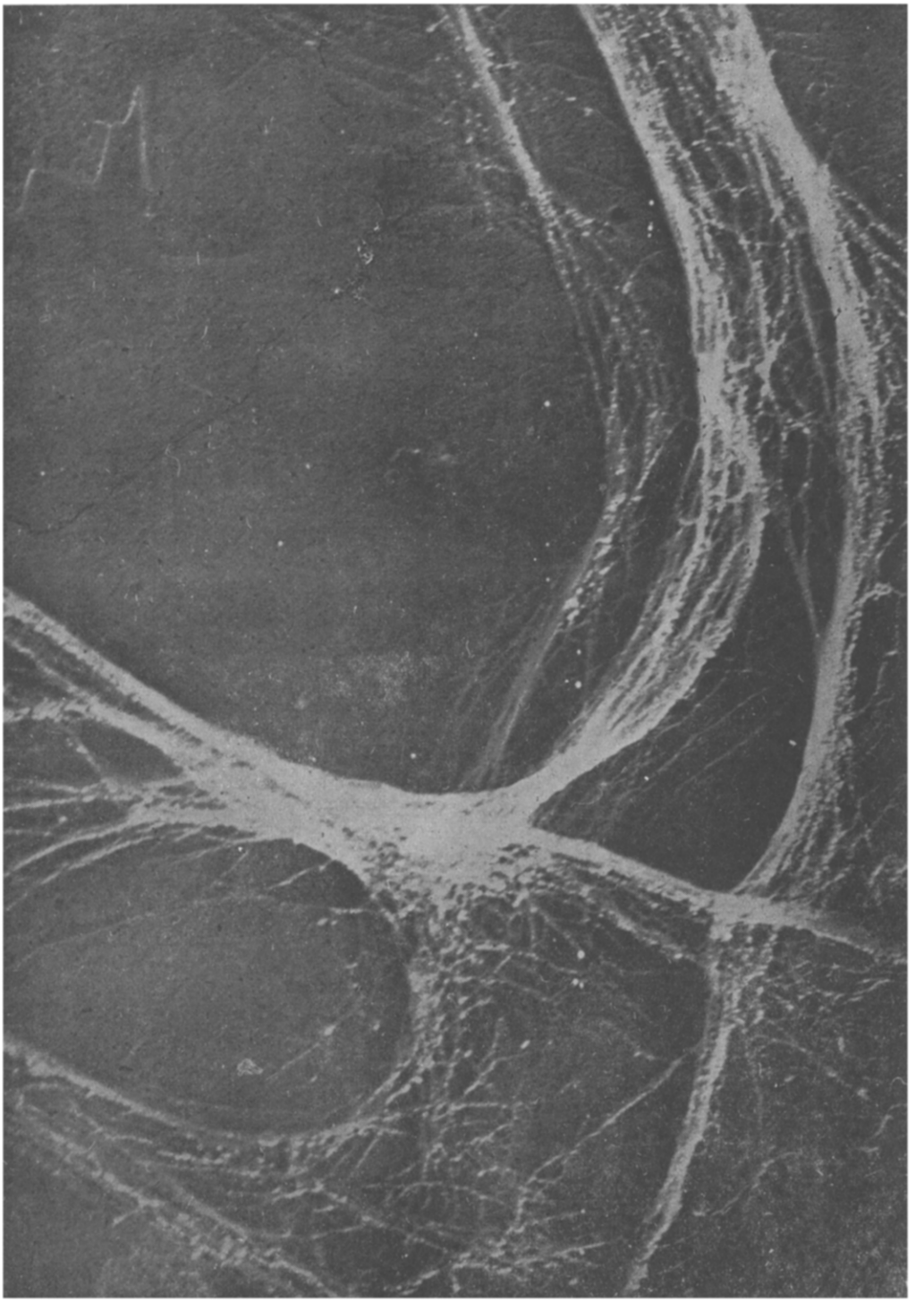

Fig. 3. Electron micrograph of crystallised myosin treated with super-sound waves prepared with gold by the shadow-casting technique. Magnification 40000 ×.

The sedimentation diagrams mainly exhibited the presence of one component. However, the material was not homogeneous since after the sedimentation had gone on for some time a smaller component appeared which sedimented more quickly but could impossibly be separated from the main component (Fig. 4). Its concentration and sedimentation constant could not be determined. This effect was more pronounced for myosin which had been crystallised twice (Fig. 5). It is possible that we have here some denatured material since the presence of the lesser component is more marked in the case of myosin which has been crystallised twice. At the second crystallisation the myosin was exposed to a more severe treatment with base in order to remove the last remains of actin.

The sedimentation constant was dependent upon the concentration (Fig. 6). It could be extrapolated to the concentration c = 0, where the sedimentation constant was $S_{20} = 7.2$*. This is the same sedimentation constant as we found for one of the components in the EDSALL-GREENSTEIN solutions. The extrapolated sedimentation constant was the same for both once and twice crystallised myosin.

Phosphate buffer

A series of investigations were carried out in phosphate buffers. We investigated myosin partly dissolved in phosphate buffer alone, 0.1 M KH_2PO_4 + K_2HPO_4 at p_H 7.6, and partly in 0.1 M KH_2PO_4 + K_2HPO_4 with 0.5 M potassium chloride at p_H 7.0.

The sedimentation diagrams now also depicted a main component, but the more rapidly sedimenting component as was observed in veronal buffer was not so pronounced.

The sedimentation constant was dependent upon the concentration. A calculation was made of the concentration dependence of the sedimentation constant, using the concentration of myosin which can be calculated from the increment of the index of refraction and the areas of the sedimentation diagrams. Such an investigation leads to the same result for the value of S at c = 0 as in the case of veronal buffer, namely $S_{20} = 7.2$. The concentration dependence was greater when 0.5 M potassium chloride was present than with phosphate solution only.

Addition of ATP to the myosin solutions did not lead to any change in the sedimentation diagrams.

DETERMINATION OF THE DIFFUSION CONSTANT

Determination of the diffusion constant was carried out with once crystallised myosin in veronal buffer. Owing to the great sensibility to metals only glass cells could be used.

The experiment was carried out at 15° C and the diffusions lasted for 5–7 days.

The diffusion curves obtained were skew, indicating that we had very long molecules or molecules with a considerable solvent layer.

Corrected for the skewness they gave a diffusion constant of $D = 0.5 \cdot 10^{-7}$. This is a very low value and if it is used for calculation of the ideal sedimentation diagram a "schlieren" curve is obtained.

THE MOLECULAR WEIGHT OF MYOSIN

A determination of the partial specific volume has also been carried out with a

* The sedimentation constants are calculated in Svedberg units (10^{-13}).

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2 % solution. (Prof. C. DRUCKER has kindly carried out this determination for us). We obtained a value of 0.74, nearly the same as one obtains for other proteins.

From these data it is possible to calculate the molecular weight of myosin with the use of the SVEDBERG molecular weight formula.

$$M = \frac{R\,T\,S_{20}}{D_{20}(1 - V_\varrho)}$$

If the obtained values are inserted one finds a molecular weight of $1.5 \cdot 10^6$. The frictional ratio which can be calculated from the formula

$$f/f_0 = 10^{-8}\left(\frac{1-V_\varrho}{D_{20}^2 \cdot S_{20} V}\right)^{1/3}$$

is in this case: $f/f_0 = 5.5$. This is a very large value for a protein. The frictional ratio f/f_0 is a function of the shape of the particle and its hydration.

EFFECT OF UREA ON MYOSIN

Earlier WEBER AND STÖVER[4] considered to have found that on addition of urea to their myosin solutions the myosin dissociated into particles with a molecular weight of 10^5. In this investigation the molecular weight was determined by the osmotic method.

GUBA[2] has found that on treating myosin with urea the viscosity diminishes to "the value of globular protein".

We have found that on addition of 6 M urea the viscosity decreased to $^1/_3$ of the original value. If the urea was removed again after about one hour by dialysing against the usual solvent, 0.5 M potassium chloride and veronal buffer, the original viscosity was again obtained. If we allowed the myosin to stand with urea for a longer time the viscosity diminished more and more when determined after the urea was removed by dialysis, and after about seven days it was about $^1/_3$ of the original value.

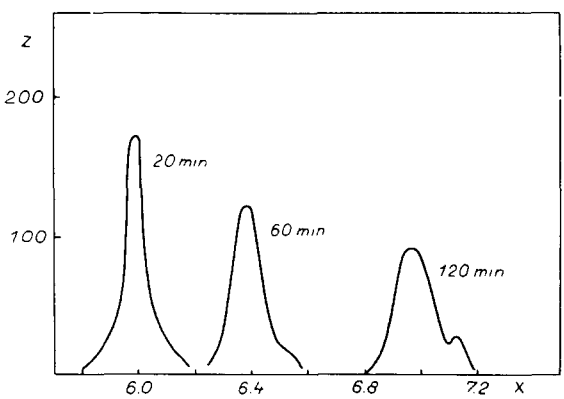

Fig. 4. Sedimentation diagram of once crystallised myosin in 0.5 M KCl (K-veronal-acetate buffer pH 6.8).

An ultracentrifugal analysis showed that the myosin became more and more polydisperse in the course of time. Fig. 7 shows a sedimentation diagram for a myosin solution which was treated with urea for one hour. The diagram exhibits only one component. The extrapolated sedimentation constant was about: $S_{20} = 16$. A determination of the diffusion constant gave a value of $D_{20} = 1.2 \cdot 10^{-7}$. These data give the same molecular weight as in the case of untreated myosin but the frictional ratio f/f_0 is now 2.5, *i.e.*, considerably lower, showing that certain changes took place with the myosin.

After seven days' treatment of the myosin with urea a sedimentation constant was obtained which showed that we had a very polydisperse substance (Fig. 8). Parts of the myosin particles had obviously been split off. The main component with $S_0 = 16$

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was still evident but material with a lower rate of sedimentation appeared as well. Often a component was observed with a sedimentation constant of about 2–3. It is possible that this part of the material corresponds to the proteins of SZENT-GYÖRGYI[5].

On dialysing away the urea against buffered 0.5 M KCl solution the solutions generally became very polydisperse. It proved to be almost impossible to obtain completely reproducible results. In urea the experiments were also very difficult to carry out owing to the large urea concentration. A test was made according to STRAUB to determine viscosimetrically if there was any myosin in the solutions which could react with actin and from the more highly viscous actomyosin. The results appear in Table I.

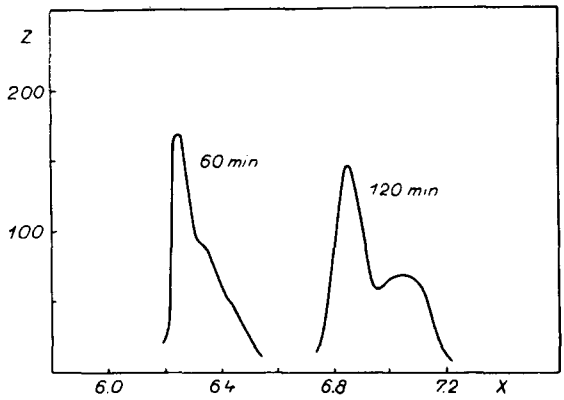

Fig. 5. Sedimentation diagram of twice crystallised myosin in 0.5 M KCl (K-veronal-acetate buffer pH 6.8).

TABLE I

VISCOSIMETRIC INVESTIGATION OF MYOSIN TREATED WITH UREA

Time in urea	Solution	η spec. Before addition of urea	η spec. 6 M urea	η spec. After dialysing away the urea
	M*	0.25	0.065	0.23
1*h*	M + A*	0.87	0.064	0.38
	M + A + ATP*	0.33	0.064	0.38
	M	0.28	0.058	0.18
24*h*	M + A	0.81	0.059	0.29
	M + A + ATP	0.36	0.058	0.29
	M	0.28	0.064	0.11
3*d*	M + A	0.87	0.065	0.21
	M + A + ATP	0.33	0.065	0.21
	M	0.26	0.063	0.068
7*d*	M + A	0.81	0.064	0.128
	M + A + ATP	0.37	0.064	0.126

Viscosity of the actin solution $\eta_{spec.}$ 0.07.

* M = Myosin A = F-Actin ATP = adenosintriphosphate

The experiments show that we certainly obtained a smaller increase of viscosity on addition of F-actin. Had this increase of viscosity depended upon the F-actomyosin the viscosity would have decreased again on the addition of adenosintriphosphate (ATP). But as this is not the case the increase of viscosity cannot be due to the formation of ordinary actomyosin.

The difference between our values and those of WEBER AND STÖVER is due to the

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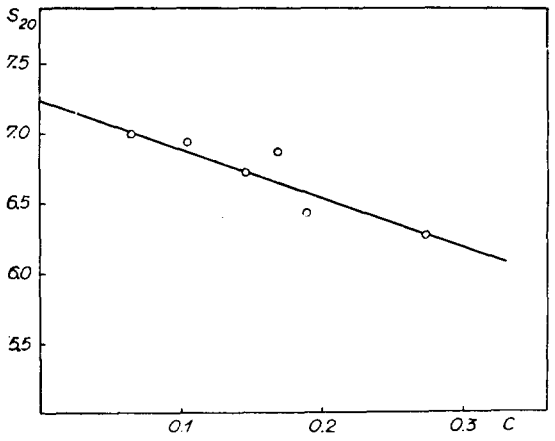

Fig. 6. The dependence upon the concentration of the sedimentation constant for crystallised myosin (The concentration is given in per cent).

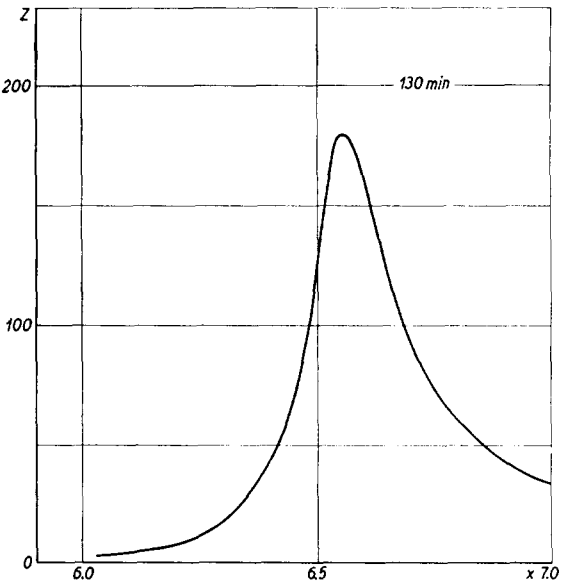

Fig. 7. Sedimentation diagram of crystallised myosin in urea. Urea (6 M) added one hour before the beginning of the experiment (p_H 6.8).

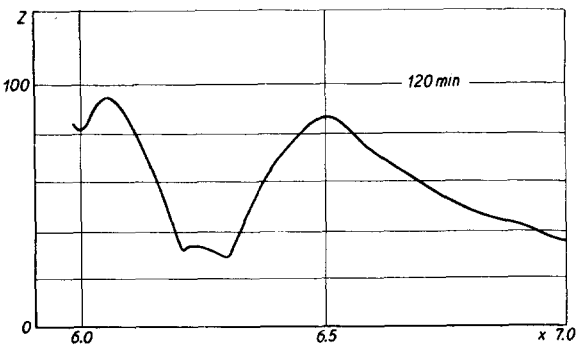

Fig. 8. Sedimentation diagram of crystallised myosin in urea. Urea (6 M) added seven days before the beginning of the experiment (p_H 6.8).

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fact that the osmotic method only gives one value, an average molecular weight, while the ultracentrifuge gives an analysis of the solution. If, therefore, as in this case we have a mixture of myosin and some degradation products, the osmotic method gives an average value which has no physico-chemical meaning either.

We wish to express our thanks to Prof. THE SVEDBERG for enabling us to carry out this work. The investigation has been supported financially by "Svenska Naturvetenskapliga Forskningsrådet".

SUMMARY

Our experiments show that crystallised myosin is accompanied by a rather small amount of impurities. Electron micrographs indicate that the myosin crystals are of anisotropic liquid nature. The myosin particles here appear to be aggregated to form long fibrils.

An ultracentrifugal determination of the molecular weight gave a value of $1.5 \cdot 10^6$.

The crystallised myosin is partly broken down into smaller fragments on treatment with urea for a length of time. On treating with urea for a short time the molecular weight is unaltered although other properties change; among others the myosin loses its power to form actomyosin.

RÉSUMÉ

Nos expériences montrent que la myosine cristallisée est accompagnée d'une quantité assez faible d'impuretés. Les micrographies électroniques indiquent que les cristaux de myosine sont des cristaux liquides anisotropes. Les particules de myosine se montrent alors agrégées en longues fibrilles. La détermination par ultracentrifugation du poids moléculaire fournit une valeur de $1.5 \cdot 10^6$.

La myosine cristallisée est partiellement dissociée en fragments plus petits par traitement avec de l'urée pendant un temps suffisant. Le traitement par l'urée pendant un temps court ne modifie pas le poids moléculaire, quoique certaines propriétés de la myosine changent. C'est ainsi que la myosine perd son pouvoir de former de l'actomyosine.

ZUSAMMENFASSUNG

Unsere Versuche zeigen, dass kristallisiertes Myosin von einer ziemlich kleinen Menge Verunreinigungen begleitet ist. Elektronenmikrografien zeigten, dass die Myosinkristalle anisotropen Flüssigkeitscharakter haben. Die Myosinteilchen sind hier offensichtlich so aufeinandergehäuft, dass sie lange Fibrillen bilden. Eine ultrazentrifugale Molekulargewichtsbestimmung ergab einen Wert von $1.5 \cdot 10^6$.

Durch Behandlung mit Harnstoff während längerer Zeit wird Myosin zum Teil in kleinere Bruchstücke aufgespalten. Bei Harnstoffbehandlung von kurzer Dauer bleibt das Molekulargewicht unverändert, obwohl andere Eigenschaften sich ändern; unter anderem verliert Myosin die Fähigkeit, Aktomyosin zu bilden.

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