that the two $\alpha 1$ chains are not identical; that is, $\alpha 1^B$ (and therefore $\alpha 1^A$ also) may be a mixture of two components with different amino acid compositions.

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On the Molecular Weight of Myosin. II*

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ABSTRACT: The number-average molecular weight of myosin, measured osmometrically, has been found to be *ca.* 4.7×10^5 g mole^{-1}. The weight-average molecular weight, inferred from the equilibrium distribution when the same preparations were in an ultracentrifugal field, was found to be 4.8×10^5 g mole^{-1} using midpoint analysis and 5.1×10^5 g mole^{-1} using whole column analysis.

These values appear to be consistent with chemical determinations of minimum molecular weight. It is speculated that molecular weight estimates of the order of 6×10^5 g mole^{-1} are characteristic of preparations which have been exposed to molar concentrations of salts.

In order to consider the substructure and enzymatic properties of myosin, it is necessary to know its molecular weight, with an uncertainty of 20% or less. This is not easy, partly because the myosin system is sensitive to conditions imposed during preparation, and partly because the molecular weight is high. Excepting the early report by Portzehl *et al.* (1950), the many physical estimates now available are weight-average molecular weights ($\bar{M}_w$), usually obtained by sedimentation, sometimes by light scattering. Because osmometric and preparative techniques have greatly improved since 1950, it seemed opportune to attempt again to measure the number-average molecular weight ($\bar{M}_n$) of myosin. On the same preparations used for osmometry we have also measured $\bar{M}_w$ by sedimentation to equilibrium, and we have listed molecular weight estimates based on chemical stoichiometry toward substrates and modifiers of myosin adenosine triphosphatase (ATPase).[1] All data taken together assure that the average molecular weight of rabbit skeletal myosin as used in most laboratories is less than 5×10^5 g mole^{-1}, very probably $4.6–4.8 \times 10^5$ g mole^{-1}. Because

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[1] Abbreviations used: ATPase, adenosine triphosphatase.

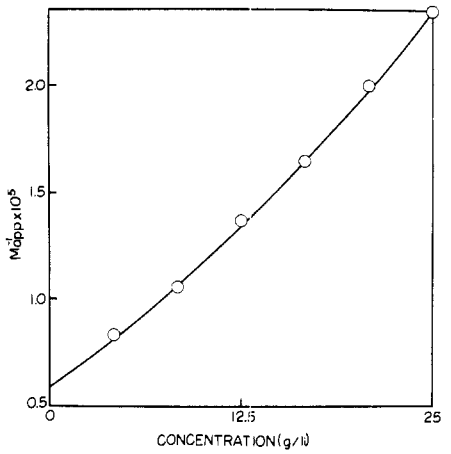

FIGURE 1: Reciprocal of apparent number-average molecular weight of polystyrene standard sample 705 (NBS) as a function of concentration. Polystyrene in benzene at 25°. The line is $[M_n(0)]^{-1} = 0.592 \times 10^{-5} + (4.96 \times 10^{-7})c + (8.23 \times 10^{-9})c^2$.

results in this area of research may depend a great deal on technique, we have reported many details which we hope will be helpful in reproducing our work.

Experimental Details

Preparation and Characterization of Myosin. All operations with myosin were performed in a cold room at 4°; all solutions were sorted at 0°; all centrifugations were performed in all-plastic bottles in a Spinco preparative ultracentrifuge Model L-2 with rotor temperature at 0°; all surfaces were prechilled before coming into contact with myosin. The dorsal muscles only of 2 or 3 exsanguinated New Zealand white rabbits were quickly excised and immediately placed on stainless steel surfaces at 0°. They were freed of visible fascia and fat, cut into small chunks, and finely minced in an electrically driven Latapie mincer. Approximately 250 g of minced tissue was extracted, with slow stirring for exactly 10 min, with three times their weight of 0.3 M KCl, 0.05 M histidine, 0.2 mM ATP, pH 6.80. The suspension was centrifuged at 6500 rpm for 10 min in the No. 19 rotor, and the supernatant was filtered in a Büchner funnel through Whatman No. 1 paper pulp, avoiding bubbling by operating slowly and by arranging a glass conduit from the funnel drain to the floor of the receptacle. The filtrate was diluted with ten times its volume of water. The resultant flock usually coalesced within 30 min; on rare occasions no coalescence had occurred by 45 min, in which case centrifugation was commenced anyhow, but with awkwardly large volumes. At this point the clear supernatant was decanted, and the hard-packed precipitate was collected by centrifugation at 6500 rpm for 10 min in the No. 19 rotor. This precipitate was dissolved by stirring in either 0.3 M or 0.4 M KCl, with *ca.* 0.015 M histidine, pH 6.8. The solution was then centrifuged at 27,000 rpm for 30 min in the No. 30 rotor. The steps from the flocculation onwards were repeated two more times except that the last dissolution was with 0.6 M KCl, 0.05 M Tris, pH 8.00. The resulting solution was centrifuged for 1 hr at 47,000 rpm in the No. 50 rotor; at this point there was never more than a trace of pellet or of lipid scum. After the solution was dialyzed for *ca.* 16 hr, with four changes (40 ml of solution to 500 ml of solvent which had been filtered through a 100-mμ Millipore filter), it became the stock solution, and usually had a concentration of 1–2.5 g%. Subdilutions were generally made with dialysate, using pipets of at least a few milliliters in volume. Protein concentrations were measured by a modified Lowry procedure (Gellert *et al.*, 1959). The preparations used had enzymatic activities near 5 μmoles of P_i g^{-1} sec^{-1} when studied in 0.6 M KCl, 0.05 M Tris, 10 mM $CaCl_2$, at 25° and pH 8.00; they all tested negatively for myosin B contamination using a sensitive turbidimetric method (Rice *et al.*, 1963). Myosin was analyzed chromatographically according to Asai (1963). In most cases the measurements to be reported were made one or two days after the day on which the preparation was made, and in no case were they made on preparations older than one week.

Osmometry. All measurements of osmotic pressure were performed in a Mechrolab Model 503 high-speed membrane osmometer. The noise in a steady recorder trace has an amplitude well within ±0.01 cm of water column height. Aqueous systems (myosin, bovine serum albumin) were measured at 5° using membranes of type B 19, and the polystyrene–benzene system was measured at 25° using membranes of type 08. When the concentration of the solution under study had to be changed, say from *a* to *b*, it could be shown that readings following flushing with *b* coincided with measurements obtained after flushing with solvent, checking solvent level, and then flushing with *b*.

The concentration-dependent reciprocal number-average molecular weight, Π/RTc, where Π is the osmotic pressure, was actually computed as $(\rho/RT)(h/c)$, where ρ (g cm^{-3}) is the density of solvent in the elevator of the osmometer, h (cm) is the solvent column height, and c (g $l.^{-1}$) is the solute concentration. For measurements with myosin, bovine serum albumin, and polystyrene, (RT/ρ) l. cm $mole^{-1} \times 10^{-4}$ was 2.2199, 2.199, and 2.820, respectively. It was assumed that the apparent reciprocal number-average molecular weight, $[\bar{M}_n(c)]^{-1}$, was given by

$$[\bar{M}_n(c)]^{-1} = [\bar{M}_n(0)]^{-1} + A_2c + A_3c^2 \qquad (1)$$

Fits of this equation to data yielded the number-average molecular weight, $\bar{M}_n(0)$ g $mole^{-1}$, the second virial coefficient, A_2, and, when desired, the third virial coefficient, A_3. To facilitate comparison with other work, A_2 is reported in dimensions of mole ml g^{-2} (rather than mole l. g^{-2}, as given by our plots).

The osmometer was validated by measurements with precharacterized substances. (i) Bovine serum albumin, purified chromatographically (by Dr. Herbert

Sober) and characterized as regards weight-average molecular weight ($\bar{M}_w$ = 7.27–7.92 × 10^4), was kindly supplied to us by Professor H. K. Schachman. This substance was dissolved directly in 0.1 M NaCl and 0.01 M CH_3COONa (isoionic condition, pH 5.15) and studied in the concentration range 2.3–5.7 g $l.^{-1}$. Its $\bar{M}_n(0)$ proved to be 7.26 × 10^4. (ii) Polystyrene, designated as Standard Sample 705 by the National Bureau of Standards, was dissolved in spectral grade benzene, and was studied in the concentration range 4.167–25 g $l.^{-1}$. The data were well fitted by the equation

$$[\bar{M}_n(c)]^{-1} = 0.592 \times 10^{-5} + (4.96 \times 10^{-7})\, c + (8.23 \times 10^{-9})c^2 \quad (2)$$

thus yielding $\bar{M}_n(0)$ = 1.688 × 10^5 (Figure 1), to be compared with $\bar{M}_n(0)$ = (1.709 ± 0.0058) × 10^5, given by the National Bureau of Standards.

Sedimentation. Sedimentation equilibrium was studied in a Spinco Model E analytical ultracentrifuge, using the AnJ rotor controlled at or below 4°. Observation was by Rayleigh interference optics, employing a symmetrical upper aperture mask of 0.36-mm slit width and Wratten No. 15 and Bausch and Lomb second-order 546-mμ interference filters. At the equilibrium speeds of 4327 and 5227 rpm no rotor precession was discernible.

Equilibrium distributions were studied in circular 4-channel or rectangular 3-channel cells (Yphantis, 1960, 1964), and the calibration of initial protein concentration in fringe units was made in double sector synthetic boundary cells. Cells were filled using gas-tight syringes (Hamilton Co., Whittier, Calif.) with Teflon-coated barrel tips. To avoid errors arising from adsorption of protein on cell surfaces (Yphantis, 1960), cells were, in some instances (preparations 67 and 68), in preliminary contact for 4 hr with the solution to be studied, then rinsed twice, and finally filled with a fresh aliquot of solution. Multiple channel cells contained Fluorochemical FC 43 (Beckman Instrument Co.) and myosin solution on the solute side, and solvent or FC 43 + solvent on the solvent side. When it was desirable to locate the white light fringe in the interference pattern, 1,3-butanediol was added to the solvent (Richards and Schachman, 1959). Synthetic boundary cells contained 0.14 ml of myosin solution on the solution side and 0.42 ml of solvent on the solvent side.

Interference patterns were read in a modified Nikon comparator (see Trautman, 1956, and Richards and Schachman, 1959). In order to obtain sufficient data (data from at least 16 fringes) for extrapolations to be described below, it was necessary to read quarter and half fringes as follows. The horizontal position of each light and dark fringe was read at the proper base-line setting. The base-line was then moved up or down one half of a fringe unit. Again, the position of each light and dark fringe was tabulated, yielding two horizontal readings for each half fringe. Quarter fringe positions were obtained by resetting the base line first one quarter and then three quarters of a fringe unit away. This method of reading fractional fringes decreased the errors arising from localized optical and cell base-line distortions.

The equilibrium distributions in myosin preparations 57, 60, 63, and 67 were observed in columns 0.08–0.10 cm high, and were achieved by bringing the rotor directly to equilibrium speed. The times required to attain equilibrium were calculated by eq 11 of Van Holde and Baldwin (1958). For myosin the quantity defined by these authors as α always lies between 1.0–2.6; consequently $F(\alpha)$ has its asymptotic value, 0.67. Except in preparations (*e.g.*, 67) which were oversped and undersped, thus making the equation inapplicable, a comparison may be made between calculations of t_{eq} and the times (in hours) when experimentally no further changes in pattern were detectable.

Prepn	Calcd t_{eq}	No further change by
57	19 hr	24–35 hr
60	23 hr	24–38 hr
63	23 hr	23 hr

The distributions in myosin preparation 68 were studied in columns 0.20 cm high. Following the technique of Richards (1960), a base-line picture was taken at 5227 rpm, then the rotor was accelerated to 6995 rpm and held at this speed for 6 hr; next it was decelerated to 3657 rpm and held there for 1 hr; finally it was accelerated to 5227 rpm until equilibrium obtained. Equilibrium was considered to be established if successive photographs taken at intervals of 4 hr or more showed no differences in distribution.

Observations in synthetic boundary cells were made for the purpose of correlating protein concentration (determined chemically or by calculation from known dilution factors) with number of interference fringes. An average of 10 studies showed 38 ± 0.5 fringes to correspond to a myosin concentration of 1% or 10 g $l.^{-1}$.

Using preparation 68, we investigated loss of myosin from solution during an equilibrium run. A white light base-line photograph was taken as soon as the rotor reached equilibrium speed. After completing the run the cell contents were redistributed and a second white light base-line photograph was taken. Comparison of these photographs showed a 6% loss of material during the equilibrium run. A similar loss occurred during synthetic boundary experiments by simulating such an experiment in a double-sector cell. Myosin solution was placed in the right channel (but no FC 43), and solvent with a calculated amount of 1,3-butanediol in the left channel. The rotor was accelerated to 5000 rpm and a white light base-line photograph was taken; the rotor was then accelerated to 23,000 rpm (the highest speed we used to form synthetic boundaries), and then decelerated to 4000 rpm (at which speed the boundaries spread) and held thus for 4 hr. Finally the run was stopped, the cell contents were redistributed, and the rotor was accelerated again to 5000 rpm for the second white light base-line photographs. A comparison of the two base-line photographs indicated a 5% loss of

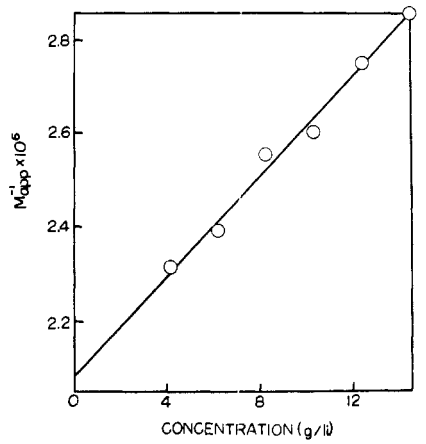

FIGURE 2: Reciprocal of apparent number-average molecular weight of myosin preparation no. 67 as a function of concentration. Myosin no. 67 in 0.6 M KCl, 0.05 M Tris buffer at pH 7.0, 5°. The line is $[\bar{M}_n(0)]^{-1} = 2.081 \times 10^{-6} + 0.531 \times 10^{-7} c$.

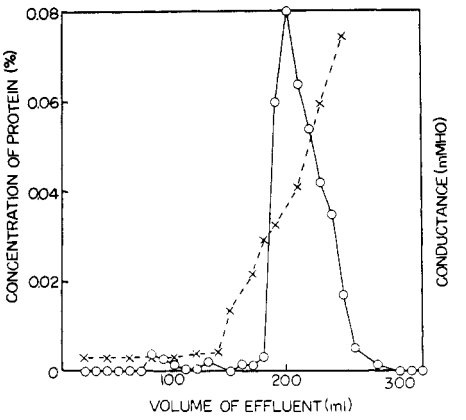

FIGURE 3: Elution profile of myosin preparation 68 chromatographed on DEAE-cellulose according to the method of Asai (1963).

myosin from solution; within experimental error, this is the same loss suffered in the equilibrium experiments.

Our data were analyzed in two ways. (i) Experiments in short (0.08–0.10 cm) columns were such as to warrant (Van Holde and Baldwin, 1958; Yphantis, 1960) equating the equilibrium concentration at the midpoint of the column with the original concentration, c_0 g l.$^{-1}$. Moreover, if $\bar{x}$ is the radial distance from the axis of rotation to the center of the column, then $(dc/dx)_{\bar{x}}$ can be approximated from the difference in fringe number between two points equidistant from the center of the column (Yphantis, 1960). Then

$$\bar{M}_w(c_0) = [RT/c_0\bar{x}\omega^2(1 - \bar{v}\rho)](dc/dx)_{\bar{x}} \quad (3)$$

where $\bar{v}$ is the partial specific volume of myosin, $\bar{M}_w$ is the apparent weight-average molecular weight at concentration c_0, and the other symbols have the meanings already assigned to them. Extrapolation to $c_0 = 0$ from a plot of $[\bar{M}_w(c_0)]^{-1}$ *vs.* c_0 now yielded the weight-average molecular weight, $\bar{M}_w$. (ii) Accurately in the case of sector-shaped cells (Lansing and Kraemer, 1935), and to a high degree of approximation in the case of rectangular cells

$$\bar{M}_w(c_0) = [2RT/\omega^2(x_b^2 - x_m^2)(1 - \bar{v}\rho)](c_b - c_m)/c_0 \quad (4)$$

where subscripts *b* and *c* refer to the cell bottom and cell meniscus, respectively. To capitalize on this relationship one must evaluate $c_b - c_m$ and c_0. This evaluation can be made either by (a) identifying the hinge point [the section of the cell at which the concentration remains at its original value (Archibald, 1947)], using white light (Richards and Schachman, 1959), counting the number of integral fringes to points near *m* and *b*, and finally extrapolating linearly or quadratically to find c_m and c_b on the assumption that in both extreme regions $d(\log c)/d(x^2)$ = constant, or (b) reading total difference in number of integral fringes between points near to *m* and *b*, extrapolating quadratically to find $c_b - c_m$ (as above), substituting this estimate of $c_b - c_m$ in the conservation of mass relationship (Ginsburg *et al.*, 1956; Richards and Schachman, 1959) to obtain $c_0 - c_m$ [or $(c_0 - c_b)$], using c_0 from synthetic boundary cell results to obtain c_m (or c_b), then proceeding recursively, each time using a more refined extrapolation. The digital computer program which automates method b is due to Teller (1965).

In all calculations with our data we have adopted the new measurement of $\bar{v}$ (0.720) reported by Kay (1960) since it was measured under conditions which correspond more closely to our own than those of Parrish and Mommaerts (1954). In discussing the molecular weight estimates of others we have quoted what their estimates would have been had they taken $\bar{v}$ to be 0.720.

Results

Osmometry. Of the five myosin preparations studied, the osmotic pressure of one (66) showed an inexplicable dependence on concentration, and the measurements were discarded; for the remaining preparations (63, 64, 67, and 68, the linearized form of eq 1 held very well (see Figure 2). Least-squares fits to the data yielded the results reported in Table I. Since osmometry of proteins can be seriously complicated by contamination with nondialyzable substances of lower molecular weight, *e.g.*, in this case, with proteins of molecular weight lower than that of myosin, we estimated the upper limit of such contamination from DEAE-cellulose chromatograms (total contamination) and velocity sedimentation experiments using interference optics (nonbound contamination). For preparation 68 such results are illustrated in Figures 3 and 4, respectively, and they indicate that the total contamination is 2.6% and the nonbound contamination is about 1%.

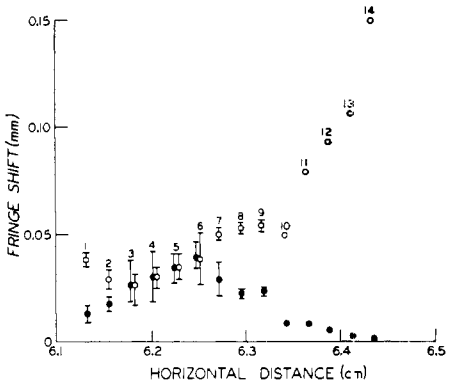

FIGURE 4: Plot of concentration difference between 0.38% myosin 68 and solvent as fringe shift in millimeter units *vs.* horizontal distance on the cell. Full circles are means of measurements on solvent, and open circles on solution; range of many readings is indicated by vertical bars. Successive points 3, 4, 5, and 6 can be made to coincide, and are assumed to constitute a base line. Points 11, 12, 13, . . etc. are assumed to constitute the "foot" of the myosin peak. Therefore, the vertical separations at points 7, 8, 9, 10 may reflect concentration of low molecular weight contaminants. The greatest of these (0.04 mm, at point 10) corresponds to a contamination of about 1%.

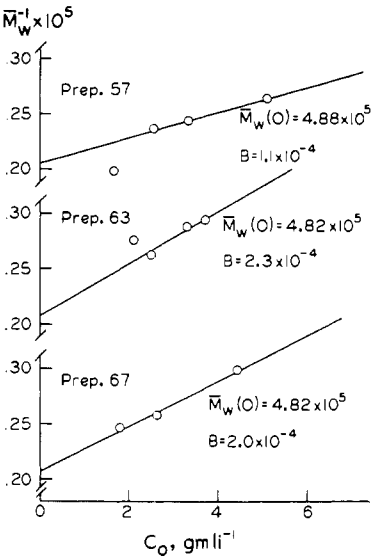

FIGURE 5: Molecular weight data from the sedimentation of three different myosin preparations, analyzed by method i (see text). Solvent same as described under Figure 2.

TABLE I: Osmometry and Sedimentation.[a]

Prepn No.	Mol Wt ($\times 10^{-5}$, g mole^{-1}) Osmometry	Mol Wt ($\times 10^{-5}$, g mole^{-1}) Sedimentation	Second Virial Coefficient ($\times 10^4$ ml g^{-2}) Osmometry	Second Virial Coefficient ($\times 10^4$ ml g^{-2}) Sedimentation
57		4.88[b]		0.55[b]
63	4.76	4.82[b]	0.87	1.1[b]
64	4.76		0.87	
67	4.80	4.82,[b] 5.15[c]	0.53	1.0,[b] 0.70[c]
68	4.67	5.15[c]	0.68	0.70[c]

[a] All measurements were performed in 0.6 M KCl, 0.05 M Tris, pH 7.0, 5°. [b] Method i (see text). [c] Method ii (see text).

Sedimentation. It was assumed that the concentration-dependent apparent reciprocal weight-average molecular weight, $[\bar{M}_w(c)]^{-1}$, was given by

$$[\bar{M}_w(c)]^{-1} = [\bar{M}_w(0)]^{-1} + Bc \qquad (5)$$

For the assumption in eq 5 to be consistent with that in eq 1, it is necessary that $B = 2A_2$. Fitting eq 5 to data thus yielded values of $\bar{M}_w(0)$ g mole^{-1} and of B; from values of B were calculated values of A_2, again in mole ml g^{-2}. The data from preparations 57, 63, and 67 (Figure 5) were obtained from the midpoint method (i). Data from preparation 67 in columns 0.01 cm high (equilibrium time, 22 hr) and from preparation 68 in columns 0.20 cm high (equilibrium time, 5.5 days) were analyzed by method ii (Figure 6).

In the course of applying method ii, c_m (in fringe units) was calculated by independent approaches (a) and (b). In five of the six comparisons the two approaches yielded the same value, within experimental error (0.2 fringe unit); in the sixth comparison the difference between the two estimates was outside experimental error by only 0.06 fringes. Such close agreement indicates that the correct value of c_0 was used in applying method ii.

Discussion

Our data (Table I) indicate that $\bar{M}_n(0)$ is nearly constant from preparation to preparation in the range 4.67–4.76 $\times 10^5$ g mole^{-1}, and it only remains to consider the possible effect of low molecular weight contaminants. This can be illustrated with data pertaining to preparation 68, for which we have reported that $\bar{M}_n(0) = 4.67 \times 10^5$ g mole^{-1}. Using the method of light scattering, Asai (1963) estimated that $\bar{M}_w$ of the low molecular weight contaminant was *ca.* 1.5×10^5 g mole^{-1}, so we may guess that $\bar{M}_n$ was 1×10^5 g mole^{-1}. If all the low molecular weight contaminant (2.6% of the protein recovered in chromatography) was actually bound to myosin molecules, then the true $\bar{M}_n(0)$ of the latter was 4.56×10^5, while if the nonbound contaminant was 1% of the total protein (estimated from velocity sedimentation profile), then the

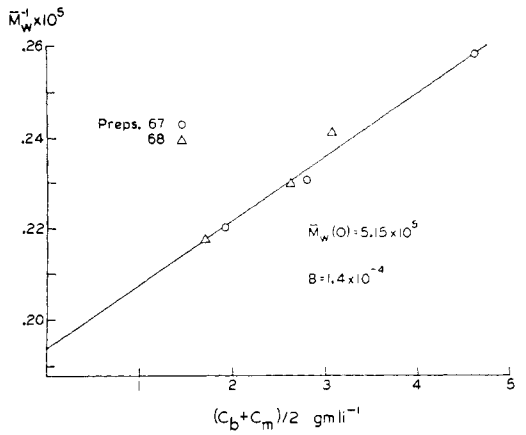

FIGURE 6: Molecular weight data from the sedimentation of two different myosin preparations, analyzed by method ii (see text). Solvent same as described under Figure 2.

TABLE II: Representative Physical Measurements of the Molecular Weight of Myosin.[a]

Method	No $(NH_4)_2SO_4$ in Prepn	$(NH_4)_2SO_4$ Used
Archibald	4.2[b]	
	3.8[c]	
		6.0[d]
	4.6–4.8[e]	
	5.03[f]	
	4.78[g]	
Equilibrium sedimentation		5.95[h]
	4.6–5.0[i]	
Light scattering	4.9[j]	
	5.3[k]	
		6.2[l]
Sedimentation-diffusion	5.0[m]	

[a] Values cited are $\bar{M}_w \times 10^{-5}$ g mole^{-1}. Values have been recalculated on the assumption that $\bar{v} = 0.720$. [b] von Hippel *et al.* (1958). Value determined at 0.5% myosin on assumption that concentration dependence was slight. [c] Mommaerts and Aldrich (1958). Experimental value was 3.8×10^5 g mole^{-1} but anticipated concentration dependence brings this to cited value. [d] Kielley and Harrington (1960). [e] Lowey and Cohen (1962). [f] Mueller (1964). [g] Luchi *et al.* (1965). This preparation did use $(NH_4)_2SO_4$, but in conjunction with the other salt extractants of the Marshall procedure. [h] Woods *et al.* (1964). [i] Connell and Mackie (1964). [j] Holtzer and Lowey (1959). [k] Holtzer *et al.* (1961). [l] Gellert and Englander (1963), as corrected and recalculated by Tomimatsu (1964). [m] Laki and Carroll (1955).

true $\bar{M}_n(0)$ was 4.78×10^5 g mole^{-1}. The uncertainty arising from this effect is therefore small, and we may safely assume that in multiples of 10^5, $4.6 < \bar{M}_n < 4.8$. On the other hand, our measurements of $\bar{M}_w(0)$ in Table I make it very likely that $4.8 < \bar{M}_w < 5.1$.

The foregoing conclusions are reenforced by all recent studies of the weight of myosin which combines with a mole of various inhibitors and substrates of myosin adenosine triphosphatase (ATPase)[1]; *e.g.*, the studies on the modification of ATPase by trinitrobenzenesulfonate (Kubo *et al.*, 1965), the studies on the binding of ATP and inorganic pyrophosphate (Nanninga and Mommaerts, 1960; Tonomura and Morita, 1959; Gergely *et al.*, 1959), and the determination of the intermediate of myosin ATPase reaction (Kanazawa and Tonomura, 1965; Tokiwa and Tonomura, 1965; Imamura *et al.*, 1965).

Generally speaking, our measurements of A_2 (Table I) fall in the range reported by others, and our osmometric measurements are roughly consistent, though slightly lower (different concentration range employed) than our sedimentation measurements. As in the work of other authors (Table II), however, measurements of A_2 show far greater variability than does extrapolated molecular weight. Whether this is an inherent artifact or expresses a variable interparticle interaction differing from preparation to preparation, we cannot say.

Taking into account the precision of contemporary measurements, we feel that the troublesome discrepancy is that between the many recent $\bar{M}_w(0)$ measurements similar to the $\bar{M}_n(0)$ and $\bar{M}_w(0)$ reported here [Table II, especially Holtzer and Lowey (1959)] and the measurements of Kielley and Harrington (1960) and Woods *et al.* (1964). It seems to us unlikely that this discrepancy arises from a far-reaching metric or analytic superiority of one group over the other, but as early expressed by the present authors, and expressed in print by Holtzer *et al.* (1961), it is conceivable that the discrepancy arises from differences in myosin preparation. Estimates of $\bar{M}_w(0) \sim 6 \times 10^5$ g appear to correlate[2] with ammonium sulfate fractionation of myosin; it is possible that treatment with very high concentrations of ions predisposes myosin toward aggregation (see, *e.g.*, Connell, 1960; von Hippel and Wong, 1964; Warren *et al.*, 1966).

If we assume that whether *in vitro* or *in situ* populations of myosin molecules are intrinsically polydisperse, then specification of their $\bar{M}_n(0)$ and $\bar{M}_w(0)$ is about all that can be done presently. In this sense, we conclude that some number between 4.5×10^5 g mole^{-1} and 5.0×10^5 g mole^{-1} best represents the average molecular weight of myosin as prepared by most investigators. However, the discovery by Kielley and Harrington (1960) that myosin can be dissociated into subunits of

[2] Luchi *et al.* (1965) used $(NH_4)_2SO_4$ fractionation in preparing myosin from dog heart and nevertheless reported $\bar{M}_w(0) = 4.78 \times 10^5$ mole^{-1}, but they followed the procedure of Marshall which also involves LiCl and several novel manipulations.

molecular weight *ca.* 2×10^5 g mole^{-1}, and the frequent observation of low values of $\overline{M}_w$ (*e.g.*, 3.8×10^5) at the meniscus during sedimentation in the present investigation may support the suggestion of Laki (1965) that the molecular weights obtained by physical methods reflect the association of fundamental, nonstatistically distributed subunits into a distribution which tends to crest very sharply at a weight corresponding to a particular *n*-mer.

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