STUDIES ON THE ANOMALOUS VISCOSITY AND FLOW-BIREFRINGENCE OF PROTEIN SOLUTIONS

I. General Behaviour of Proteins Subjected to Shear

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INTRODUCTION

The experiments to be described in this communication and in succeeding communications in the same series originated from a belief that further knowledge of the shapes of molecules, especially proteins, may throw light upon the nature of morphological and histological shapes, and the changes which they undergo. We were especially interested in the formation of the neural tube in the amphibian embryo, where this, the first, morphological change in development, consists of a tenfold lengthening of the cuboidal ectodermal cells and nuclei to form the cells of the neural plate. The question arises whether such morphological transformations may not depend upon, or be accompanied by, an increase in the number, size, or axial ratio, of anisometric protein particles present.

As far back as 1912 Koltzov suggested that protein particles of a highly anisometric character might have an important part to play in maintaining cell structure. At that early period his view could only be based upon logical deductions from the Emil Fischer polypeptide chain hypothesis of protein constitution. But by the time of his further review (Koltzov, 1928), several different lines of attack were combining to demonstrate the fundamental correctness of his ideas. The x-ray examination of the cellulose of plant cell walls (Sponsler, 1925; Sponsler and Dore, 1926) was already beginning to show the oriented state of the elongated cellulose micelles, and the similar studies of the fibre proteins of textile strands, hair, and the like (summarised in the book of Astbury, 1933) followed not long afterwards. About the same time, the work of von Muralt and Edsall (1930 *a*) on the principal protein of muscle tissue, the globulin myosin, in which the method of flow-birefringence was first employed in physiology, demonstrated that the micelles of this protein are in fact highly elongated, in accordance with the shape of the muscle cells themselves.

Numerous other methods have been used to investigate the shape of protein particles, notably (1) the Mie-Siedentopf effect (sparkling of the Tyndall beam when long particles are present) used by Szegvari (1923, 1924); (2) the Gans effect (depolarisation of the Tyndall beam) used by Wöhlisch and Belonoschkin (1936); (3) the Majorana effect (birefringence caused by orientation of particles

in electromagnetic fields) used by Bergholm and Björnståhl (1920); (4) the dielectric dispersion method of Oncley (1938); (5) the freezing speed method (Freundlich and Oppenheimer, 1925; Püllen, 1933); and (6) the calculations of Neurath (1939) from diffusion constants and relative viscosities. All these methods study particles which are at rest, or moving only very slowly. But there are also (7) the change of Tyndall beam intensity when the sol is sheared, if long particles are present (Dieselhorst, Freundlich, and Leonhardt, 1916); and (8) the anomalous dependence of viscosity on shear force (Rothlin, 1919). Perhaps the most promising approach at the present time is (8) the method of simultaneous measurement of anomalous viscosity and flow-birefringence in a modified form of the coaxial (Couette) viscosimeter. The theory of this method was first worked out by one of us with Robinson (see Robinson, 1939), who applied it to tobacco mosaic disease virus nucleoprotein[1] and other proteins. In the present work we have concentrated on the measurement of flow-birefringence and anomalous viscosity, as indications of molecular asymmetry.

On the whole subject of protein particle shape in relation to living organisation, a rather large literature has now grown up, and it cannot be reviewed in this place. The remarkable properties of polarity and symmetry which eggs and embryonic cells possess, the phenomena of determination of spatial axes of embryonic structures at different points in time, etc., etc., have led to the conception of a "cyto-skeleton" or framework of highly dynamic nature (*cf.* Peters, 1929; Needham, 1936, 1942; Seifriz, 1936; Frey-Wyssling, 1938). On approaching such possibilities for the first time, one is apt to be impressed by the very large difference of scale between the largest protein particle, however anisometric it may be, and the smallest histological form, but it must be remembered that readily breakable and re-formable chains may exist, the conditions for existence of which must include the shape of the ultimate particles of which they are composed. It is not perhaps irrelevant to refer, in this connection, to events at a higher level. In human technology we are aware of numerous instances where the final (morphological) form has been influenced by the nature of the building materials, *e.g.* the stockades of Saxon churches, the dependence of apsidal construction upon the use of bricks, and the functional possibilities permitted by steel and concrete. There are numerous indications that anisometric particles may exist in eggs, and embryonic, as well as adult, cells. Birefringence has long been known in amoeboid pseudopodia, filopodia, and axopodia (Engelmann, 1875, 1881; MacKinnon, 1909; Schmidt, 1937*a*), in chloroplasts (Weber, 1937), and in chromosomes (Nakamura, 1937; Kuwada and Nakamura, 1934)—see the books of Schmidt (1924, 1937*b*). Birefringent phases in echinoderm egg cytoplasm have been reported by Schmidt (1936) and Moore and Miller (1937). Pfeiffer (1936, 1937) observed optical anisotropy and true anomalous flow when naked cytoplasm is caused to pass at different pressures through a capillary tube. Pollister (1941) has described orientations of mitochondria in embryonic cells which suggest the orientation of elongated submicroscopic particles in the cytoplasm, while Hobson (1941) has demonstrated an orienta-

[1] Hereinafter referred to as TMD virus.

tion of molecules, both protein and lipoidal, in the intact chick embryo by the aid of polarisation microscopy. Egg proteins, too, have given indications of their anisometric character, *e.g.* avian ovoglobulin (Böhm and Signer, 1931), avian livetin (Needham and Robinson, 1937), and a fraction from the echinoderm egg (Mirsky, 1936).

No doubt the most striking instance of elongated particle shape in proteins is the case of the plant viruses, especially the TMD virus (Bawden, Pirie, Bernal, and Fankuchen, 1936; Bernal and Fankuchen, 1941) where the particles have an axial ratio of the order of 100. It has since been possible to obtain actual photographs of these elongated particles with the aid of the electron microscope (Kausche, Pfankuch, and Ruska, 1939). The tendency of elongated molecules to form liquid crystals, tactoids, etc., is well known, and is especially marked in the case of these viruses. The existence of paracrystalline phases within the living cell is therefore of much importance for cell architecture, as has often been pointed out (as by Przibram, 1926; Rinne, 1931; Needham, 1936). The spindle in cell division is probably a tactoid (Bernal, 1939). Moreover, important biological substances other than proteins show flow-birefringence and anomalous viscosity, *e.g.* the polymerised particles of sodium thymonucleate (Signer, Caspersson, and Hammarsten, 1938; Greenstein and Jenrette, 1940*a*). The chromosomes themselves are built up on the chromonema thread (Muller, 1941) of nucleoprotein fibre molecules (Koltzov, 1928; Wrinch, 1936; Schultz, 1941) and are highly extensible, like myosin, though not fully elastic (Buck, 1942).

The biological significance of the phenomena to be reported in this series of papers may therefore be summarised as follows: We are concerned with: (1) the location of protein fractions showing elongated particle shape, and the participation of these in the architecture of the living cell, both embryonic and fully developed; (2) the mutual interactions of substrates and enzymes, when the latter are themselves elongated particles, involving changes, reversible or irreversible, in the configuration of the enzyme micelle; (3) the formation of the elongated molecules and micelles by the living cells—the processes by which they are "spun;" (4) the formation of microscopically visible fibres, as *e.g.* in connective tissue.

General Principles of the Methods

The first step towards a hydrodynamical treatment of the viscosity of colloidal solutions was the Einstein equation (Einstein, 1906, 1911), usually cited in the sterile form: $\eta = \eta_0(1 + F\ V)$ where η is the viscosity of the suspension, η_0 that of the dispersion medium, and F is a factor equal to 2.5 for spherical particles; without any description of its derivation. The equation was calculated for spherical particles large enough for Brownian motion to be negligible and for concentrations small enough to ensure absence of any sort of mutual interference. In a velocity gradient, the spheres rotate carrying with them a region of disturbed flow in the surrounding liquid dispersion medium. This dissipation of energy over and above that used in maintaining stream line flow,

results in an apparent overall viscosity of the suspension higher than that of the solvent. The viscosity of the solvent is unchanged; only its conditions of flow are affected by the presence of the particles.

When we consider anisometric particles, it is clear that their interference with normal stream line flow will be greater than that due to an equal volume of spherical particles. Further, it is clear from Fig. 1 that the maximum torque is exerted upon the particle when lying athwart the stream lines, and that the position of minimum dissipation is with the long axis of the particle in the direction of streaming. Earlier workers assumed that rod-shaped particles were orientated irreversibly in this direction by flow, but although this state is approached in certain aged sols in which the particle length is very great, continu-

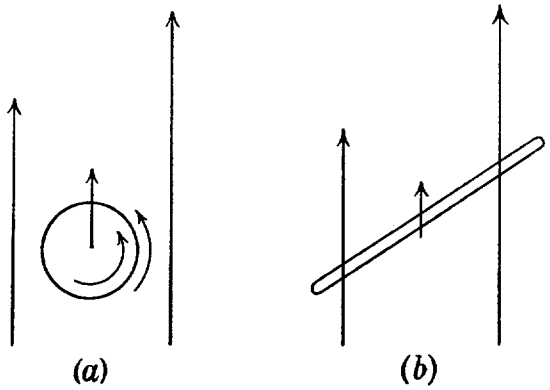

FIG. 1. Motion of particles in streaming flow. The length of arrows is proportional to streaming velocity.

ous disorientation also occurs owing to the finite cross-section of the rods and to Brownian motion.

Jeffery (1922) and Eisenschitz (1933) extended the hydrodynamical treatment to ellipsoidal particles assuming a steady precessional movement without irregularities due to Brownian motion. Jeffery determined the position of minimum dissipation of energy but his results are not in accord with the experimental results obtained from measurements of flow-birefringence. His position of equilibrium for minimum dissipation of energy is for the rods to be rotating about their long axes with these parallel to the axes of the (Couette) viscosimeter. Taylor (1923) confirmed this experimentally for particles with small axial ratio. Increasing the length of the particles, however, causes them to deviate from this position, and with increasing rate of shear, their plane of rotation approaches that shown in Fig. 1; *i.e.*, at right angles to the axis of the instrument (Guth, 1936). It may be noted that there will always be a very small vertical velocity gradient in the Couette apparatus which would explain

this difference of behaviour between very long thin fibrils and Taylor's ellipsoids with much smaller axial ratio; *e.g.*, 3.

The treatment most used, however, is a simple extension of the Einstein equation consisting of replacement of the V term by one which takes into account the larger effective volume of an anisometric particle rotating in the velocity gradient. Staudinger (1932) takes this effective volume to be a flat disc of diameter equal to the particle length and of thickness equal to the particle breadth. It would seem that an additional factor should be added for energy dissipated in the region of liquid in disturbed flow around the "disc" of each particle, but this factor will be small compared with that in the "disc" itself. Actually, of course, Staudinger's disc is an idealised picture of the integral of a number of motions each of which is a sector of such a disc but which are not all in one plane. Staudinger's attempt to justify his equation on hydrodynamical grounds by extension of the Einstein equation is obviously unsound but Huggins (1939) later derived an equation which reduced to Staudinger's for the special case of long *kinked* particles. Kuhn (1932) has also considered viscosity and flow-birefringence for cases where there is no preferred orientation of the particles, but he attributes the birefringence to the strain caused in a model particle by the pull on the two ends moving at different velocity. Although strain-birefringence may be found in gels under shear, orientation-birefringence has been established in so many cases that we must consider it in more detail. Boeder (1932) first discussed the theory which is outlined below and a number of other workers (Haller, 1932; Guth, 1936; Guth and Simha, 1936; Peterlin, 1939) have made important contributions, (review by Eirich, 1940).

The two actually occurring cases considered so far in this paper are: (*a*) anisometric particles so large that, even at low velocity gradients, orientation occurs with the long axis of the particles along the stream lines, *i.e.* the position of minimum dissipation of energy and therefore of minimum viscosity and of maximum birefringence. Many earlier workers implicitly assumed that flow-birefringence and apparent viscosity increased together whereas it is obvious that the two properties must move in the opposite sense with change of rate of shear, as it has been proved experimentally that they do (Lawrence, 1937; Robinson, 1939); (*b*) anisometric particles so small that realisable shear rates are insufficient to overcome the disorientation caused by Brownian motion, so that they progress with irregular precessional movements.

Consider now (*c*) the behaviour of anisometric particles of intermediate length in a velocity gradient. In the Couette viscosimeter, Fig. 1 (*b*) will represent a plane at right angles to the axis of the instrument. The rod-shaped particle lying athwart the velocity gradient is rotated since its two ends are moving with different velocities. The overall motion of the particle is therefore a precessional one—rotation plus linear motion along the stream lines.

The particle will turn until its long axis coincides with the stream lines. If its cross-sectional area is sufficiently small there will be no further couple exerted on it by the flowing liquid. Against this hydrodynamical orientation, however, is Brownian disorientation. If streaming were stopped at this point, disorientation by Brownian motion would follow. For a spherical particle, Einstein has given the equation:

$$\frac{A^2}{t} = \frac{RT}{4\pi N r^3 \eta}$$

where A is one-third of the square of the mean angle of rotation in time t, r is the radius of the spheres, η the viscosity of the liquid dispersion medium, and the other terms have their usual significance. It is clear that Brownian disorientation may be assessed as a rotatory diffusion constant, D, which is given by $D = \frac{RT}{8\pi\eta r^3}$ while for rods we get

$$D = \frac{3KT}{8\pi\eta}\left(\frac{\log l/r - 0.8}{(l/2)^3}\right)$$

(Burgers, 1938). A sol containing anisometric particles is therefore in a state of equilibrium between hydrodynamic orientation and Brownian disorientation. Orientation will increase with particle size (axial ratio) and with rate of shear. Disorientation will decrease with particle size (axial ratio) and with viscosity of the dispersion medium but will *increase* with rise of temperature on account of both increased heat motion of the particles and reduction of viscosity of the medium in which they rotate (Langmuir, 1938 *b*; Robinson, 1939; Lawrence, 1940).

When an anisometric particle is rotated by Brownian motion in a clockwise direction (Fig. 1), the velocity gradient will oppose its rotation. In the converse direction, the effects will be additive. The particles will therefore no longer have their minimum angular velocity in the stream line direction but in a position displaced from it in the clockwise sense. At low rates of shear, the particles will need to rotate through a considerable angle before the velocity gradient force balances the rotation diffusion one. At higher rates of shear this angle will fall. We see therefore that the angle of preferred orientation will vary with velocity gradient or rate of shear. This direction of orientation is denoted by the angle ψ. Fig. 2 shows the appearance of the field when a sol of anisometric particles is sheared in a coaxial cylinder viscosimeter and viewed along the axis by polarised light with Nicols crossed. The four black "brushes" mark the positions where the optic axes of the particles are parallel to the planes of polarisation of the Nicols. ψ is defined as the larger of the two angles made by the radius, on which the brush lies, with the planes of polarisation. Clearly it must lie between 45° and 90°.

At this stage, we must note that there are three different types of flow-birefringence. There is: (*a*) *Strain-birefringence* which is found in elastic gels (Kunitz, 1930) and in certain liquids at very high rates of shear (Clerk-Maxwell, 1874; Kundt, 1881; Umlauf, 1892; Vorländer and Walter, 1925). Stokes showed that this photoelastic effect arises from axes of pressure and

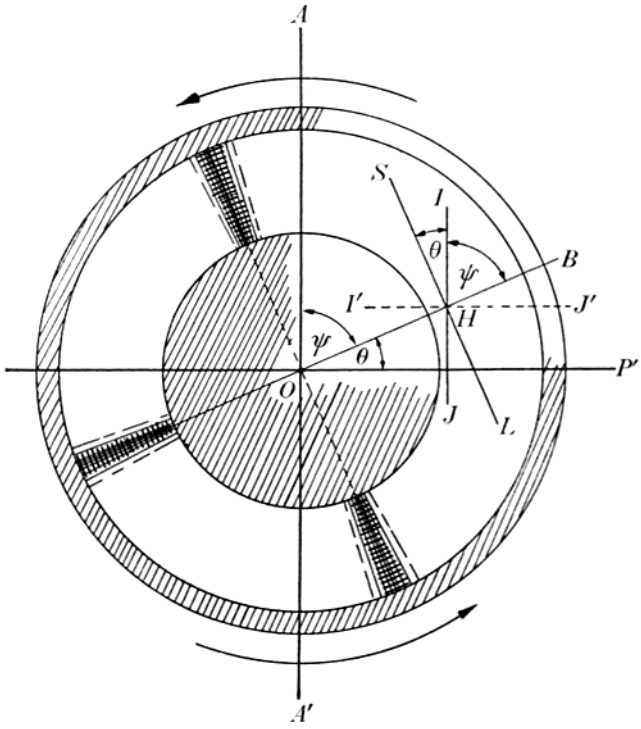

FIG. 2. The black cross of isocline. If AA' and PP' are the planes of polarisation of the optical system, then for OB to be a dark brush, a particle with its centre at H must have its optic axes in the directions IJ and $I'J'$, parallel to AA' and PP'. SL, the perpendicular to the radius OB, is the direction of the stream lines. The geometry of Ψ and θ is then fixed as shown.

tension perpendicular to each other and inclined at 45° to the stream lines. The measured angle of isocline should always be, therefore, 45°, and this has been experimentally confirmed. Such photoelastic birefringence in transparent plastic models has been used to study strains in engineering structures (Coker and Filon, 1931). (*b*) *Form-birefringence* (Wiener, 1912) which arises from the orientation of optically isotropic rod-shaped particles, the size of which is small compared with the wave length of light. Such a system in flow shows birefringence provided that the refractive index of the particles is different

from that of the dispersion medium. (*c*) *Intrinsic birefringence*, which is due to orientation of optically anisotropic particles. Form-birefringence disappears when the refractive index of solvent and solute are identical. For example, measurements (at rest) have shown that this does not occur, either with gelatin (Ambronn, 1915) or with myosin (Stubel, 1923; Weber, 1933, 1934). Hence the particles must be optically anisotropic.

To correlate viscosity with particle shape, according to the theoretical treatment outlined above, it is necessary to deduce a distribution function for the position of the particles with respect to the stream lines. The expression of Boeder (1932) cannot be integrated and he gave graphical solutions in the form of curves. Robinson (1939) made the very important advance of using his experimental flow-birefringence data as a measure of the distribution of the particles and, hence, of their contribution to the dissipation of energy by precessional motion. He arrives at the expression:

$$\eta/\eta_0 = 1 + \tfrac{1}{4}Vf^2\left[1 - \frac{\Delta}{\Delta_{max.}}(1 - \cos.^2\psi)\right]$$

where Δ and $\Delta_{max.}$ are the observed and maximum intensities of birefringence, f being the ratio of particle length to particle radius. If we neglect the term in square brackets and place it at unity, we get Kuhn's equation less the arbitrarily added Einstein 2.5 V term. If we evaluate the term in square brackets in Robinson's equation, we find that it can vary from 2 to zero. The approximate form $\eta/\eta_0 = 1 + \frac{Vf^2}{4}$ can therefore be used for an estimate of f.

We may now sum up the position. Three ranges of particle size must be recognised: (*a*) Very long thin particles which are nearly completely oriented by moderate rates of shear. These are rather rare, *e.g.* "aged" sols of V_2O_5 (Freundlich, Stapelfeld, and Zocher, 1924; Freundlich and Schalek, 1924). (*b*) Intermediate sized long thin particles whose behaviour can be studied over the whole range of ψ from 45° to 90°. TMD virus (Robinson, 1939) and mercuric sulphosalicylic acid (Hatschek, 1928) are good examples. (*c*) Small elongated particles the behaviour of which in the viscosimeter never passes random orientation. This is the largest class, comprising many polymers, although some of these have been found to show flow-birefringence, *e.g.* methyl methylacrylate (de Rosset, 1941), polystyrenes (Signer and Gross, 1933; Signer, 1936).

All that has been said above assumes that only one parameter changes, *e.g.* axial ratio of the particle. When, however, we meet with other changes, such as the degree of dispersion of the sol, aggregation and disaggregation, changes of intermicellar forces, etc., the situation becomes very much more complicated, and a full analysis of any given case is as yet not always possible. A

beginning has been made by considering the effects of polydispersity (Sadron, 1937, 1938; Sadron and Mosimann, 1938). The effects of electrical fields in counteracting orientation due to shear (Björnståhl, 1935; Björnståhl and Snellman, 1937) may be of use in analysing the more complicated cases.

As for the adhesions between particles which intermicellar forces bring about, we know that something of the kind exists in sols of the TMD virus, because hexagonal packing occurs in the plane at right angles to the length of the particles, if the concentration is high. For dilute sols the forces concerned must always be small, and sound hydrodynamical treatment may ignore them. Adhesion forces act in two ways. Weak attachments will be broken by moderate shearing, and will show themselves only as a high initial anomaly or very small yield-point, if the solution is sufficiently concentrated for a continuous meshwork to be built up on standing. Alternatively, adhesion forces may act by building up micelles or bundles of particles which cannot be broken by moderate shearing. Such bundles need not bear any geometrical similarity to the constituent particles. Thus a bundle of fibrils may become, by lateral aggregation, as broad as it is long, and so approximate to a spherical particle. On the other hand, there may be any intermediate degree of elongation, ending in the unlikely case of end-to-end packing, but including sliding parallel extension and closure.

Coming now to the type of the instrument used, it is important to note that the coaxial viscosimeter (Couette, 1890) is essential for a number of reasons. (*a*) The variation of rate of shear is very small across the annulus, as compared with that across the capillary in the Ostwald viscosimeter. Where viscous anomaly is present, this is a serious matter, for the liquid will be subject to a much higher rate of shear near the walls of the capillary than near its centre; calculations of mean rates of shear under these conditions have no physical meaning. (*b*) Steady equilibrium flow conditions are reached in the coaxial, but not in the capillary, viscosimeter. (*c*) Optical observations in the coaxial cell will indicate when random orientation equations no longer apply, and furnish important alternative information about the status of the particles. (*d*) Observations in the coaxial cell can be continuous so that changes in the rheological and other physical properties can be followed moment by moment, and compared, if desired, with simultaneously proceeding chemical changes.

The value of the Couette type of viscosimeter was not overlooked by earlier workers on the physical chemistry of the proteins, especially those who studied flow-birefringence in the coaxial cell, but they reported a lack of success in using it as a viscosimeter (von Muralt and Edsall, 1930*a*, pp. 339, 363; 1930*b*, p. 852). We believe that their difficulties were due to lack of appreciation of the important part played by the surface film of protein solutions in the Couette viscosimeter as normally employed (see below, p. 219).

Definitions and Units

The intensity of flow-birefringence in a protein sol subjected to shear stress is a function of the number of anisometric particles present (protein concentration), the degree of their own optical anisotropy, the perfection of their orientation (the resultant, for a given axial ratio, of the opposing forces of shearing stress and thermal disorientation), the depth of the solution through which the light beam passes, and the other conditions governing particle shape and size such as pH and salt concentration. We express it here in terms of $\Delta°$: the angle through which the analysing Nicol must be rotated to extinguish the plane-polarised light emerging from a $\frac{1}{4}$-wave plate.[2] The double refraction itself can be obtained at once from this figure by means of the following relations: $\frac{\Delta°}{180°} = \Delta p$ the phase-difference in wave lengths between the two components of the elliptically polarised light; and $\frac{\Delta p \cdot \lambda}{S} = N_e - N_0$ the double refraction (the difference between the two refractive indices, N_e for the extraordinary and N_0 for the ordinary beam), where λ is the wave length of the light source, and S the depth of solution under examination, expressed in the same units (Ambronn and Frey, 1926).

The angle of isocline, ψ, is by definition the larger of the two angles which the cross of isocline makes with the crossed planes of polarisation of the polarising and analysing Nicols. It is here interpreted as a measure of the degree of perfection of the orientation of the anisometric particles in the stream lines under shear stress. It varies between 45° for nil orientation and 90° for perfect orientation.

The relative viscosity, η/η_0, is the ratio between the mirror-deflection responses of the suspended central cylinder to the torques exerted by the solution under examination, and by distilled water or equivalent electrolyte solution, respectively, at the same temperature.

Anomalous viscosity is the departure of relative viscosity from independence of the shear rate. Unfortunately no satisfactory means has yet been devised for its quantitative expression.

[2] Monochromatic plane-polarised light passing through a birefringent medium is transformed into elliptically polarised light. Now the planes of vibration of the two components (fast and slow) of the light passing out of the streaming sol are inclined at a certain angle to the original plane of polarisation of the light from the lower Nicol. The Bravais $\frac{1}{4}$-wave plate, used as a Senarmont compensator, by virtue of its own birefringence, converts the elliptically polarised light back into approximately plane-polarised light. The analyser therefore has to be rotated through a certain angle ($\Delta°$), proportional to the phase difference of the two components of the elliptically polarised light, to give approximate extinction, *i.e.* to allow the cross of isocline to manifest itself at a new position, and this occurs at 45° to the former.

Technique

For the physical measurements in the present work we used two pieces of apparatus: (*a*) a small cell placed on the stage of a polarising microscope, and (*b*) a Couette viscosimeter arranged for simultaneous optical determinations.

The Microscope Cell.—With the cell on the microscope stage, measurements of flow-birefringence intensity and angle of isocline are readily made. The cell is of glass, fitted into a brass tube forming part of a pulley and ball race; the bottom is also of glass. The central stationary pillar is a glass rod, held in position by three struts from a brass cover attached to the base by three bolts and nuts. The dimensions of the cell, which holds 0.85 ml. when full, are given in Table I for comparison with other apparatus. Illumination is provided by a G.E.C. Osira laboratory type monochromatic sodium lamp, giving pure light of wave length 590 λ and the outer wall of the cell can be rotated at speeds up to 600 R.P.M. by a geared electric motor with suitable rheostats, using a rubber belt.

The Swift-Dick fixed-stage polarising microscope was provided with a ¼-wave plate between the ocular and the analysing Nicol. Owing to the depth of the annular space in the cell, above the polarising Nicol and stage lens, it was necessary to remove the ocular normally present and insert a telescopic tube, mounting objective and ocular, of some 3 inch focal length. This raised the height of the whole microscope, but the analysing Nicol holder and the ¼-wave plate easily fitted on to the top of the telescopic tube. The ¼-wave plate was kept permanently in position, its accuracy of setting being checked from time to time. The flow-birefringence intensity could then immediately be found by noting the angle through which the analyser had to be rotated in order to obtain the narrowest and darkest cross at 45° to the former cross of isocline.

The angle of isocline was obtained by placing a pointer just above the annular meniscus at 90° to the polariser. Then, with the analyser crossed to give extinction, the coupled Nicols are turned together through the longest possible range till one of the brushes of the cross of isocline centres on the pointer. This angle is the angle of isocline.

Comparison of Shear Rates.—The shear rate of a coaxial cell is its most important characteristic. It is derived from the formula

$$2\Omega \frac{R_1^2 R_2^2}{r^2(R_2^2 - R_1^2)}$$

where Ω is the speed of revolution in radians per second; R_1 the radius of the inner, and R_2 that of the outer, cylinder; and r the radius of a circle half-way across the annular space. Values which show how our apparatus compared with other instruments will be found in Table I.

The Coaxial Viscosimeter.—The coaxial viscosimeter is a smaller form of the instrument designed by one of us and used by Robinson (1939). As no description has yet been published, some details are given here. It is one of the Couette type but with the essential new feature that simultaneous measurements may be made of flow-birefringence and viscosity. The two properties are therefore measured under formally identical flow conditions (see below), a requirement persistently ignored by most workers in this field, who have measured one property in the coaxial cell and

the other in the Ostwald capillary viscosimeter in which the flow conditions are quite different and may not reach hydrodynamical equilibrium.

The design aims at maximum steadiness of flow combined with ease of dismantling and reassembly without loss of precision (see Fig. 3). It consists of four units, each

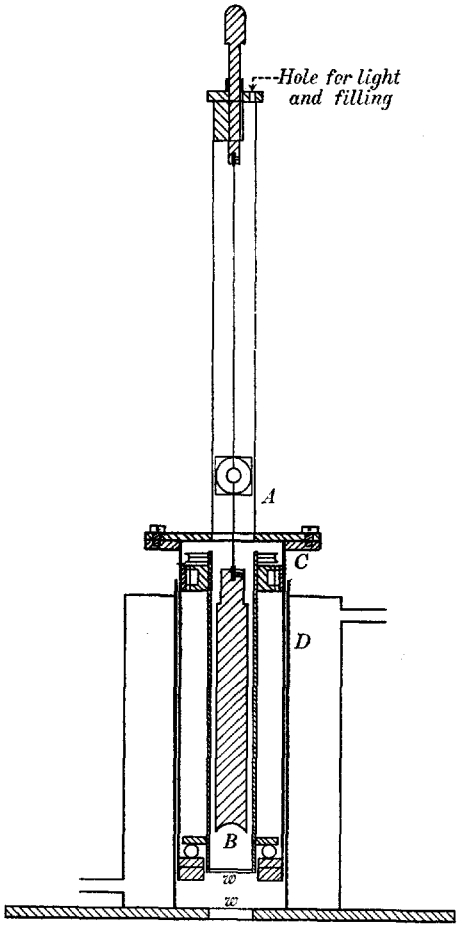

FIG. 3. Construction of viscosimeter.

of which is constructed and trued up on the lathe before assembly into the instrument, the deviation of which from a truly coaxial set-up is negligible. The units consist of: *A*, inner hanging cylinder (see Taylor, 1936), torsion head and its supporting tube mounted on the top plate of the instrument with or without a space permitting access at this point: *B*, rotating cylinder, the top of which is fixed inside a roller bearing, while near to the bottom it carries the upper plate of a ball-bearing. After removal of *A*, *B* can be lifted out. *C* is the supporting cylinder for *B*, which makes a tight

sliding fit in it (shown in the diagram as a finite clearance): D is the water jacket and base of the instrument. The correct position of C in D is fixed by a small set-screw (not shown in the diagram). The suspension wire is soldered into very small chucks which slip into the ends of the hanging cylinder and the torsion head where they are fixed by set-screws. The clearance between the bottoms of the two cylinders is adjustable by moving the rod in the torsion head and its magnitude can be determined by lowering this rod until the suspension wire sags and then raising the rod to any desired level. The base of the instrument and the bottom of the rotating cylinder are provided with glass windows (w) through which the polarised light passes upwards and emerges through a cut away space in the torsion head block. No glass window was fitted here since this space is useful as access to the annulus for removing bubbles, filling, and emptying, etc. By using a thin glass tube with a fiducial mark to coincide with the top of the torsion head, the liquid in the annulus can be adjusted to any level required. The base plate is provided with levelling screws.

The dimensions of the instrument are a compromise between the theoretically best and limitations imposed by the small amounts of liquid available. To allow for end-correction at the bottom of the cylinders, the clearance needs to be large compared with the width of the annulus. The hemispherical form of the bottom of the hanging cylinder is provided to enclose an air bubble and so further reduce end-correction (*cf.* Mallock, 1888). The length of the annulus needs to be considerable to get sufficient torque on the inner cylinder at low rates of shear. The result is that R_1 and R_2 are rather small but the clearance between them is thin enough to ensure a small variation of the rate of shear across the annulus. There is a practical lower limit to the width of the annulus below which sufficient illumination of the field is not obtained for the observation of the flow-birefringence. The diameters of the two cylinders and other measurements, with the calculated shear rates, are given in Table I. In the original instrument, the two cylinders were made of stainless steel but chromium-plated brass was used successfully later. We did not meet with spurious birefringence arising from reflections off these surfaces (*cf.* Frey-Wyssling and Weber, 1941), partly because our light beam was very nearly parallel, and partly because protein solutions are so highly light-absorbing. We have not thought it worth while to introduce any correction for this, since the main function of our data is in relative comparisons.

In use, the instrument's outer cylinder can be evenly rotated at speeds varying from 0.3 to 200 R.P.M., while the lagged jacket allows of accurate temperature control. This was carried out by letting water at known temperature flow through the apparatus; it comes in a lagged tube a short distance from a large volume Warburg manometer tank electrically heated and controlled by a thermostat and relay. Most of the experiments here reported were made at 20°C. The roller bearings and ball race of the rotating outer cylinder (the space between B and C in Fig. 1) operate in medicinal paraffin oil, and this lubricating medium also fills the whole of the inner jacket space, including that which intervenes between the glass bottom of the instrument and the glass bottom of the rotating cylinder. It is, of course, essential that the bearings should be absolutely free from rust or other particles, and from time to time, even when no visible particles can be detected, it is necessary to remove the rotating cylinder and bearings, soak them in benzene, and polish the races with a small amount of mild abrasive such as brasso.

The drive for the rotating cylinder is a matter of much importance. We obtained excellent results by using a ½-h.p. electric motor and a stand of adjustable pulleys

TABLE I

*Comparison of Properties of Coaxial Cells**

Cell	Type	Inner cylinder used	R_1	R_2	r	Width of annulus	For 100 R.P.M. Ω	Shear rate	Height of column of liquid sheared
			cm.	*cm.*	*cm.*	*cm.*	*radians /sec.*	*cm./cm./ sec.*	*cm.*
von Muralt and Edsall (1930)	Optical only	I	0.91	2.0	1.45	1.09	10.5	10.4	9.9
		II	1.30	2.0	1.65	0.70	10.5	22.6	9.9
		III	1.80	2.0	1.90	0.20	10.5	98.0	9.9
Böhm and Signer (1931)	Optical only	—	2.30	2.495	2.40	0.195	10.5	131.0	4.7
Sadron (1936)	Optical only	I	2.42	2.55	2.485	0.13	10.5	199.0	9.8
		II	2.50	2.55	2.525	0.05	10.5	445.0	
		III	2.53	2.55	2.54	0.02	10.5	1360.0	
Robinson (1939)	Optical and viscosimetric	—	1.73	2.86	2.30	1.135	10.5	18.8	20.7
Present work: Microscope cell	Optical only	I	0.15	0.295	0.222	0.145	10.5	13.1	At first 1.5 Later lengthened to 3.0
		II	0.245	0.295	0.270	0.05	10.5	55.9	
Couette	Optical and viscosimetric	I	0.40	0.785	0.593	0.192	10.5	7.7	At flood level 10.0 At low level 8.0
		II	0.64	0.785	0.713	0.072	10.5	44.0	

* *Cf.* Edsall, 1942, p. 275.

similar to those used for Warburg manometer shakers. From the motor to the final pulley in the stand the connections were round-section leather belts, from the final pulley to the drive-wheel (which at every revolution was made to sound a bell) the connection was stout string, and from the drive-wheel to the apparatus itself, the connection was a length of cotton seven times the distance between the wheels and yet knotted only in one place. Suitable pulleys for adjusting the passage of the cotton-

drive were found (in the absence of small pulleys on ball bearings) in the pulleys used for the drives of dentists' drills; these are excellently machined, and one is provided on a ratchet mechanism as an idler for giving the exact degree of tautness required. If such a cotton belt is kept free from oil by an occasional cleaning with ether, it gives (as no other type of drive in our experience did) smooth enough running for acceptable readings on the galvanometer scale.

The mirror on the torsion wire, the lamp illuminating it, and the scale on which the image shone, were of normal galvanometer pattern. The torsion wire itself was at first of steel, but later on, when, as will be described below, it became essential for the hanging cylinder to be completely immersed in the solution under examination, the wire broke through corrosion too frequently and phosphor bronze was used instead, with excellently reproducible results. It is necessary to calibrate the behaviour of every wire with pure distilled water; with steel wires we sometimes, though by no means always, found a double instead of a single linear relation between speed of rotation (shear force) and deflection (response to torque). Instead of a single line from the origin at a certain angle, throughout the range, the relation would follow such a single line until a certain speed was reached, and thenceforward follow another single line at a lesser angle with the abscissa. This, however, did not occur till fairly high speeds were reached, was not readily detectable with the experimental solutions themselves, and has not been observed with phosphor bronze wires.

For varying the speed it was found convenient to have a rheostat conveniently placed for the observer at the galvanometer scale, so that the result for any speed desired could be read from rest up to 2.4 R.P.M., then from 2.4 R.P.M. up to 6.0 R.P.M., from 6.0 to 15 R.P.M. and so on, the belting and pulleys being changed only at these intervals. It was also found convenient to suspend the galvanometer scale from a runway made of curtain railing thus permitting some lateral movement, but with a locking device to keep it at any desired place. Unlike galvanometer technique, the constant manipulations of the cylinders affect the position of the mirror on the suspension wire, and its exact angle relative to the light beam cannot be easily controlled.

The optical equipment used for measuring the flow-birefringence of solutions in the viscosimeter is shown diagrammatically in Fig. 4. The light source is a 3000 watt B.T.H. projector lamp, enclosed in a box of asbestos and tin, and cooled by a powerful electric fan. This illumination is the minimal adequate, and an even stronger light source would be desirable, owing to the great scattering of light by protein solutions. The light is conducted horizontally through a black tube (since such lamps must burn vertically), concentrated slightly by a biconvex lens, and reflected up into the instrument by a plane mirror, the 45° setting of which can be exactly adjusted. This part of the apparatus is contained within a small but stout wooden table which supports the instrument above, while below it rests upon a length of tree trunk about 1 foot 6 inches high. Pads of Sorbo rubber intervene between the table and the base, and between the base and the floor; these insulate the whole structure fairly effectively from stray vibrations in the building. Above the plane mirror is a shelf on which rest two pyrex Corning filters (Nos. 978 and 349), giving a band of wave lengths centering on the sodium line (550–630). The light next passes into a large polarising Nicol, taken from a mineralogical lecture room type lantern; the carriage of this is capable of wide rotation and provided with a handle and pointer to a scale just below the

viscosimeter. Just above the lenses of the polariser there is a small planoconvex lens. By this means a powerful beam of polarised light ascends over nearly the whole area of the annulus, but the viscosimeter is placed slightly excentrically in order to obtain

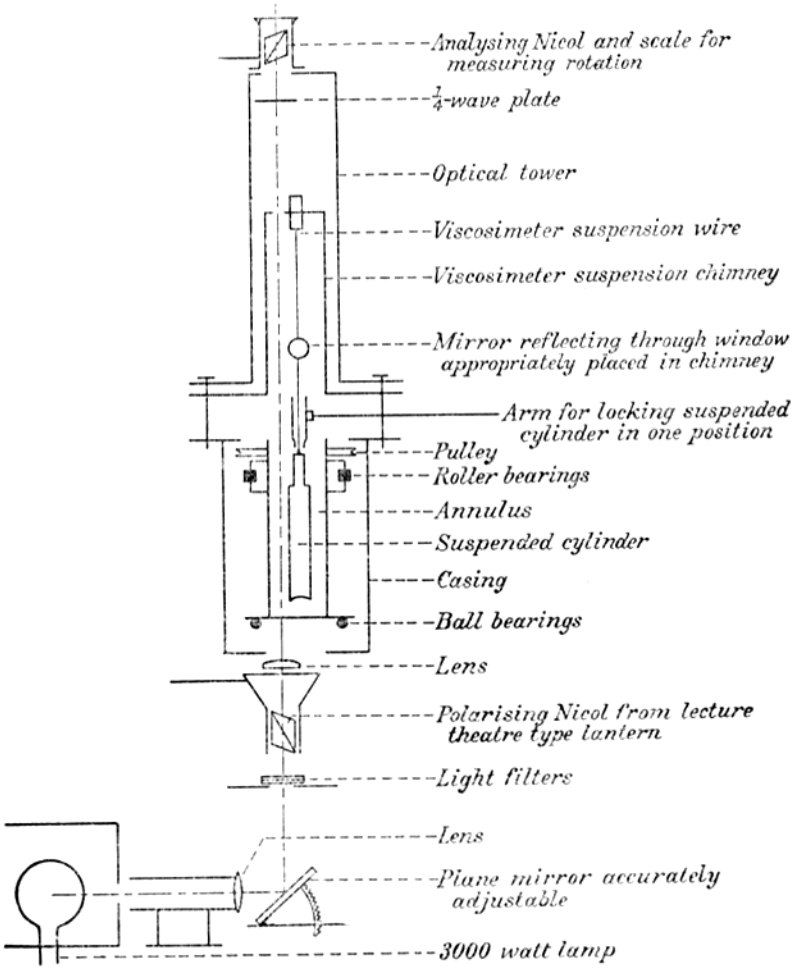

FIG. 4. Diagram of optical arrangements in the viscosimeter.

more perfect illumination of one half its disc. The light ascending in the viscosimeter's annulus is not perfectly parallel, but very nearly so. At the meniscus it passes out into the chimney of the suspension, at the top of which there is a window so arranged as to enable the observer to see a good deal more than half of the whole disc and annulus, and hence three of the four brushes of the cross of isocline. Outside the brass suspension wire chimney, and attached like it by the same brass bolts to the lower parts of the instrument, there is another chimney or tower made of stout

cardboard tubing, and bearing fitted to its upper end the ¼-wave plate and the analysing Nicol with its scale of measurement of rotation.

One further component of the apparatus remains to be described. It is usually necessary, when measuring flow-birefringence, to employ a higher speed of rotation than when measuring anomalous viscosity; it is therefore necessary to have some braking mechanism whereby the central cylinder can be maintained in a stationary position. It may be said at this point that only in the case of the TMD virus, in the work of Robinson (1939), has it ever proved possible to measure the flow-birefringence and viscosity of a protein strictly simultaneously. No other protein so far studied, even myosin, has sufficient specific flow-birefringence intensity. At the same time, it is obviously a great advantage to take the optical and viscosimetric measurements upon the same sample of protein, at unchanged concentration and temperature, and in the presence of the same added substances, etc. The suspension wire of the hanging cylinder, therefore, passes through a short glass tube, as shown in the diagram, the lower surface of which is ground. This tube is secured in a brass holder electroplated with silver, which in turn is attached to a rod passing horizontally out of the apparatus, and capable of being carefully raised and lowered by an adjacent rack and pinion mechanism. When it is desired to fix the hanging cylinder in one position, this glass tube is lowered so that it holds the cylinder tight with an annular but regular pressure; the shear force can then be increased as much as is necessary to obtain good flow-birefringence readings without any danger of straining the suspension wire.

Degree of Accuracy Obtainable in Readings.—The brushes of the cross of isocline, under good conditions, are quite sharp. In the microscope cell the accuracy obtainable in the readings was $\pm 2°$ over the major part of the range but towards the ends (below 10° and above 100°) the experimental error was somewhat higher. In the viscosimeter the accuracy obtainable in the readings was rather less, $\pm 5°$ over the major part of the range.

Comparison of Readings in Microscope Cell and Viscosimeter.—Convenient speeds of rotation are some 8 times higher in the microscope cell than in the viscosimeter (300 to 500 R.P.M. as against 50 R.P.M.), but the depth of solution is some 3.3 times less (1.5 to 3.0 cm. as against 8 to 10 cm.). The shear rate in the viscosimeter as generally used (cylinder II) is 3.36 times that in the microscope cell as generally used (cylinder I). Hence the flow-birefringence readings in the two instruments on the same myosin sample are not very far apart; it is necessary, however, to use more dilute sols in the viscosimeter when viscosimetric readings are simultaneously undertaken.

General Types of Effect Observed

The general types of effect observed follow from the theoretical introduction given above. Fig. 5 reduces the facts to their simplest form (*cf.* Lawrence, 1937). In Fig. 5 *a* and 5 *b* the deflection of the mirror, δ, on the suspension wire responding to the torque to which the hanging cylinder is subjected, is plotted against the shear force applied to it, which is proportional to the speed of rotation of the outer cylinder in revolutions per minute. The lines marked *W* represent the deflections given by water alone at the experimental temperature, or by whatever salt solutions are used as controls for the dilute protein

sols. Line A represents that given by proteins with approximately spherical molecules or particles. The flow here is normal or Newtonian, and the relative viscosity (shown as A_1 on the accompanying diagram, Fig. 5 *c*) is therefore constant whatever the shear force. Proteins with anisometric molecules, particles, or aggregates, however, give lines such as that shown by line B in

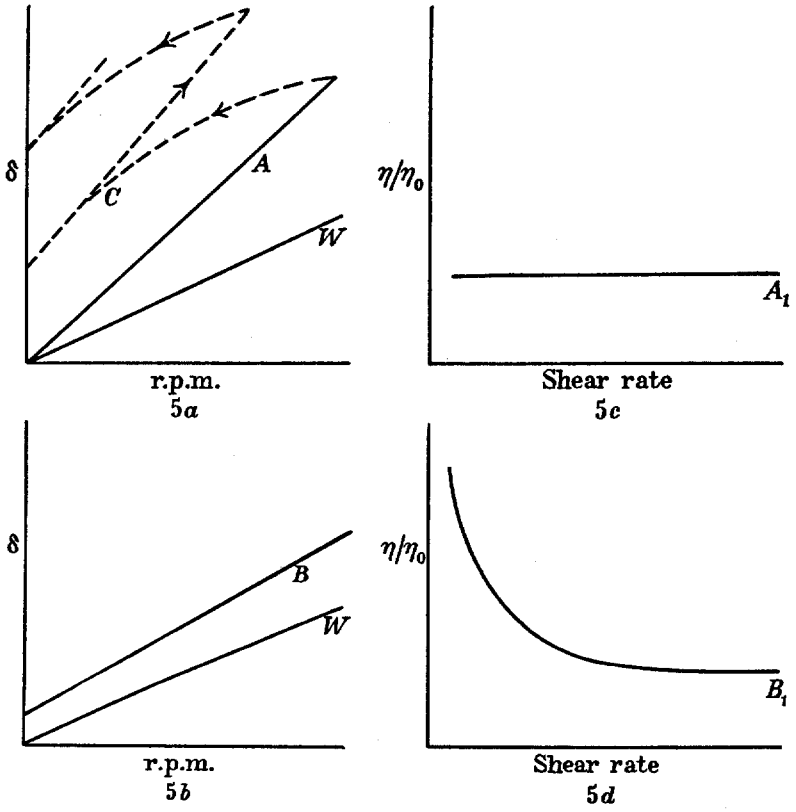

FIG. 5. General types of effects observed.

Fig. 5 *b*, the readings being abnormally high at low shear rates and falling off as high rates are approached. Such *anomalous flow* means that the relative viscosity varies with the rate of shear (as in B_1 on the accompanying diagram, Fig. 5 *d*), falling from a very high value to a constant or approximately constant level when perfect orientation of the long particles in the stream lines is attained. These two cases are the simplest met with, as will be seen from the references to specific proteins in the following paper.

Other proteins exhibit more complex behaviour. Thus a series of protein deflection readings may first follow a line similar to A in Fig. 5 *a*, but when slow

speeds are again applied, an "anomalous return" is obtained, cutting the ordinate at a higher level. The cycle can then be repeated, with a return to the ordinate (rest) at a higher level still. It should follow then that by continuous rotation of the outer cylinder at constant speed, the deflection should continuously increase instead of remaining constant, and this is accordingly found to happen (see below). After a definite time this "stretching" process comes to an end, and no further rise in deflection is observable. We undoubtedly have to deal here with the gradual formation of a monolayer or multilayer on the air-water interface of the solution. This will be fully discussed in what follows (p. 220).

We have not so far found any protein which showed anomalous flow initially, followed by anomalous return and polyfilm formation; the probable significance of this fact will become clear later.

It is of interest that something like our "anomalous return" was observed by Hatschek (1913) in gelatin sols and described by him as a "hysteresis" effect. Later Ostwald (1927) came near to what we believe to be the correct explanation, though he did not actually give it, in his suggestion that the effect was due to film formation at the surface of the inner cylinder.

In the irreversibility of the phenomenon and its non-repeatability on the same sample of protein, this "stretching effect" is reminiscent of the compression of protein films by piston oil in the experiments of Li and Wu (1932) extended by Langmuir and Waugh (1940). A compressed film gradually contracts because weakly hydrophobe groups are forced into solution, then later, when released, cannot expand again to its original area; and several cycles of this kind may be followed. In our case the shearing rotation of the surface film seems to invite more and more protein molecules into it, perhaps because they are better packed therein than normally. Eventually the maximal surface area is covered with a maximally thick film.

The coaxial viscosimeter is, of course, not indispensable for the detection of anomalous flow, since solutions may be forced through capillary viscosimeters under different pressures or suctions and hence at different average shear rates. The capillary method suffers indeed from severe theoretical deficiencies, as described above. But it is important to note that the capillary method cannot measure the changing relative viscosity from moment to moment, and without the coaxial viscosimeter it would therefore have been impossible to examine the "stretching" effect just described, and hence to make a separate analysis of film and bulk viscosity.

More remarkable is the following phenomenon. We have frequently observed that if, after the "stretching" process has proceeded a short while or has come to a conclusion, the protein solution be removed from the viscosimeter and filtered, upon being put back it will give a picture like that of Fig. 5 *b*, curve *B*; *i.e.*, anomalous flow. It is extremely probable that this is due to

the spinning off of anisometric protein particles from the surface film, or to their liberation from it once having been formed there, when the solution is removed from the apparatus. This may possibly throw some light on how anisometric molecules are formed in the living cell.

A word may be added here about the assessment of anomaly. During the actual observations we found it impossible to predict how the diagram would appear, and it is usual to obtain either marked anomaly on the graph or definitely normal flow, but there is a class of cases where the line describing the set of deflection points cuts the ordinate so near the origin that its status cannot readily be determined. We have not chosen any of these uncertain cases for discussion in this paper.

It should also be remarked that in the following papers in the series, when comparing the relative viscosities of solutions showing anomalous flow, we always chose shear rates by which the relative viscosity had sunk to its minimal and constant plateau.

Film and Bulk Viscosity of Protein Solutions

The property which most proteins have of forming monolayers and multilayers at the air-water surface is well known, and any attempt to study protein solutions in the coaxial apparatus must necessarily reckon with it. The "stretching" phenomenon just described, *i.e.* the continuous rise in deflection when the solution is rotated in the annulus at constant speed, until a final value is reached, was soon found to depend upon the presence of the surface film. If the inner cylinder, instead of being partially immersed (as has nearly always been the practice in coaxial viscosimeters hitherto)—see Fig. 6 *A*—is entirely immersed, so that the suspension wire alone passes through the surface film—see Fig. 6 *B*—the "stretching" effect is never observed.

This is illustrated in Fig. 7. Curve *a* shows the behaviour of a 0.10 per cent solution of freshly dialysed crystalline ovalbumin, rotated continuously at 11.9 R.P.M. at low level. After about 15 minutes the deflection had reached a maximum and, as usual, remained there. Curve *b* shows the same solution at flood level. No "stretching" took place, until at the point marked with the arrow sufficient of the solution was removed to bring it down to low level, after which there followed on further rotation precisely the same effect as before. It is probable therefore that not only is a solid multilayer formed, but also, since the surface area of the meniscus must, owing to centrifugal force, be very slightly larger during the rotation than at rest, the multilayer buckles when the rotation ceases. In any case it is clear that the torque on the wire alone is quite insufficient to manifest the properties of the surface film.

These facts suggest that at the low level position, we have to deal with surface or film viscosity, while at the flood level position we have to deal with bulk viscosity. This is further demonstrated by the following experiments.

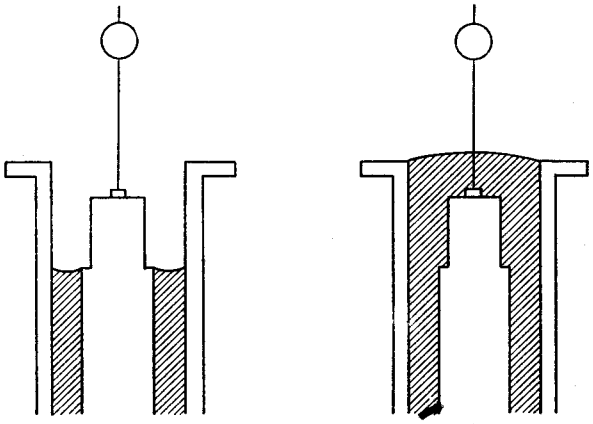

FIG. 6. Conditions in viscosimeter for film and bulk viscosity measurements.

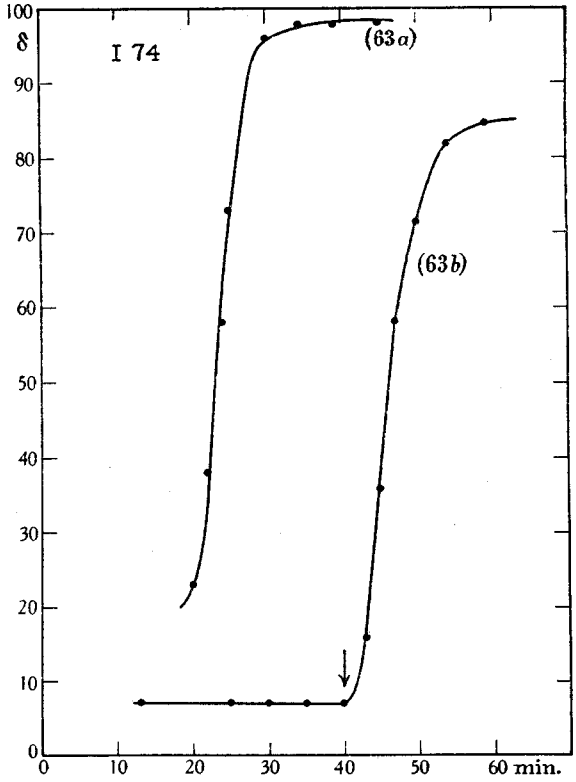

FIG. 7. "Stretching" effect of ovalbumin surface film; building of multilayer.

In the first place we may keep the film and the shear rate constant while varying the bulk torque. To do this we must immerse the same suspended

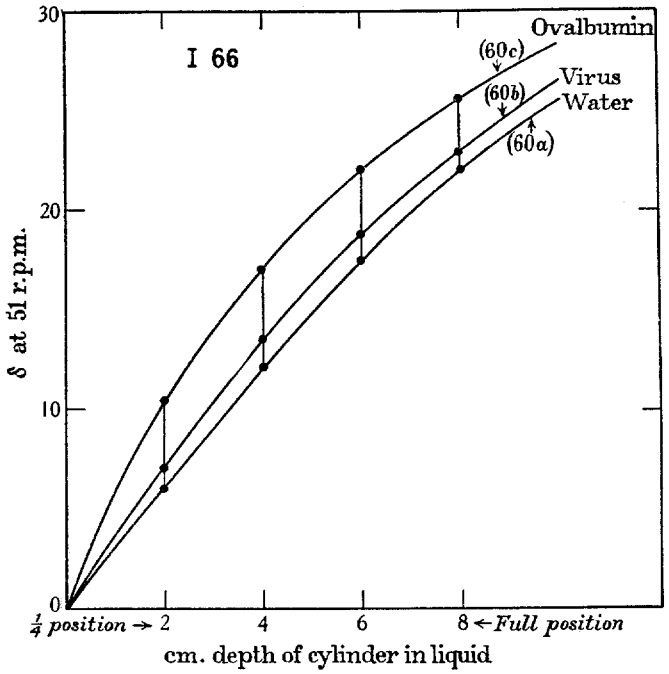

FIG. 8. Variation in bulk torque with film and shear rate constant.

TABLE II

Deflections of Inner Cylinder Mirror under Varying Bulk Torque by Virus Nucleoprotein and Ovalbumin

Cm. of cylinder I immersed in solution	Solution	Deflections above water value	
		TMD virus	Ovalbumin
	ml.	*mm.*	*mm.*
8 (normal low level)	13.39	2.0	7.0
6	10.55	2.5	9.0
4	7.66	3.0	9.5
2	4.81	2.5	8.5

cylinder in different amounts of solution. The results of one of several such experiments are shown in Fig. 8. The deflections observed while running at 51 R.P.M. and with the cylinder immersed to different extents, are plotted for distilled water, 0.025 per cent TMD virus, and 0.03 per cent crystalline ovalbumin, all at 20°C. As can be seen from the plot and from the figures in Table II

the difference between the water values and those of the two protein samples is substantially the same to whatever depth the cylinder is covered, yet the bulk phase and hence its torque is progressively less in amount. Had the bulk phase been the main determining factor, the difference would have been expected to show a regular decrease. One may therefore conclude that although the bulk torque of the water alone does of course decrease as the cylinder is less and less immersed, the torque of the protein viscosity is effected entirely through its surface film. In order to measure bulk phase protein viscosity, therefore, conditions must be found in which the surface film does not play an important part. Flood level provides such conditions.

The second experiment is to keep the film and the bulk torque constant but to vary the shear rate. To do this we must use suspended cylinders of different diameters—in this case, cylinders I and II, of 0.8 and 1.2 cm. diameter respectively, in each case at flood level. If the surface film were here responsible, or intervening to any extent, agreement between the viscosity values would not occur. The line plotted through the deflections read with cylinder II for a protein solution might not fall very much or even at all when cylinder I was used, and would certainly not fall to exactly the correct place. Results obtained in two typical experiments are shown in Fig. 9 for a protein showing normal flow (0.1 per cent crystalline ovalbumin) and in Fig. 10 for a protein showing anomalous flow (0.078 per cent flow-birefringent rabbit myosin). The figures in Table III indicate that the relative viscosity at any rotation speed appears substantially the same whichever cylinder is used. It is interesting to notice that the results with the small cylinder are consistently slightly lower than those with the large cylinder, whereas if the surface film had been playing any part, the error would have been in the opposite direction. It may therefore be concluded that the surface film plays absolutely no part when the flood level position of the suspended cylinder is used; *i.e.*, that the torque on the suspension wire is quite negligible as compared with the bulk torque under these conditions.

In Fig. 10 the curves of relative viscosity have been inserted. It is interesting to notice how the greater shear rate of the larger cylinder with its narrower annulus orients the particles in the stream lines about 2 R.P.M. earlier than that of the smaller cylinder, with its broader annulus.

One may therefore assume with fair confidence that under low level conditions we have to deal with the flow of fibrillar or plate-like particles within the surface film. The presence of such films was often verified, by noting the behaviour of talc motes scattered on the surface of the solution in the apparatus. Conversely, under flood level conditions we have to deal with the flow of particles within the bulk phase. In conformity with this, the whole of our experience has shown that at flood level all protein solutions have to be much stronger than at low level; *e.g.*, of the order of 0.1 per cent protein as against

0.001 per cent protein. The multilayers formed at the stronger concentrations are too tough and rigid for the range of the instrument.

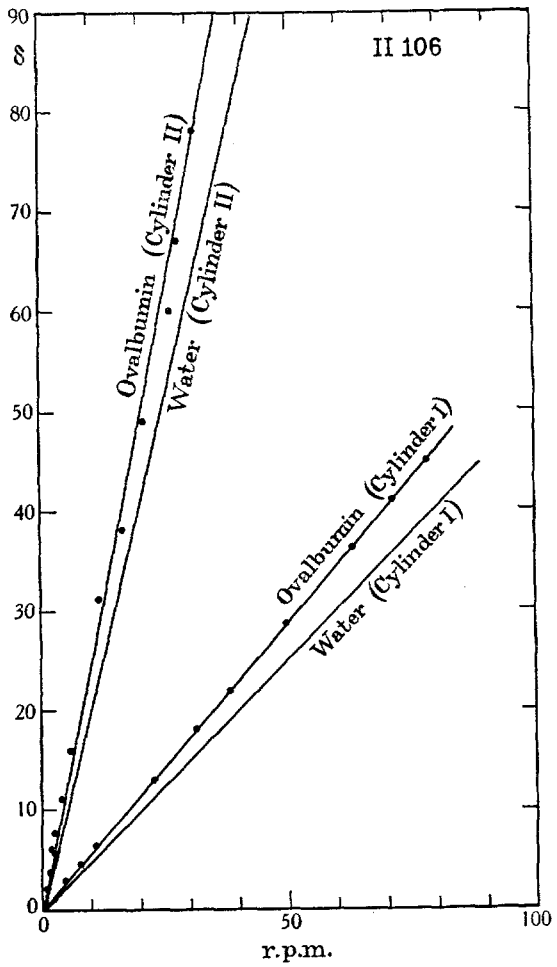

FIG. 9. Variation in shear rate with film and bulk torque constant (a) flow diagram for ovalbumin.

It may be added that there are theoretical grounds for believing in the correctness of the above conclusions. Surface viscosities of protein solutions vary over a wide range, but a value of 50 surface poises could reasonably be taken for ovalbumin solutions as used here. In this case, it may be calculated that the surface film has a viscosity 300 times as great as that of the bulk phase. Experiments of an analogous kind to those described above have been done

by Crisp (1942) who determined the change of log decrement with depth of immersion of a surface viscosimeter of the swinging vane type. Here again the surface viscosity was of the order of more than 100 times that of the bulk.

Calculations moreover may be made comparing the radii of suspension wire and cylinder (in this case 0.003 and 0.40 cm. respectively). For a given

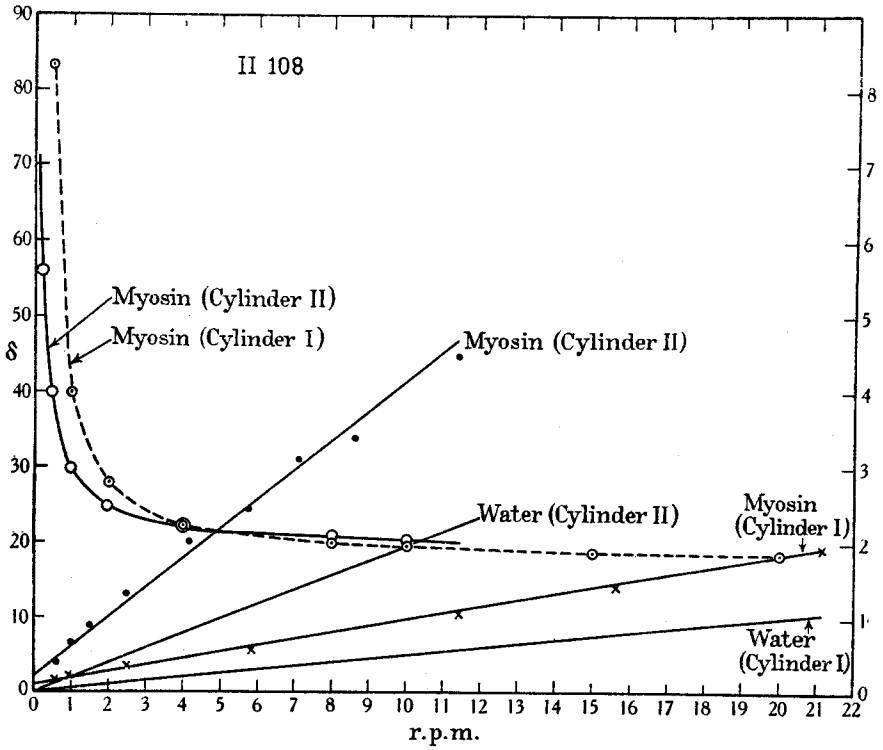

FIG. 10. Variation in shear rate with film and bulk torque constant (b) flow diagram for myosin.

protein solution, the torque on the cylinder would be 13,900 times that on the wire.

It is not easy to understand how the particles move with respect to one another in orienting to shear stress within an apparently relatively solid surface film, but as may be seen from Fig. 11, the relative viscosity of such films falls with increasing shear rate in a slow and regular way, considerably slower, indeed, than in the case of bulk measurements. If the film were to break away along the edge of its attachment to one or the other of the coaxial cyl-

inders, the initial torque on the hanging cylinder would be very high, but the deflection would instantly return to zero, or rather, to the bulk level for water as soon as the breakage occurred, and no slowly falling curve would appear. If the film were to break up into isolated floes, the apparent relative viscosity

TABLE III*a*

Relative Viscosities of Myosin and Ovalbumin at Varying Shear Rates; and Ratio of the Torques

	Speed of rotation	Relative viscosity	
		Small cylinder (I)	Large cylinder (II)
	R.P.M.		
Ovalbumin	10	1.21	1.26
	20	1.17	1.22
	30	1.16	1.20
	40	1.14	1.18
Myosin	4	2.25	2.25
	8	2.00	2.14
	10	1.96	2.13

TABLE III*b*

Experiment No.		R.P.M.	Ratio between the two torques
II. 106	Water	Average	4.40
	Ovalbumin	10	4.36
		20	4.38
		30	4.36
II. 108	Water	Average	4.05
	Myosin	4	4.00
		8	4.30
		10	4.25

would decline in a series of jerks, yet this again does not happen. We may have to have recourse to explanations involving high viscosity liquid crystalline flow or possibly plastic flow in two dimensions (*cf.* Scott Blair, 1938; anonymous, 1942). That molecules may move relatively to one another within films is known from the old experiments on saponin bubbles which assume curious shapes when the air contained within them is withdrawn (Lawrence, 1929, p. 110); and anomalous viscosity such as we have found for

protein monolayers has also been found for hydrocarbon chains in monolayers by Fourt and Harkins (1938).

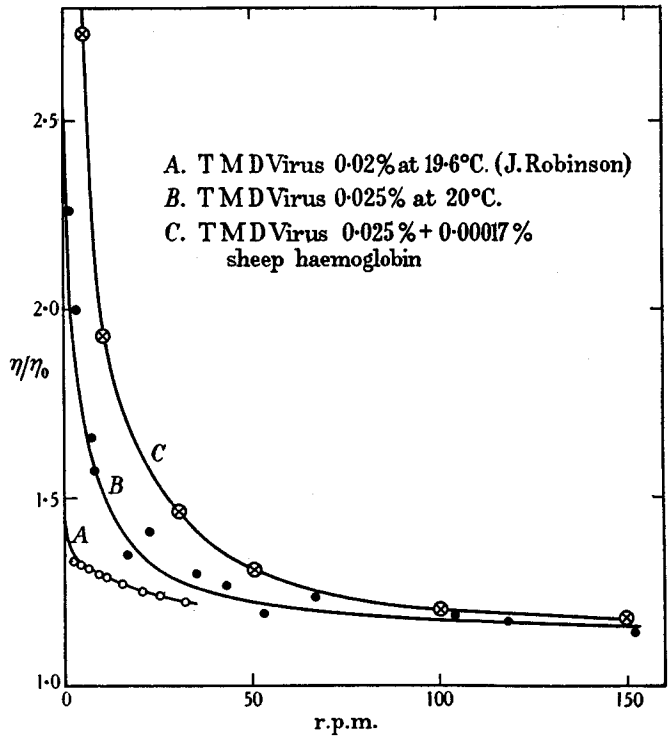

FIG. 11. Anomalous and relative film viscosity of virus nucleoprotein in presence and absence of methaemoglobin.

Relation of Optical and Viscosimetric Properties

On theoretical grounds flow-birefringence must be accompanied by anomalous viscosity—in our experience it always is. When we have found anomalous viscosity without flow-birefringence it has always been in the surface film, the thickness of which is, of course, insufficient to allow of the observation of flow-birefringence by our present methods. Nevertheless, proteins may exist which will show bulk phase anomalous viscosity without flow-birefringence; we have found mention of only one in the literature, the liver nucleoprotein of Greenstein and Jenrette (1940 *b*), whose measurements were made with capillary viscosimeters. Such cases might conceivably be due to anisometric but quite isotropic particles of the same refractive index as the medium; under such conditions flow-birefringence would not appear until they were examined in a medium of different refractive index. Or again, if the protein

particles had a tendency to linear aggregation when at rest, and if the end-to-end bonds between them were very weak, shearing might pull them apart altogether (depolymerisation) so that orientation and fall of viscosity would first be seen but flow-birefringence would never appear. It is also formally true that anomalous viscosity is a more sensitive, if less certain, method of determining the anisometry of particles than flow-birefringence, since the former method attends to the borderline of complete randomness while the latter attends to the borderline of complete orientation.

A more important point is that in our experiments with myosin, there is not an exact correspondence between the attainment of the minimal viscosity plateau and the maximal birefringence plateau. In a 0.1 per cent rabbit myosin sol at neutral pH in the viscosimeter, for example, the relative viscosity will fall to its minimal plateau level by about 6 R.P.M., but the flow-birefringence will not be accurately readable under about 30 R.P.M.

Two possible types of explanation suggest themselves for this fact. It may be that the particles of the myosin sol at rest resemble a bunch of pencils held together by an elastic band. Shearing stress first orients them, until an apparent minimal viscosity plateau is reached; but further shearing stress then pulls out the particles by making the molecules slide over one another until maximal particle length has been attained; this corresponds to the birefringence maximum. Factors which will make the molecules slide on one another would thus acquire considerable importance, having a significance similar to that "of plasticisers;" *e.g.*, camphor added to celluloid, or dibutyl phthalate to synthetic resins. It is possible that the effect of adenosine triphosphate, described in the third paper of this series (Dainty *et al.*, 1944), might be of this kind. Alternatively, the myosin particles may be aperiodic coils, which shearing stress will first orient, and then proceed to stretch out.

The second type of explanation would suggest that the main part of the anomalous viscosity is due to intermicellar forces which are broken by the shear stress. In this case the minimal viscosity plateau would be only apparently parallel with the abscissa and would really be falling imperceptibly until the shear force corresponding to maximal birefringence is reached. We have not observed any signs of such behaviour.

After we had formulated the former "parallel aggregation and disaggregation" hypothesis for myosin, we found that it had already been suggested for the very curious case of sodium caseinate by Nitschmann (1938) and Nitschmann and Guggisberg (1941). Sodium caseinate is exceptional in the opposite sense to Greenstein and Jenrette's liver nucleoprotein, for it shows flow-birefringence without anomalous viscosity. Its lack of anomalous viscosity had been noted long ago by Rothlin (1919). Such behaviour could be explained if the protein consisted of rather short micelles, not very orientable at the low shear rates of anomalous flow measurements, but capable of great exten-

sion and hence becoming highly asymmetric at the higher shear rates of flow-birefringence measurement.

A rather strong argument in favour of the conception of the micelle reversibly extensible either by parallel sliding units or by coiling and uncoiling springs, is that Wöhlisch and Belonoschkin (1936) using the Gans effect (depolarisation of the Tyndall beam) could not find any great difference between the globular corpuscular and the fibrillar corpuscular proteins. It is true that the average depolarisation constant (θ_0) for myosin and ovoglobulin was greater than for ovalbumin and serum globulin (0.039 and 0.045 as against 0.025 and 0.031 respectively), but the scatter of readings showed much overlap (ranging from 0.030 to 0.052 for the two former proteins and 0.020 to 0.056 for the two latter), and none approached the value for the inorganic V_2O_5 sol of 0.131. The authors themselves pointed out that their measurements were done on myosin sols at rest, and suggested that under shear force an *Entknäuelung* or unravelling takes place.

So also the differences between the two types of protein as shown in the freezing speed method (Freundlich and Oppenheimer, 1925) were in the same direction as indicated by flow-birefringence studies, but of a very small order. Elongated particles accelerate, and spherical ones retard, the speed of propagation of ice formation in a supercooled liquid, as against pure water. But Püllen (1933) found a retardation of only 15 per cent for serum albumin, ovalbumin, and myogen, and an acceleration of only 2 per cent for myosin and ovoglobulin, though deviations of up to 50 per cent were expected. Here again the measurements were made on sols at rest. But that myosin micelles *are* to some extent elongated when at rest is not in doubt, since anomalous viscosity measurements start from rest.

The principal theoretical and quantitative treatment of the unravelling factor is that of Sakurada (1938) but it was applied only to celluloses and isoprene polymers. The proteins still await precise treatment from this point of view.

Interference by Other Proteins

It is interesting to consider whether the simultaneous presence of other, relatively spherical, protein particles, exerts an inhibitory influence on the appearance of flow-birefringence and anomalous viscosity. On thermodynamical principles, such a spherical particle should knock an anisometric particle into stream line orientation for every one which it knocks out. But the possibility of intermicellar attachments (Pedersen, 1940) warns us that the relatively spherical particle might form with the anisometric particle a combination which, even though loose and transient, would give the whole an unorientable configuration.

Empirically, there is a marked effect. Workers on virus nucleoproteins (*e.g.*,

Pirie, 1939) have informed us that only a small admixture of non-birefringent protein greatly reduces their flow-birefringence. The subject requires a specialised investigation, but we have carried out a few experiments which are worth mention. For example, a 0.63 per cent TMD virus solution (of the preparation used at that time by us) gave a flow-birefringence in the microscope cell of 145°, but on being tested at the same concentration in the simultaneous presence of 0.17 per cent crystalline methaemoglobin (which, as will be seen later, shows evidence of approximately spherical particles), the flow-birefringence fell to 95°. This was, of course, a bulk effect.

Similar effects were found for film viscosity at low shear rates. Fig. 11 shows an experiment in which the relative viscosities of TMD virus are plotted, in the presence and absence of crystalline sheep methaemoglobin. Curve *A* describes the data of Robinson (1939) for the bulk viscosity of 0.02 per cent virus at 19.6°C., with a shear rate of 30, as against our 7.7. Curve *B* describes our data for 0.025 per cent virus at 20°C. in the film, and Curve *C* the same in the presence of 0.00017 per cent methaemoglobin. It will be seen that whereas the sample of TMD virus alone reaches its minimal level at approximately 50 R.P.M., the sample of virus mixed with methaemoglobin does not reach it till perhaps 90 R.P.M. It does therefore seem likely that the presence of even a small amount of protein of which the particles are roughly spherical will interfere with the orientation of elongated protein particles in the stream lines.

The behaviour of the strongly flow-birefringent polymerised sodium thymonucleate in the presence of other substances (Greenstein and Jenrette, 1941, 1942; Greenstein, 1942) forms an interesting parallel with the above. Most proteins and amino acids added to thymonucleate cause a small fall in its flow-birefringence and its anomalous flow, but with some organ extracts (liver, tumour, milk, serum, and even plant tissue) the fall is prolonged and severe. Since this effect can be inhibited by prior heating of the organ extracts, Greenstein and his collaborators, assuming a disaggregation, speak of a "thymonucleo-depolymerase." Their results seem, however, also to be compatible with a change in shape of the elongated micelles so that they become less orientable, either by a reduction of axial ratio by contraction or by the formation of clumps. The effects do not appear to be reversible.

DISCUSSION

It will be more profitable to postpone the general discussion of the effects of shear on protein solutions until after the specific results have been described —the reader is therefore referred to the discussion in the following paper.

The opportunity may, however, be taken here of saying a few further words about the problem from which the whole of the present work originated, namely

the change in shape of the neural cells during the formation of the neural plate in the amphibian embryo. It is at present impossible to identify the factor which is most important in these cell shape changes; it may involve (*a*) the fibrillar micelles in the cytoplasm, (*b*) the structure of the ectoplasmic layer, (*c*) the structure of the cell membranes.

The fibrillar micellar "cyto-skeleton" is proving difficult to demonstrate precisely, either by granule movement observations (Howard, 1932) or by orientation of cell inclusions (Pollister, 1941; Waddington, 1942) or by polarisation microscopy (Hobson, 1941) though Cowdry (1914) describes the filamentous mitochondria in the neural cells as oriented parallel to their long axes, and Waddington and Picken (1941) have reported birefringence in the piriform cells of the amphibian blastopore. At the same time we are bound to admit that something of the kind must be present, since cytoplasm shows unmistakable viscous anomaly (Pfeiffer, 1937) and thixotropy (Fauré-Fremiet, 1930, 1934). It may be that the ectoplasmic layer is responsible for the cell shape changes, for its important rôle in cell division has long been recognised. Here experiments with high pressures (see Cattell, 1936) might help us, for Marsland (1939) found that high pressures (*e.g.* 600 atmospheres) liquefy the ectoplasmic layer in the dumb-bell-shaped cleaving egg, causing recession to the spherical form; significantly, high pressures also liquefy gelatin gels (Posnjak, 1912) which are known to be thixotropic (Freundlich and Abramson, 1927). On the other hand, Brown, Hamburger, and Schmitt (1941) who have recently shown by density measurements that the early local hydration theory of Glaser for neurulation is unlikely to be true, incline to the view that the cell surfaces are of prime importance, the "attractive" forces between molecules in the adjoining cell surfaces of prospective neural tissue cells increasing so that the area of contact is actively increased. These views, which coordinate with the fundamental work of Holtfreter (1939) on the surface "affinities" of embryonic tissues, are elaborated in an interesting review by Schmitt (1941) with special reference to lipoprotein complexes. Waddington (1942) has since pushed the matter a little further by determining the surface tension necessary to spread amphibian embryo cells into a surface film; this varies with different parts of the embryo at different developmental stages.

As will be seen below, our contribution to the question lies in the fact that in the amphibian embryo there is a protein or group of proteins in the total euglobulin class which spreads instantaneously into a surface film having the property of anomalous flow. Its molecules must therefore readily pass into the fibrillar state. The evidence for this will be found in the following paper (Lawrence, Miall, Needham, and Shen, 1944). The union of all these facts into a coherent picture of morphological change at neurulation is, however, a matter for the future.

SUMMARY

1. A coaxial viscosimeter which permits the simultaneous determination of relative and anomalous viscosity and of flow-birefringence is described. Flow-anomaly and flow-birefringence are regarded as characteristic of elongated micelles and molecules.

2. Such methods have been applied to dilute solutions of proteins. The conditions under which the coaxial (Couette) viscosimeter measures the viscosity of the bulk phase and the surface film phase respectively have been investigated and are described.

3. The general behaviour of protein solutions subjected to shear is summarised.

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REFERENCES

The references in this paper will be found together with those of the next at the conclusion of the second paper of this series.