DETERMINATION OF THE MOLECULAR WEIGHT OF MYOSIN

INTERFERENCE-OPTICAL MEASUREMENTS DURING THE APPROACH TO ULTRACENTRIFUGAL SEDIMENTATION AND DIFFUSION EQUILIBRIUM*

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Determinations of the molecular weight of myosin have, so far, not led to results that have found general acceptance. The value of 850,000[1,2] often cited as the standard figure (*e.g.*[3–5]), was based upon a sedimentation constant of $7.1 \cdot 10^{-13}$ (see also[6,7]), a diffusion constant of $0.9 \cdot 10^{-7}$, and a partial specific volume of 0.74. These results became doubtful when higher figures for the diffusion constant[8,9], and lower ones for the sedimentation constant were reported[8–11]. The uncertainties increased when PARRISH AND MOMMAERTS[8] described dynamic anomalies in the sedimentation behavior of such a nature that no explicit answer would seem obtainable, while LAKI AND CARROLL[9] noted time-dependent changes occurring in myosin in the temperature range normally employed in sedimentation analysis.

The light-scattering method, apart from yielding the reproducible result of 1600 Angstrom for the molecular length[12,13], has likewise failed to contribute reliable weight data. RUPP AND MOMMAERTS[13] discovered progressive changes in turbidity at room temperature; but even when those were prevented, the results were highly erratic, permitting only the conclusion that the molecular weight would be 650,000 or less; a lower range was obtained by HOLTZER[14,15].

In the present work (preliminary publication[16]), we have succeeded in obtaining consistent molecular weight values from studies on the approach toward the sedimentation and diffusion equilibrium according to the ARCHIBALD[17] theory, on the basis of measurements of the displacement of Rayleigh interference fringes.

METHODS

Preparations

Myosin was prepared in crystalline form (SZENT-GYÖRGYI[18]), from rabbit skeletal muscle with the routine procedures of this laboratory[19]. It was dissolved in a medium of the following composition: 0.4 *M* KCl, 0.018 *M* KH_2PO_4, 0.027 *M* K_2HPO_4, total ionic strength 0.5, pH 6.8, in which the protein appears to be molecularly dispersed with a minimal tendency toward aggregation[13]. All samples appeared ultracentrifugally homogeneous. The concentration of the protein in solution, after dialysis against an excess of solvent, was determined refractometrically or by the Kjeldahl procedure according to HILLER, PLAZIN AND VAN SLYKE[20], modified by the use of sodium thiosulfate[21] instead of zinc dust for the reduction of mercuric compounds which would bind ammonia during the distillation.

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Ultracentrifugal sedimentation

Sedimentation studies were done in the Spinco model E analytical ultracentrifuge, equipped with provisions for measuring and regulating the temperature during the run. This regulation consistently proved reliable within a few hundredths of a degree even over periods of several days in the low speed runs. In conventional sedimentation studies with the schlieren-optical system and phase plate, two samples at different concentrations were run simultaneously, one being contained in a wedge-window cell.

Interference-fringe observations were done in the same instrument, provided with an optical system for recording Rayleigh fringes from a double-sector cell, containing solvent in the one and myosin solution in the other limb. The meniscus in the solvent column stood about 1 mm higher than that of the solution, and a perfectly arc-shaped interface at the bottom of the solution column was created by introducing 0.1 ml of Dow Corning No. 555 silicone oil[22]. These runs were performed at low rotor speeds, obtained by means of the standard speed-setting arrangement and a 1:3 speed reduction. Since the speeds employed were reached within a few minutes after starting, and the runs extended over thousands of minutes, it was deemed unnecessary to apply a zero-time correction as practiced by KLAINER AND KEGELES[23]. Before the experiment, the rotor was brought close to the desired temperature, and was then left to cool or warm, in the rotor chamber, to the experimental temperature (5° or 20°) predetermined by the regulating circuit.

Evaluation of fringe patterns

The ARCHIBALD *method.* Fringe patterns (Fig. 1) were measured by means of a travelling microscope, after careful alignment of the plate by matching the central reference fringes (transmitted through holes in the counterbalance cell, above and below the solution fringes) with the direction of measurement. The cross hairs of the microscope were then set to coincide with the central fringe through the solution which, in our experiments of relatively short duration, always ran without deformation over a considerable distance corresponding to the plateau-region of the sedimentation diagram. The total fringe number n_o corresponding to the original concentration c_o, was determined either in a separate run at higher speed (or sometimes by acceleration after a low-speed run of short duration), sufficient to completely remove the boundary from the meniscus; or by means of the synthetic boundary cell at low speed, permitting a few hours of boundary spreading for sufficient separation of the fringes. In the former case, marked sedimentation having occurred, the observed fringe number at time t, n_t, was decreased because of the dilution due to sedimentation and was corrected with the formula $n_o = n_t (x_t/x_o)^2$; the magnitude of this correction was of the order of 5%. In the synthetic boundary cell, the dilution correction was computed by the formula $n_o = n_t e^{2s\omega^2 t}$; for runs at about 4000 r.p.m. during a few hours, this correction was unnecessary since it was well within the accuracy with which the total fringe number was estimated.

Fig. 1. Example of fringe pattern traced from original photograph; concentration changes in ARCHIBALD type experiment: Myosin 0.5%, 5°, 60 h sedimentation at 4,196 r.p.m. The distance between the two vertical marks above the pattern corresponds to 1 cm in the cell. For measurement, the plate is aligned along the line a-a with the aid of the reference fringes ref. Measurement of fringe positions is along the line d-d, beginning from the meniscus m to the bottom b.

Beginning at the top of the pattern (Fig. 1), the positions of the meniscus, of all fringes, and of the bottom interface were measured within 2 to 3 μ on the plate, corresponding to 1 μ in the cell. This precision was illusory, since the location of the fringe maxima involved subjective judgement. The real precision was, in general, satisfactory for the top part of the diagrams, but in the bottom part the fringes were crowded, and it was often advisable to proceed pairwise.

The ARCHIBALD treatment requires expression of these measurements into relative concentrations $n_{x,t}/n_o$ as a function of distance x. In all our experiments, there was a plateau region in the cell characterized by the conditions:

$$\frac{dn}{dx} = 0, \quad \frac{dn}{dt} \neq 0$$

so that the concentration here is not equal to the original concentration as seems implied in Fig. 1 or ARCHIBALD's paper[17]. Instead, the concentration in the plateau region is given by:

$$n_{p,t} = n_o e^{-2s\omega^2 t}$$

for the application of which we have used a sedimentation coefficient valid for the concentration, temperature and rotor speed of the experiment in question. As a rule, this coefficient was obtained

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from the same or a similar run, with the procedures described below. However, the correction factor is so small that no significant error is introduced by estimating the value of s on the basis of other information available. The concentration in the plateau region being determined, it is clear that at a point x, which is i_x fringes above or below the plateau, the concentration will be

$$\frac{n_x}{n_0} = \frac{n_p - i_x}{n_0} \text{ or } \frac{n_p + i_x}{n_0}, \text{ respectively.}$$

Further treatment was done by numerical tabulation as is illustrated (but for omission of the initial columns which convert the measurements on the plate into distances from the center of rotation) in Table I. The last column provides the quantity $\frac{1}{rn_x}\frac{\Delta n}{\Delta x}$. The value δ which this quantity assumes upon extrapolation to the top or bottom meniscus is used for the calculation of the molecular weight:

$$\delta = \frac{M(1 - \bar{v}\varrho)\omega^2}{RT}$$

An example of such an extrapolation, derived from a series of measurements at different time intervals, is given in Fig. 5. According to the ARCHIBALD theory, and to actual experimentation[22-24], curves like these change in the course of time and eventually approach horizontal lines with a constant value δ as equilibrium is reached. The accuracy of the extrapolation increases *pari passu*. However, in the case of myosin it is not convenient to extend the runs so far since it can be calculated (ARCHIBALD[25]) that in this case several weeks would be required to reach equilibrium.

TABLE I

EXAMPLE OF TABULATION OF DATA FOR CALCULATION ACCORDING TO THE ARCHIBALD METHOD

Experiment MI-17: 0.5 % myosin, 5°, 24 h sedimentation at 4,190 r.p.m.; $n_0 = 18.68$, $n_p = 18.54$; top part of pattern only*.

x	Δx	$n_p - i$	$\frac{n_p - i}{n_0}$	$\frac{\Delta n}{\Delta x} \cdot \frac{1}{n_0}$	x_n/n_0	$\frac{1}{x_n} \cdot \frac{\Delta n}{\Delta n}$
6.1274	(meniscus)					
6.1474	0.0153	13.54	0.7249	3.497	4.453	(0.7863)
6.1620	0.0193	14.54	0.7785	2.772	4.797	0.578
6.1851	0.0231	15.54	0.8320	2.316	5.146	0.450
6.2143	0.0292	16.54	0.8855	1.832	5.503	0.333
6.2603	0.0460	17.54	0.9391	1.163	5.880	0.198
6.54		18.54	0.9926			

* The first fringe position represents a partial fringe distance only; the value resulting from it is not used for the extrapolation toward δ.

Determination of the sedimentation coefficient

The GUTFREUND-OGSTON *method.* The ARCHIBALD theory also provides further calculation by which the sedimentation coefficient can be obtained from the same measurements. We found, however, that the location of the fringe maxima was not carried out accurately enough to allow these additional evaluations. On the other hand, GUTFREUND AND OGSTON[26] have devised a simpler procedure to calculate the sedimentation coefficient in cases where no definite boundary is formed, and KLAINER AND KEGELES[23] have combined their method with the ARCHIBALD principle. We find that the interference method permits the same approach in a considerably simpler fashion, since the concentration distribution in the cell is obtained explicitly, rather than in differential form.

The same figures were used as for the ARCHIBALD procedure, up to the values of n_x/n_0 (Table II). To obtain n_x/n_0, n_p was needed as before, and was calculated by means of a value of s obtained from other knowledge. If no such figure is at all available, one can first proceed by using n_p and n_0 interchangeably in the computation of a preliminary value of s, which is then employed to go through the same calculations rigorously. In most experiments, the difference between n_0 and n_p was of the order of 1%, so that either successive approximation, or the use of a value of s obtained otherwise, are fully satisfactory.

In analogy with the procedure of GUTFREUND AND OGSTON, a plot was made of the function

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TABLE II

EXAMPLE OF TABULATION OF DATA FOR THE COMPUTATION OF SEDIMENTATION CONSTANT

Same experiment as in Table I

x	$\frac{n_p - i}{n_0}$	$\frac{n_p - n_x}{n_0}$	$x\left(\frac{n_p - n_x}{n_0}\right)$	
6.1274				Result of integration by planimetry:
6.1474	0.7249	0.2677	1.6444	$\frac{Q't}{n_0} = 0.1468$
6.1620	0.7785	0.2141	1.3193	
6.1851	0.8320	0.1606	0.9933	$\log\left\{1 - \frac{2}{x_0^2}\cdot\frac{Qt}{n_0}\right\} = -0.00340$
6.2143	0.8855	0.1071	0.6656	
6.2603	0.9391	0.0535	0.3349	
6.54	0.9926	0.0000	0.0000	

$\frac{x}{n_0 x_i}\int^{x_p}\frac{dn}{dx}dx$ against x. In our case, these points are obtained quite directly, since $\frac{1}{n_0 x_i}\int^{x_p}\frac{dn}{dx}dx$ simply equals $\frac{n_p - n_x}{n_0} = \frac{n_p}{n_0} - \frac{n_x}{n_0}$. By graphical integration of the curve between x_0 and an arbitrary abscissa x_p in the plateau region, we obtained $\frac{Q't}{n_0} = \frac{1}{2}x_0^2(1 - e^{-2s\omega^2 t})$, from which s was calculated according to GUTFREUND AND OGSTON. This can either be done for one exposure at a single time, or for various moments in the course of a run (Fig. 2).

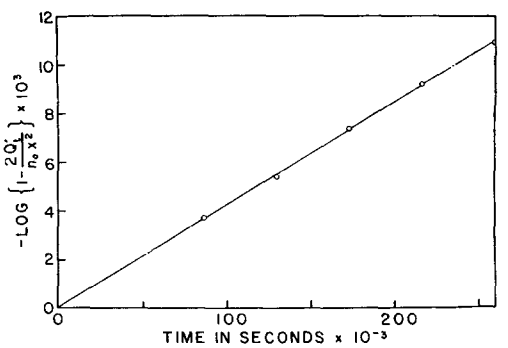

Fig. 2. Determination of the sedimentation constant with the GUTFREUND-OGSTON procedure. Plot of $-\log\left\{1 - \frac{2Q't}{n_0 x^2}\right\} = \frac{2\omega^2 ls}{2.303}$, obtained as in Table II, as a function of time.

The diffusion coefficient

When δ and s are both known, the diffusion coefficient can be obtained by the expression[17]: $D = w^2 s/\delta$. However, this does not constitute a separate determination of D. To provide for such an independent measurement, we have measured the spreading of the fringes in a run with the synthetic boundary cell at 4.190 r.p.m. at which rotor speed the boundary-sharpening effect[8] should be negligible. The time interval over which diffusion could be observed was limited by the height of the cell and the approach to sedimentation equilibrium; good measurements at 0.5% concentration were obtained by hourly observation for 6 h. No claim is made that this procedure is advantageous in comparison to the employ of stationary diffusion apparatus. At the same time it may be noted that the presence of a density gradient in a centrifugal field may stabilize the system to such an extent as to compensate for some of the disadvantages of the method; with regard to speed, the method seems quite superior. The zero-time correction was usually high, since the formation of the boundary was not perfect.

The evaluation of the patterns was done in analogy with the method of SVEDBERG AND PEDERSEN[27] for the light-absorption method (Table III and Fig. 4). The calculations were based on measuring the displacements, μ, of points corresponding to various relative concentrations 0.1, 0.2 etc., expressed as fractions of the total concentration. These displacements were read from

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plots of concentration *vs.* distance, or, alternatively, from such plots on probability function paper (Keuffel and Esser, No. 358-23). In the latter case, an ideal diffusion boundary appears as a straight line. In our experiments, the line was slightly curved corresponding to skewness, but was still advantageous because it could be drawn with greater accuracy than a traditional ogive.

TABLE III

EXAMPLE OF TABULATION OF DATA FOR ESTIMATION OF DIFFUSION CONSTANT FROM BOUNDARY SPREADING

Experiment MI-29, 0.5 % myosin in synthetic boundary cell at 4,190 r.p.m.; diffusion for 3 h at 5°. Fringe positions plotted to determine the distances μ between the center of the boundary and points of relative concentration c_{rel} 0.1, 0.2 etc.; y = probability function from Table I in SVEDBERG AND PEDERSEN[27]. Total fringe number 19.80

c_{rel}	u	$\bar{u}^2/4y^2$
0.1; 0.9	0.0497; 0.0527	$7.68 \cdot 10^{-4}$
0.2; 0.8	0.0320; 0.0335	$7.58 \cdot 10^{-4}$
0.3; 0.7	0.0197; 0.0207	$7.39 \cdot 10^{-4}$
0.4; 0.6	0.0098; 0.0098	$7.45 \cdot 10^{-4}$
		Average $7.52 \cdot 10^{-4}$

RESULTS

The sedimentation and diffusion coefficients

The results by PARRISH AND MOMMAERTS[8] on the sedimentation anomalies of myosin, which were the original reason for reinvestigating in this field, were affected by uncertainties regarding the actual rotor temperature during the measurements. Without attempting to cover the full scope of their observations, we have repeated certain crucial ranges, with continuous regulation and measurement of the rotor temperature. Different from the earlier work which was restricted to the temperature range 15 to 30°, the present measurements were done at 6° and 20°. Consequently, the anomalies might be expected to be somewhat less. The results may be summarized as follows: at 59,780 r.p.m., the temperature dependence of $s_{20,w}$, although suggested, was not statistically significant, and the most probable value at zero concentration equals $(6.08 \pm 0.10) \cdot 10^{-13}$. At 29,500 r.p.m., there was a significant increase of $s_{20,w}$ with rising temperature, and the most probable value near 0° is $(6.10 \pm 0.10) \cdot 10^{-13}$. The rotor-speed dependence was not statistically significant at 6°, and the probable value of $s_{20,w}$ for $c = 0$ at this temperature was $(6.18 \pm 0.10) \cdot 10^{-13}$. At 20°, the rotor-speed dependence, a decrease of s with increasing angular velocity, was pronounced; the extrapolated value of $s_{20,w}$ at $w = 0$ and $c = 0$, might be about 6.5. These sample experiments are in complete accord with those of PARRISH AND MOMMAERTS. A considerable program of measurements would be required for a complete description of the phenomena, but it does appear as if the anomalies play a relatively small role at low temperature, and it seems unlikely that the valid magnitude of $s_{20,w,c=0}$ would be much below the given figures. A similar conclusion is reached from our measurements with the GUTFREUND–OGSTON procedure, an example of which is in Fig. 2. Table IV gives all results of s so obtained, and also contains values for the diffusion constant obtained with the relation $D = \omega^2 s/\delta$. The s and D values obtained in the single experiments in Table IV are only approximate since such separate determinations of s may be inaccurate by 10 % or more, while those of D are in addition afflicted by the possible error of δ. However, the results from the serial experiments (Table IV,

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TABLE IV

COMPILATION OF MOLECULAR WEIGHTS OBTAINED WITH THE ARCHIBALD METHOD

Also tabulated are the sedimentation constants calculated from the same runs, and the diffusion constants computed form s and δ. The s and D values are corrected for water at 20°, but are valid for the concentrations of the respective experiments

Concentration %	*Temperature* °C	*Rotor speed* r.p.m.	*Duration* hours	$s_{20,w} \times 10^{13}$ at finite concentration	$D_{20,w} \times 10^{7}$ at finite concentration	*Molecular weight* × 10^{-4} Top	Bottom
0.5	20	8,210	4			37.9	36.0
				3.1	0.8		
			6			37.2	38.4
0.38	20	8,210	5			37.5	—
				3.3	0.8		
			6			39.0	—
0.25	20	8,210	4			39.3	37.8
				4.0	1.0		
			6			38.6	38.6
0.5	5	4,196	10½			—	38.0
				2.6	0.7		
			23			37.1	—
0.38	5	4,196	24	2.6	0.6	41.2	37.0
0.25	5	4,196	15	3.3	0.8	—	39.4
0.5	5	4.196	24			39.4	37.5
				2.9	0.8		
			42			35.2	37.5
0.5	5	4,196	42	3.1	0.8	38.5	37.0
0.38	5	4,196	30	3.2	0.74	38.5	38.4
			Average of all single values			38.4	37.8
0.5	5	4.196	12 to 72	3.33	0.82	38.5	38.0
0.5	5	4,196	24 to 72	3.51	0.89	37.5	36.5

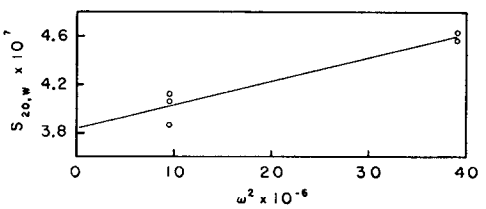

Fig. 3. Sedimentation constant of myosin in 0.5% solution at 5° as a function of angular velocity.

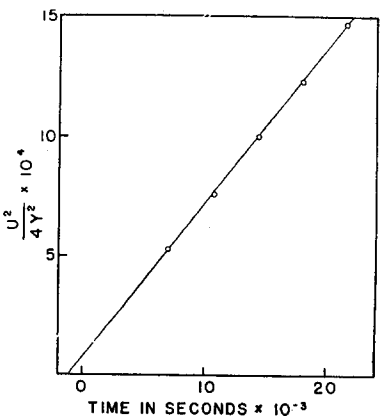

Fig. 4. Boundary spreading in synthetic boundary cell, as in Table III. Plot of $\bar{u}^2/4y^2$ as a function of time. The diffusion coefficient is the slope of the line.

bottom) are somewhat more exact. To compare the results for s with those obtained from direct measurements of boundary displacements, we refer to Fig. 3 which gives the rotor-speed dependence of $s_{20,w}$ measured at low temperature and at finite concentration. It is seen that the values listed in Table IV (bottom) are not out of line with these results. Table IV also lists the diffusion coefficients that are conjugate with

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the experimentally obtained s and δ. In addition, a few direct determinations of D were performed as described (Fig. 4), yielding figures of $1.04 \cdot 10^{-7}$ and $0.95 \cdot 10^{-7}$ at 0.5 % and at 1 % myosin respectively.

Molecular weight

In most experiments aiming at a molecular-weight determination with the ARCHIBALD procedure, one or two fringe pictures were taken after arbitrary times of sedimentation. These were evaluated separately, so that values for δ were obtained from single ARCHIBALD plots, both for the top and bottom interface. It was estimated that the uncertainty in each individual extrapolation was of the order of 5 %. The molecular-weight values so obtained are collected in Table IV. The experiments represent a certain variation in concentration, rotor speed and temperature. The data are fully consistent, within the limits of accuracy. In all cases, approximately the same value is obtained from the top and the bottom of the cell, as would be required for a pure substance not engaged in association or dissociation equilibrium (see below). The average of all these single experiments amounted to 382,000. In other extensive experimental series (*e.g.* Fig. 5), measurements were made at regular intervals, and the curves were used jointly to obtain δ with greater certainty by simultaneous extrapolation as advocated by ARCHIBALD[17]. These series led to molecular weight values likewise listed in Table IV. Combining all our results, we shall accept a figure of 380,000 as the experimental value of the molecular weight.

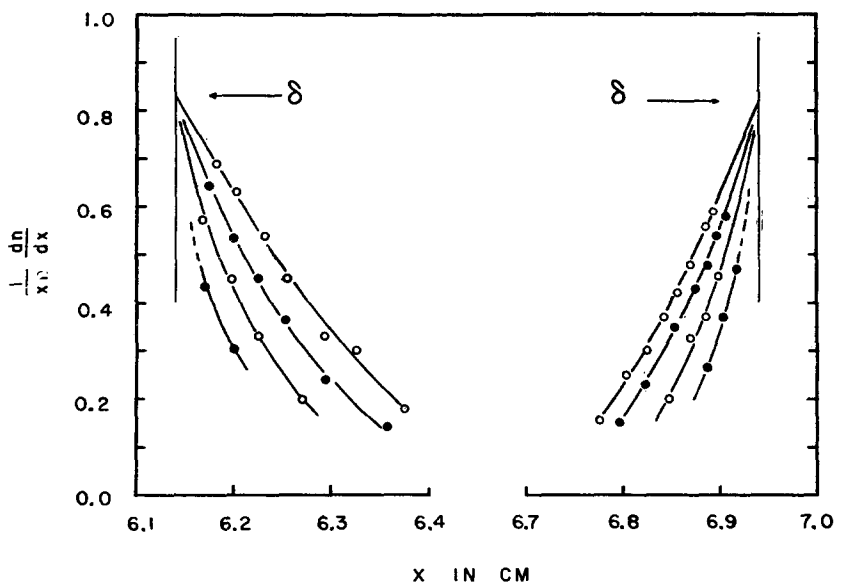

Fig. 5. Example of ARCHIBALD plots obtained from measurements after 12, 24, 48 and 72 h of sedimentation at 4,196 r.p.m. at 5°, 0.5 % myosin. Abscissa: distance in cm from axis of rotation.

DISCUSSION

Since this study represents the first application of the interference-fringe method for the study of the concentration gradients with the ARCHIBALD procedure, it will be

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advisable to discuss first those technical limitations encountered which limit the accuracy of the results. Limitations originate from the imperfect rotor-speed constancy and the relative inaccuracy with which the fringe positions were measured. The need for a reasonable number of fringe shifts limits the applicability to concentrations not much below 0.25 %; measurements at 0.5 % were more nearly optimal. Significant inaccuracy resulted also from the arbitrariness with which the curves (Fig. 5) were drawn. While these approach straight lines near the meniscus and bottom, the ARCHIBALD theory contains no explicit analytical expression for the actual course of the curves, and arbitrary free-hand extrapolations had to be resorted to. The scatter of the values in Table IV illustrates the extent of these uncertainties.

The value of 380,000 for the molecular weight of myosin, obtained as a result of this study, is in marked disagreement with any experimental figure published so far, although close to the one anticipated by LAKI AND CARROLL[9]. While extensive research would be required to explain all discrepancies, the following tentative considerations may be offered.

In the evaluation of sedimentation velocity and diffusion data, the kinetic anomalies recorded by PARRISH AND MOMMAERTS have caused much ambiguity. However, our present data suggest that these anomalies may become small at low temperature; the valid sedimentation coefficient may be 5.8 to $6.0 \cdot 10^{-13}$. With such figures, one would still obtain molecular weight close to 500,000 unless the available diffusion coefficients were also in error, as LAKI AND CARROLL have considered. Our orientating measurements do indeed indicate that the diffusion coefficient may be somewhat higher than was previously measured. Concerning the deviating and erratic results obtained with the light-scattering method, we now propose that these were due to a methodological factor. GERGELY[28] has found that dilution of an actomyosin solution into a larger volume of solvent may cause partial aggregation; the same may occur with myosin. We have no explanation for the higher molecular weights obtained by the osmotic pressure method[23] and must leave the explanation of this discrepancy to future investigation.

The majority of our determinations has been performed at a relatively high concentration, mostly 0.5 %. Although the elementary theory does not foresee a concentration dependence for the molecular weight obtained from equilibrium measurements, it is likely that, when activities instead of concentrations are considered, the results at finite concentrations are affected by the same interaction constant B that appears in the theory of osmotic pressure or, at twice the numerical value, in light scattering. There is, indeed, a trend in our results (Table IV) to yield somewhat lower values in the bottom of the cell where the concentration is higher than at the meniscus. From the slope of the light-scattering plots[13] it may be estimated that the apparent molecular weight at 0.5 % concentration may be depressed by about 10 %, but the real difference is likely to be less if aggregation had occurred in those scattering measurements. Our few measurements with the ARCHIBALD method at lower concentrations likewise exclude a significant concentration dependence. Until these factors have been investigated more exhaustively we shall, for the purpose of discussion, accept a molecular weight of 420,000. The fact that this is just one half of the value which resulted from several earlier studies[1,2] gives rise to the thought that under certain circumstances myosin dimers may arise, just like higher paucimers have been detected[6].

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The molecular weight here proposed* agrees quite closely with the sum, 424,000, of the one H- and two L-meromyosins which are assumed to constitute the myosin molecule (LAKI AND CARROLL[9]). Furthermore, adding the accepted length-values of 1 heavy and 2 light meromyosins[9] one obtains 1540 Å, in agreement with the 1600 Å[13] experimentally found for myosin. However, the molecular weights and dimensions of these constituents themselves may not be fully accurate and will be reinvestigated.**

In a recent paper, NANNINGA AND MOMMAERTS[29] have reported that one mole of ATP, in its physical and enzymic interaction with actomyosin, reacts with 504,000 g of this protein complex. The proportion of actin and myosin in actomyosin is still not known with certainty, and may not be same in all cases, but is probably about 1:3 or 1:4. Hence, the molecular weight of myosin compares fairly accurately with the presumable weight of the myosin moiety of the actomyosin that reacts with one mole ATP. Myosin, therefore, would have one active center for the interaction with nucleotides.

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SUMMARY

Procedures have been described for the use of an interference-fringe optical system in the ultracentrifuge, for the determination of molecular weights according to ARCHIBALD, and of sedimentation coefficients according to GUTFREUND AND OGSTON, as well as of diffusion constants.

Applied to myosin, this method yields a molecular weight of 380,000, which may have to be corrected to about 420,000 because of interaction effects. This corresponds to the amount of myosin which, as actomyosin, reacts with one mole of adenosine triphosphate.

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* *Note added in proof*

In an appendix to another paper, M. F. MORALES, A. J. OSBAHR, H. L. MARTIN AND R. W. CHAMBERS (*Arch. Biochem. Biophys.*, 1957) mention that, according to P. VON HIPPEL AND H. SCHACHMAN, the major component in an unfractionated myosin-actomyosin mixture has a molecular weight of the order of 450,000. According to a personal communication by Dr. MORALES, this was obtained with the ARCHIBALD method, presumably by extrapolation to the meniscus.

** *Note added in proof*

According to a communication by Dr. J. GERGELY, the dimensions of the meromyosin molecules may indeed be quite different from the current values in the litterature, and the myosin molecule may contain only one of each of the constituent molecules.

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Short Communications

The properties of algal and sperm flagella obtained by sedimentation

Flagella from the alga *Polytoma uvella* have been reported by the author to contain about 0.6% ribonucleic acid phosphorus[1]. This was in contrast with the tails of fish sperm, from which this substance appeared to be absent. The reason for the difference was not apparent, but it now seems that the algal nucleic acid was cytoplasmic in origin. Previously, flagella material had been isolated from a suspension by precipitation with alcohol and acetic acid, and in the electron microscope this suspension seemed reasonably pure. If, however, the flagella are isolated by sedimentation, the nucleic acid phosphorus, on a lipid-free basis, measured by u.v. absorption, falls to 0.22% ± 0.04% (6 determinations), and if the intact cells are shaken with 0.5 *M* sucrose instead of distilled water and two or three drops of chloroform, this value falls again to 0.10% ± 0.02% (4 determinations). The small residual phosphorus is not considered significant.

Other properties of flagella obtained by sedimentation are compared in Table I with those of the original precipitated material. Methods are as described previously with the exception that cystine was determined by the slightly more sensitive and more convenient Fleming reaction as used by VASSEL[2].

Thus lipid, which is left behind during precipitation, is present in the sedimented flagella. The rather high value of 20% for *Polytoma* flagella can hardly arise from the very thin sheath but must come more probably from the matrix and/or the fibrils themselves.

The cystine content of flagella appears to be 1% or less. Lower values were obtained from sedimented material but hydrolysis, especially in the presence of carbohydrate, destroys a proportion of the cystine. More carbohydrate was present in the sedimented *Polytoma* preparations than in material obtained by precipitation, and it seems likely that the lower cystine values obtained in the former case were due to a greater breakdown of this amino acid during the preliminary hydrolysis. Hydrolyses were all performed in a HCl–formic acid mixture[3], which would reduce but certainly not eliminate the destruction of cystine.