Properties of Actomyosin before and after Treatment with Ion-exchange Resins: Influence of pH *and Ionic Strength on Molecular Shape of a Structural Muscle Protein.*

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Actomyosin before and after treatment with ion-exchange resins is characterised by ash content, analysis, solubility, qualitative flow birefringence tests, electrophoretic migration, light scattering, and intrinsic viscosity. An attempt to explain the properties of the protein in terms of a reversible polymerisation reaction, the solute being taken to be a rigid structure, was incompatible with results of light-scattering and viscosity measurements. A consistent explanation can be given if it is postulated that actomyosin, in dilute solution, is composed of flexible chains, the shape of which depends on pH and ionic strength of the solvent. The order of magnitude of the average distance between the chain ends is estimated.

It is important to know whether filamentous structural muscle proteins, in dilute solution, can be regarded as (*a*) rigid structures or (*b*) flexible chains capable of marked changes of configuration under appropriate chemical conditions. These macromolecular ampholytes have been frequently investigated (for references see Bailey, *Adv. Protein Chem.*, 1944, **1**, 289; Weber and Portzehl, *ibid.*, 1952, **7**, 161), but a convincing decision between the two possibilities has not been made. The present work deals with actomyosin (for nomenclature, see *idem*, *ibid.*), because it is a representative filamentous muscle protein and may play a significant rôle in the process of muscular activity. If actomyosin is prepared according to standard methods (*e.g.*, Greenstein and Edsall, *J. Biol. Chem.*, 1940, **133**, 397) it retains about 1% of ash (see Dubuisson, *Arch. Int. Physiol.*, 1941, **51**, 136) and it is sparingly soluble, between pH 4·0 and 9·0, in water or in salt solutions of ionic strength $\mu \leqslant 0{\cdot}3$. It will be shown in this paper that the ash content of actomyosin can be reduced to less than 0·02% by treatment with ion-exchange resins, and that the demineralised protein is easily soluble in water of pH $< 4{\cdot}8$ and $> 6{\cdot}5$. Owing to the increased solubility, comparative tests, including light-scattering experiments, could be carried out upon actomyosin dissolved in both pure water and potassium salt solutions. The results favour hypothesis (*b*), the biological implications of which were pointed out by Meyer (*Biochem. Z.*, 1929, **214**, 272) and later by others.

The present conclusions relate to dilute solutions of actomyosin and are not relevant for the problem of protein structure in concentrated solutions, or in the dry state, where the molecules may be arranged in helical shape. No evidence is available to show that such configurations play a rôle in dilute sols; moreover, the light-scattering and the hydrodynamic treatment of helices have not yet been developed and they are not considered here. The molecular weight or length of actomyosin has not been determined accurately but their order of magnitude could be computed. These results relate to "unfractionated" protein, made by a technique outlined below. It is not suggested that this material and actomyosin prepared by other techniques are of similar size and shape. The light scattering by solutions of actomyosin has so far only been measured by the transmission method. Investigations dealing with the angular distribution of scattered light and with "fractions" of actomyosin in solutions of various simple salts will be described in other papers.

Experimental

Reactions with Ion-exchange Resins.—Actomyosin was extracted from the hind-leg muscles of rabbits, according to Singher and Meister (*J. Biol. Chem.*, 1945, **159**, 419; Singher, Ph.D. Thesis, Harvard University, 1944, "method 4"). In most experiments the sol–gel conversion of the protein was brought about by controlled dialysis at 0°; the outer solution, three times renewed, was water or potassium veronal buffer ($\mu = 0{\cdot}0025$) of pH = 7·8; the ratio v = total volume of outer solution/volume of protein sol was 20—50, and the time, t, of the dialysis was 7—15 hr.

The gel was centrifuged (3000 ***g***.) at 0° for 1 hr. and redissolved in a potassium chloride–bicarbonate buffer of pH 7·8 (μ_{KCl} and μ_{KHCO_3} of the sol being respectively 0·500 and 0·0400), a second precipitation being brought about by dialysis as described. The centrifuged, fully swollen gel (I) was redissolved in the specified potassium chloride–bicarbonate buffer and precipitated by prolonged dialysis against five-times renewed water at 0° ($v = 200$; $t = 50$—60 hr.), the centrifuged gel being designated by (II). The measurements to be described were done not later than 5 days after the preparation of these gels, all protein preparations being stored at 0°. The water was distilled from an all-glass apparatus; control tests showed that no significant change of the properties of actomyosin occurs if the water had been redistilled from an all-silica still of the type described by Weber (*Z. Naturforsch.*, 1949, **4**b, 124).

The following ion-exchange resins were used : A = Amberlite IR-100 or IR-120, strong acids in the hydrogen form; B = Dowex-2, a strong base in the hydroxide form; C = Dowex-2 in the acetate form; D = Amberlite IR-4, a weaker base in the carbonate form. The resins were washed with pure water immediately before use, until the specific conductance of the filtrate was less than 10^{-6} ohm^{-1} cm.$^{-1}$, at 20°. In each run a suction-dry aliquot portion of the washed resin was tested for dry weight and total exchange capacity. In preliminary tests (I) was stirred with a mechanical mixture of A and B; under these conditions insoluble isoelectric actomyosin is formed which cannot easily be separated without considerable loss. In other tests (I) was treated successively with (1) B and A; (2) A, B, and A; (3) A and D; (4) C, A, and B; the concentration and time conditions being given in Table 1. The first resin treatment caused

TABLE 1. *Experimental conditions in reactions between actomyosin and ion-exchange resins.*

Sequence of application of resin	G. of acto-myosin in 100 cm.^{3} of reaction mixture	G. of dry ion-exchanger per g. of dry actomyosin				Time of contact (min.) between ion-exchanger and actomyosin				pH of solution after treatment with resin			
		A	B	C	D	A	B	C	D	A	B	C	D
B, A	0·3—0·9	10—90	10—70	—	—	30—180	45—720	—	—	4·1—4·2	10·5—11·3	—	—
A, B, A	0·5	11(55) *	11	—	—	75(210) *	5	—	—	3·5	4·5	—	—
A, D	0·3—0·4	55—220	—	—	24—55	20—60	—	—	20	3·5	—	—	4·2
C, A, B	0·3	40	40	30	—	30	5	75	—	4·0	4·2	8·1	—

* Numbers in parentheses relate to second resin treatment.

dissolution of the protein, and no precipitation occurred in the later stages, so the separation from the resins could be done by filtration. These reactions occurred in a static system at 0°, with stirring, except in the last stages of procedure (4), in which the sol was passed through a bed of the resin under conditions similar to those prevailing in chromatographic adsorption. Atmospheric carbon dioxide had no significant influence, as established by control experiments in which regeneration of the resins, interaction with (I), filtration, and washing of the residue with water were done in an atmosphere of pure nitrogen. Procedure (4), in which the pH remained within the range 4·0—8·1, was selected for the preparation of the demineralised actomyosin sols, used for all the following experiments. After the final filtration, the sol was centrifuged and stored in silica containers at 0° (sol III).

TABLE 2. *Composition of actomyosin gels.*

Treatment of acto-myosin with ion exchanger	Sample obtained from	No. of samples analysed	Equiv. of KCl per 100 g. of fully swollen centrifuged gel	C, %	H, %	N, %	K, %	Cl, %	Ash, %
				in dry gel					
	Gel (I)	11	~0·02	47·1 ± 0·6	7·3 ± 0·2	13·6 ± 0·4	10·9 ± 0·4	3·6 ± 1·4	>2
—	Gel (II)	2	<0·001	50·9 ± 0·5	7·2 ± 0·3	15·8 ± 0·4	<0·1	1·1 ± 0·5	1·5 ± 0·5
+	Sol (III)	8	—	49·7 ± 0·6	7·3 ± 0·3	15·5 ± 0·4	—	1·6 ± 0·6	<0·02

Analytical and pH *Measurements.*—The concentration of sols or gels with respect to actomyosin was determined by the micro-Kjeldahl method with the precautions described by Chibnall, Rees, and Williams (*Biochem. J.*, 1943, **37**, 354), and 15·8% nitrogen content being

assumed. This is the mean of figures given by Greenstein and Edsall (*loc. cit.*), Dubuisson (*loc. cit.*), Szent-Györgyi (*Acta Physiol. Scand.*, 1945, Suppl. 25), and of those given in col. 7 of Table 2, the latter analyses relating to actomyosin which had not been treated with organic solvents before the drying operation (0·5 mm. pressure, first at room temperature and finally at 80°). Potassium was estimated according to Tisdale and Kramer after digestion according to Abelin (*Helv. Chim. Acta*, 1941, **24**, 611), other analyses being done according to Pregl. The potassium chloride content of gels (I) and (II) was estimated conductometrically or by pH determination after interaction with resin A.

The pH measurements were made with a Cambridge Instrument Company meter in conjunction with the vessel shown in Fig. 1. In the electrometric titrations the reference electrode was in contact with the magnetically stirred sol. If, on the other hand, a solution of actomyosin in water, of specified pH, but free from potassium chloride, was required, suction was applied at (6) and, by operation of tap (7), the sol could be introduced into the burette (5), which was removed and quickly connected, by means of the standard joint, with the head of a viscometer or with an optical or conductivity cell. The opening (8) was closed, the reference electrode was pushed down, thereby bringing it in contact with the remaining sol in the vessel, and the pH was determined. The vessel was swept by nitrogen, so that no significant uptake of carbon dioxide occurred during the few seconds between the removal of (5) and the

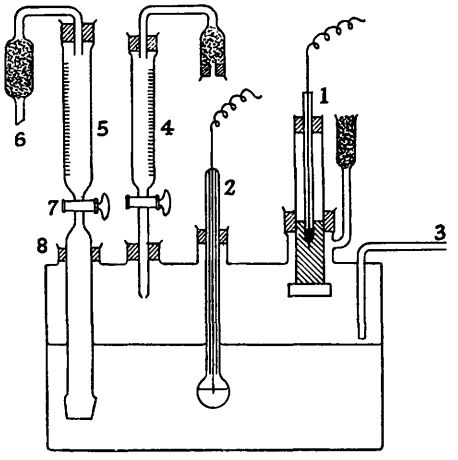


FIG. 1. *Reaction vessel used for* pH *measurements and for preparation of actomyosin sols.*

(1) Saturated potassium chloride calomel reference electrode.
(2) Glass electrode.
(3) Inlet tubing for flushing vessel with pure nitrogen.
(4) Microburette for addition of potassium hydroxide or hydrochloric acid solutions.
(5) Burette used for the transfer of protein sols.

closing of (8). A mechanical mixture of thoroughly washed ion-exchange resins A and B was suspended in the lower part of a burette, which was fitted to the opening (8). By percolating distilled water through the resin mixture a protein sol could be diluted with a known volume of pure water, without simultaneous uptake of carbon dioxide.

Qualitative Flow-birefringence Tests.—These were carried out according to Edsall and Mehl (*J. Biol. Chem.*, 1940, **133**, 409).

Electrical Conductivity.—Sols and gels were introduced into cells the constants of which were respectively 0·0430 and 1·48 cm.$^{-1}$. The platinum electrodes were lightly platinised, and the construction of the cells was such that dilution could be done in the cell, without uptake of atmospheric carbon dioxide. The bridge arrangement for measuring the resistance was similar to that described by Shedlovsky (*J. Amer. Chem. Soc.*, 1930, **52**, 1793).

Electrophoretic Migration.—This was measured in a Tiselius apparatus. The sols were centrifuged at 12,000—20,000 ***g*** (0°) and equilibrated, at 0°, against the relevant buffer solutions. Boundaries were photographed with red light, Ilford H P 3 plates being used.

Light Absorption and Scattering.—The experiments were done with the help of a Unicam SP. 500 spectrophotometer, turbidities being determined at least at five wave-lengths (350—700 mμ) by the transmission method (Doty and Steiner, *J. Chem. Phys.*, 1950, **18**, 1214) with the necessary precautions as to slit-width adjustments and with a set of optical cells, varying in length from 0·2 to 10 cm.; some of the cells could be evacuated and the sols could be introduced without bringing them into contact with atmospheric carbon dioxide. The sols, containing not more than 0·6% of actomyosin, had been centrifuged to constant turbidity at 20,000—40,000 ***g*** (0°). In order to avoid a possible contamination by small particles stirred up during the removal of the centrifuge cups and decantation, a final gravity filtration (0°) through sintered-glass

filters was carried out, the concentration of the sol and the porosity of the plate being adjusted in such a way that about 50 cm.^{3} of sol passed the filter in 12 hr., the ratio of surface of filter plate to volume of sol, initially on top of the filter, being 10 cm.$^{-1}$. If the centrifugation of the sols is done at concentrations above 0·6%, or if filtration through sintered-glass filters of smaller porosity is carried out, a fractionation occurs and sols are obtained which contain an actomyosin the weight average molecular weight of which is substantially smaller than that specified below.

Refractivity.—The increments in Table 5 were determined with a differential refractometer having a limiting sensitivity of $\pm 1 \times 10^{-6}$ (McEwan and Pratt, unpublished work). The centrifuged and filtered sols were carefully equilibrated against buffers, the refractive indices of which were measured in a Pulfrich refractometer, mercury, sodium, and cadmium lamps being used. The refractive indices of water were taken from Landolt and Börnstein's tables.

Viscometers.—These were of the type described by Fox, Fox, and Flory (*J. Amer. Chem. Soc.*, 1951, **73**, 1901), two instruments of the following significant dimensions were used :

	Capillary, cm.		Bulb, cm.^{3}			Capillary, cm.		Bulb, cm.^{3}	
Viscometer	Length	Diameter	Upper	Lower	Viscometer	Length	Diameter	Upper	Lower
1	17·1	0·1509	4·780	4·033	2	24·0	0·06796	3·430	3·176

The kinetic-energy correction, determined by calibration with water or sucrose solution, was less than 2% of the total viscosity, even at the highest rate of shear. Flow times were determined with a calibrated stop-watch, the viscometer being placed in a thermostat, the temperature of which was constant to within $\pm 0{\cdot}01°$. In carrying out experiments with protein sols at pH >4, both limbs of the viscometer were connected with soda–lime tubes, the carbon dioxide being removed from the empty viscometer by a stream of nitrogen. Burette (5) (Fig. 1) was fitted to the wide limb of the viscometer by way of a T-piece connection. From the moment of introducing the sol into the viscometer until temperature equilibrium was established the sol was kept in continuous motion by sucking it into one of the bulbs and letting it flow down, thereby reducing the influence of thixotropic effects. In runs in which the flow time of the first reading was somewhat greater than that of consecutive readings Bailey and Perry's procedure (*Biochim. Biophys. Acta*, 1947, **1**, 507) was adopted. In determining intrinsic viscosities, the concentration of the sols was adjusted in such a way that the thixotropic effects become negligible at least at the three most dilute solutions. The maximum rate of shear at the wall of the capillary (referred to below as the rate of shear) was calculated as by Fox, Fox, and Flory (*loc. cit.*).

Results

Analytical, Solubility, Flow-birefringence, and Electrochemical Measurements.—The analytical composition and some solubility and flow birefringence properties of actomyosin, before and

TABLE 3. *Comparison of some properties of actomyosin before and after treatment with ion-exchange resins; temp.*, 20—25°.

Treatment of actomyosin with ion exchanger	pH range	Solvent	Solubility *	Flow birefringence
—	<4	Water, buffer, or salt solns.	+	—
+			+	—
—	4·0—4·8 *	Water	—	
+			+	—
—	4·0—4·8 *	Acetate buffer (μ = 0·02—0·1)	—	
+			+	—
—	4·0—4·8	Salt solns. (μ = 0·05—0·8)	—	
+			—	
—	4·8—6·5	Water, acetate buffer, or salt solns.	—	
+		(μ = 0·05—0·8)	—	
—	6·5—9·0 *	Water	—	
+			+	+
—	6·5—9·0	Salt solns. with or without buffer (μ<0·3)	—	
+			—	
—	6·5—9·0	Salt solns. with or without buffer	+	+
+		(μ>0·35<1)	+	—
—	>9	Water, buffer, or salt solns.	+	—
+			+	—

* + Indicates that no precipitate is formed at 20°, after 24 hr., the concentration of actomyosin being 0·6%; — indicates that the gel-sol conversion is not complete under the same temperature and time conditions, even if the reaction mixture contains only 0·05% of actomyosin.

after treatment with ion-exchange resins, are shown in Tables 2 and 3. Starting materials for these runs were gel (I) or sol (III). The salts referred to in col. 3 of Table 3 are the chlorides of potassium, sodium, calcium, and magnesium; pH values >6·5 were adjusted by veronal or phosphate buffers. Sol (III) does not give a precipitate on addition of acetic acid–potassium acetate buffers within the specified range of pH values and ionic strengths, but precipitation occurs if a phthalic acid–potassium phthalate buffer of pH 4·0 and $\mu = 0{\cdot}030$ is added. The pH ranges 4·0—4·8 and 6·5—9·0 are marked in Table 3 with an asterisk, because they are those in which treatment with ion-exchange resins affects the solubility of actomyosin in the most marked manner. Sol (III) is not detectably flow-birefringent, under the conditions of these qualitative tests, which were done at low rates of shear; flow birefringence could easily be observed, however, if a sufficient amount of potassium hydroxide is added to sol (III) to bring the pH within the range 6·5—9·0; the effect being detectable even if the sol contains only 0·1% of actomyosin. If a strongly flow-birefringent, salt-free sol of pH 6·5—9·0 containing 0·6% of actomyosin is made 0·50M with respect to potassium chloride, the optical anisotropy disappears. Before treatment with the resin, a 0·6% solution of actomyosin in 0·50M-potassium

FIG. 2.

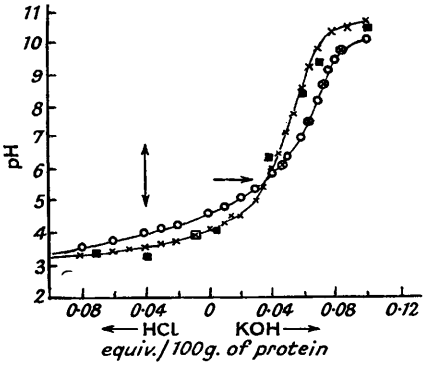


FIG. 3.

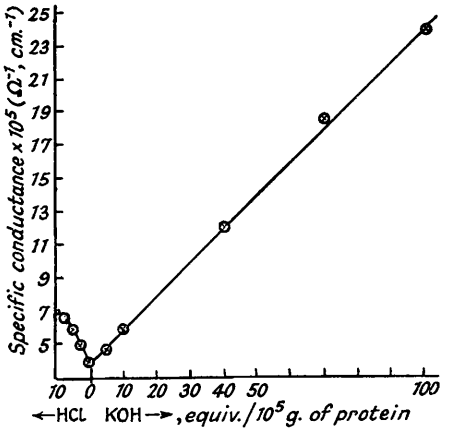


FIG. 2. *Electrometric titration of actomyosin sols before and after treatment with ion-exchange resins.*

×, ○, ⊗, ⊠, After treatment with ion-exchange resins; starting material sol (III); temp. 18·0°. Initial and final protein concentration 0·150 and 0·132 g./100 cm.³ of solution.

×, Solvent, water; ○, □, solvent, 0·500M-KCl.

⊗, Sol (III) was brought to pH 10·5; after 5 min. at this pH the lower pH values were reached by back-titration with HCl.

⊠, Sol (III) was brought to pH 3·1; after 5 min. at this pH the higher pH values were reached by back-titration with KOH. ×, ○, KOH or HCl directly added to Sol (III).

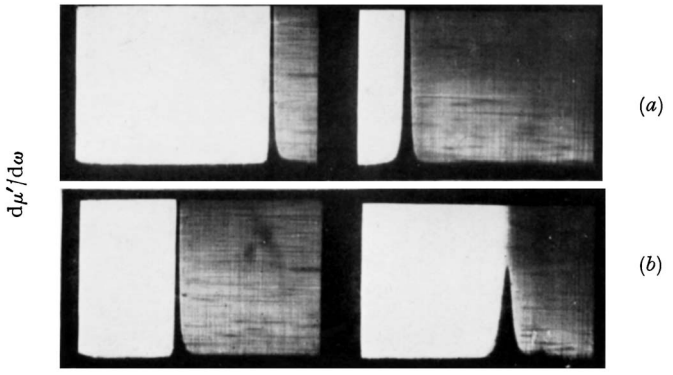
■, Actomyosin which had not been in contact with ion-exchange resins; these results are taken from Dubuisson and Hamoir (*Arch. Int. Physiol.*, 1943, **53**, 313); it has to be taken into account that in these tests the starting material was the isoelectric protein.

FIG. 3. *Conductometric titration of actomyosin after treatment with ion-exchange resins. Starting material: sol* (III). *Temp.*, 0·0°.

chloride is strongly flow-birefringent (cf. Greenstein and Edsall, *loc. cit.*; Edsall and Mehl, *loc. cit.*; Edsall, *J. Biol. Chem.*, 1930, **89**, 289).

Results of electrometric titrations are shown in Fig. 2. Practically identical values were obtained in experiments in which the protein concentration was 0·0520 or 0·635 g./100 cm.³ of sol, or in which the reaction mixture was 0·0204 and 0·102M with respect to potassium chloride. The " intersection points " in these runs corresponded to pH 5·5 or 5·6; the mean of these values, and of that indicated in Fig. 2 by a horizontal arrow, is 5·6. In the case of actomyosin not treated with ion-exchange resins, an " intersection " point of pH 5·5 has been observed (Dubuisson and Hamoir, *loc. cit.*). The vertical arrow in Fig. 2 indicates the approximate pH range in which actomyosin is insoluble. With the protein which had been treated with ion-exchange resins, and in the absence of salts, precipitation and re-solution occur if one passes from pH < 4·8 to > 6·4; this, however, does not give rise to a break in the electrometric or

FIG. 4. *Electrophoretic pattern of actomyosin sols after treatment with ion-exchange resins. Temp.*, 0·5°.



The width of the band, ω, is plotted against the refractive index gradient $d\mu'/d\omega$; potential gradient, 1·24 v/cm.; duration of test 1·5 hr. in (*a*) and 19 hr. in (*b*). Ascending boundary (protein migrates to anode, into the buffer) on left; descending boundary (protein migrates away from buffer) on right. Actomyosin concentration 0·55 g./100 cm.^{3} of solution. pH = 6·60. $\mu_{KCl} = 0{\cdot}335$; $\mu_{K_2HPO_4} = 0{\cdot}0750$; $\mu_{KH_2PO_4} = 0{\cdot}0250$.

[*To face p.* 1349.

conductometric titration curve. Typical results of the latter measurements are in Fig. 3. From these and similar conductometric analyses it can be calculated that sol (III) cannot contain more than 2×10^{-4} mole of free acetic acid per litre, this upper limit being obtained by assuming that the contribution of the protein conductivity is negligible.

The electrophoretic pattern of actomyosin prepared from sol (III) is shown in Fig. 4. The starting solution had been brought to pH 6·60 by addition of potassium hydroxide, potassium chloride and phosphates being subsequently added. A similar pattern was observed with actomyosin which had not been in contact with ion-exchangers. Electrophoretic migration velocities of the principal protein component are in Table 4; the specified inaccuracies of the

TABLE 4. *Electrophoretic migration velocity of actomyosin before and after treatment with ion-exchange resin; temp.*, 0·5°.

Treatment of actomyosin with ion-exchanger	pH	Solvent	Protein concn. (g./100 cm.³ of sol)	Electrophoretic migration velocity × 10^5 (cm.²/v-sec.)
—	6·46	Buffer *A*	0·22	2·26 ± 0·02
	6·46	Buffer *A*	0·30	2·60 ± 0·05
+	6·60	Buffer *B*	0·55	3·24 ± 0·02

Buffer *A* = KCl ($\mu = 0{\cdot}500$), K_2HPO_4 ($\mu = 0{\cdot}0206$), KH_2PO_4 ($\mu = 0{\cdot}0161$).
Buffer *B* = KCl ($\mu = 0{\cdot}335$), Na_2HPO_4, ($\mu = 0{\cdot}0750$), KH_2PO_4 ($\mu = 0{\cdot}0250$).

figures in the last column were computed by taking into account both ascending and descending boundaries.

Turbidities and Refractivities.—To facilitate presentation of results and subsequent discussion, the following relations are mentioned:

$$\tau = (1/d)\ln_e J_0/J = 2{\cdot}30\varepsilon \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$(\tau/HC)_{c\to 0} = MQ_{\text{exp.}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$$(\tau/HC)_{\substack{c\to 0 \\ \lambda\to\infty}} = M \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$H = (32\pi^3/3N\lambda^4)[(\mu' - \mu_0')/C]^2\mu_0'^2 \text{ and } H\lambda^4 = H'. \quad . \quad . \quad . \quad . \quad (4)$$

$$-\frac{\mathrm{d}\log(\tau)_{c\to 0}}{\mathrm{d}\log\lambda} + \frac{\mathrm{d}\log H'}{\mathrm{d}\log\lambda} = 4 - \frac{\mathrm{d}\log Q_{\text{exp.}}}{\mathrm{d}\log\lambda} = 4-\beta \quad . \quad . \quad . \quad . \quad (5)$$

$$\mathrm{d}\log Q_{\text{theor.}}/\mathrm{d}\log\lambda = \beta' \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where τ is the turbidity of the actomyosin sols, d the optical path-length, J_0 and J the intensity of incident and transmitted light, C the solute concentration, M the weight-average molecular weight of the solute, N Avogadro's number, λ the wave-length of the light, and μ' and μ_0' the refractive indices of solution and solvent, respectively; $Q_{\text{exp.}}$ and $Q_{\text{theor.}}$ are particle dissipation factors, the former to be experimentally determined from the wave-length dependence of τ and H' and the latter to be calculated from analytical expressions due to Doty and Steiner (*loc. cit.*) and Bucche, Debye, and Cashin (*J. Chem. Physics*, 1951, **19**, 803). Equation (5) is identical with (17) of Doty and Steiner's paper if one neglects $\mathrm{d}\log H'/\mathrm{d}\log\lambda$; in Doty and Steiner's numerical calculations the term has been taken into account. The dependence of β' on the molecular dimensions becomes negligibly small if the scattering particles are sufficiently large, but the limiting values, β'_∞, depend markedly on the shape of the solute. For this reason a comparison between β and β'_∞ is useful, as shown below, but it does not enable one to compute molecular weight or dimensions. These parameters can be estimated from (3), in conjunction with an extrapolation method proposed by Cashin and Debye (*Phys. Review*, 1949, **75**, 1307), in which $(HC/\tau)_{c\to 0}$ is plotted against $(\mu_0'/\lambda)^2$, thereby obtaining the intercept for $(\mu_0'/\lambda)^2 = 0$. Insertion of the reciprocal of this intercept in (2) gives $Q_{\text{exp.}}$, and comparison with the appropriate value of $Q_{\text{theor.}}$ enables one to calculate the dimension of the solute, the choice of the model to be used for a calculation of $Q_{\text{theor.}}$ being facilitated by comparison between β_∞ and β.

Results of typical experiments showing the dependence of τ/c on the concentration of actomyosin are in Fig. 5; from these and similar measurements the term on the left-hand side of (2) can be estimated. The shape of the graphs in Fig. 5 is typical for all the wave-lengths and solvents. Measurements, similar to those shown by *d* in Fig. 5, were done with actomyosin prepared from four rabbits, and the following values for $\tau/2{\cdot}30C$ ($C = 5{\cdot}00 \times 10^{-4}$ g./cm.³ of sol) were obtained: 32, 35, 36, 34 cm.²/g. In order to calculate β, (2) is written in the form

$$\log\{(\varepsilon/hc)_{c\to 0}\} + q = \log Q_{\text{exp.}}$$

TABLE 5. *Refractive-index increments of actomyosin sols; temp.*, 20°.

Solvent	pH	Wave-length (mμ)	Refractive-index increment (cm.³/g.)	Solvent	pH	Wave-length (mμ)	Refractive-index increment (cm.³/g.)
Water	4·26	578	0·191 ± 0·002	Buffer *C*	8·26	578	0·203 ± 0·002
		546	0·193 ± 0·002			546	0·205 ± 0·002
		436	0·195 ± 0·002			436	0·208 ± 0·002
Buffer *A*	6·46	578	0·193 ± 0·003				

Buffer *A*: as Table 4.
Buffer *C*: KCl ($\mu = 0{\cdot}500$), $KHCO_3$ ($\mu = 0{\cdot}0400$).

where $h = H \times 3N/32\pi^3$ and q is independent of λ. For the sake of simplicity in plotting, a quantity A is defined by

$$A = \log\{(\varepsilon/hc)_{c\to 0}\} + 15{\cdot}00 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

It will be seen that β is the slope of the straight line obtained by plotting A against log λ. Typical graphs of this kind are in Fig. 6. For calculations of h in (7) for different wave-

FIG. 5.

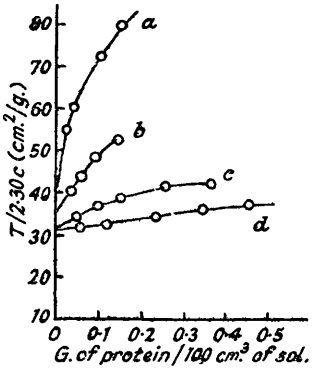


FIG. 6.

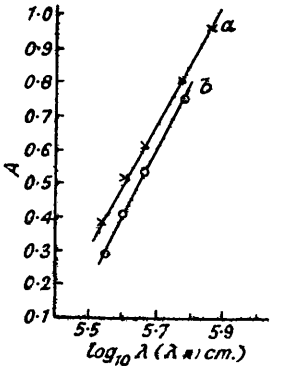


FIG. 5. *Reduced turbidities of actomyosin sols; temp.*, 20°; *wave-length of light*, 589 mμ.

(*a*) Before treatment with ion-exchange resins; starting material gel (I); solvent buffer *A* (see Table 4); pH 6·46; $\mu = 0{\cdot}537$.
(*b*), (*c*), and (*d*) After treatment with ion-exchange resins.
(*b*) Sol (III) brought to pH 6·46 by addition of KOH; KCl and phosphates subsequently added; solvent and μ as in (*a*).
(*c*) Sol (III) brought to pH 8·80 by addition of KOH; no salt or buffer added.
(*d*) Sol (III); pH 4·26; no salt or buffer added.

FIG. 6. *Light-scattering of actomyosin sols at different wave-lengths; temp.*, 20°.

The significance of the symbol A is given in (7), above, ε/ch being expressed in g. (*a*) and (*b*) relate to the same sols as those specified in the legend of Fig. 5.

TABLE 6. *Results of light-scattering and refractive-index measurements.*

No.	Treatment of acto-myosin with ion-exchanger	Solvent	pH	Ionic strength	$\left(\frac{\tau}{2{\cdot}30C}\right)_{c\to 0}$, in cm.²/g. (λ = 589 mμ)	$\left(\frac{10^8 HC}{\tau}\right)_{c\to 0}$, in g.⁻¹ (λ = 589 mμ)	Weight-average molecular weight × 10⁻⁸	$\beta = \frac{\Delta \log Q}{\Delta \log \lambda}$	Particle dissipation factor, $Q_{exp.}$ (λ = 589 mμ)
1 *	—	Buffer *A*	6·46	0·537	42 ± 3	3·6 ± 0·2	2 ± 1	1·8 ± 0·3	0·13 ± 0·05
2	—	Buffer *C*	8·26	0·540	55 ± 5	2·7 ± 0·2	2 ± 1	1·5 ± 0·3	0·19 ± 0·08
3	+	Water	4·08	—	31 ± 2	4·2 ± 0·2	1·3 ± 0·4	1·7 ± 0·3	0·18 ± 0·05
4 *	+	Water	4·26	—	30 ± 3	4·1 ± 0·4	1·4 ± 0·5	1·6 ± 0·3	0·18 ± 0·06
5	+	Buffer *D*	4·00	0·100	41 ± 3	3·3 ± 0·2	3·0 ± 1	2·0 ± 0·4	0·10 ± 0·03
6	+	Buffer *A*	6·46	0·537	35 ± 3	4·2 ± 0·4	2·0 ± 0·5	1·7 ± 0·3	0·12 ± 0·04
7	+	Buffer *C*	8·26	0·540	34 ± 2	4·3 ± 0·3	2·0 ± 0·5	1·8 ± 0·3	0·12 ± 0·04
8	+	Water	7·88	—	45 ± 3	2·9 ± 0·2	2·0 ± 0·5	1·8 ± 0·3	0·17 ± 0·04
9	+	Water	8·88	—	32 ± 3	4·0 ± 0·4	2·0 ± 0·5	1·7 ± 0·3	0·13 ± 0·04

Buffers *A* and *C* as Tables 4 and 5; buffer *D* = acetic acid–potassium acetate.
* These experiments were done in duplicate.

lengths, the refractive index increments were determined at different concentration of actomyosin in the range 0·103—0·412%. The results of these measurements are in Table 5; in no case could a detectable concentration dependence of the increments be observed. These measurements were done with actomyosin after treatment with ion-exchangers: comparison with unpublished work of Dr. P. Johnson shows that the interaction with the resins has no significant influence on the increment. The increments shown in Table 5 were converted into other wave-lengths, within the range specified before, by an interpolation or extrapolation method similar to that of Perlman and Longsworth (*J. Amer. Chem. Soc.*, 1948, **70**, 2719). The various β values in col. 9 of Table 6 were calculated from graphs similar to those shown in Fig. 6. The weight-average molecular weight, M, of actomyosin is listed in column 8 of Table 6. These values were deduced from the intercept of such graphs as those shown in Fig. 7. The slopes of these graphs, $S = (\lambda/\mu_0')^2(HC/\tau)_{c\rightarrow 0}$, are $0{\cdot}62 \times 10^{-16}$ and $0{\cdot}77 \times 10^{-16}$ cm.²/g. The mean S value of all the experiments listed in Table 6 is $(0{\cdot}6 \pm 0{\cdot}2) \times 10^{-16}$ cm.²/g. No significant relation could be detected between the numerical value of S and pH, ionic strength, or other chemical conditions.

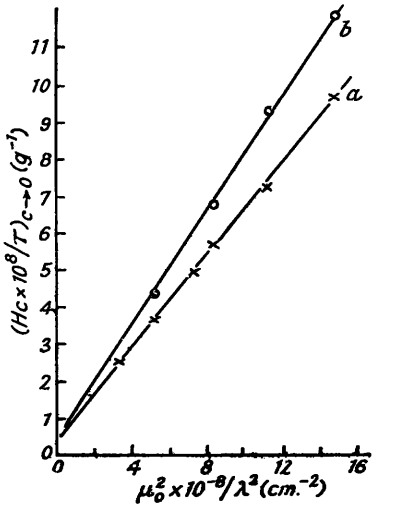


FIG. 7. *Typical graphs used for estimation of weight-average molecule weight of actomyosin.*

(*a*) and (*b*) relate to the sols specified in the legend of Fig. 5.

Light Absorption.—All the sols of actomyosin are characterised by a light-absorption maximum at 275 mμ, in agreement with previous observations relating to the protein which had not been in contact with ion-exchangers (Schauenstein and Treiber, *J. Polymer Sci.*, 1950, **5**, 145; Laki, Bowen, and Clark, *J. Gen. Physiol.*, 1949/50, **33**, 430). The band is due to electronic transitions, an effect which is assumed not to play a significant rôle at $\lambda > 350$ mμ. The assumption is justified by a consideration of the amino-acid composition of actomyosin (cf. Bailey, *loc. cit.*) in conjunction with empirically well-established relations between chemical structures and light absorption.

Viscosities.—The reciprocal observed viscosities of solutions of actomyosin, $1/\eta$, were plotted, at each concentration of the protein, against the rate of shear, γ, the graph thus obtained being

TABLE 7. *Range of protein concentration and rate of shear in which measurements for calculation of intrinsic viscosity of actomyosin sols were carried out; shear-rate dependence of viscosity.*

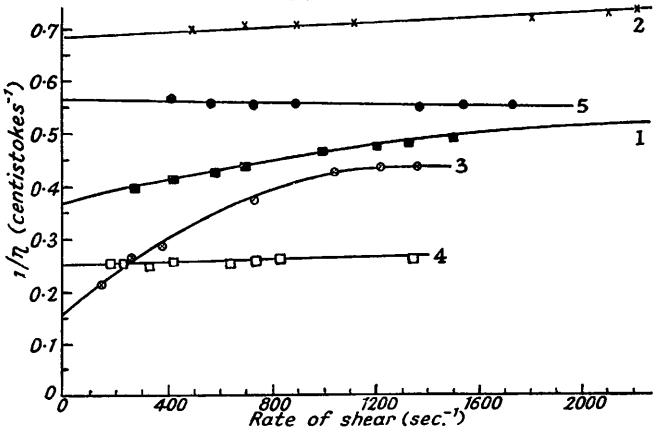
Treatment of actomyosin with ion exchanger	Solvent	pH	Ionic strength	Protein concentration, C (g./100 cm.³ of sol)	Rate of shear (sec.$^{-1}$)	$10^4\alpha$ (sec./centistoke) for C = 0·100 and 20·0°
—	Buffer *A*	6·46	0·537	0·0300—0·553	282—2708	
	Buffer *C*	8·26	0·540	0·0865—0·693	106—2708	
+	Buffer *A*	6·46	0·537	0·0580—0·279	501—2708	
	Buffer *E*	7·77	0·501	0·0433—0·173	50—2860	
	Buffer *D*	4·00	0·100	0·0470—0·284	464—2780	<1
	Soltn. of KCl	2·90	0·100	0·0800—0·640	406—2710	
	H_2O	2·50	—	0·0800—1·28	367—2710	
		3·50	—	0·0940—0·377	464—2760	
		4·00	—	0·0560—0·450	184—2592	
		4·26	—	0·0280—0·450	207—2680	
		6·50	—	0·0230—0·460	531—2860	3 ± 1
		7·20	—	0·0150—0·292	50—2860	>3
		8·20	—	0·0164—0·0655	148—2732	6 ± 2
		9·30	—	0·0160—0·262	188—2488	<1
		11·50	—	0·0608—0·288	403—2833	

For composition of buffers, see Tables 4—6 and legend to Fig. 1.

extrapolated to $\gamma \longrightarrow 0$, thereby estimating the relevant viscosity, η', at zero rate of shear. The reduced viscosities, $(\eta'/\eta_0 - 1)/c$ or $(\ln \eta'/\eta_0)/c$ (where η_0 is the viscosity of solvent), were plotted against c, the concentration of actomyosin, extrapolation to $c \longrightarrow 0$ enabling a comput-

ation of the intrinsic viscosity, $[\eta]$. Typical results of measurements showing the influence of γ and c are shown in Figs. 8 and 9, and the various values of $[\eta]$, together with a few reduced viscosities, are shown in Fig. 10. These $[\eta]$ values were calculated from measurements carried

FIG. 8. *Influence of rate of shear on viscosities of actomyosin sols; temp.*, 20·0°. *Protein concentrations*, c, *in g./100 cm.³ of solution.*



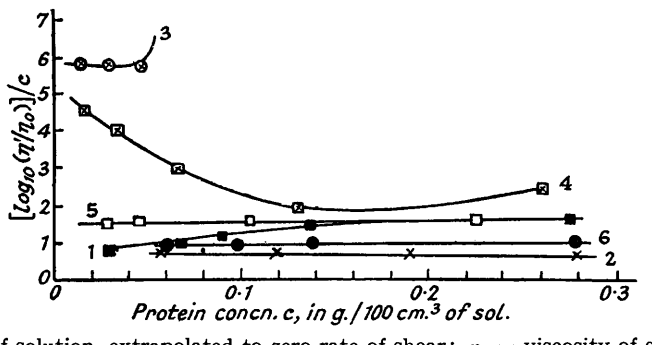
(1) Before treatment with ion-exchange resins; sol prepared from gel (I), solvent buffer *A* (Table 4), pH 6·46; $\mu = 0{\cdot}537$; $c = 0{\cdot}276$.
(2)—(5) After treatment with exchange resins.
(2) Sol (III) brought to pH 6·46 by addition of KOH; KCl and phosphates subsequently added. Solvent, pH and μ as in (1); $c = 0{\cdot}276$.
(3) Sol (III) brought to pH 7·20 by addition of KOH; no salt or buffer added; $c = 0{\cdot}0655$.
(4) Sol (III); pH 4·26; no salt or buffer added; $c = 0{\cdot}450$.
(5) Sol (III) brought to pH 11·5 by addition of KOH; no salt or buffer added; $c = 0{\cdot}288$.

out in the range of concentration and rates of shear given in cols. 5 and 6 of Table 7; the last column of the Table contains the value of the quantity defined by

$$\alpha = [\mathrm{d}(1/\eta)/\mathrm{d}\gamma]_{\gamma \to 0} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

which was estimated from the initial slope of such graphs as those shown in Fig. 8. If solutions of actomyosin of pH 11·5 or <4·0 are treated with appropriate ion-exchange resins, or if the

FIG. 9. *Concentration dependence of viscosity of actomyosin sols; temp.*, 20·0°.



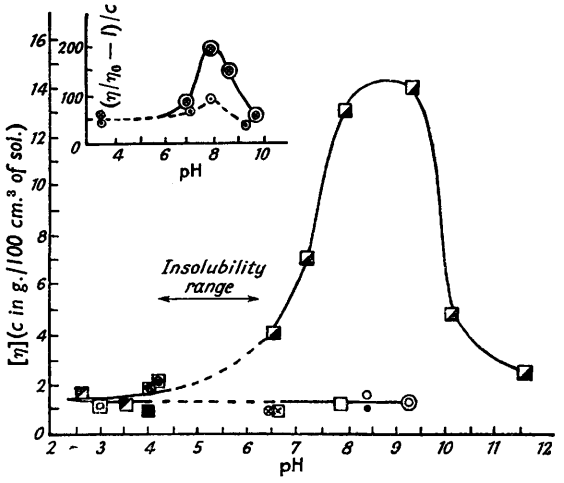
η' = viscosity of solution, extrapolated to zero rate of shear; η_0 = viscosity of solvent.
(1) Before treatment with ion-exchange resins. Solvent buffer *A* (Table 4); pH 6·46; $\mu = 0{\cdot}537$.
(2)—(6) After treatment with ion-exchange resins.
(2) Solvent, pH, and μ as in (1); (3) pH 7·20; (4) pH 9·30; (5) pH 4·26; (6) pH 11·5. In (3)—(6) the solvent was water, no salts or buffers being added.

required quantity of potassium hydroxide is added, the pH can be brought back to the range 6·5—9·0. The $[\eta]$ values of these latter sols were not significantly different from those of sols whose pH had not been above 8·5 or below 4·0.

Reaction of Actomyosin with Ion-exchange Resins.—These do not substantially alter the weight-average molecular weight, *M*, or the intrinsic viscosity, $[\eta]$, of the protein (as shown by ref. nos.

1, 2, 6, and 7 of Table 6 and by the experiments at pH 6·46 and $\mu = 0{\cdot}539$, represented in Fig. 10), the electrometric titration curve, the pH of the " intersection point " shown in Fig. 2, or the electrophoretic migration velocity. The concentration dependence of the reduced turbidities (curves *a* and *b* in Fig. 5) and the flow birefringence in 0·5M-potassium chloride decrease on treatment with the resins, while the solubility in water increases.

FIG. 10. *Influence of* pH *on intrinsic viscosities of actomyosin sols; temp.* 20·0°



Symbol ..... ⊗ ⊠ ○ ● □ ■ ◎ ⊡ ◩ ⊕ ◪ (pH 6·5—10) ◪ pH (11·5)

Accuracy of [η] ..... ± 0·1—0·2 ± 1—2 ± 0·2

⊗ and ○ Actomyosin not treated with exchange resins; all other symbols relate to actomyosin after treatment with resins.

⊗ and ⊠ Solvent buffer *A* (Table 4); $\mu = 0{\cdot}537$.
○ and ● Solvent buffer *C* (Table 5); $\mu = 0{\cdot}540$.
□ Solvent = solution of KCl; $\mu = 0{\cdot}500$; K–veronal buffer; $\mu = 0{\cdot}013$.
■ Solvent buffer *D* (Table 6); $\mu = 0{\cdot}100$.
◎ Solvent = solution of KCl, $\mu = 0{\cdot}600$; K_2CO_3, $\mu = 0{\cdot}030$; $KHCO_3$, $\mu = 0{\cdot}040$ (buffer *E*).
⊡ Solvent = solution of KCl; $\mu = 0{\cdot}100$.
◪ No salts or buffer added, pH adjusted by addition of HCl to sol (III).
⊗ Sol (III) without addition of salts or buffers.
◪ No salts or buffers added; pH adjusted by addition of KOH to sol (III).

Inset: Influence of pH *on reduced viscosities of actomyosin sols; temp.*, 0°.

No salts or buffer added; pH adjusted by addition of KOH or HCl to sol (III); protein concentration, *c*, in g./100 cm.³ of solution.

⊛ $c = 0{\cdot}105$; rates of shear 194—719 sec.$^{-1}$.
◉ $c = 0{\cdot}0525$; rates of shear 576—857 sec.$^{-1}$.

DISCUSSION

Possibility of Polymerisation Reactions.—The actomyosin sols can be divided into groups: (*a*) solvent water, pH 8·0 and 9·5, (*b*) solvent 0·5—0·6M-potassium chloride, pH 7·8, 8·3, and 9·2; (*c*) solvent water, pH 11·5; (*d*) pH <5. It will be seen from Figs. 8, 9, and 10 that the intrinsic viscosities, [η], of sols (*a*) are 13 ± 2 and 14 ± 2, while those of sols (*b*) and (*c*) are between 0·8 and 2·5, the influence of rate of shear and of protein concentration being relatively small in the last case. If actomyosin, in dilute solution, were taken to be a rigid structure, stabilised by hydrogen bonds or salt bridges,* an interpretation of the change of properties on passing from sols (*a*) to (*b*) and (*c*) could be attempted, by assuming the occurrence of a reversible polymerisation reaction, the [η] value of polymerised actomyosin being large. The protein being represented by an

* The salt bridge of actomyosin, not treated with ion-exchange resins, may be due to, *inter alia*, polyfunctional ionic impurities.

ellipsoid of rotation, of partial specific volume 0·75, and solvation being neglected, it follows from Simha's formula (*J. Phys. Chem.*, 1940, **44**, 25; cf. also Kuhn, Kuhn, and Buchner, *Ergebn. exakt. Naturwiss.*, 1951, **25**, 39) that the axial ratio of the proteins in sols (*a*) and (*b*) is respectively 175 and 37; if end-to-end alignment of the ellipsoids occurred, the molecular weight of the solute in sols (*a*) should be about 470% larger than that of the actomyosin in sols (*b*). The experimentally determined molecular weights (ref. nos. 6—9, Table 6) show that the difference, if any, is at the most 60%, and it is concluded that the high intrinsic viscosity of sols (*a*) is not due to a polymerisation reaction. If the protein is represented by a cylindrical rod, the argument against the occurrence of a polymerisation reaction is similar (for reference relating to the hydrodynamic treatment of rods, see Kuhn, Kuhn, and Buchner, *loc. cit.*).

Qualitative Interpretation of Results of Viscosity Tests.—It is assumed that actomyosin, in dilute solution, is composed of flexible chains which take up an extended configuration

FIG. 11.

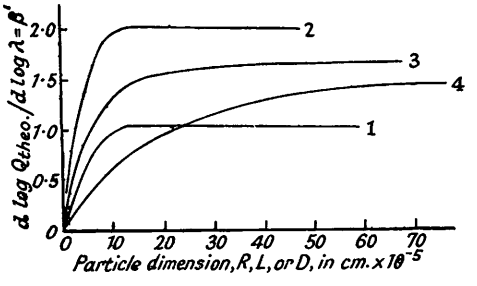


FIG. 12.

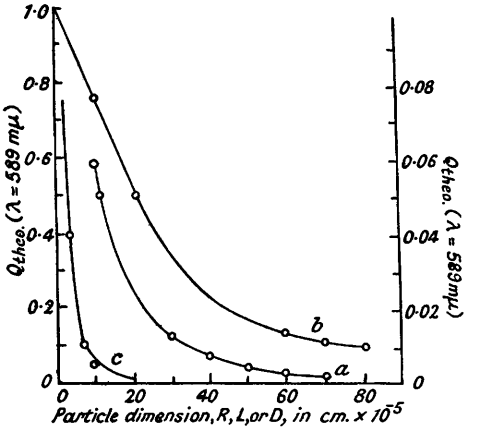


FIG. 11.

d log $Q_{theor.}$/d log $\lambda = \beta'$ (see equation 6) plotted against particle dimensions.
D = diameter of sphere; R = root-mean-square distance between ends of coiled chain; L = length of thin rod.
(1) Thin rod. (2) Sphere. (3) Monodisperse coil. (4) Polydisperse coil. The analytical expressions for (1)—(3) are given by Bueche, Debye, and Cashin (*loc. cit.*); the expression for (4) is due to Doty and Steiner (*loc. cit.*).

FIG. 12.

$Q_{theor.}$ for $\lambda = 589$ mμ, on ordinate, plotted against particle dimension, D or R on abscissa; D = diameter of sphere; R = root-mean-square distance between ends of coiled chains.
(*a*) = Monodisperse coil, relates to right-hand ordinate; (*b*) = polydisperse coil, and (*c*) = sphere, both relate to left-hand ordinate. In all three cases $Q_{theor.}$ decreases on passing on abscissa from left to right.

if side-chain groupings repel each other, owing to a negative electrostatic charge. Such effects are made responsible for the high [η] values of sols (*a*), but it is not to be supposed that the pH of maximum intrinsic viscosity coincides with the equivalent point (cf. Fuoss, *Discuss. Faraday Soc.*, 1951, **11**, 125). On addition of salts, buffer, or excess of potassium hydroxide, an increased binding of counter ions will take place, thereby decreasing the net charge on the protein and reducing the electrostatic repulsion. This screening of the charge must lead to more compact chain configuration, possibly of higher statistical probability, and therefore the [η] values of sols (*b*) and (*c*) are small. It follows from (15) (below) that a decrease of [η] from 13·5 to 1·4 corresponds to a contraction of the protein chain of about 100%.

Some previously investigated proteins (for references, see Pauli and Valko, " Elektrochemie der Kolloide," Vienna, 1929, chapter 44) or synthetic polyampholytes (Alfrey, Fuoss, and Morawetz, *J. Amer. Chem. Soc.*, 1952, **74**, 438) are characterised by viscosity

maxima on each side of the isoelectric point. In the case of actomyosin a second maximum has not been observed, the $[\eta]$ values of sols (*d*) being small compared with those of sols (*a*). This can be explained by assuming that the binding of chloride counter-ions by positively charged actomyosin ions is sufficiently large, even in the absence of added potassium chloride, to prevent a significant electrostatic repulsion of the side-chain groupings of the proteins, and an increase of the average distance between the chain ends. The bonds between counter-ions and actomyosin ion, $I^{m(+)}$ or $I^{m(-)}$, will be partly ionic and partly covalent in character, like simple salt links (cf., *e.g.*, Bell and Prue, *J.*, 1949, 362, where references to previous work will be found); and disperion forces, depending on the polarisability of the counter-ions and of the side-chain groupings of the protein, have also to be taken into account. The relative importance of the various contributions is not known, but the binding of simple counter-ions by the ions of natural or synthetic polyelectrolytes, $P^{q(-)}$, is well established (Scatchard, Scheinberg, and Armstrong, *J. Amer. Chem. Soc.*, 1950, **72**, 533; Huizenga, Grieger, and Wall, *ibid.*, 1950, **72**, 4228). It is possible that such equilibria as

$$n\mathrm{Cl}^{(-)} + \mathrm{I}^{n(+)} \rightleftharpoons \mathrm{I}^{n(+)} \ldots . n\mathrm{Cl}^{(-)} \quad . . . . . \quad (9)$$

$$m\mathrm{K}^{(+)} + \mathrm{I}^{m(-)} \rightleftharpoons \mathrm{I}^{m(-)} \ldots . m\mathrm{K}^{(+)} \quad . . . . . \quad (10)$$

$$q\mathrm{K}^{(+)} + \mathrm{P}^{q(-)} \rightleftharpoons \mathrm{P}^{q(-)} \ldots . q\mathrm{K}^{(+)} \quad . . . . . \quad (11)$$

are characterised by constants of greatly different numerical values and it would not be justified, therefore, to postulate that electrostatic screening by counter-ions and viscosity properties must be similar on both sides of the isoelectric point of actomyosin.

Fuoss (*loc. cit.*) discussed the concentration dependence of the reduced viscosity of synthetic polyelectrolytes and explained the typical "concave-up" shape frequently observed. Graphs (3) and (4) in Fig. 9 are of a different shape, which may be due to stronger binding of counter-ions by the actomyosin, corresponding to a marked difference in the position of equilibria (10) and (11).

Order of Magnitude of Molecular Dimensions.—Numerical values of β', defined by (6), for various dimensions of scattering particles are shown by the graphs in Fig. 11. It will be seen that the limiting values, β'_∞, relating to sufficiently large solute molecules are respectively 2·0, 1·7, 1·45, and 1·0 for spheres, monodisperse coiled chains, polydisperse coiled chains, and thin rods. The experimentally determined β values, $1{\cdot}7 \pm 0{\cdot}3$ (mean of figures in col. 9 of Table 6), are not compatible with β'_∞ (rod) and can only be reconciled with graphs 2, 3, or 4 of Fig. 11 if D (diameter) $> 8 \times 10^{-5}$ cm. for spheres, R (root-mean-square distance between ends of coiled chain) $> 15 \times 10^{-5}$ cm. for monodisperse coils, or $R > 40 \times 10^{-5}$ cm. for polydisperse coils. $Q_{\text{theor.}}$ decreases with increasing size of the solute, as shown, for a specified wave-length, in Fig. 12, and therefore the lower limits of D and R require upper limits for $Q_{\text{theor.}}$. These upper limits ($\lambda = 589$ mμ) are as follows:

$$Q_{\text{theor.}} < 0{\cdot}08 \text{ (sphere)} \quad . . . . . \quad (12)$$

$$Q_{\text{theor.}} < 0{\cdot}03 \text{ (monodisperse coil)} \quad . . . . . \quad (13)$$

$$Q_{\text{theor.}} < 0{\cdot}25 \text{ (polydisperse coil)} \quad . . . . . \quad (14)$$

$Q_{\text{exp.}}$ ($\lambda = 589$ mμ) is $0{\cdot}14 \pm 0{\cdot}04$ (mean of figures in last column of Table 6); this value cannot be reconciled with (12) or (13) but is compatible with (14), and it follows that the sphere and monodisperse coil models are also ruled out. Comparison of $Q_{\text{exp.}}$ with graph (*b*) in Fig. 12 shows that the root-mean-square end-to-end distance, R, of actomyosin, represented as a polydisperse coil, is $(60 \pm 10) \times 10^{-5}$ cm. The accuracy of these R values is not sufficient to distinguish between extended and contracted protein molecules. Under the conditions of these experiments, length changes of about 100% appear to occur (cf. above) but these are not sufficient to bring about a transition from a coiled chain to a rod-like configuration.

It appears of interest to estimate the R values of these actomyosin preparations with the help of a different method, involving the use of the following relation.

$$R = \{[\eta]M\}^{\frac{1}{3}} \times B \quad . . . . . \quad (15)$$

where $[\eta]$ is the intrinsic viscosity, in terms of g. of solute per 100 cm.^{3} solution, M is the molecular weight of the solute, and B is a constant. This formula applies to flexible chains like macromolecules; it is not suggested, however, that in the present case the hydrodynamic thickness of the solute is of low molecular dimensions. If one assumes that the chain molecule is sufficiently long, it can be shown (cf. Kuhn, Kuhn, and Buchner, *loc. cit.*, p. 103) that the numerical values of the constant are as follows: $B = 7{\cdot}3 \times 10^{-8}$ (Kuhn, Moning, and Kuhn, *Helv. Chim. Acta*, 1953, **36**, 747), $B = 6{\cdot}5 \times 10^{-8}$ (Kirkwood and Riseman, *J. Chem. Physics*, 1948, **16**, 573; 1949, **17**, 442; Wilson, *ibid.*, p. 217), $B = 7{\cdot}8 \times 10^{-8}$ (Flory and Fox, *J. Polymer Sci.*, 1950, **5**, 745). On introduction in (15) of $7{\cdot}2 \times 10^{-8}$ for B, 2×10^8 for M (see col. 8, Table 6), and 13·5 (sols *a*) and 1·4 (sols *b*) for $[\eta]$, it follows that R is respectively 10×10^{-5} and 5×10^{-5} cm. These values are smaller than the dimension estimated from light scattering but of the same order of magnitude.

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