STUDIES ON THE STRUCTURAL PROPERTIES OF ACTIN, MYOSIN, ACTOMYOSIN, AND THE INTERACTION WITH ADENOSINE TRIPHOSPHATE

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Abstract

The muscle proteins actin, myosin, and actomyosin have been investigated. Their electrophoretic behavior was examined. The molecular weights by light scattering and dissymmetry coefficients were also determined for each protein. From the last two quantities, it has been possible to show that the random coil is the best structure to assign to most of the proteins involved. Values of the root mean square separations of such random coils have been found.

The effect of adenosinetriphosphate on these properties has been determined, and a mechanism proposed to account for the changes observed. The mechanism postulates the existence of an actomyosin complex of high molecular weight. This complex molecule dissociates in the presence of ATP, not into actin and myosin, but into complex molecules of smaller size. The experimental evidence for this mechanism is presented.

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INTRODUCTION

In 1939, Engelhardt and Ljubimova (1) discovered that the enzyme adenosinetriphosphatase is either the muscle globulin, myosin, itself, or some substance very closely associated with it. Since then much research has been done on the nature of this interaction between myosin and adenosine triphosphate, or ATP as we shall abbreviate it from now on.

A great deal of this research was done on muscle fibrils and on artificial "threads" prepared by squirting a myosin solution into water through a fine capillary. Results obtained on these threads were confusing, since Engelhardt reported an extension in the presence of ATP, while Szent-Gyorgyi (2) found a contraction. For the purpose of this work we shall confine ourselves to a review of experiments made on solutions of the protein.

Needham (3) then discovered that the viscosity and the flow birefringence of myosin solutions dropped on addition of ATP. He further observed that this drop was not permanent, but that the viscosity started rising almost at once. It returned to its original value in a period varying from twenty minutes to several hours. Since Edsall (4) had previously shown that myosin was a long fibrous molecule, this drop and rise were interpreted by Needham as a contraction of the myosin molecule and subsequent relaxation.

Since myosin is the main component of muscle tissue, the implications of this "contraction" have much interest. There exists now a possible link between the effect of ATP on a solution of myosin and the action of muscle in the body.

The next important step in the investigation of myosin occurred when Szent-Gyorgyi (2) succeeded in fractionating myosin into two proteins. By investigating the supernatant from which myosin had been precipitated, he found it still had enzyme activity. Since myosin is soluble only in salt solutions of around 0.5 molar, there should be no activity left in the very dilute supernatant. Upon further work, he found a new protein which was water soluble, and enzymatically active. He was able to show that it was also a component of the precipitated myosin, in which it was associated with some other protein. F. Straub, working in Szent-Gyorgyi's laboratory, isolated this other protein. Szent-Gyorgyi then said that the "myosin" of all previous investigators was a complex of these two proteins. He named the water soluble one, myosin, as the enzyme activity was still associated with it. The inactive fraction was called actin. This latter protein exists in two forms, depending upon the salt concentration. The form in water has a low viscosity and was called G (for globular) actin, while the form in salt solution, which has a high viscosity, was called F (for fibrous) actin. The complex formed when the two were mixed was called actomyosin.

For the balance of this thesis, we shall adopt this nomenclature, except that the myosin fraction shall be generally denoted as actin free, or AF myosin, to prevent confusion with previous work.

Actin free myosin is a protein which is soluble in water, but is precipitated by addition of a slight amount of salt (0.025 M). It redissolves as the salt concentration is increased to around 0.3 molar. It has fairly high viscosity, the intrinsic viscosity being 2.4. The enzyme activity seems to be present only in this part of the actomyosin complex. Szent-Gyorgyi states that AF myosin can be

obtained in the form of crystals, although the exact nature of this crystallinity is doubtful.

G-actin is soluble in water and has a low viscosity, the intrinsic viscosity being less than 0.1. On addition of salt the intrinsic viscosity is increased to around 2.0, this being the fibrous form. There is no enzyme activity present in either material.

On mixing F-actin and AF myosin, a resulting solution of intrinsic viscosity greater than 2.4 is obtained. The value of the viscosity is dependent on the amount of actin present, but it is of the range reported by Edsall, Needham, and others for the solutions they refer to as myosin.

When ATP is added to AF myosin solutions, no change in the viscosity of the preparation is noted, although the enzyme activity is still present. When ATP is added to the actomyosin complex, the viscosity drops to a value approximately equal to the corresponding AF myosin solution. Szent-Gyorgyi interprets this as a dissociation of the actomyosin complex into F-actin and AF myosin. The viscosity of the solution after adding ATP appears to be the same no matter how much actin is present. Szent-Gyorgyi thus explains by a dissociation the experimental evidence which Needham interpreted as contraction.

Riseman and Kirkwood (5) have proposed a mechanism based on phosphorylation and dephosphorylation of the protein, producing an extension of the molecule. The ATP increases the charge of the molecule by donating phosphate groups to the -OH sites on amino acid residues of the myosin. This would produce an elongation in the molecule, which cannot easily explain the observed viscosity and flow birefringence effects. However, if the latter effects are due to

dissociation into F-actin and AF myosin, then this mechanism may be applicable to the AF myosin molecule.

Binkley (6) has given a mechanism which accounts for contraction by interaction of the -SH groups in the molecule with neighboring phosphorylated hydroxy amino acid side chains to form a thio ether linkage. This predicts contraction in the absence of ATP and relaxation in the presence of ATP.

Thus we have several theories to account for the interaction of ATP with actomyosin. We shall now briefly discuss some of the recent work done on the enzyme properties of the protein, and then examine the state of the physical measurements made upon it.

There have been many attempt to separate the enzyme activity from the myosin molecule, other than in the sense of Szent-Gyorgyi's work. Price and Cori (7) claimed to have effected such a separation, but Cori (8) retracted the claim. Recently Polis and Meyerhof (9) and Kielley and Meyerhof (10) have apparently succeeded in doing this. Their procedure, based on fractionation with lanthanum salts, allows them to prepare samples of greater activity than normally found, and to obtain an ATPase solution not containing myosin. The latter paper states that there seem to be two enzymes, and only one of them can be thus separated.

Singher and Meister (11) have made an investigation of the effect of concentration of both enzyme and substrate on the activity. They found that, above a minimum value of the ATP concentration of about 0.004M, there was no further effect with increasing concentration for myosin concentrations of around 1%.

Singer and Barron (12) demonstrated that the enzymatic activity

is towards the sulfhydryl group. Mommaerts (13) and Braverman and Morgulis (14) have studied the effect of various ions, especially calcium and magnesium on the enzyme activity. Mommaerts reports that he was able to destroy the ATP activity without destroying the viscosity. Magnesium inhibits the enzyme activity while it promotes extension of the recovery time. Calcium has the opposite effect. The samples which had no enzyme activity but exhibited a viscosity drop, did not show a recovery in the viscosity. However, Mommaerts expressed dissatisfaction with this experiment.

The above indicates that there is much work to be done on the enzyme activity and much is dependent on the results obtained in this work. However, as this thesis is concerned mainly with the effect of the ATP interaction upon the size and shape of the actomyosin molecule, we will now proceed to a review of the work done on these properties.

The earliest work done on the molecular characteristics of myosin is that due to Edsall (4), who extensively investigated the flow birefringence of myosin solutions and the effect of various denaturing agents upon it. The material with which he worked was probably actomyosin containing around 5% actin. He concluded that the molecule was a long cylinder, and for an axial ratio of 100, calculated the length as 1000 mμ for a molecular weight of around a million. This value of the molecular weight was obtained by Weber (15) through osmotic pressure measurements.

Binkley (16) has observed the flow birefringence of AF myosin, of actin, and of actomyosin in the presence of ATP. He interprets

the fall of flow birefringence as due to dissociation.

Ziff and Moore (17) attempted to separate ATPase from actomyosin by electrophoresis with negative results. They also obtained a molecular weight by ultracentrifuge data of 3,900,000 at a concentration of 0.3%. Dubuission (18) observed the electrophoresis of actomyosin and reported a split into three components which he called α, β, and γ. He later claimed to have fractionated them (19). However, the electrophoresis patterns shown for the fractionated material are so steep that possible resolution would not be shown. Szent-Gyorgyi (20) states that he checked this fractionation and found that the α component was actomyosin and β component a rather actin free myosin.

Snellman and Erdos (21) have recently reported the mobility of actin free myosin. They have also found, as stated by Petersen (22), that the molecular weight of actin free myosin is 1,500,000 in the ultracentrifuge. Actomyosin was observed to have "many components".

X-ray diffraction work has been done by Astbury (23) indicating that the structure of the fiber is like that of β-keratin.

Munch-Peterson (24) reports that ATP causes expansion of the area of a myosin film spread on a salt solution surface.

Quite a considerable amount of electron microscope work has been done recently on actomyosin and its components. Jakus, Hall and Schmidt (25), working with the same actomyosin as Edsall, found average lengths of 410 mμ and average widths of 12 mμ. Later, Jakus and Hall (26) obtained pictures of AF myosin and actin. They report the length of AF myosin as 400-600 mμ and its width as 20-40 mμ. The actin pictures showed a very long fibrous material in salt solution.

Under conditions which should give G actin, they obtained pictures which were interpreted as indicating G-actin is so small as to be unresolved. Szent-Gyorgyi (27) reports similar results by Rozsa on actin. Reed et al (28) also obtained such results with actin, and further report that actomyosin in the presence of ATP gives small globules. This they consider due to dissociation.

Oster (29), however, obtained the same actomyosin-ATP pictures and interprets them as showing a contraction of the molecule. He also determined the change in light scattering dissymmetry in the presence of ATP. There was an increase of around 30%. This is also interpreted as showing contraction.

The experiments described in the following sections were undertaken in an attempt to clarify some of the confusing aspects of the problem. It was hoped that by electrophoresis, major changes in charge should become evident. Also, any dissociation might be discovered. The light scattering experiments should give information both on the shape, and molecular weight of the proteins. Taken together, we should be able to answer the question of whether dissociation, or change in shape causes the drop in viscosity and flow birefringence.

EXPERIMENTAL PROCEDURES

I. Preparation of proteins.

In the following are described the methods used to prepare the various proteins used in this investigation. The procedures used have been gone into in some detail due to the fact that the preparations are difficult. Slight variations in the manner of preparation can cause large changes in the properties and yields of the resulting materials.

All work was carried out in the cold room at a temperature of 4° C, except where specifically indicated otherwise. The preparations were stored in the cold and used as rapidly as possible, although in the type of experiments performed delays of several days were unavoidable.

Actomyosin and actin are the easiest to prepare and seem to denature less readily than actin free myosin. The preparation for the latter is difficult under the best conditions and the yield is very low. Szent-Gyorgyi (20) has recommended an alternate method using an ammonium sulfate fractionation which may improve the yield. This method was not used, however, as it was desired to reproduce the material on which the majority of Szent-Gyorgyi's work was done.

1. Actomyosin:

Actomyosin was prepared by the method of Greenstein and Edsall (30), with slight modifications.

The rabbit was anesthetized by injection of 10 ml. of a saline solution containing two grains of nembutal into the ear vein. The rabbit was killed by withdrawing blood from the heart with a large

hypodermic syringe. Thus the animal died without any struggle. This is necessary as struggle destroys the ATP present and makes extraction of the protein difficult, reducing the yield obtained.

The animal was skinned, and the muscle quickly excised and packed in dry ice. Generally only the hind leg muscles were taken, although on occasion, the hind and front leg muscles were also used. The time elapsed from death to packing in dry ice was never greater than 15 minutes.

The muscle was left in dry ice for varying lengths of time, sometimes as long as two weeks, with no apparent changes in the properties of the extracted protein. This agrees with the observation of Singher and Meister. (11).

Upon removal from dry ice, the muscle was allowed to thaw at 4° C for approximately an hour. By this time, although still well frozen, it could be cut into pieces about the size of a marble and run through a meat grinder. The resulting mass was placed in ten volumes of buffer solution containing 0.5 moles of potassium chloride and 0.04 moles of sodium bicarbonate per liter with a pH of 7.8. The buffer solution was at 4° C.

The muscle pulp was stirred slowly in this solution for periods varying from one hour to twenty-four hours. The length of the extraction depends on the viscosity desired for the final solution . The longer one extracts, the more actin is taken from the pulp and the higher the viscosity of the preparation. Most of the work done in this thesis was on material from extraction periods of six hours.

At the conclusion of the stirring period, the muscle pulp was filtered off by means of cheesecloth. The resulting turbid solution was filtered by suction through a centimeter thick layer of filter paper pulp on a Buchner funnel. The filtration is slow, but results in a clear orange solution. The depth of the color depends on the amount of blood remaining in the muscle after bleeding the animal to death.

The pH at this point was still in the neighborhood of 7.8. Dilute (1:16) acetic acid was now added slowly until the pH falls to 7.0. This pH is observed by taking samples, allowing them to warm to 10° C and measuring the value at this temperature on a Beckmann type G pH meter.

The protein solution was now diluted with 8-10 volumes of cold distilled water of pH 7. There resulted a large quantity of flocculent white precipitate. The pH of this solution was checked and adjusted if necessary. Then it was allowed to stand overnight to permit settling.

The supernatant was siphoned off and the residue centrifuged down. The precipitate was washed three times with cold distilled water of pH 7 and then dissolved by bringing the salt concentration to 0.5 - 0.6 molar by addition of powdered potassium chloride. This solution was generally clear, colorless and strongly opalescent. The concentration was usually around 1.5%, which is a yield of 3% of muscle weight. The longer the extraction time, the more turbid this solution becomes.

The material may be purified by dilution with 2-3 volumes of

water, centrifuging and redissolving. Needham (3) states that once precipitated material gives better response to ATP and this was generally used.

The actomyosin solutions so prepared were stored in the cold and used within 3-5 days. There is evidence that its properties probably remain constant for two weeks, but the quicker the material is used the more reliable are the results.

2. Actin free myosin:

The procedure used for this preparation is that developed by Szent-Gyorgyi (2). All water used in the preparation and in the experiments on this protein was redistilled from a Barnstead conductivity water still. The rabbit was killed, the muscle excised and packed in dry ice as before. The muscle was cut up and run through the meat grinder within two hours of death.

The ground muscle pulp was placed in a 4° C solution of phosphate buffer of ionic strength 0.15 and pH 6.5 which contained an additional 0.3 moles of potassium chloride per liter. Three volumes of buffer were used for each gram of muscle. This solution was stirred gently by hand for 10 minutes. Then it was poured into water at room temperature, four volumes being used for each volume of buffer. This solution was strained through cheesecloth to separate off the muscle pulp, and then stirred slowly at room temperature for two hours. The pulp was reserved for the actin preparation.

At the end of this time, a flocculent precipitate could be observed forming in the turbid solution. The stirring was stopped

and the solution centrifuged as rapidly as possible at room temperature. The supernatant was cooled in an ice bath. The entire volume had generally been put through the centrifuging and cooled back to 4° C within 90 minutes after stopping the stirring.

This clear solution was then stirred vigorously mechanically while 1.5 volumes of cold water were added from a separatory funnel at a rate such that it required 10 minutes to complete. The myosin precipitated in the form of fine threads or needles which had a distinctive sheen. The "crystallinity" of these particles was not investigated.

The suspension was allowed to stand for two hours, and centrifuged down after decantation of the supernatant. The precipitate is dissolved in 2 M potassium chloride solution and usually stored overnight at this point.

The preparation was reprecipitated by adding four volumes of cold water with vigorous stirring. After two hours standing, the precipitate was centrifuged down, and dissolved by addition of 0.02 M potassium carbonate solution containing phenophthalein. Excess carbonate solution was added until a rose color was retained by the solution. The pH at this point was 8.3. Then four ml. of 2 M potassium chloride solution was added for each gram of myosin present.

For every ml. of potassium chloride solution added above, 50 ml of room temperature water was added with vigorous stirring. If rose color disappeared, it was reestablished with carbonate

solution. There resulted a small amount of flocculent precipitate which was centrifuged down at room temperature. The supernatant was cooled in an ice bath. The residue was treated with half as much potassium chloride as before, water added and recentrifuged. The combined cooled supernatants were stirred strongly while adding 1% acetic acid until the pH fell to 7.0. The precipitated myosin was separated on the centrifuge and dissolved in 2 M potassium chloride solution. Typical yield from 250 grams of muscle was 30 ml of a 0.8% solution, or a yield of 0.1%.

3. Actin:

Actin was prepared by the method of F. Straub as described by Szent-Gyorgyi (2). The muscle pulp residue from the myosin preparation was placed in four volumes of 0.4% sodium bicarbonate solution and stirred for twenty minutes. It was then filtered through cheesecloth and pressed out. The pulp was then placed in an equal volume of solution containing 0.5 moles of sodium bicarbonate and 0.05 moles of sodium carbonate per liter. This solution was allowed to stand for ten minutes and then diluted with ten volumes of 0.0005 M calcium chloride. This was allowed to stand for ten minutes, and then centrifuged at room temperature. The muscle pulp was then suspended in three volumes of acetone, strained through cheesecloth and pressed out. The pulp was again suspended in an equal volume of acetone, strained and pressed out, after standing twenty minutes. It was then suspended in an equal weight of acetone, containing 5 ml of 0.1 M sodium carbonate per liter, strained and pressed out. The last step was repeated and the muscle pulp was spread on filter paper and air dried in the cold for two days. It was then stored in

a desiccator over calcium chloride until needed.

To extract the actin from the dried muscle pulp, the pulp was mixed with twenty volumes of carbon dioxide free water. This mixture was allowed to stand for twenty minutes, and then filtered on a Buchner funnel. The resulting solution was usually slightly turbid, and contained around five milligrams of actin per milliliter.

The solution was purified by adding 1 M acetic acid-acetate buffer of pH 4.6 until a precipitate was formed. This was centrifuged down and dissolved in 0.1 M sodium carbonate. The actin was reprecipitated, centrifuged, and redissolved.

4. Adenosine triphosphate:

The ATP used was obtained from Armour & Company as the dibarium salt and later from Schwarz Laboratories as the free acid. The dibarium salt was converted into the sodium salt by dissolving with a stoichiometric amount of sulfuric acid and then neutralizing with sodium hydroxide. The resulting barium sulfate was filtered off.

The free acid was dissolved in water or buffer solution and the pH readjusted with sodium hydroxide to the value desired.

When using the dibarium salt it was usually necessary to lyophilize in order to obtain solutions of the necessary concentration.

II. Analytical methods:

The protein concentrations were determined by the nesslerization method of Koch and McMeekin (31), the solutions being compared on a Fisher electrophotometer. A nitrogen factor of 6.00 was used for actomyosin (Bailey (32)), 6.63 for actin, and 6.20 for AF myosin. The last two were taken from Szent-Gyorgyi (2).

The enzyme activity of the preparations was checked by the method used by Singher and Meister (11), with the phosphorus analyses being made by the method of Sumner (33).

Viscosities were run in a modified Ostwald pipette, with a water flow time at 2.0° C of around 90 seconds. The viscosities were run in the thermostat bath of the electrophoresis apparatus, although checks were usually made at room temperature on the experiments, showing the drop in presence of ATP.

III. Electrophoresis:

The electrophoretic analyses were done on a Longsworth-Tiselius type apparatus, manufactured by the Klett Company. The cells were made by the Pyrocell Corporation. The boundary was observed by schlieren methods and the refractive index gradients recorded on 9 x 12 cm. plates. The plates were projected in an enlarger and traced. From the tracings, mobilities and relative concentrations were determined.

The solutions to be run in the apparatus were dialysed for three days statically, changing buffer each day; or, alternately, dialysed 24 hours, stirring the dialysing bag in the buffer, and then 24 hours statically with a change of buffer.

In all runs except the actomyosin-ATP runs the procedure was standard and no unusual errors should have occurred. In the actomyosin runs with ATP present, it was impractical to dialyse an actomyosin-ATP solution as the ATP would be dialysed off. The difficulty of obtaining ATP in large quantities prevents using it freely in all the buffer solutions. The ATP was added just prior to placing the protein solution in the cell, and although the volume of the ATP solution was kept as low as possible and the solution was prepared in buffer, there probably are present errors in the mobilities found, due to the presence of the undialysed solution.

One run was made in a compartmented, fractionation cell which permitted ATP solution to be placed in front of the rising, and behind the descending boundaries. This procedure eliminated

any conflicting ATP-buffer boundaries.

The current used was around 25 milliamperes, but due to low resistance of the buffer used, the heating effects were still slight enough so that no convection resulted.

Conductances were measured on a Jones conductivity bridge using a Jena glass cell with truncated platinum electrodes sealed directly to the glass.

Mobilities were calculated from the following equation

$$\mu = K \frac{d}{Rit}$$

μ is the electrophoretic mobility in cm/sec/volt, d is distance boundary moved, i is current, R the specific resistance, and t the time of the run. K is a constant which is characteristic of the cell.

For the cell used, the values of K were 2.768 for the left hand channel and 2.757 for the right hand channel.

IV. Light scattering measurements:

The light scattering instruments used in this investigation were those described by Blaker, Badger and Gilman (34). There are three types of measurements required to get a complete picture of a large molecule such as myosin. These are dissymmetry measurements, 90° scattering measurements, and determination of refractive index increment. They will be discussed individually.

1. Dissymmetry coefficient measurements:

Light from a mercury lamp (GE - AH-4) was filtered to isolate the line at 5461 Å, and then passed up through the bottom of a cylindrical cell. The cell has a window in the side which allows the scattered light to impinge on a strip of photographic film. This film is bent in a semicircle in the plane of the vertical axis of the cell, and has a series of step wedges interposed between it and the cell. Between each step wedge is a blank section. Thus on the exposure described above, light is recorded only on alternate sections of the film. At completion of the first exposure, the step wedges are displaced, and light from the same source is let on the cell through a window which is parallel to the vertical axis of the cell, and at 90° from the first mentioned exit window. Thus a record of 90° scattering is recorded side by side with the angular scattering. The strip is developed and the steps compared photometrically. There is obtained a value for the ratio of the intensity of the scattering at various angles to the intensity scattered from the same solution at 90°. The angular range covered is limited by the interference effects of the walls

of the cell and so is only from 52 to 124 degrees for the camera used. Values of the above ratio are determined at twelve angles in this range by the one exposure. A plot is made of this relative intensity, normalized to 1 at 90°. The ratio of the value at 60° to that at 120° is called the dissymmetry coefficient, q, and is determined from the plot. q is a function of the concentration and is usually extrapolated to zero concentration to obtain the intrinsic dissymmetry, [q]. Interpretation of this quantity will be discussed later.

2. 90° scattering measurements:

Light from the same type source described above is passed up through a cylindrical cell which has a small window allowing escape of the light scattered at 90° to a 931 A electron multiplier phototube. A small fraction of the incident light is reflected to another phototube and the outputs of the two tubes are balanced against one another by means of a potentiometer arrangement. By use of a sliding holder the scattered light may be polarized either vertically or horizontally, and thus the depolarization obtained.

The reading obtained above is compared on the instrument with light scattered from a sealed tube of carbon disulfide. The absolute value of the ratio of 90° scattered light intensity to the intensity of the incident light is known for this material and so a value is obtained for the solution under investigation.

The variation of this intensity with concentration is determined by adding increments of a concentrated protein solution

to the solvent in the cell, weighing and measuring the intensity as above. It is necessary in order to obtain accurate values, to remove all the dust from the solutions used. This is done by centrifuging for twenty minutes in a field 32,000 times that of gravity. Similar technique was used on the solutions for which dissymmetry measurements were made.

3. Refractive index increment determination:

This was carried out in a differential refractometer of the type described by P. Debye (35). The light source was again the filtered mercury arc lamp, and the deflection in the light passing through the protein solution from that passing through the reference buffer solution was measured on a Spencer eyepiece micrometer.

4. Molecular weight calculation:

The extinction coefficient, h, may be determined from the above obtained intensity by the equation:

$$h = \frac{16\pi}{3} \frac{i}{I_o}$$

where i is the intensity of the light scattered through 90°, and I_o is the intensity of the incident light.

h is related to the molecular weight by the formula

$$h = \left\{ \frac{\frac{32\pi^2 n^2}{3\lambda^4 N_o} \left(\frac{\partial n}{\partial c}\right)^2 c}{1/M + \frac{2Bc}{RT}} \right\} \frac{6+3\rho}{6-7\rho}$$

where

n is the refractive index of the solution

c is the concentration in grams per gram of solution

$\partial n/\partial c$ is the refractive index increment of the solute

λ is the wave length of the incident light

N_o is Avogadro's number

M is the molecular weight of the solute

B is a constant which describes the deviation of the system from van't Hoff's law

R is the gas constant

T is the absolute temperature

ρ is the depolarization of the scattered light

Combining the above two expressions and rearranging, we obtain

$$M = \frac{\lambda^4 N_o}{2\pi^2 n^2 \left(\frac{\partial n}{\partial c}\right)^2 (c/i/i_{cs_2})_{c\to 0} (I_o/i_{cs_2})}$$

These expressions are valid only for molecules small compared with the wave length of the light.

Using a value for I_o/i_{cs_2} of 4.4×10^{-5}, and wave length of 5461A, the equation reduces to

$$M = \frac{12.0}{\left(\frac{\partial n}{\partial c}\right)^2 \left(c/i/i_{cs_2}\right)_{c\to 0} n^2}$$

V. Buffer composition:

In the electrophoretic runs the buffer used was a barbital-hydrochloric acid solution of the following composition for three liters:

36.54 grams of diethyl barbituric acid

8.1 grams of sodium hydroxide

67.1 grams of potassium chloride

100 milliliters of 1 N hydrochloric acid.

The pH was adjusted to 7.85 with hydrochloric acid.

The phosphate buffer used in two of the electrophoretic runs was prepared as follows for twelve liters.

138.3 g disodium hydrogen phosphate duodecahydrate

5.9 g sodium dihydrogen phosphate monohydrate

268 g potassium chloride

The pH of this solution was 7.6.

For light scattering work, the buffers were similar to the above, but pH and ionic strength were varied somewhat. For the low pH barbital buffers, it was necessary to reduce the amount of diethyl barbituric acid to half that above, due to its slight solubility.

Experimental Results

I. Electrophoresis:

In table I are assembled the mobility data obtained for the proteins investigated. All the material used in these runs was less than a week old and generally three days old at start of the run. A number of preliminary runs made without regard to age of preparation are omitted. Despite this aging, little change in the electrophoretic properties was noted.

It will be observed that in some cases the mobility of either α or γ component of actomyosin is missing. This is due to the fact that the boundary in question is occasionally masked by the main β peak. The notation used to describe the peaks is that suggested by Dubuission (18). It is in accordance with general electrophoretic practice of designating the leading peak by α, and so on.

All runs were made at current of 25 milliamperes and for a duration of 25 hours, except for the actin run which was made at 15 milliamperes for 2 hours.

The mobility values for actomyosin-ATP in phosphate buffer are lower than the values in barbital buffer. This is to be expected in light of the work reported by Longsworth (36) on ovalbumin. Also the values obtained by Dubuission (18) and Ziff and Moore (17) indicate that the mobility is lower in phosphate buffer. This effect is due to the divalent character of the buffer ions.

It will be noted that the difference between the mobility for actomyosin and that obtained for actomyosin in the presence of ATP

is of the order of 0.1 mobility unit. This is within the probable experimental error of 5%. It is also possible that a larger experimental error is present in the actomyosin-ATP runs due to the impractibility of dialyzing. This point is discussed further in the experimental procedures section.

Table I

protein		$[\eta]$	$\mu \times 10^5$ descending	$\mu \times 10^5$ rising	concentration mg/ml	pH
actomyosin	α	3.4	3.04	2.96	3.40	7.82
	β		2.86	2.85		
	γ		2.66	2.57		
actomyosin	α	3.4	----	3.02	3.06	7.85
	β		2.93	2.86		
	γ		2.72	2.63		
actomyosin	α	3.4	3.15	----	5.0	7.86
	β		2.85	2.99		
	γ		2.50	----		
actomyosin-ATP	α	2.3	3.04	3.02	2.6	7.89
	β		2.81	2.84		
	γ		3.72	2.34		
actomyosin-ATP	α	2.3	2.93	2.93	0.7	7.85
	β		2.72	2.86		
	γ		2.52	2.72		
actomyosin-ATP	α	2.3	2.68	2.76	3.84	7.59
	β		2.52	2.70		
	γ		2.34	2.49		
AF myosin		2.1	2.66	2.74	3.10	7.77
AF myosin		2.1	2.53	2.59	3.10	7.77
actin		0.2	5.70	6.13	7.02	7.87
ATP			8.97	9.10	1.02	7.93

All runs were in 0.4 molar barbital buffer, except the last actomyosin-ATP run which was in phosphate buffer, and the actin run which was in 0.1 molar barbital buffer.

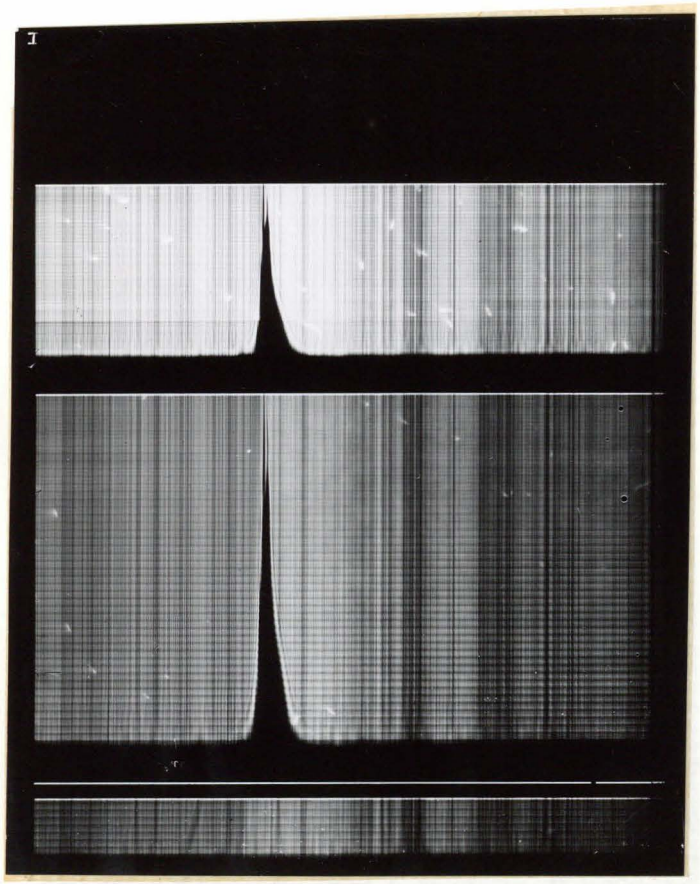
Plates I, II, and III show the rising and descending boundaries obtained for actomyosin, actin free myosin, and actin. The relative concentrations of α, β, and γ in actomyosin are 25%, 65%, and 10%.

Plate IV shows a typical pattern of an actomyosin ATP run. This plate was taken early during the run, when the fast moving boundary was well out, but had not yet disappeared by diffusion. This fast moving boundary had a mobility of the order of either actin or ATP. It was necessary to devise an experiment to determine which one it was.

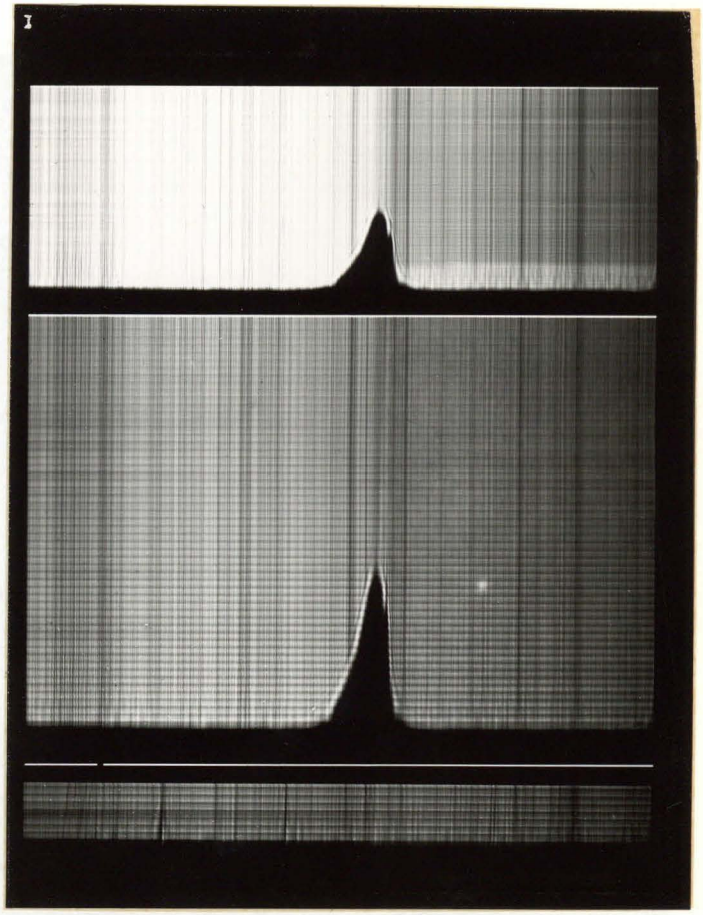
First, a run was made, and after a suitable time, the current was stopped and the leading, fast boundary was drawn off with a needle attached to the compensating device. This was analyzed for phosphate and for nitrogen. The values obtained indicated that the peak contained phosphate, and the N:P ratio was about that expected for ATP.

A more conclusive experiment was performed, however, when a fractionation type cell was made available. The two lower sections of the cell were filled with actomyosin-ATP solution, and the two upper channels with ATP solution in buffer. Thus a boundary was formed at the middle dividing plate between actomyosin-ATP-buffer solution and ATP-buffer solution. If the doubtful boundary was due solely to ATP, it should be eliminated by this procedure. The run could not be allowed to go as long as usual, as it was calculated that the ATP-buffer boundary formed at the upper dividing plate would overtake the actomyosin boundary in about 600 minutes.

Plate V was taken at 500 minutes and clearly shows that no peak has moved away from the actomyosin boundary. This appears to show



Rising

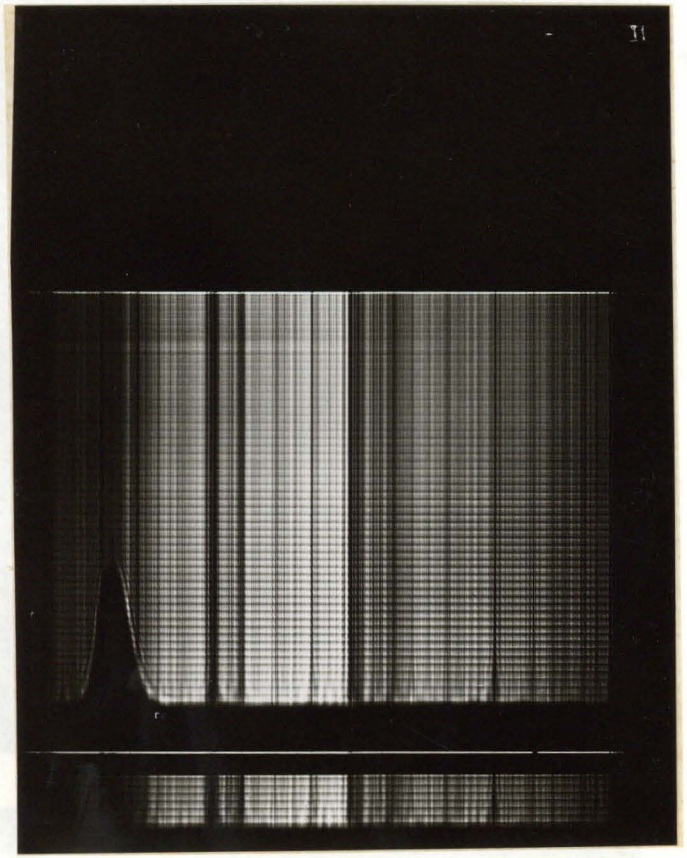


Descending

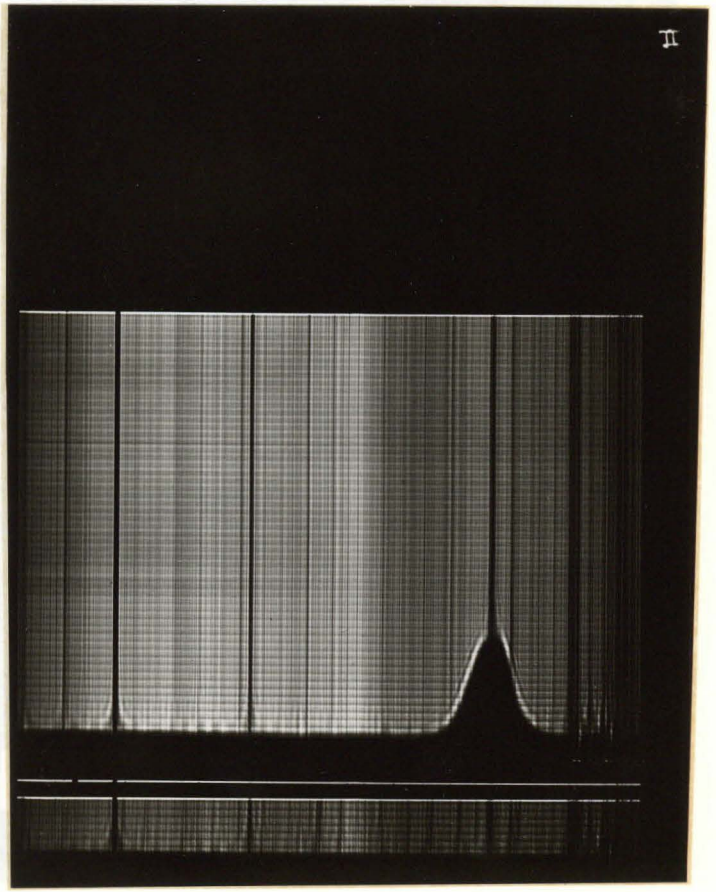
Upper: Reduced gear ratio
Lower: Normal gear ratio

Actomyosin

Plate I



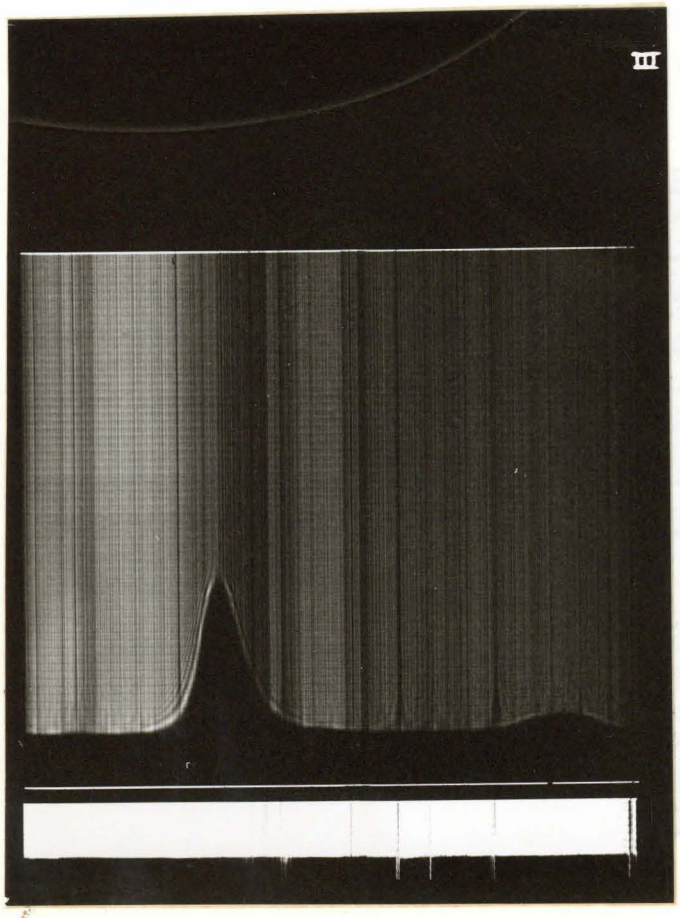
Rising



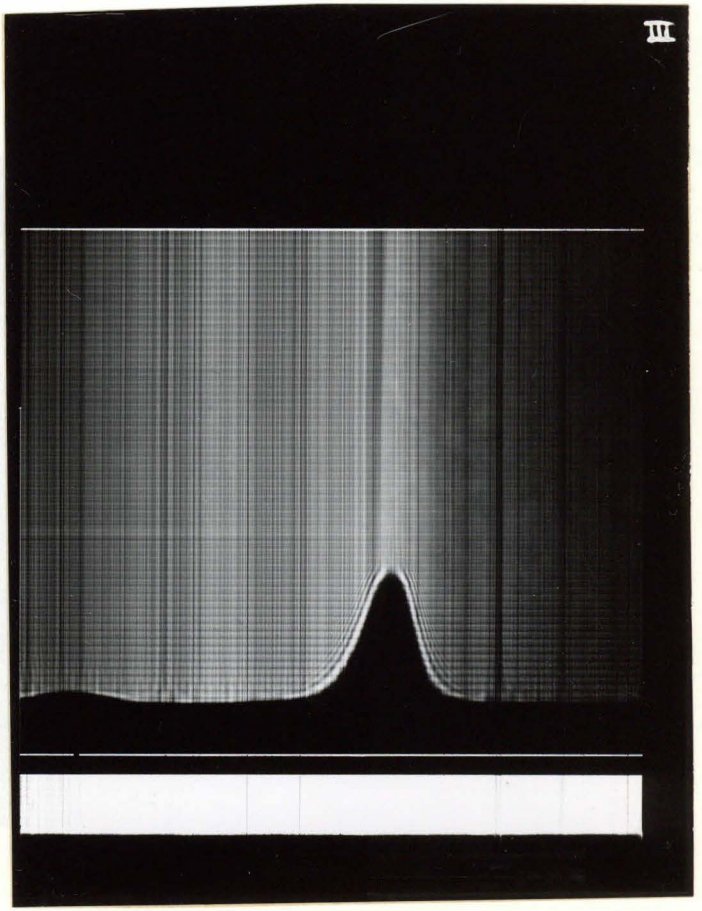
Descending

Actin-free Myosin

Plate II



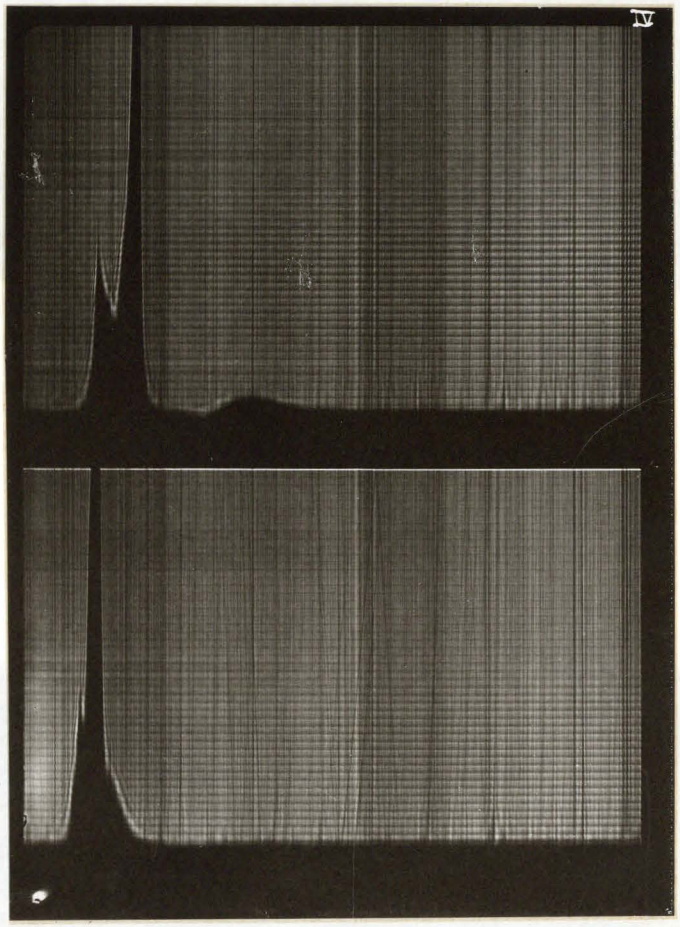
Rising



Descending

Actin

Plate III

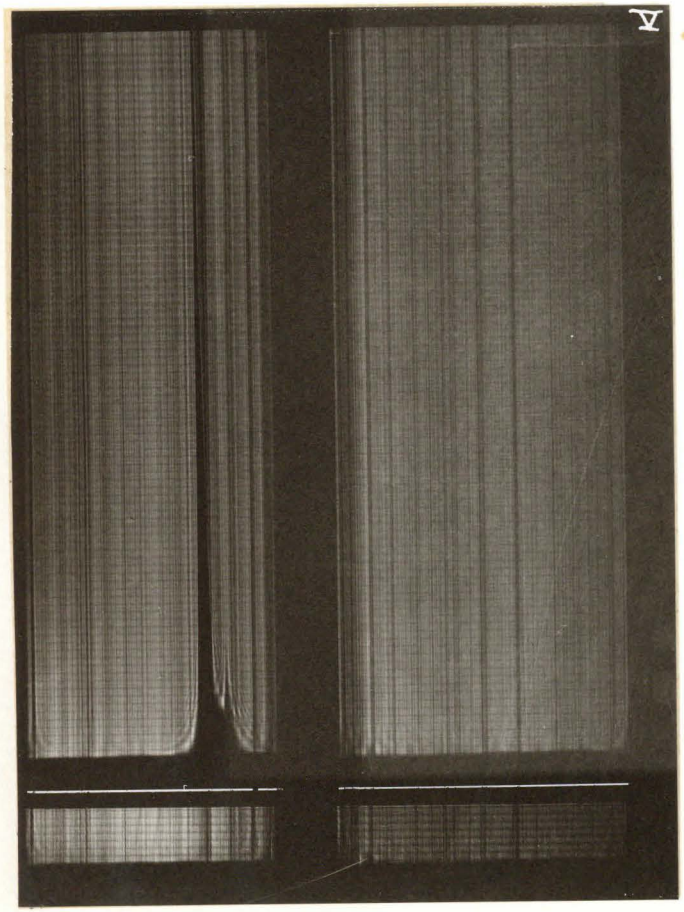


Descending

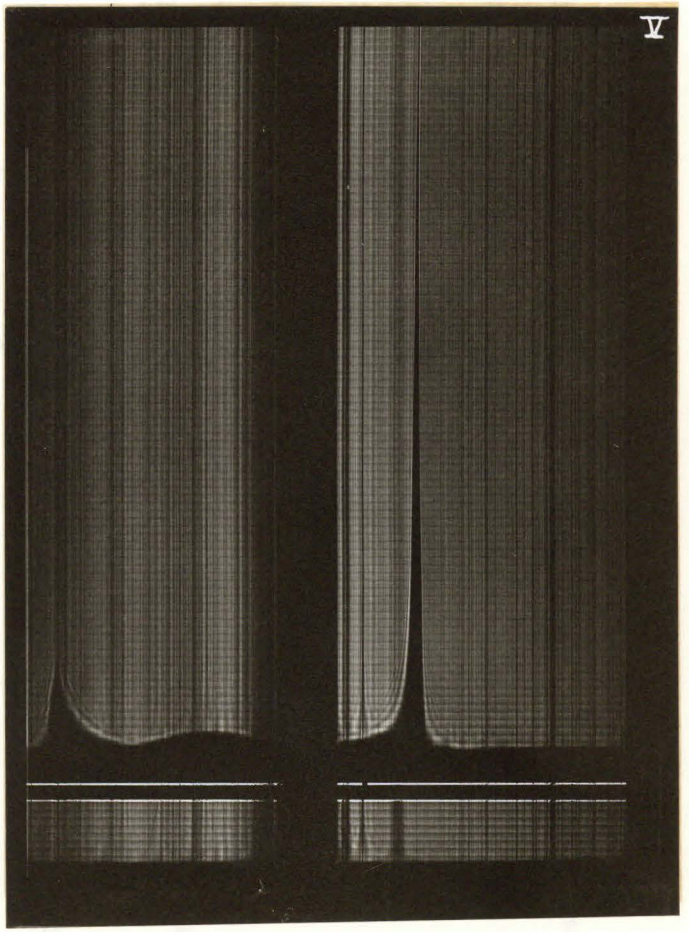
Lower: 29 minutes
Upper: 195 minutes

Actomyosin-ATP

Plate IV



Rising



Descending

Actomyosin-ATP

Plate V

definitely that the boundary in question was due to ATP alone.

Table II gives best values for the mobilities of the various components.

Table II

protein		$\mu \times 10^5$ cm/sec volt descending	pH
actomyosin	α	3.05	7.85
	β	2.86	
	γ	2.6	
actomyosin-ATP	α	2.98	7.85
	β	2.76	
	γ	2.6	
AF myosin		2.60	7.77
actin		5.70	7.87

II. Light scattering dissymmetry measurements:

The readings obtained from the film strip by a densitometer were converted into relative densities and tabulated as in Table III. Column 4 shows the reference 90° step used for each angle. The densitometer readings for this step, on each side of the corresponding angular step, were averaged. This average was then interpolated into the densitometer readings for the angular step, and a fractional value obtained which was entered into column 3. The values in column 5 represent the differences in densities of column 3, and 4, using a calibration graph for the step wedges. The sixth column is obtained by taking the antilogarithm of the density difference. This column is the relative intensity.

These relative intensities are plotted versus the angle and from the plot a value of the relative intensity at 90° is found. This is set equal to one and the rest of the values converted to this scale. These normalized values are plotted against the angle as shown in Figure 1.

This figure shows two such runs on the same solution, indicating the scatter obtained between check runs. From the value of the intensity at 60° to that at 120° the dissymmetry coefficient is calculated.

The data from all the runs made was treated in this fashion and in Table IV are listed the dissymmetry coefficients obtained for the conditions and concentrations indicated.

In the earlier, unbuffered actomyosin runs, the change of the dissymmetry coefficient with time was followed and the results

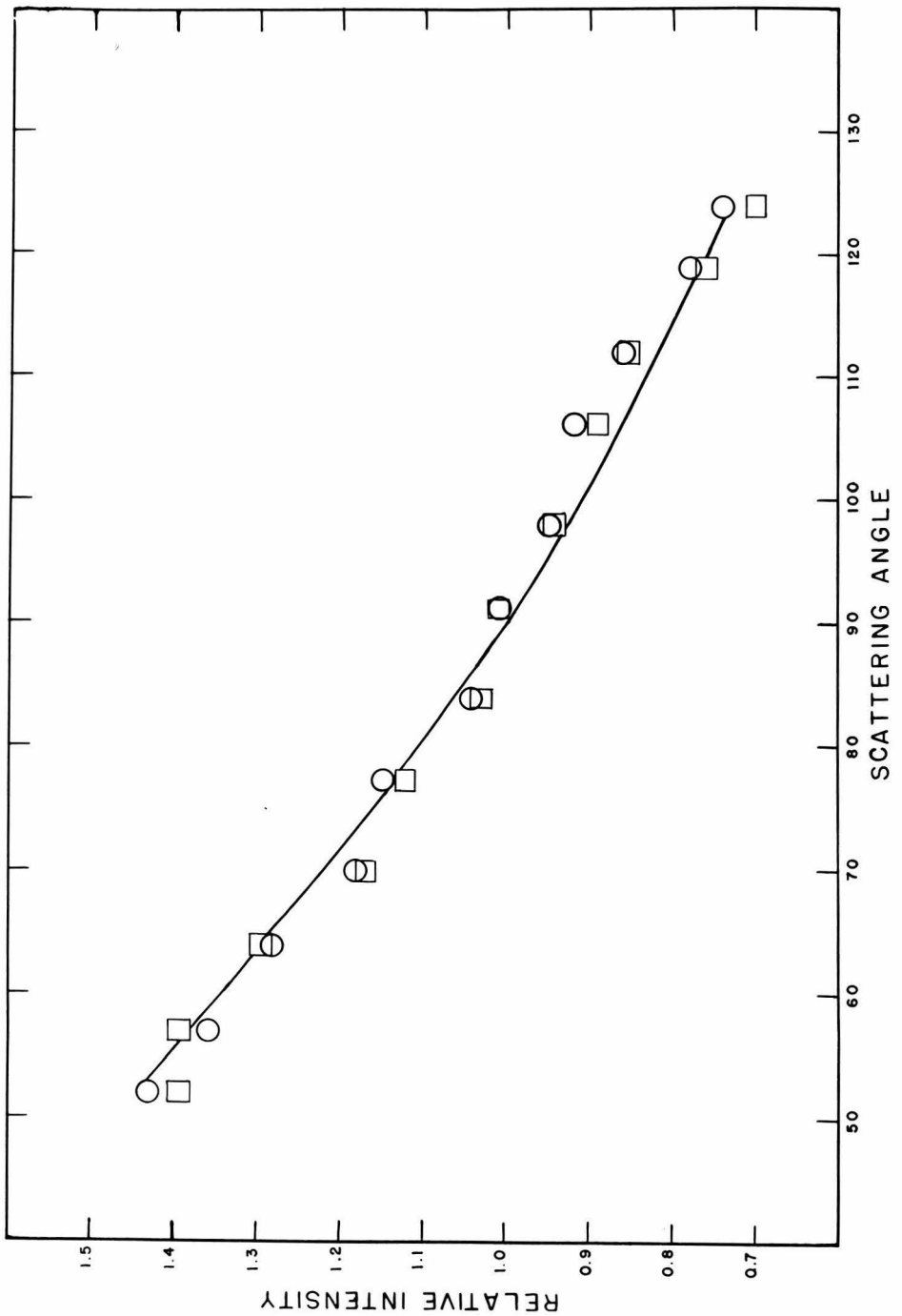

RELATIVE INTENSITY
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
SCATTERING ANGLE
50
60
70
80
90
100
110
120
130

FIGURE 1

indicate a slight recovery.

The values of the dissymmetry coefficient were plotted against concentration and the intercept at zero concentration is taken as the intrinsic dissymmetry coefficient. A typical plot is shown in figure 2.

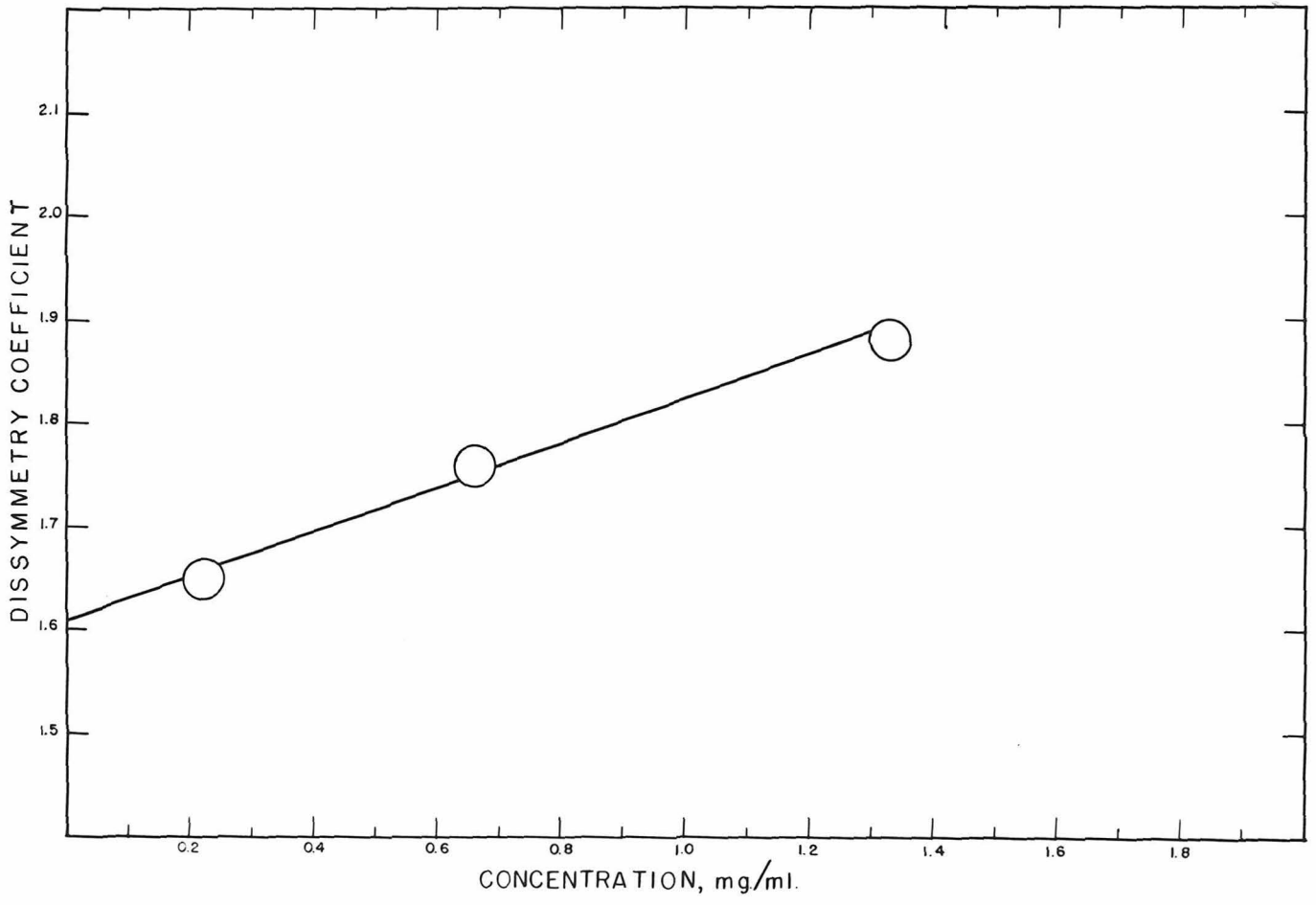

DISSYMMETRY COEFFICIENT
2.1
2.0
1.9
1.8
1.7
1.6
1.5
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
CONCENTRATION, mg./ml.

FIGURE 2

Table III

actomyosin - ATP in barbital buffer. concentration 0.67 mg/ml

aperture	angle	angular step	reference step	$D_\Theta - D_{90°}$	$\frac{I_\Theta}{I_{90°}}$
3	124	1.25	2	-0.120	0.76
4	119	1.40	2	-0.095	0.80
5	112	1.65	2	-0.055	0.88
6	106	1.85	2	-0.025	0.94
7	98	1.92	2	-0.014	0.97
8	91	1.11	1	0.011	1.03
9	84	1.20	1	0.025	1.06
10	77	1.47	1	0.069	1.17
11	70	1.53	1	0.080	1.20
12	64	1.78	1	0.119	1.31
13	57	1.93	1	0.142	1.39
14	52	2.08	1	0.164	1.46
				90°	- 1.02

Table IV

protein	$[\eta]$	q	concentration mg/ml		Remarks
actomyosin	3.0	1.93	1.02	6.0	0.5M KCl
actomyosin-ATP	2.3	1.72	1.08	6.0	0.5M KCl
		1.78			16 hours later
actomyosin B	4.0	2.10	0.90	6.1	0.5M KCl
actomyosin B-ATP	2.3	2.36	0.95	6.8	0.5M KCl
		2.24			28 hrs. later
		2.26			67 hrs. later
actomyosin	3.2	2.01	0.17	6.80	0.4M phosphate buffer
		2.26	0.41	6.80	
		2.38	0.84	6.80	
actomyosin-ATP	2.3	2.86	0.76	6.80	0.4M phosphate buffer
		2.70	0.30	6.80	
actomyosin	3.2	2.18	1.10	6.98	0.4M barbital buffer
		1.91	0.44	6.98	
actomyosin-ATP	2.3	1.65	0.22	6.50	0.4M barbital buffer
		1.76	0.66	7.00	
		1.88	1.33	7.00	
A-F myosin	2.1	1.36	1.14	6.75	0.4M phosphate buffer
		1.39	0.45	6.75	
		1.25	0.26	6.75	
G-actin	0.1	2.52	0.25	6.5	water
		2.82	0.70	6.8	
		2.98	1.27	6.7	
F-actin	2.0	1.72	1.03	7.7	0.1M bicarbonate buffer
		1.61	0.47	7.7	
actin	0.2	1.39	0.60	7.3	0.1M phosphate buffer

Table V

protein	[q]	pH	Remarks
actomyosin	1.72	6.98	0.4M barbital buffer
actomyosin	1.96	6.80	0.4M phosphate buffer
actomyosin-ATP	1.61	7.00	0.4M barbital buffer
actomyosin-ATP	2.60	6.80	0.4M phosphate buffer
A-F myosin	1.26	6.75	0.4M phosphate buffer
G-actin	2.44	6.7	water
F-actin	1.56	7.7	0.1M bicarbonate buffer

Table V is a summary of intrinsic dissymmetry coefficients for the proteins investigated.

The error in q is probably around 5% so values given for [q] are valid to within 0.05.

III. Light scattering molecular weights:

The intensity readings obtained as described in the procedure section were tabulated and corrected as indicated in table VI. The depolarization, ρ, was plotted versus the concentration and the intercept at zero concentration was used to calculate a value for the Cabanne's factor. Figure 3 shows a typical plot. The variation is large, but the correction factor is small.

The corrected intensity was divided into the concentration and the resultant ratio was plotted versus concentration. Figure 4 is a typical plot. The intercept at zero concentration is used to calculate the molecular weight using the formula given before.

The refractive index increments determined on the differential refractometer were 0.192 for actin, 0.164 for AF myosin and 0.159 for actomyosin.

This calculated molecular weight is still uncorrected for the dissymmetry of the molecule. The correction will be discussed later. Table VII gives uncorrected values of the molecular weight.

The probable error in these values is about 20%.

On both types of measurements, in the presence of ATP, the time elapsed in the run was less than the recovery time of the viscosity drop. It was further checked on the molecular weight run by adding additional ATP at the end with little effect.

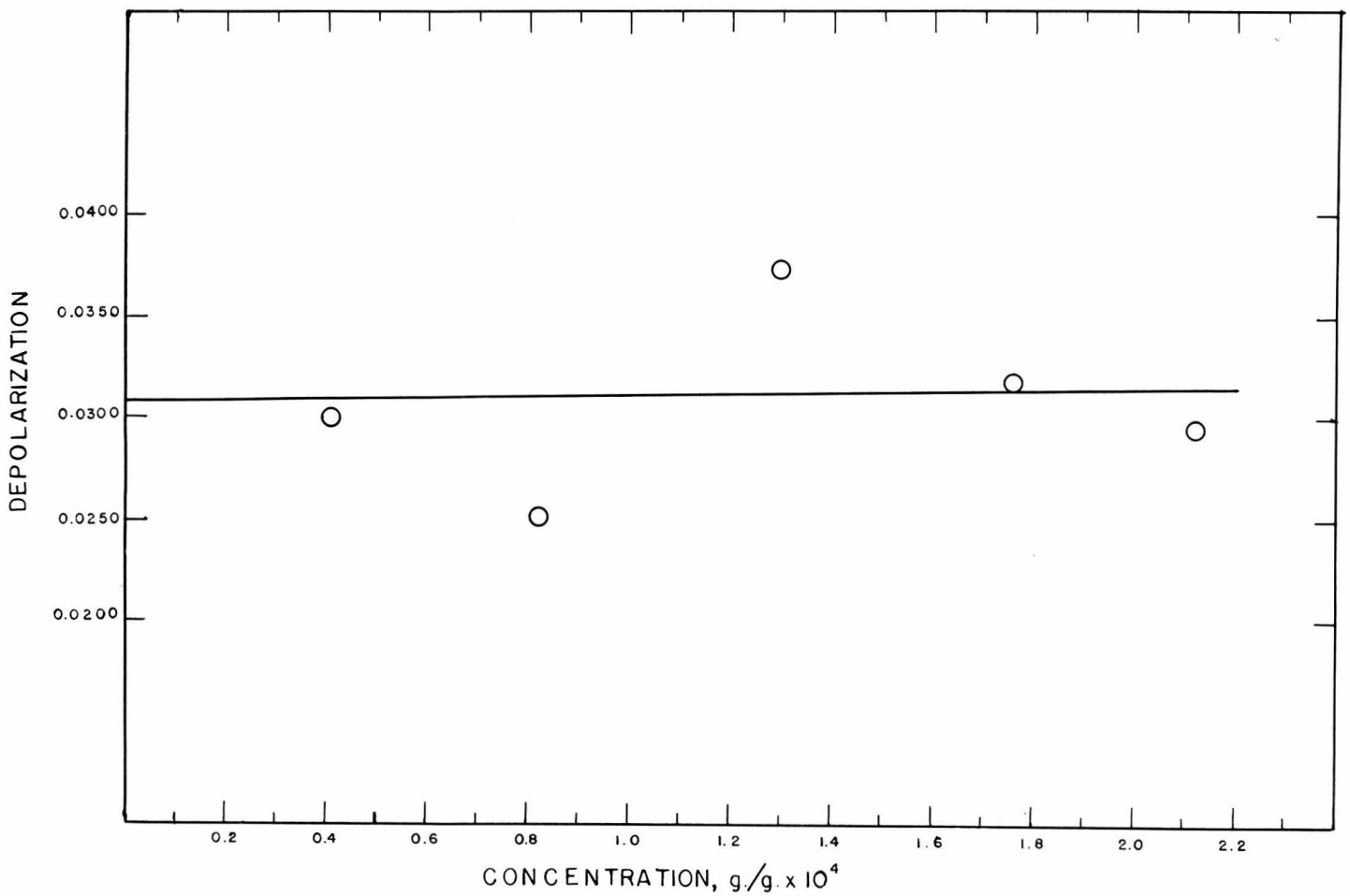

DEPOLARIZATION
0.0400
0.0350
0.0300
0.0250
0.0200
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
CONCENTRATION, g./g. x 10⁴

FIGURE 3

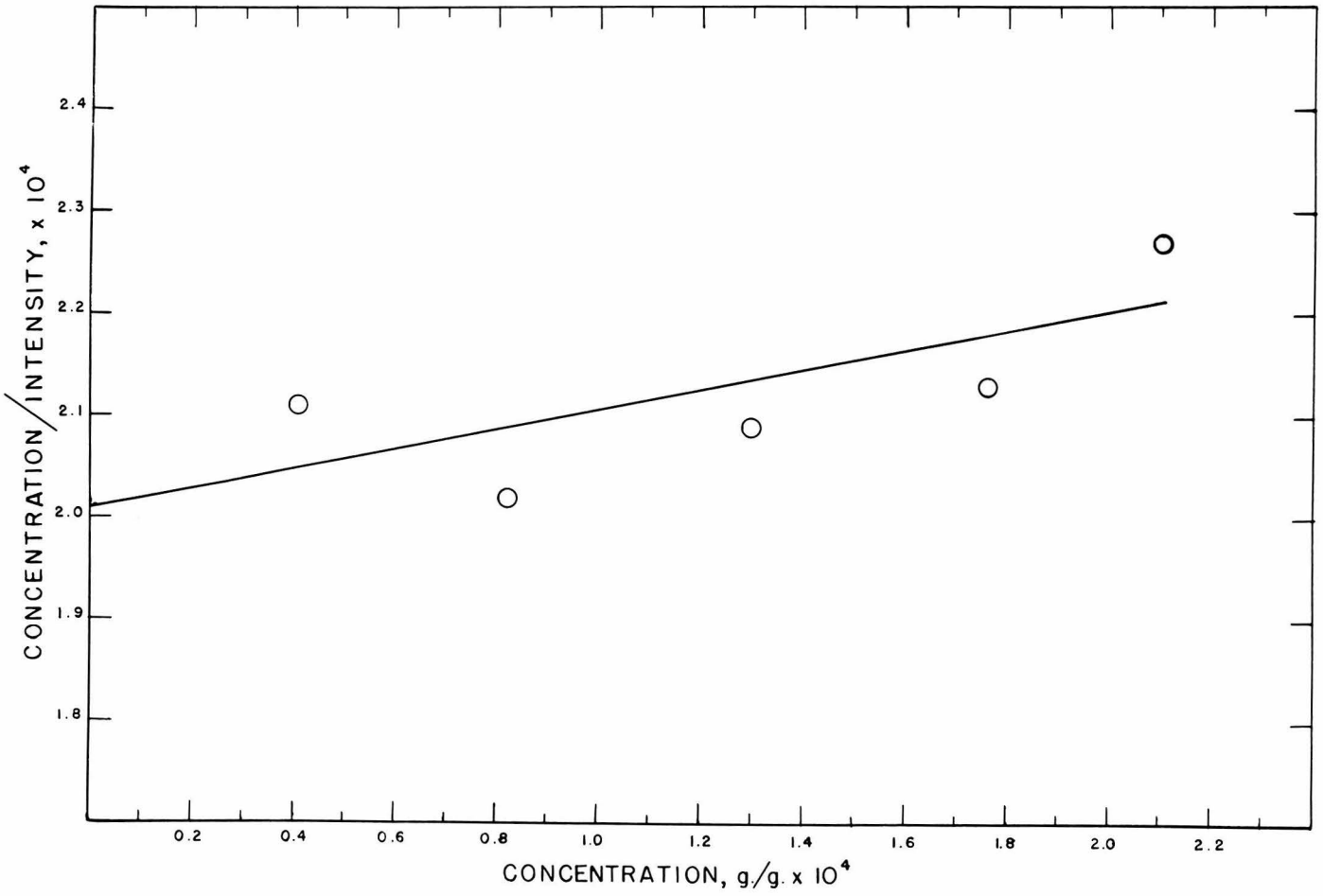

CONCENTRATION/INTENSITY, x 10⁴
2.4
2.3
2.2
2.1
2.0
1.9
1.8
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
CONCENTRATION, g./g. x 10⁴

FIGURE 4

Table VI

Actomyosin-ATP in phosphate buffer

	I	II	III	IV	V
1. concentration g/g x10^4	0.41	0.82	1.30	1.76	2.10
2. relative intensity, solution	0.307	0.530	0.758	0.974	1.077
3. relative intensity, solvent	0.103	0.103	0.103	0.103	0.103
4. line 2 - line 3	0.204	0.427	0.655	0.871	0.974
5. ρ	0.0301	0.0252	0.0374	0.0318	0.0295
6. $\rho]_{c\to o}$		0.0308			
7. $\frac{6-3\ \rho]_{c\to o}}{6\ 7\ \rho]_{c\to o}}$		0.95			
8. corrected relative intensity	0.194	0.406	0.622	0.827	0.925
9. concentration/relative intensity x10^4	2.11	2.02	2.09	2.13	2.27

$$c/_i]_{c\to o} = 2.01 \times 10^{-4}$$

$$M = \frac{12}{2.01 \times 10^{-4}(0.159)^2(1.337)^2}$$

$$= 1.33 \times 10^6$$

Table VII

protein	Molecular weight uncorrected $x10^{-6}$		pH	Remarks
actomyosin	3.82	3.0	6.3	0.5M KCl
actomyosin	3.79	3.0	6.8	0.4M phosphate buffer
actomyosin-ATP	1.33	2.3	6.9	0.4M phosphate buffer
actomyosin-ATP	1.75	2.3	7.2	0.4M barbital
actomyosin B	12.0	4.0	6.8	0.5M KCl
A-F myosin	1.05	2.1	6.8	0.4M phosphate buffer
G-actin	0.238	0.1	6.8	water
F-actin	0.490	2.0	7.5	0.1M bicarbonate buffer
Actin	0.490	0.2	7.8	0.1M phosphate buffer

Discussion

I. Treatment of light scattering results.

In order to interpret the light scattering data, it is necessary to apply a correction to the molecular weight. The molecular weights were calculated on the assumption that the molecule was small as compared with the wave length of light. In the proteins considered here, they are not. Therefore there arises an interference effect due to scattering from different regions of the molecule. This causes the scattering envelope around the molecule to become asymmetric, and reduces the total intensity of scattered light. This asymmetry gives rise to the dissymmetry coefficient, and the reduction in intensity scattered at 90° causes the molecular weights calculated in the preceding section to be low.

Debye (37) has derived equations which give the intensity as a function of the scattering angle, θ, the wave length of the incident light, and the length of the particle for three models: a sphere, a random coil, and a long thin rod. From these equations the intensities needed to calculate the dissymmetry coefficient and the correction factor may be obtained.

Figure 5 and figure 6 were calculated from these equations. From figure 5, the length of the particle may be determined from the experimental value of the dissymmetry coefficient. From figure 6, the correction factor to multiply the previously calculated molecular weight may be obtained from the length. This length is the diameter of the sphere model, the root mean square separation of the ends for the coil, and the length of the rod.

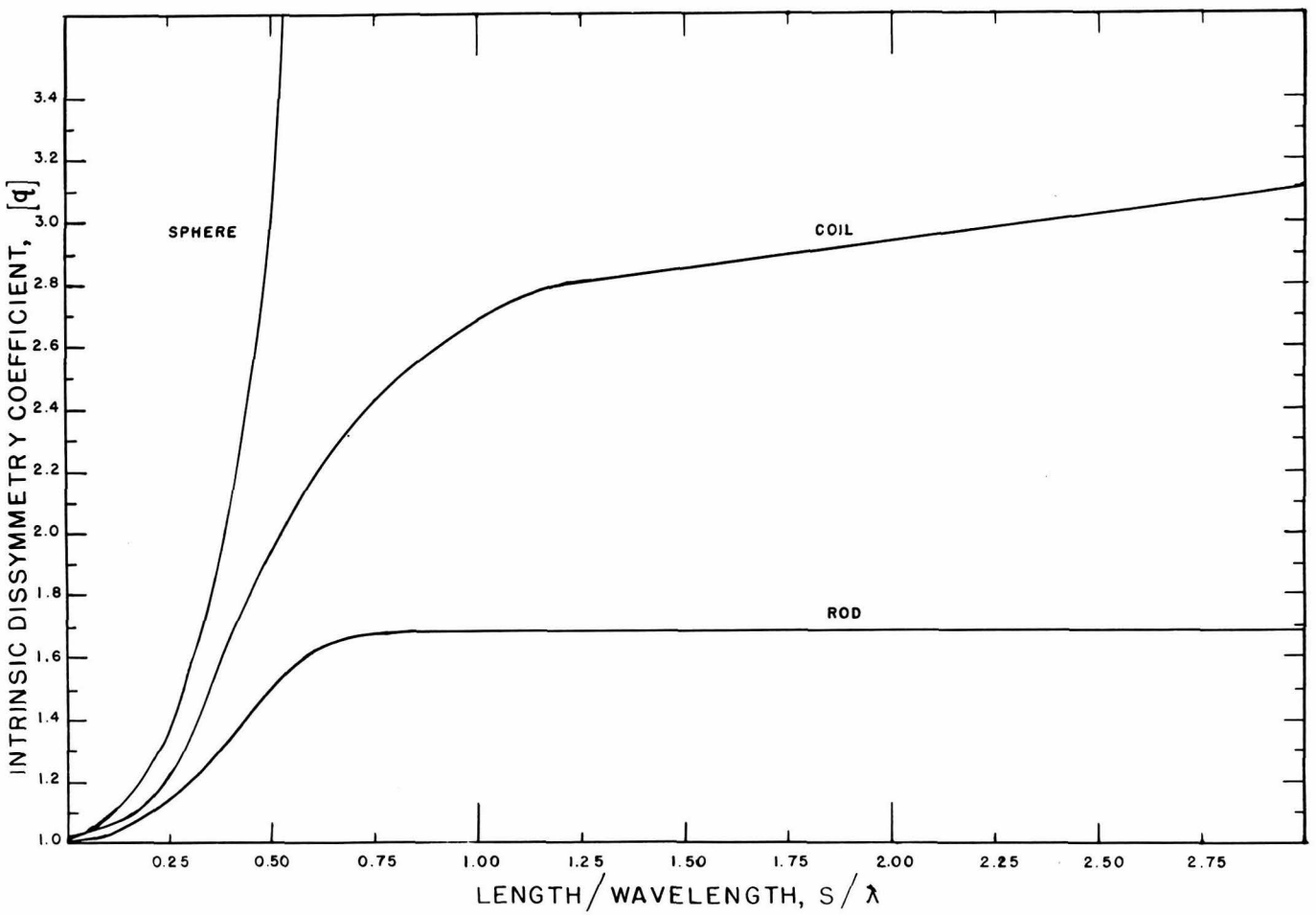

INTRINSIC DISSYMMETRY COEFFICIENT, [q]
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
SPHERE
COIL
ROD
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
LENGTH/WAVELENGTH, S/λ

FIGURE 5

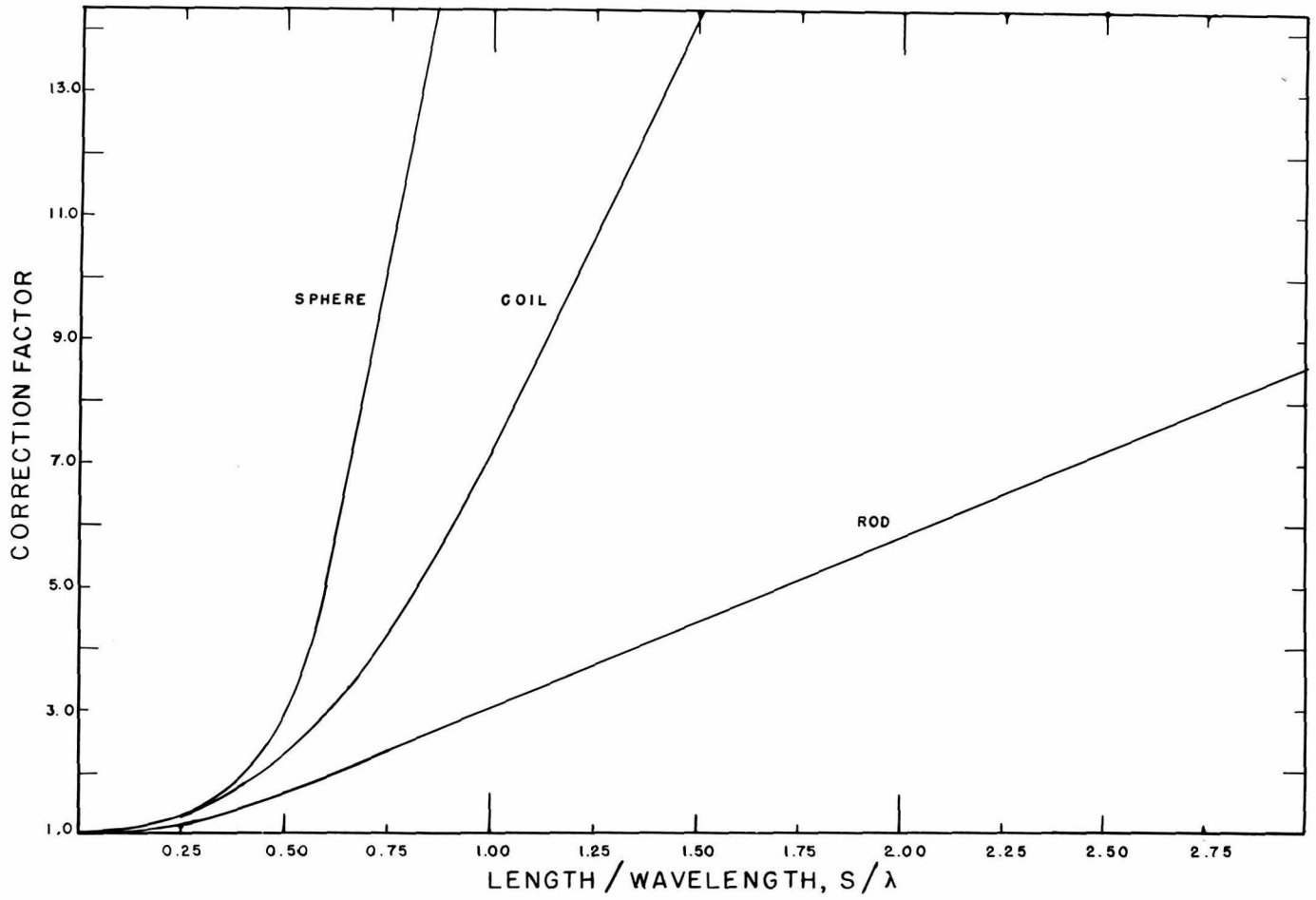

CORRECTION FACTOR
13.0
11.0
9.0
7.0
5.0
3.0
1.0
SPHERE
COIL
ROD
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
LENGTH / WAVELENGTH, S/λ

FIGURE 6

Therefore, it is possible to obtain a length and a correction factor from the dissymmetry coefficient, if a model is chosen for the molecule concerned.

In table VIII are given the molecular weights calculated in the preceding section, and the determined dissymmetry coefficients. The corresponding values for lengths, correction factors, and corrected molecular weights are also given for each of the three possible shapes.

II. Determination of shape of molecule

To decide which of these shapes is the best approximation to the molecules investigated, we can compare the corrected molecular weight with the length determined by the dissymmetry coefficient for each model. This can be done by calculating the dimension required from the molecular weight using the geometry of the model.

For the sphere, this is simple; the equation connecting the two quantities being

$$M = \frac{\pi D^3 N}{6V} = 4.20 \times 10^{23} D^3$$

where D is the diameter of the sphere, M is the molecular weight, N is Avogadro's number, and V is the partial specific volume of the protein. The last quantity is taken to be 0.75 as this is the value generally used for all proteins and was used for myosin by Edsall (4).

For the rod, we need not only the length, but the diameter, of the cylinder, to obtain a molecular weight. By using the results of Mehl, Oncley, and Simha (38), we can get the axial ratio, A, from

Table VIII

	AFmyosin	F-actin	G-actin	actomyosin barbital buffer	actomyosin phosphate buffer	actomyosin — ATP barbital buffer	actomyosin — ATP phosphate buffer
molecular weight$\times 10^{-6}$	1.05	0.490	0.238	3.79	3.79	1.75	1.33
[q]	1.26	1.56	2.44	1.72	1.96	1.61	2.60
S/λ sphere	0.22	0.30	0.45	0.34	0.39	0.32	0.37
S/λ coil	0.27	0.37	0.76	0.42	0.51	0.39	0.90
S/λ rod	0.36	0.55	-	1.0	-	0.59	-
D , mμ	90	123	185	139	159	131	151
R , mμ	110	152	312	171	208	159	368
L , mμ	147	226	-	408	-	241	-
correction factor:							
sphere	1.20	1.50	2.40	1.70	1.95	1.60	1.84
coil	1.35	1.70	4.30	1.95	2.40	1.80	5.75
rod	1.32	1.75	-	3.15	-	1.85	-
corrected molecular weight:							
sphere	1.26	0.735	0.572	6.45	7.40	2.80	2.45
coil	1.42	0.832	1.02	7.40	9.10	3.15	7.65
rod	1.39	0.856	-	11.9	-	3.24	-

the viscosity. The resulting equation is

$$M = \frac{\pi L^3 N}{4A^2 V} = 6.31 \times 10^{23} \frac{L^3}{A^2}$$

where L is the length as determined from the dissymmetry coefficient, and A is the axial ratio from viscosity.

For the random coil, Kirkwood and Riseman (39) have shown that

$$b^3 = \frac{2267\, M_0}{(6\pi^3)^{\frac{1}{2}} N} \lim_{z \to \infty} \frac{[\eta]}{z^{\frac{1}{2}}}$$

where b is the effective bond length, M_0 is the molecular weight of one unit of the coil, z is the ratio of the total molecular weight to the molecular weight of one unit, and $[\eta]$ is the intrinsic viscosity. They also give the relation

$$R = bz^{\frac{1}{2}}$$

for the root mean square separation of the ends. Rearranging and assuming that we are at the limiting value of $[\eta]/z^{\frac{1}{2}}$, we get

$$M = \frac{(6\pi^3)^{\frac{1}{2}} N R^3}{2267[\eta]} = 3.62 \times 10^{21} \frac{R^3}{[\eta]}$$

From the above equations we have calculated the lengths from the experimental molecular weights, and compared them with the lengths determined experimentally. This comparison is also presented by obtaining the molecular weights from the experimental lengths, and comparing with the experimental molecular weights. These comparisons are given in table IX.

The agreement for the coil model is good for AF myosin, and

Table IX

	AF myosin	F-actin	G-actin	actomyosin barbital buffer	actomyosin phosphate buffer	actomyosin ATP barbital buffer	actomyosin ATP phosphate buffer
$[\eta]$	2.1	2.0	~0.1	3.0	3.0	2.3	2.3
A	66	64	~10	80	80	69	69
R from $[q]$, mμ	110	152	312	171	208	159	368
R from $[\eta]$ and M	94	77	19	183	196	126	169
L from $[q]$, mμ	147	226	-	408	-	241	-
L from A and M	212	177	-	494	-	290	-
D from $[q]$, mμ	90	123	185	139	159	131	151
D from M , mμ	14	12	11	25	26	19	18
$Mx10^{-6}$ for coil:							
experimental	1.42	0.832	1.02	7.40	9.10	3.15	7.65
from $[q]$ and $[\eta]$	2.30	6.34	11.0	6.03	10.8	6.30	78.5
$Mx10^{-6}$ for rod:							
experimental	1.39	0.856	-	11.9	-	3.24	-
from $[q]$ and A	0.46	1.77	-	6.7	-	1.85	-
$Mx10^{-6}$ for sphere:							
experimental	1.26	0.735	0.572	6.45	7.40	2.80	2.45
from $[q]$	300	780	2,650	1,120	1,680	950	1,450

actomyosin in barbital and phosphate buffer. For actomyosin-ATP in barbital buffer, there is about the same amount of agreement for both coil and rod models. For actomyosin-ATP in phosphate buffer, and for G-actin there is no good agreement with any of the models. In the G-actin case, this may be due to a poor value for the intrinsic viscosity. F-actin appears to give the best results with the rod shape.

The conclusion reached was that the random coil is probably the best representation for the molecules involved here, and in the discussion which follows that model shall be used.

III. Interpretation of electrophoresis patterns.

We would now like to see how our results agree with the theories of actomyosin-ATP interaction. Looking first at the electrophoretic patterns, we note that there are no major changes in either the peaks present or the mobilities of the components when ATP is added. If complete dissociation occurs, as Szent-Gyorgyi states, then an actin peak should move away from the main peaks rapidly, as its mobility is twice as large. If there was a large amount of contraction or lengthening in the components, there should be a mobility change.

Riseman and Kirkwood (5) have predicted that the change in the total charge of the molecule when ATP is added should be around one hundred electron charges. It is possible to obtain an idea of the magnitude of the charge on the actomyosin molecule by using the equations developed by Gorin (40), based on the Henry solution of the problem of a charged particle moving through a dielectric medium. If we use the model of a long cylinder, which is the closest of those developed by Gorin to the actual molecule, we get the relation:

$$\mu = \frac{2\,Q\,C}{\eta_o(L-2a)}\left[\frac{K_0(\varkappa a+\varkappa r)}{(\varkappa a+\varkappa r)K_1(\varkappa a+\varkappa r)} + \ln\left(\frac{a+r}{a}\right)\right]$$

where μ is the electrophoretic mobility, Q is the charge on the molecule, η_o is the viscosity of the solvent, L is the length of the cylinder, a is the radius of the cylinder, r is the average radius of the ions in the medium, C is a factor depending on the dimensions of the molecule, and K_0 and K_1 are Bessel functions which are related to the Bessel functions of the third kind, $H_n^{(1)}$, of Jahnke and Emde (41) by the equation

$$K_n(z) = \tfrac{1}{2}\pi i \exp\left(\tfrac{1}{2}n\pi i\right) H_n^{(1)}(iz)$$

$\varkappa$ is the quantity usually appearing in Debye-Huckel theory, and is 0.2050×10^8 for the solvent used.

Using 4π as the value of C, 410 mμ for L, 7 mμ for a, 0.25 mμ for r, and 0.167 for η_o ; we obtained the equation

$$Q = 300\,\mu$$

for Q in electronic charges. This value is good to around 20%.

From this for actomyosin at pH 7.8 the total charge is around 900 units. Thus a change of 100 units, as predicted by Riseman and Kirkwood, would cause a change in the mobility of 0.30×10^{-5} cm/volt sec.

The difference observed was 0.10×10^{-5}. This is not significant since the possible error was 0.20×10^{-5}. This lack of change could possibly be explained on the basis that the viscosity would change with the change in shape and this effect might oppose the change due to charge.

We must conclude, however, that the electrophoretic evidence reveals no great changes occurring as would be expected by the viscosity change. This lassitude may be due to the low temperature (2° C) at

which electrophoretic runs are made. However, the viscosity drop is obtained at this temperature, although its recovery to the original value is extremely slow, being of the order of days rather than hours. Needham (3) also reports a flow birefringence drop at this temperature.

Turning now to the light scattering data, we see that the molecular weight drops, but not to the value of the molecular weight of AF myosin, which would be expected if the simple dissociation theory of Szent-Gyorgyi held. In barbital buffer, the drop is larger than in phosphate buffer. The dissymmetry coefficients also change differently in the different buffers. In barbital, there is a slight drop, while in phosphate, there is a marked increase. It is this latter result which was observed by Oster (29) and interpreted as evidence of a contraction occurring.

IV. Proposed mechanism to explain results.

Thus we still seem to have contradictory results in that electrophoresis shows no change, while light scattering indicates large changes, but of an unpredicted nature. It is possible to connect this evidence if we resort to a new explanation of what occurs when ATP is added. It is with some reluctance that the following mechanism is proposed, as it can only be a crude approximation to the true events. However, it does account for the experimental evidence in a fairly satisfactory manner, and so probably has some connection to reality.

Confining ourselves for the present to the experiments run in barbital buffer, let us assume that dissociation does occur, but not in the manner stated by Szent-Gyorgyi. Instead of his complete dissociation into F-actin and AF myosin, we shall postulate that the complex molecule breaks into n particles in the presence of ATP. Thus if the

original complex actomyosin molecule contains x molecules of F-actin and y molecules of AF myosin, then the dissociated complex molecules contain x/n molecules of F actin and y/n molecules of AF myosin.

To be able to handle this mechanism simply, we shall assume that there is only one type of complex molecule originally, and that upon dissociation it becomes n segments which are all the same. It is further assumed that no F actin or AF myosin is lost or gained from the complex type molecules during this process.

By use of graphs given by Szent-Gyorgyi (2), an estimation of the amount of actin in the actomyosin complex can be made from the viscosity of the preparation. For the material used here, the complex solution contains 7% F-actin and 93% AF myosin. This ratio corresponds to 12 F-actin molecules to 88 AF myosin molecules in the solution.

For these relative proportions of molecules, it is reasonable to assume that all the actin molecules will be in the complex molecule, while some of the myosin molecules may be unattached to it. This is supported by absence of any actin pattern in the electrophoresis of normal actomyosin.

On the basis of the above assumptions, we can now calculate the molecular weight of a mixture of N_c grams of actomyosin complex and N_m grams of AF myosin. Since the molecular weights concerned are weight averages, we have

$$M = \frac{N_c M_c + N_m M_m}{N_c + N_m}$$

Now $N_c = n_c w_c$ and $N_m = n_m w_m$ where the n's are the numbers of molecules and the w's the weights of the molecules of each type.

Taking the determined molecular weights of F actin and AF myosin as $8 \times 10^{+5}$ and 1.4×10^6, and using the weight percentages of 7% and 93%, we can calculate the number of actin and myosin molecules in one gram of total actin and myosin. We get 5.28×10^{16} molecules of actin and 40.1×10^{16} molecules of myosin. Then

$$n_c = \frac{5.28 \times 10^{16}}{x}$$

and

$$n_m = 40.1 \times 10^{16} - 5.28 \times 10^{16} \frac{y}{x}$$

as the number of complex actomyosin and free AF myosin molecules, respectively.

Now,
$$w_c = \frac{8 \times 10^5}{6.03 \times 10^{23}} x + \frac{1.4 \times 10^6}{6.03 \times 10^{23}} y$$

$$w_m = \frac{1.4 \times 10^6}{6.03 \times 10^{23}}$$

$$M_c = 6.03 \times 10^{23} w_c$$

Rewriting our expression for the resultant molecular weight,

$$Mx = \frac{Mx}{10^6} = 0.55 x^2 + 0.192 xy + 0.168(y^2-y) + 1.30 x$$

Rearranging,

$$y^2 - y + 1.14 xy = \left(\frac{M_o}{0.168} - 7.74\right) x - 0.327 x^2$$

Now using the molecular weights obtained in the absence and in the presence of ATP, we obtain two equations:

$$y_1^2 - y_1 + 1.14\, x_1 y_1 = 36.3 x_1 - 0.327\, x_1^2$$

$$y_2^2 - y_2 + 1.14\, x_2 y_2 = 11.0 x_2 - 0.327\, x_2^2$$

where the subscript 2 is the solution containing ATP.

Table X gives the values of y obtained for the corresponding value of x. Also given are the values of x for which the maximum number of AF myosin molecules will be in the complex.

Using now the assumption that the splitting is into n equal segments, we have

$$y_2 = \frac{y_1}{n} \; ; \qquad x_2 = \frac{x_1}{n}$$

We can solve the two equations above simultaneously, and obtain x and y as functions of n. Then trying integral values of n, we can get the following results:

$$n = 2; \quad x_1 = 0.24 \; ; \quad y_1 = 3.3$$

$$n = 3; \quad x_1 = 7.6 \; ; \quad y_1 = 12.6$$

$$n = 4; \quad x_1 = -9.6 \; ; \quad y_1 = 24.1$$

In table X are also tabulated the values of x_1/n and y_1/n for the various integral values of n. From both of these devices, it is seen that the best results are with n = 3. A better fit in the table could be obtained with n = 2.5, but for the crude theory presented here, this is not important.

Therefore we have determined fairly well the number of molecules into which our complex must dissociate in order to satisfy the

Table X

	x_1	y_1	x_2	y_2	$\frac{x_1}{2}$	$\frac{y_1}{2}$	$\frac{x_1}{3}$	$\frac{y_1}{3}$	$\frac{x_1}{4}$	$\frac{y_1}{4}$	$\frac{x_1}{5}$	$\frac{y_1}{5}$
	1	5.9	1	3.2	1	3.9	1	3.0	1	2.5	1	2.0
	2	7.8	2	4.0	2	5.0	2	3.9	2	3.2	2	2.7
	3	9.1	3	4.4	3	5.8	3	4.4				
	4	10.1	4	4.6	4	6.4	4	4.7				
	5	11.0	5	4.8	5	6.9						
	6	11.7	6	5.0	6	7.1						
	7	12.3	7	5.0								
	8	12.8	8	5.1								
	9	13.3	9	5.0								
	10	13.7	10	5.0								
	11	14.0	11	4.9								
	12	14.3	12	4.8								
Maxima	28	16.2	8	5.1	13	8.1	9	5.4	7	4	5	3

experimental results. From the values of x and y determined with this, we can calculate the molecular weight of the complex and the relative percentages of complex actomyosin and free AF myosin molecules present. The complex molecular weight has the value 24 x 10^6, which drops to 8 x 10^6 upon dissociation. The percentage of actomyosin complex present is 27% and that of the unassociated AF myosin 73%.

Before examining the rest of the experimental results in the light of this mechanism, let us look a little closer at one of the above steps. In using the experimental value of the molecular weight, it was assumed that the correction factor was independent of the amount of the complex present. Using now a weight averaged correction factor, and determining a value of the complex correction factor from the above results, we can get a better equation. However, upon solving it, the above values are changed only in that x_1 is lowered to 7.3. So this simple improvement does not appreciably change the results.

We will now proceed to apply this mechanism, using the values obtained from the molecular weight determination, to the other experimental results.

Examining the electrophoretic experiments, we see that no actin peak would be expected to appear upon addition of ATP. Also no great changes in mobility could be expected as the dissociation was said to be into equivalent segments. An interesting result is found when we examine the relative concentrations of the components in the electrophoretic pattern. It is observed that the α component has a relative concentration of 25%, which is close to the value of 27% found above for the amount of actomyosin complex present. This peak is always

quite steep in the patterns, which indicates that the diffusion constant is probably low. Thus we are able to identify this peak as the high molecular weight.actomyosin complex and assume the β and γ components are free AF myosin. The mean mobility found for these last two components is of the order of magnitude of the mobility of AF myosin, which is additional support. Furthermore, Szent-Gyorgyi has reported (20) that he has analyzed Dubuission's fractions (19) and that the α component was actomyosin and the β component a fairly actin free myosin. Thus the electrophoretic evidence is in strong agreement with the proposed mechanism.

Turning next to the viscosity data, we find that it will be necessary to obtain a value for the length of the complex molecule. This is derived from the viscosity of the actomyosin solution, and the result divided by three and substituted in the equation to get the viscosity of the actomyosin solution containing ATP. The equations used connecting viscosity, molecular weight and length are those obtained earlier from the Kirkwood-Riseman theory.

Thus, as viscosity is a weight average quantity,

$$[\eta]_o = 0.27[\eta]_c + 0.73\ [\eta]_m$$

for the original actomyosin solution. Using 3.2 for $[\eta]_o$, and 2.1 for $[\eta]_m$, we get $[\eta]_c$. Then using the relation

$$[\eta]_c = \frac{3.62 \times 10^{21} R^3}{M} \quad \text{where M is } 24 \times 10^6$$

we find that R is 345 mμ. Using this value in

$$[\eta]_c' = \frac{3.62 \times 10^{21} (R/3)^3}{8 \times 10^6}$$

we get the value of $[\eta]_c'$ for the dissociated complex. Placing this in a weight average equation similar to the one above, we obtain

$$[\eta_\bullet]_b' = 0.27\,[\eta]_c' + 0.73[\eta]_m$$

from which we find $[\eta]_o'$ is 2.1. This is in fair agreement with the experimental value of 2.3.

We can also treat the dissymmetry coefficients in a similar manner, using weight average values* The value of the dissymmetry coefficient found from

$$[q_o] = 0.27[q_c] + 0.73[q_m]$$

is used in conjunction with figure 5 to get a length, which is then divided by three and used to get the value of $[q_c']$. This last is placed in the following equation, and $[q_o']$ obtained.

$$[q_o'] = 0.27\,[q_o'] + 0.73[q_m]$$

Using 1.72 for the original solution, a value of 1.53 for the final solution in the presence of ATP is obtained. If the values of R found in the viscosity calculation are used here, the value of $[q]$ is still lower, around 1.4.

The agreement in this last calculation is not as good as before, and it may be possible that after dissociating, the complex molecules are stretching. Such an elongation would give a better fit in the last two calculations, and would not affect the others. It would also bear out the proposed mechanism of Riseman and Kirkwood (5). However, the present state of the theory and results does not justify its being carried out to such a conclusion with any validity.

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*See Appendix

One other additional piece of evidence for the proposed mechanism is the following. If we calculate the percentage of F-actin in the solution which would give the maximum average molecular weight, we obtain the result as 27%. This solution would have all the AF myosin in the complex form, and should have an average molecular weight of 24×10^6. The actomyosin B on which one run was made had about this concentration of actin, and a value of 29×10^6 was obtained for its molecular weight. Also Szent-Gyorgyi states (2) that the maximum in the viscosity curve for mixtures of actin and myosin occurs at around 40% which is greater than the above maximum. This is to be expected since as the actin concentration increases, the molecular weight of the complex may be expected to increase.

V. Consideration of results in phosphate buffer.

So far we have dealt only with the results in barbital buffer. It is now necessary to examine the phosphate results and attempt to explain them on the basis of this mechanism.

The main point of difference in phosphate buffer is the large increase in the dissymmetry coefficient upon adding ATP. Without consideration of the balance of the data, this increase could be interpreted as a stretching of the random coil. However, such a stretching would not produce a viscosity drop.

If we now use the type of mechanism which was successful in the barbital case, we find that the viscosity drop and molecular weight data can be reconciled if one assumes that the length of the dissociated complex is about 0.6 of the length of the original complex.

The barbital results gave this ratio as one-third. However, the large increase in dissymmetry coefficient is still unexplained.

Other possible mechanisms were tried, including a coiling up of the AF myosin molecules to spheres, reducing their contribution to the viscosity to essentially zero. No better agreement was obtained. Variation of the amount of complex present also failed to improve the picture.

Oster (29) obtained the same change of dissymmetry coefficient as reported above, working in phosphate buffer only. He decided that this change was evidence for contraction of the molecule. This was based on the assumption that none of the three curves in figure 5 will give a good representation for the molecules. He believes that the original shape is that of a coil which is stretched out to nearly a rod. This molecule would have a q versus S/λ curve lying above but near the curve given for the long rod in figure 5. When ATP is added, there is violent contraction, and the q versus S/λ curve is now changed to a position near the sphere curve.

It would be possible to adapt this concept into the dissociation with change in length of segments mechanism, but in the present state of the evidence, it is not considered to be fruitful. We can certainly explain the results on such a basis, but since the whole question of what happens in figure 5 when one is off one of the curves has not been investigated, it is perhaps dangerous to speculate too far in this direction.

There is another possible explanation of the behavior in phosphate buffer. In the enzyme reaction accompanying the viscosity

drop, ATP is split into adenosine diphosphate and phosphate ions. The course of the enzyme reaction is followed by determining the amount of inorganic phosphate present. If we carry out experiments in phosphate buffer, while no noticeable effect may occur in the viscosity drop, we may get unusual results due to some special interaction of the many phosphate ions present with the dissociated complex. The interaction may be large enough to invalidate the simple use of the dissymmetry coefficient in determining lengths and in applying correction factors to the molecular weight. This idea has some support in the fact that none of the models give agreement with the phosphate results as presented in table IX.

VI. G-F transformation.

Now let us look briefly at the molecular weights obtained for G and F actin. The G-actin results, again, did not fit any model, and this may invalidate them somewhat, although this may also be due to the inadequately determined value for the intrinsic viscosity. However the molecular weights are of the same order of magnitude for both G and F actin. This would cause one to believe that the G-F transformation is a matter of a change of shape, rather than a polymerization as stated by Szent-Gyorgyi. Thus the globular form of the molecule would be spread out into a rod in the presence of salt ions.

VII. Summary and conclusion

We can now conclude this discussion by a summary of the interpretation of the experimental results. In barbital buffer, the results are all explained fairly well by the proposal of a mechanism

for the dissociation of a complex molecule into segments which are still actomyosin. These segments may change shape after dissociation. Values for the molecular weights, and sizes of these complex particles have been estimated.

The simplest way to check the mechanism would be to carry out ultracentrifuge experiments, which should give direct evidence of the different molecular weights estimated.

The phosphate buffer results have not been satisfactorily explained, although several possible explanations are given. A new interpretation for the G-F transformation has been obtained.

It is felt that the proposed mechanism, while still requiring additional investigation, represents a truer picture of the actual events than the simple AF myosin-F-actin dissociation proposed by Szent-Gyorgyi.

Appendix

The expression used to determine the value of $[q_0]$ is only approximate. The correct expression is

$$[q_0] = \frac{0.27\, I_c^{60^\circ} + 0.73\, I_m^{60^\circ}}{0.27\, I_c^{120^\circ} + 0.73\, I_m^{120^\circ}}$$

In the range of q's used in the calculations made in the thesis, the values of $[q_0]$ obtained by the approximate expression are nearly the same as those obtained by the above correct equation.

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Committee: Professors Badger, D.H. Campbell, Christy, Kirkwood (Chairman), Niemann, Pauling, Swift.

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1. The viscosity measurements of Szent-Gyorgyi (1) plus the work reported in the thesis indicate that the molecular weight of actomyosin increases with actin content up to a certain point and then decreases. Molecular weight studies as a function of actin content would be helpful in determining the nature of the actomyosin complex. The effect of ATP on these higher actomyosins would also be of interest.

2. Szent-Gyorgyi (1) states that the G-F actin transformation is catalyzed by magnesium ions and inhibited by calcium ions. This same type of antagonism occurs in the enzyme activity of actomyosin and has been investigated by Mommaerts (2). Bailey et al (3) have reported experiments on the enzyme activity of actomyosin, using sulfhydryl agents. The effect of these agents upon the G-F actin transformation should give information on the nature of this change.

3. Buchtal et al (4) have reported that dried myosin threads (which are enzymatically inactive) show a contraction when ATP is applied. If they are previously treated with acetyl choline, this contraction is reduced by one-half. In view of the apparent importance of acetyl choline in some nerve processes, an examination of its effect on myosin solutions and on the ATP interaction would be of interest.

4. Spreading coefficients should be more sensitive to changes in charge caused by ATP interaction than ordinary mobility measurements. They could be used to detect the change in charge predicted by Riseman and Kirkwood (5).

5. Mommaerts (2) has reported a calcium-magnesium antagonism in the enzyme reaction and in the viscosity drop of actomyosin with ATP. Snellman and Erdos (8) have reported that calcium myosinate (actin free) has a positive rather than negative mobility at pH 7. An investigation of the effect of these ions on electrophoretic pattern and on light scattering data should shed light on the nature of the interaction.

6. Cullwick (6) states that special relativity transformations predict a magnetic field should exist only when a charge is moving relative to a ring, and not when the ring moves relative to the charge. It can be shown that special relativity does give magnetic fields in both situations.

7. The theory of electrophoretic mobility has been handicapped in its development of lack of good experimental data on a relatively simple system. Such a system would be an amphoteric polymer. Such a polymer

could be well characterized as to composition by polymer methods, and the physical dimensions determined by light scattering and other methods. Then a series of electrophoretic runs could be made, varying pH, buffer, and concentration. This might permit correlation of electrophoretic measurements with a well determined structure.

8. A possible method of preparing an artificial antibody would be to electrodialyze a serum globulin solution in a strong field. The protein would be forced onto the surface of the membrane, and spread out. Then a precipitating antigen would be allowed to pass through the system. Thus changes in the structure of the spread out globulin might be effected. At the end of a certain period, the current could be reversed and the protein molecules pulled off of the membrane. The precipitating antigen could be removed by addition of excess hapten. This method has the advantage of permitting use at any temperature.

9. McElroy (7) states that ATP causes a flash of light to appear when added to a water extract of firefly lanterns. The extract contains the enzyme luciferase and the substrate, luciferin. It would be interesting to observe whether the ATP causes a structure change in this system, as in the actomyosin system.

10. A conductometric investigation of the amphoteric polymer, vinyl pyridine-acrylic acid, should yield information on the properties of the polymer which would be of value. The investigation would be carried out along the lines used by Fuoss and Strauss (9) on polyvinyl pyridine.

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