REVIEW ARTICLE

MOLECULAR SIZE AND SHAPE

A Review of the Light-scattering Method applied to some Important Biological and other Macromolecules. Part II.

By N. Robinson, M.Sc., Ph.D., A.R.I.C.

Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, Brunswick Square, London, W.C.1.

It is only within the last generation that it has been possible to study biological and other macromolecules on a new and more fundamental level. This has been achieved by measurements of rates of diffusion, viscosity, sedimentation, and the development of electron microscopy, X-ray diffraction and light-scattering. The study of biological systems in terms of microscopic units has thus now become one of molecular architecture.

In Part I of this review (published in the March issue) the theoretical and practical aspects of the light-scattering method and its application to the study of the physico-chemical characteristics of some important biological compounds were discussed. Other biopolymers and substances having physiological activity and also studies of reaction mechanisms which involve changes in molecular size and shape are reviewed in Part II. Substances of more general chemical interest investigated by the light-scattering method are also mentioned.

Biopolymers and Substances having Physiological Activity

Muscle Protein

An important group of proteins which have been studied extensively are myosin, actin and actomyosin.

Several investigators have attempted to characterise the myosin molecule but the results so far are inconclusive; one difficulty is probably related to the readiness with which myosin aggregates, particularly in dilute salt solutions. Mommaerts[75] examined a very pure myosin by light-scattering and obtained a molecular weight of 850,000; the length of the molecule, using the rod model, was 1,500 Å. Portzehl[76] reported that the molecular weight by sedimentation-diffusion was 858,000 and by osmotic pressure 840,000; both are in close agreement with light-scattering, but values for the length of the molecule were higher (2,000 to 2,400 Å).

A much lower light-scattering molecular weight of 530,000 was obtained in 1956 by Holtzer and Lowey[77], who also found that the rod-shaped molecule had a length of 1,650 Å (verified by viscosity and sedimentation) which was greater than Mommaerts' value (1,500 Å) by the same method. These workers reported that part of the difficulty of obtaining accurate measurements could be ascribed to spontaneous, temperature-dependent, side-to-side molecular aggregation. Their rabbit muscle protein was extracted, analysed and investigated within 60 hours of the animal's

death; evidence of dimer formation was apparent after keeping for a longer time. Recently, Rupp and Mommaerts[78] have also suggested that myosin solutions have an important ageing effect and temperature dependence. Measurements at a suitable temperature gave a molecular weight of 650,000 and a rod-shape molecular length of 1,600 Å, indicating a diameter of approximately 25 Å and axial ratio of 60. Blum[79] found that the length of the myosin molecule changed with the pH of the solution or on addition of adenosine triphosphate (ATP); the kinetics and mechanisms in muscle action based on Blum's and other light-scattering results have been discussed by Morales and Botts[80].

Preparations of actin appear to be rather ill-defined, making physical characterisation uncertain. Johnson and Landolt[81] studied actin by light-scattering and obtained a molecular weight of 130,000 but suggested that this value could be approximate only, on account of the polydispersity of the preparations. Whilst this value approaches that obtained by Snellman, Erdos and Tenow[82] (150,000) from ultracentrifuge and diffusion measurements, osmotic pressure and fluorescence polarisation studies of Tsao[83] gave a value of 70,000, suggesting that the earlier preparations were in dimeric form.

Johnson and Landolt[84] investigated the transformation of G-actin to F-actin by the addition of salts. On addition of 0·1M potassium chloride and 0·001 M magnesium chloride a 15-fold increase in scattering intensity occurred and within an hour "activation" to the F-actin appeared complete. The increase in scattering intensity was accompanied by an increase in dissymmetry over the transformation process, indicating a transition from a probable spherical shape to that of a coil having a root mean square distance between its ends of 2,370 Å. In similar studies on the polymerisation of G-actin, Steiner, Laki, and Spicer[85] found from their Zimm plots that the scattering envelope of F-actin best fitted a thin, rigid rod shape; this was confirmed by an intrinsic viscosity of 3·0 in the same medium. They also showed that G-actin was polymerised by decreasing the pH, and the net negative charge could be decreased until maximum polymerisation at the isoelectric point took place. Beyond this point the particles became positively charged and the average extent of polymerisation decreased.

Steiner, Laki and Spicer also found that the size of the complex formed between F-actin and myosin was dependent on the amount of polymerisation of G-actin to F-actin before myosin interaction; Zimm plots from the light-scattering data showed that the F-actomyosin complex decreased in average length and molecular weight with increasing ionic strength or decreasing size of the actin component.

Interest in the nature of the interaction between actomyosin and ATP stemmed from the experiments of Engelhardt, Lyubimova and Meitina[86], who found that ATP caused a strengthening of actomyosin threads produced by injecting a thin stream of the concentrated protein solution into water. Later Szent-Gyorgyi[87] reported that a contraction of threads of actomyosin floating on water took place when ATP was added. It has since been found that, whilst addition of ATP to solutions of actin and

myosin does not cause any observable change in light-scattering intensity, when ATP is added to a solution of the actomyosin complex a noticeable decrease in intensity takes place. The effect of ATP on the viscosity, birefringence and light-scattering intensity of actomyosin solutions has since attracted the attention of many workers whose findings have been interpreted in different ways.

The two-fold importance of the effect has been outlined by Blum and Morales[88]. If the particles of the rabbit muscle protein extracted by the Weber-Edsall method (which Blum and Morales refer to as myosin) undergo deformation, then the theory that muscle action is mediated by a mechanically continuous structure is supported, in which case the elementary action system can be readily studied in solution. If the particles dissociated, however, this theory is weakened and the likelihood of an actin-myosin complex gains ground. The viscosity and birefringence studies of Needham and co-workers[89] indicate that the addition of ATP causes a contraction of the particles whilst the results of ultracentrifugal and viscometric investigations by Mommaerts[90], also Weber[91] and Johnson and Landolt indicate that dissociation takes place.

As previously mentioned, ATP does not cause a noticeable change in light-scattering intensity when ATP is added to solutions of actin and myosin, but the intensity does decrease when added to solutions of the actomyosin complex; the interpretations of this behaviour have likewise been at variance. Johnson and Landolt suggested that the ATP dissociated the actomyosin complex into actin and myosin and this was further

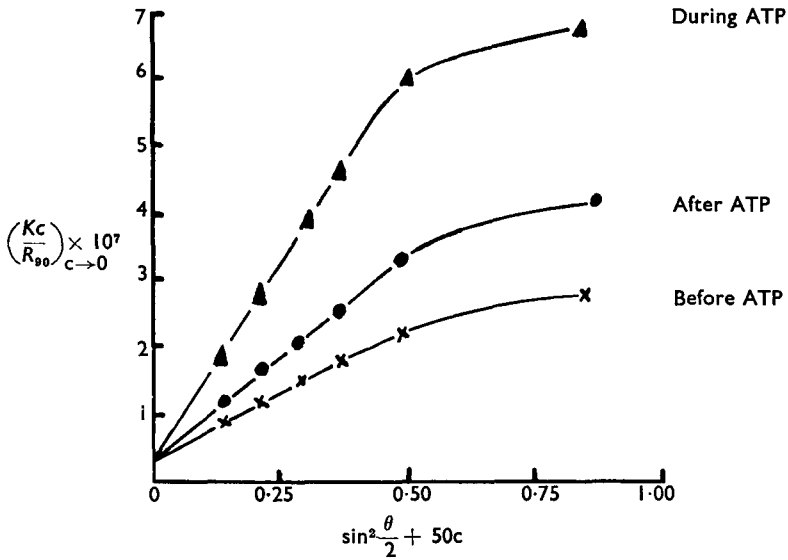

FIG. 7. Limiting curves of the Zimm plots for myosin before, during and after reaction with ATP. The curves show that the addition of ATP causes a change in shape but not in weight of the molecules (after Blum and Morales[88], *Arch. Biochem. Biophys.*, 1953, **43**, 208, with permission.)

substantiated by its effect on similar solutions. However, Jordan and Oster[92] found that the dissymmetry of the scattering envelope increased from 3·4 to 4·7 on addition of ATP and suggested that increased coiling had taken place. A reversible change in shape of the particles was also observed by Blum and Morales[88] (Fig. 7), who suggested that no dissociation into actin and myosin could have taken place. In their extensive study these workers found that the particles of their muscle protein extract were approximately cylindrical and the extension or contraction of cylinders was ATP-concentration dependent.

Tonomura, Wanatabe and co-workers[93,94], investigating the kinetics of the addition of ATP to actomyosin solutions, found that the intensity of scattered light over the whole reaction indicated three phases upon which they have based a mechanism for this reaction.

Arabic Acid

A few light-scattering studies have been made on coiling polyelectrolytes, the most interesting from the pharmaceutical viewpoint being arabic acid. The light-scattering molecular weight for arabic acid was found by Veis and Eggenberger[95] to be one million, whilst Oakley[96] obtained the number average molecular weight of 290,000 by osmotic pressure, both determinations being carried out in 0·02N hydrochloric acid when ionisation of arabic acid was at a minimum. Veis and Eggenberger suggested that the discrepancy in these values could be accounted for in two ways, namely, the solutes were polydisperse and in acid solution hydrolysis probably took place; subsequently Oakley showed that acid hydrolysis did take place to yield a small diffusible component of molecular weight less than 10,000.

Application of the Flory-Fox[97] equation (relating the extension of a coil to its molecular weight and intrinsic viscosity) to the intrinsic viscosity value of 0·329 for the undissociated acid, predicted the shape of the molecule to be a stiff coil of length 548 Å compared with the value of 1,049 Å by dissymmetry of the light-scattering envelope. It was possible that the value of the constant (ϕ) in the Flory-Fox equation was considerably less in Veis and Eggenberger's system where the extension ratio is quite high—allowing for this the two values showed fair agreement. The equivalent radius of the uncharged molecule was calculated to be 555 Å and its effective volume $7{\cdot}2 \times 10^{-16}$ cc./mol. As the concentration of arabic acid was increased Veis and Eggenberger suggested that the randomly-coiled extended molecules would contract, allowing more molecules to fill the empty space; but the closer packing resulted in an increased repulsion between molecules. The contraction process, which would be limited by the bulkiness of the molecule, would lead to an increase in the coil free-energies (gegen-ion effects neglected). The authors considered that the only alternative to allow minimum free energy to be attained would be a change of structure to the rod-shaped form possessing a lower free energy and mutual attraction energy, and also a smaller effective volume. Increasing the concentration beyond available free solvent would bring the molecules within the proximity

of short range electrostatic repulsive and other forces, resulting in the overlapping of the effective volume of one coil by another.

When the arabic acid solution was neutralised and the ionic strength of the solution further increased by the addition of sodium chloride, Veis and Eggenberger found that the reciprocal scattering (Kc/R_{90}) increased with concentration, levelled off, and then increased rapidly to produce a point of inflection. This behaviour, less marked with other polyelectrolytes, was attributed to the highly polar nature of the carbohydrate main chain of arabic acid, its large extension and stiffness, and the presence of bulky polar side chains. The angular dissymmetry of scattered light from arabic acid in the ionised state indicated a considerable extension to 2,400 Å, that is, to approximately twice the extension of the unionised molecule. The variation of the limiting interaction constant (B) with ionic strength showed that the effective volume of the arabic acid molecule also increased as the ionic strength was lowered. There was an unusual variation of the dissymmetry of scattered light with concentration of arabic acid. At different ionic strengths, the curves showed a maximum followed by a minimum (Fig. 8) and were nearly superimposable at lower concentrations, indicating a limiting contraction of the stiff branched molecules.

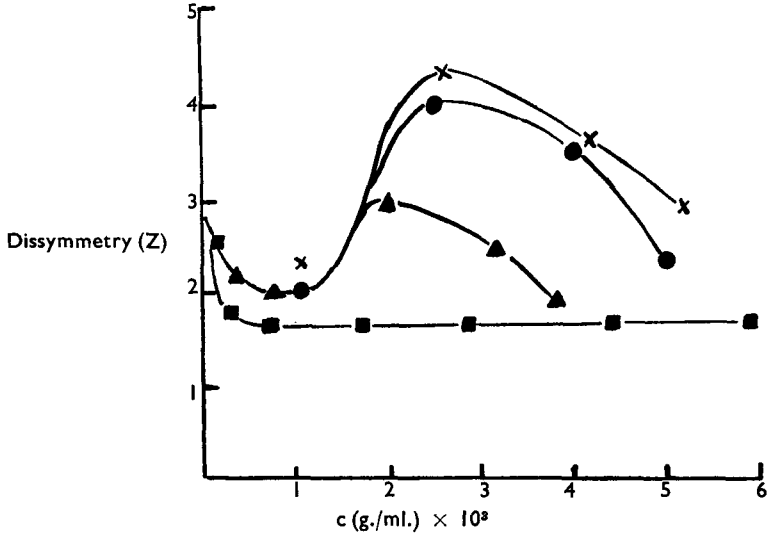

FIG. 8. Dissymmetry of scattered-light for arabic acid in solutions of increasing ionic strength (μ) (after Veis and Eggenberger[95], *J. Amer. chem. Soc.*, 1954, **76**, 1560, with permission.)

× $\mu = 0$. ● $\mu = 3{\cdot}84 \times 10^{-4}$. ▲ $\mu = 2{\cdot}65 \times 10^{-3}$. ■ $\mu = 1{\cdot}99 \times 10^{-2}$

Tobacco Mosaic Virus

The very long rod-like molecules have made tobacco mosaic virus (TMV) an interesting study and workers have gained much information about the behaviour of this virus in aqueous solutions by the light-scattering method.

Particles of TMV, having a maximum dimension at least half the wavelength of the mercury green line (5,460 Å), were found by electron microscopy (Oster[98]) to form rods of uniform thickness of approximately 150 Å and length of 2,700 Å. Older purified suspensions were found to be associated end-to-end in a manner so exact that any repetitive structure was thought to be absent.

Oster, Doty and Zimm[99] measured the dissymmetry of the angular scattering of light by TMV in pure water and buffer solutions; their results are shown in Figure 9. In polar systems the dissymmetry was

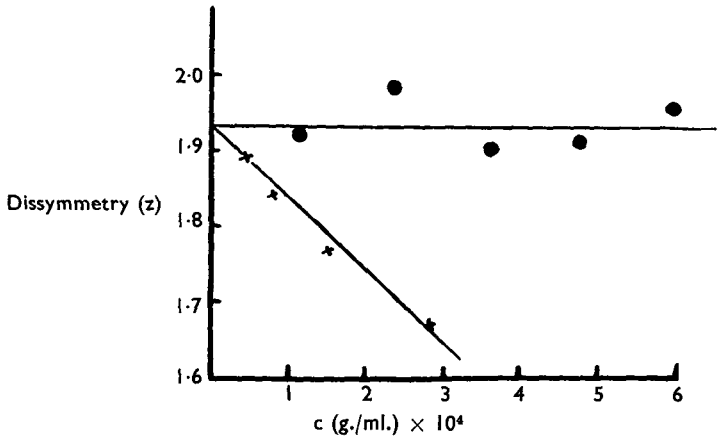

FIG. 9. Dissymmetry of scattered light as a function of concentration of tobacco mosaic virus in water and 0·1M phosphate buffer (after Oster, Doty and Zimm[99], *J. Amer. chem. Soc.*, 1947, **69**, 1193, with permission).

concentration-dependent and any change in dissymmetry was a measure of the particle interaction. In buffer solution (high ionic strength) the dissymmetry:concentration relation was almost constant, indicating a collapse of the electric double layer, resulting in very little interparticle repulsion. In pure water (low ionic strength) an increase in the concentration of the virus brought about a decrease in the dissymmetry; the intermolecular electrostatic repulsion gave rise to a large effective diameter of the particles, producing less randomness and a consequent lowering of the scattering in the forward direction. Extrapolation of the results of Oster and others gave a dissymmetry corresponding to a rod-shaped particle of length 2,700 Å in close agreement with results obtained from the electron microscope and intrinsic viscosity (2,600 Å) measurements. The molecular weight of 40 million also agreed with values calculated from the electron microscope and other methods.

The discrepancy between the scattering dissymmetry from the two solutions led Oster to reinvestigate the turbidity of TMV in water and in 0·1M phosphate buffer; his results are shown in Figure 10. The variation of turbidity with concentration was very much greater in water than in phosphate buffer; the slope of the curves clearly showed the effect

of intermolecular electrostatic repulsion. In buffer, where the interaction constant B was $6{\cdot}3 \times 10^{-7}$, the virus particles behaved (thermodynamically) like particles of twice their volume and in water (where $B = 33 \times 10^{-7}$) nearly ten times the theoretical volume ($B = 3{\cdot}4 \times 10^{-7}$). Dissymmetry measurements corresponded to a rod 2,800 Å in length, in close agreement with the earlier results. Depolarisation of the scattered light indicated that the particles were isotropic.

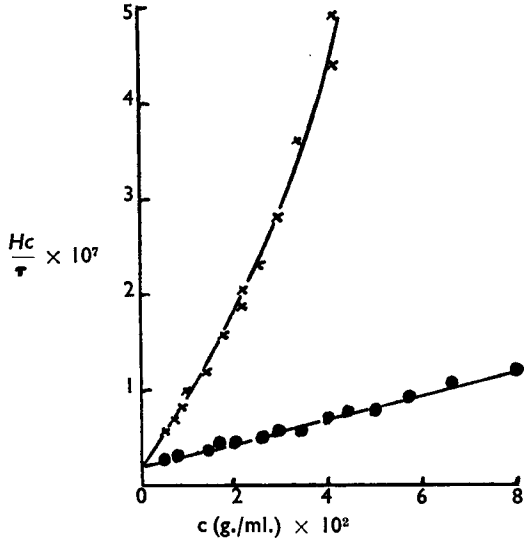

FIG. 10. Reciprocal turbidity: concentration curves for tobacco mosaic virus, showing greater intermolecular repulsion in solutions of smaller ionic strength (after Oster[101], *J. gen. Physiol.*, 1950, **33**, 445, with permission).
× = Water. ● = Buffer.

In 1937 Bawden and Pirie[100] observed that aqueous solutions of the virus, above a concentration of about 2 per cent, separated into two layers on standing for several days. Using crossed polaroid discs they found that the top layer was isotropic and the bottom layer anisotropic (permenently birefringent). Oster[101] used the light-scattering method to study the conditions for two-phase formation with regard to concentration of the virus, concentration of electrolyte and size of particles.

Oster found that in very dilute (0·004 per cent) solutions at the isoelectric point the rod-like molecules aggregated side-to-side as shown by the large dissymmetry ($z = 4{\cdot}8$) of scattered light, breaking down on dilution to a dissymmetry of 2·1. Increasing the concentration to 2·3 per cent the two reversible phases of equal volumes formed on standing for 1 day at 4°. Onsager's[102] analysis predicts that the theoretical minimum concentration for phase separation is $3{\cdot}34/BM$ for the isotropic phase and $4{\cdot}49/BM$ for the anisotropic phase. Substituting his experimental value for B, Oster found that two-phase formation should take place at a minimum concentration of 2·5 per cent, showing close agreement with his experimentally observed value.

The lower, more concentrated phase (by 40 per cent) separated to give a third gel-like phase which showed irridescence when illuminated by white light. Depolarisation of the scattered light indicated that the rod-shaped particles of TMV in the lower, more concentrated phase were not intrinsically anisotropic but that the permanent birefringence was due to orientation of the molecules with their long axes parallel; the low values in turbidity and dissymmetry arose from destructive interference in the fairly ordered system. Electron micrographs showed that the same particle size distribution existed in both phases and also confirmed the length and molecular weight of the particles obtained by light-scattering.

Oster[103] has suggested that since light-scattering of solutions, and therefore light absorptive powers, are sensitive to changes in particle size, shape and interaction, the method could be applied to the determination of the isoelectric point. Adding hydrochloric acid to a solution of TMV, the point of maximum absorption of light was obtained at a pH of 3·9, which agreed well with results obtained from a microelectrophoresis apparatus.

Oster[103,104] has also published light-scattering measurements on solutions of influenza virus and bushy-stunt virus both of which appear to be spherical with molecular weights of 322×10^6 and 9×10^6, respectively. The latter value agreed closely with estimates from ultracentrifugation[105] and diffusion[106].

Turnip-yellow virus was examined by Goring and Johnson[107] in a development study of the light-scattering experimental technique; the particles were found to be spherical with a molecular weight of $5{\cdot}7 \times 10^6$.

Casein

Despite many efforts to obtain the molecular weight of casein the values reported are at such variance that further work on this protein appears to be essential. Early molecular weight determinations were by chemical analysis. The results of Van Slyke and Bosworth[108], based on the supposition that casein molecules contained two atoms each of sulphur and phosphorus, gave a value of 4,444. This, for some obscure reason, was doubled.

Pauli and Matula[109] attempted to estimate the valency of casein by application of the Ostwald dilution rule and gave the valency as 3. The combining weight of casein for base was 1,000, which gave a molecular weight of 3,000.

Yamakami[110] investigated the molecular weight by comparing solutions of equal pressure (Bargers method). For the simplest alkali caseinates which he could prepare he concluded that the mean weight of the ions was approximately 2,000 which, for a caseinate dissociating into only two ions, indicated a molecular weight of about 4,000.

Cohn, Hardy and Prentiss[111] evaluated the molecular weight of casein from its amino acid content and hesitatingly gave a value not less than 96,000, and suggesting that it was most probably twice this value.

Svedberg, Carpenter and Carpenter[112] used ultracentrifugal methods developed in Svedberg's laboratory to study the sedimentation rate and

sedimentation equilibrium of casein prepared by Hammarsten's method. At pH 6·8 (phosphate buffer) they found that casein was a polydisperse mixture of different molecular weights. The fraction soluble in hot 70 per cent ethanol acidified with hydrochloric acid was found to be monodisperse with a molecular weight of 375,000 and a particle size of 83 to 120 Å. The value of 336,000 by osmotic pressure measurements[113] for whole casein in 6·66M urea is regarded by Halwer[114] as one of the most reliable values; urea has a dispersing effect on aggregates which he found were present in solutions of casein and could account for such wide discrepancies in reported values. Burk and Greenberg[113] obtained an even lower value of 24,500 by dissolving the casein in phenol at 42·5°.

In a study of the industrial processing of milk, two Russian workers, D'Yachenko and Vlodavets[115], used the light-scattering method to investigate the molecular weight of casein in solutions of potassium hydroxide (pH 9·0), hydrochloric acid (pH 1·5) and urea (pH 6·2) and obtained values within the limits of 27,000 to 32,000. They found that an increase in temperature in alkaline solution led to aggregation of casein particles which they attributed to traces of calcium, whilst in acid solution dissociation took place. It was suggested that the nature of temperature dependence indicated that the aggregates were linked by hydrogen bonds. In subsequent work[116] they obtained a molecular weight of casein in milk of between 2 and 8 million. This departure from the true molecular weight (approximately 30,000) was again attributed to the presence of calcium ions, which are known to be present in milk in the form of a calcium-caseinate-phosphate complex. Further work on the kinetics of variation of the molecular weight of casein with changes in concentration of calcium ions and hydrogen ions enabled an empirical relation to be calculated. Maximal molecular weights at $pCa = 1{\cdot}5$ (negative log calcium ion concentration) and pH approximately 4·6 were found and in more concentrated solutions these maxima corresponded to regions of visible coagulation.

In 1953 Halwer[114] examined α- and β-casein solutions to determine the effect of electrolytes on their size. He considered that this protein was not a "globular" or "native" protein in the sense that the terms are applied to unaltered ovalbumin, serum albumin and others but that the caseins resembled them in the denatured state.

Casein is close to its isoelectric point in pure water and is then unstable and almost insoluble; complexities in measurements then arise as a result of mutual repulsions of the unshielded charge centres. Fuoss[117] has discussed these complexities in osmotic pressure, light-scattering and viscosity measurements of synthetic polyelectrolytes. Halwer suggested that the light-scattering results obtained for α-casein at pH 7 in the absence of added electrolyte were erratic (molecular weights ranged from 25,000 to 65,000) partly because the extent of dilution to which extrapolation for a molecular weight could be accurately made was uncertain and in any case was very high. Doty and Steiner[118] found that the light-scattering results of serum albumin at pH 8 were likewise not reproducible. In the presence of an electrolyte the casein is charged and its solubility

is increased, a state exhibited by most proteins and which often determines the conditions whereby their physical properties can be investigated. The scattering of the α- and β-caseins increased with increasing concentrations of electrolytes whereas native ovalbumin, serum albumin and lactoglobulin showed independence to electrolyte concentration. The sensitivity of the caseins was akin to that of denatured ovalbumin. The extent of aggregation of the caseins was found to be greater for higher concentrations of the proteins. The two forms of casein differ in their amino acid composition[119] and other properties as shown by the time required for each material to reach a constant light-scattering intensity; on addition of potassium chloride the β-casein showed a much slower reaction. Halwer further showed that aggregation of the α-casein was reversible and that of the β-casein irreversible.

Fibrinogen

Most of the physical techniques have been applied to this important constituent of plasma (0·2 per cent w/v in blood representing 4 per cent of plasma protein content), and the most recent values for the physicochemical constants of human and bovine fibrinogen have been tabulated by Scheraga and Laskowski[120]. From their data it is apparent that the physical characteristics of human and bovine fibrinogen are indistinguishable. Discrepancies between older and more recent values are probably attributable to the states of purity. Scheraga and Laskowski suggest that at least 95 per cent clottability is necessary as a criterion of purity; such preparations are reported to be relatively homogeneous.

Earlier values for the molecular weight of bovine fibrinogen by the light-scattering method were in the region of 400,000 to 500,000, but the more recent value of 340,000, together with information from the angular scattering envelope, obtained by Katz[121,122] and others, suggests that the higher values may have been caused by some polydispersity. This value is in agreement with sedimentation-diffusion[123,124] results (330,000 to 340,000) whilst the earlier values agreed closely with those from sedimentation-viscosity[125] (440,000) and osmotic pressure[125,126] (441,000 to 580,000).

Scheraga and Laskowski regard a molecular dimension of 500 to 600 Å as the best value obtained from their collected data. By comparison, the light-scattering results of Katz and others gave a molecular dimension of 520 Å for a rod-shaped molecule, but assuming an ellipsoid the molecular dimension was 650 Å, which is high by the estimate of Scheraga and Laskowski. An axial ratio of 5 for a prolate equivalent hydrodynamic ellipsoid is reported by Scheraga and Laskoski, calculated without arbitrary assumptions about hydration or volume. In the calculations of Katz where a circular cylinder model was assumed, an axial ratio of 17 was in fair agreement with values obtained by other methods requiring the assumptions mentioned above. All these values were obtained on fibrinogen in dilute solutions. From the results of a dry sample in the electron microscope, Hall[127] calculated a rod-shaped length of 600 Å and diameter 30 to 40 Å; the length and axial ratio were in fair agreement

with the light-scattering results of Katz although there appeared to be greater polydispersity of the dry sample. Later work with the electron microscope by Hall[128] and other investigators has led to evidence of particles containing 3 or 4 small globules "strung together like beads" and having a total length approaching the dimension of the particles in solution.

In a recent investigation, Casassa[129] found that the light-scattering molecular weight of bovine fibrinogen increased by 7 per cent and the molecular length by 12 per cent in hexamethylene glycol at pH 6·2 as solvent. One explanation of the molecular weight increase was attributed to binding with glycol (in the thermodynamic sense), but the 12 per cent increase in molecular length made Casassa incline to the view that swelling of the fibrinogen molecule had taken place. The results showed, however, that hexamethylene glycol produced steric effects by reason of swelling or binding, which were likely to interfere with the clotting process. At pH 9 in salt-glycine buffer Casassa observed that aggregates of fibrinogen were formed in the presence of hexamethylene glycol or upon dilution.

Fibrinogen-Fibrin Reaction

Addition of small amounts of the enzyme thrombin to an appropriately buffered solution of fibrinogen catalyses the solution to produce a fibrin monomer. It is probable that thrombin is a proteolytic enzyme catalysing the hydrolysis of peptide bonds (see Scheraga and Laskowski[120]) The liberation of peptides probably unmasks functional groups (donors or acceptors in the hydrogen bonding process) capable of reacting to form intermediate rod-shaped polymers which undergo further lateral association and cross-linking to form a clot. The properties of the fibrin clot appear to depend on the conditions in which the clot is formed. In general, the reaction proceeds at high pH and ionic strength to form a low turbidity, relatively rigid "fine clot"; lowering the pH or ionic strength increases the turbidity and viscosity and a coarse clot is formed. Some substances are known to inhibit the clotting reaction without denaturing the two reactants.

Using hexamethylene glycol as inhibitor the flow birefringence measurements of Ehrlich, Shulman and Ferry[130] indicated that polymerisation of fibrinogen extends approximately from six to ten times the length of the fibrinogen monomer while the sedimentation constant[130] (combined with the birefringence data) corresponded to a cross-sectional area double and length ten times that of fibrinogen. Viscosity measurements were complicated by this component which had non-Newtonian flow. An independent light-scattering investigation of the intermediate polymer by Ferry, Shulman, Gutfreund and Katz[122] showed that in 0·5M hexamethylene glycol, at pH 6·2 and ionic strength 0·45, about half the protein is converted to a polymer with a weight average of fifteen times the molecular weight of the monomer, a length of 3,500 Å and width double that of fibrinogen. The polymer was found to dissociate sharply on dilution and to have stabilising forces similar to those in micelle formation.

Using urea and guanidine as solvents, Steiner and Laki[131] obtained

a light-scattering molecular weight of 540,000; the length of the extended rod-like model was 840 Å. They reported that, under conditions of high pH and ionic strength, end-to-end association predominated over side-to-side in the early stages. At pH values below 8 lateral association predominated, double and triple parallel fibrils being built up in the clotting process through association of dimers and trimers. The polymerisation process could be stopped completely by oxidation with potassium permanganate, as shown by the lack of further change in the molecular weight. Higher concentrations of potassium permanganate disrupted the aggregates. Evidence from the oxidative reaction led Steiner and Laki to conclude that end-to-end linkages were broken first, indicating a possible difference in bond type between the end-to-end and lateral associations. Light-scattering molecular weight determinations also showed that papain induced a similar clotting of fibrinogen to that shown by thrombin. Casassa[132] came to similar conclusions on the lateral association (dimerisation) and longitudinal polymerisation.

Casassa and Billick[133] studied the clotting of fibrinogen under more realistic conditions by using high concentrations of material. An estimate by ultracentrifugation of the extent of clotting showed that an 80 per cent conversion (in all probability reversible) of 0·4 per cent w/v fibrinogen solution under the given conditions took place for a concentration of 1 unit/ml. of thrombin. The angular distribution of the light-scattering intensity showed polymerisation to an intermediate polymer having a cylindrical rod-shape at least 5,000 Å in length and a mass to length ratio of 2·3 times the monomer units. The effect of variation in composition of the solute components on thermodynamic interactions was also studied. Investigators have contributed greatly to the elucidation of this reaction in recent years but the kinetics of the process is still only partially solved.

Nucleic Acids

One of the main problems in the study of the size and shape of nucleic acids has been the isolation of samples having a native structure. Degradation by depolymerisation takes place easily in acid or alkaline conditions, producing a polydisperse residue of decreased asymmetry. The extent of denaturation and consequent change in physical characteristics of such highly polymeric biological substances depends largely on the method of preparation.

Nucleic acid containing the deoxyribose sugar component (DNA) has been most extensively examined. X-ray diffraction experiments of Franklin and Gosling[134] have shown that it can exist in three main forms which are reversible, the changes in structure depending on humidity. At 75 per cent relative humidity the A form is a stable crystalline helical configuration. At higher humidities the B form is paracrystalline, showing a lower degree of order of the helixes. Each helix of this form is surrounded by a sheath of water molecules which leaves each unit free to adopt a configuration having minimum free energy, independent of its neighbouring units. At low humidities the structure is one of disorder.

Shooter and Butler[135] obtained a sedimentation coefficient by following the path of DNA in the ultracentrifuge using ultra-violet absorption but they reported that, at low concentrations, the sedimentation rate was so dependent on concentration that extrapolation to infinite dilution was not possible. Viscosity measurements and flow birefringence are dynamic methods and distortion of the very large molecules makes calculations based on models difficult. The results of Goldstein and Reichmann[136] on four samples of DNA studied by flow birefringence and light-scattering agreed for lower molecular weight samples (2 to 4 $\times$ 10^6) but higher molecular weight samples showed discrepancies in dimensions.

Very little work has been reported on diffusion studies at low concentrations, but calculations based on diffusion coefficients obtained by James[137] for calf thymus and Goodgall[138] and others for the transforming principle of *Haemophilus influenzae* gave molecular weights of 5 $\times$ 10^6 and 15 $\times$ 10^6, respectively (using a sedimentation coefficient of 20 $\times$ 10^{-13}). Fluke, Drew and Pollard[139] obtained a molecular weight of 6 $\times$ 10^6 for the DNA of pneumococcus transforming principle by electron and deuteron bombardment. Results from preparations from other sources agree more closely to the higher value by Goodgall.

The most recent estimates for the molecular weight of DNA have been by light-scattering. The molecular weight range for most specimens has been narrowed down to 5 to 8 $\times$ 10^6 (Doty[140] reported in 1955 that about 50 preparations of DNA had been examined in his laboratory). Brown, M'Ewen and Pratt[141] examined different specimens of DNA within a molecular weight range of 2·3 to 14·4 $\times$ 10^6 (see also Sadron[142]) They found that the molecular weight increased markedly with the guanine content, their highest values obtained after ageing of the DNA in the solvent for 3 to 8 days before final centrifugation. They explained the significance of the high molecular weight associated with guanine on the basis of the DNA molecule proposed by Watson and Crick[160], where, in the twin helical hydrogen-bonded structure, the 2-amino group of guanine appears to be available for binding two chains or for the binding of different parts of the same chain. Their values of parameters for calf thymus DNA are in excellent agreement with those of Reichmann, Rice, Thomas and Doty[143].

Calculations from the angular distribution of scattering intensity have been variously interpreted for DNA. Different authors have suggested that the particles are flexible and rigid rods[144], stiff coils[145,147] and branched coils[148], statistical coils[145,147] and intermediate shapes. The most recent investigations indicate that the molecules are flexible, probably closest to a coil (greatly extended double helix), their flexibility and size depending on the nature of the aqueous solvent. They are, however, known to be extremely large for their weight, that is, they are highly extended, with characteristics influencing the likelihood of a highly polydisperse system.

When the radius of gyration was plotted against molecular weight for different preparations of DNA[141,142] it was found that a uniform relation existed between the size and shape of the molecules (see Shooter[149]); the higher molecular weight preparations of Brown and

others[141] showed more compactness for their size, indicating a more highly coiled unit. Pouvet, Hermans and Vendrely[150] suggested that salt and alcohol play a predominant role in determining size and shape. This was evidenced by a 66 per cent decrease in the diameter of DNA in methanol to that in water[151]. From an analysis of work up to 1955 Doty concluded that the radius of gyration of the extended chain model was about 2,200 Å and the diameter of the volume of a DNA molecule estimated at 5,500 Å.

Reichmann, Rice, Thomas and Doty[143] examined DNA from calf thymus (molecular weight 6×10^6) and obtained a molecular shape resembling a stiff coil with root mean square end-to-end length of 5,000 Å and a contour length of 20,000 Å. These figures were corroborated by viscosity and sedimentation results. The form of angular scattering envelope depends mainly on the shape of the molecule but also to some extent on the molecular weight distribution of the sample. The observed envelope for DNA corresponded closely to a coil model. However, the envelope was also within the limits that could be reproduced by a polydisperse system alone. Assuming a very narrow molecular weight distribution, the envelope could correspond to a highly swollen symmetrical particle with a centre of high density, or to an ellipsoid or partially extended chain. Alternatively, if the envelope corresponds to a wide molecular weight distribution of randomly coiled chains, it gives the unusually high values of $M_z/M_w = 2$ and $M_w/M_n = 6$, from which it was calculated that about half of the randomly coiled chains were too stiff to be Gaussian in character. For more exact relations on shape it is therefore essential that the molecular weight distribution is known in some detail. Peterlin[152] has put forward a theory for scattering from non-Gaussian random coils where M_w/M_n is not greater than 2. This visualises a narrower range of polydispersity than existed in the experiments of Reichman, Rice, Thomas and Doty.

Recently Doty[153] has reported molecular weights of about 8×10^6 and a chain configuration described by a persistence length of 500 Å for DNA from thymus and salmon sperm. He has probably thus isolated samples of the DNA having a sufficiently narrow molecular weight distribution to apply Peterlin's theory relating the shape of the molecule to the stiffness of the chain by the persistence length parameter.

Using the light-scattering, electron microscope, and viscosity methods, Rowan, Eden and Kahler[145] found that the molecular configuration of sodium deoxyribonucleate was intermediate between a rod and a coil (molecular weight $4{\cdot}5 \times 10^6$), the molecules undergoing a marked change in size under the influence of ions. As the ionic strength of the solution increased from 0 to 0·02 the long thread-like molecules contracted from 6,800 to 4,500 Å. A similar decrease in size of this substance induced by dilute acid was found by Reichmann, Bunce and Doty[154]. From these and similar observations on later work Rowan[155] suggests that the contraction takes place when the molecule is surrounded by electrical charges of neighbouring ions. Relating his observations to the theory of Hermans and Overbeek[156] he obtained good agreement supporting the concept that

the DNA molecule is flexible enough to contract when surrounded by charged ions. Rowan[157] also suggests that similar contractions of DNA molecules *in vivo* may explain how they are able to be incorporated into small biological substances and impart to the complex changes in size and shape that chromosomes undergo during mitosis.

Alexander and Stacey[158] examined DNA from herring sperm heads under different conditions of pH and electrolyte. In 0·1M sodium chloride at pH 6·6, DNA had a molecular weight of 6×10^6 comparable with the results obtained by Reichmann and others[143] and a root mean square radius of gyration of 1,980 Å. Lowering the pH to 2·8 decreased the size of the coil with no change in molecular weight but on neutralisation the coil recovered to the original radius of gyration. Thomas and Doty[159] similarly found that the light-scattering characteristics of DNA in neutral 0·02M sodium chloride remained unchanged for two weeks, but at pH 2·6 a gradual degradation took place. Below pH 2·6 Alexander and Stacey found that an increase in molecular weight took place, indicating aggregation, but on neutralisation the original molecular weight was regained although the radius of gyration was not restored. Below pH 2·6 and in the absence of salt the molecular weight decreased to approximately half the original value, suggesting that the molecular weight of 6×10^6 represents a dimer. To investigate this possibility further, DNA was dissolved in 4M urea where a lowering of the molecular weight to $2{\cdot}7 \times 10^6$ took place (approximately half the previous value) accompanied by a slight increase (20 per cent) in the radius of gyration (2,100 Å) of the coil. Alexander and Stacey suggested that the conditions of experimentation indicated that native nucleic acid of molecular weight 6×10^6 was a hydrogen-bonded dimer consistent with the assumption that the urea split the hydrogen bonds of the twin-stranded spiral model of Watson and Crick[160].

Further parallel work by Doty and Rice[161] on a sample of DNA from thymus glands showed a contradiction in behaviour; the DNA was not split by urea. On heating samples of herring sperm and thymus gland DNA in aqueous solution another difference became apparent. The herring sperm DNA molecular weight of Alexander and Stacey decreased from 6 to $2{\cdot}5 \times 10^6$ whereas the thymus gland DNA molecule of Doty and Rice contracted without change in molecular weight. Alexander and Stacey[162] have since confirmed the work of Doty and Rice.

Geiduschek and Doty[163] studied the interaction between a low molecular weight DNA and bovine serum albumin and found no reaction in phosphate buffer at pH 7·5 and pH 6·5 but at pH 5·1 DNA bound with bovine serum albumin in a 1 : 1 ratio by weight. The complex gave a molecular weight of 3×10^6, indicating that 1 molecule of DNA bound 11 molecules of bovine serum albumin.

Alexander and Stacey[164] irradiated moist herring sperm DNA samples with gamma rays and 1·2 MeV electrons, and examined the irradiated samples by light-scattering. Equal doses of the different rays were found to produce the same amount of degradation; a minimum molecular

weight occurred at a dose of approximately $10^6 r$, indicating that the effect of radiation was one of degradation and aggregation.

Polysaccharides

The large size and asymmetrical shape of polysaccharides and the importance of the complexes which they form with proteins has stimulated interest in light-scattering studies on some of these compounds; glycogens, amyloses, dextrans, and cellulose and its derivatives have been investigated under various conditions.

Glycogens from several animal and other sources were studied by the light-scattering method by Harrap and Manners[165], who found that the values for the molecular weight ranged between 2·8 and 14·8 millions. These showed a marked deviation from values obtained by sedimentation and diffusion which were consistently lower. Small amounts of very high molecular weight material could affect light-scattering results, and for this reason the authors did not correct for dissymmetry.

Foster and others[166,167] were interested in the state of aggregation and degradation (in a sense akin to the denaturation of proteins) of amylose. An aqueous solution of amylose adjusted to pH 4 was found to have a constant turbidity for 30 days. This indicated that the aggregates were very stable and since disaggregation was a precursor to degradation, presumably the amylose remained in its native state. Foster considered that amylose possessed a helical structure stabilised largely by hydrogen-bonding, similar to that suggested for proteins (see Pauling, Corey and Branson[16]), the extent of any degradation being governed by the rate of disruption of the intramolecular hydrogen-bonds of the helical form. In a solution of normal potassium hydroxide, amylose showed a substantial reduction in turbidity with time and in solutions near neutral pH the decrease in turbidity was even more rapid. This suggested that dissociation of the aggregates preceded a transition from the "native" to the degraded amylose.

Fractional samples of native dextran show a wide range of molecular weights, the highly branched samples showing more compactness than the unbranched dextrans of the same molecular weight. Arond and Frank[169] obtained molecular weights of native dextran ranging from 12·6 to 600 millions, the corresponding radii of gyration varying from 570 to 2,930 Å. Other workers[170] found that clarified fractions of enzymatically synthesised dextrans had molecular weights three times as great as the centrifuged material. Price, Bellamy and Lawton[171] showed that the extent to which a sample of crude high molecular weight dextran underwent degradation by high energy cathode rays was reflected in a molecular weight decrease from 650 million to 29,000. Fishmann and Mastrangelo[172] suggest that the erratic results they obtained for measurements on 0·2 per cent solutions of low molecular weight clinical dextran were due to absorption by filters. In collaboration with other workers[173] they used dextran to investigate a method for evaluating the reliability of routine molecular weights determined by light-scattering. The reproducibility of light-scattering results was also investigated by Graham[174]

using a degraded dextran. He found that determinations on the same sample could be expected to fall within ± 10 per cent of the mean value 95 per cent of the time. The high degree of branching found in some branched starch polymers was investigated by Stacey, Foster and Erlander[175].

Cellulose, the most abundant of the polysaccharides, is known to have a fibrillar structure of approximately 200 Å in diameter and indefinite length. A few studies are reported for cellulose but most have been detailed investigations on the size, shape, interaction and hydrodynamic properties of the nitrate, acetate, xanthate and ester derivatives. A wide range of samples have been investigated, to which both the rod and random coil shapes have been assigned.

Badger and Blaker[176] investigated samples of cellulose trinitrate having a range of molecular weights from 9,400 to 518,000 and found that the shapes approached the stiff rod model for a degree of polymerisation less than 100 units; more highly polymerised molecules approached the random coil structure with an effective bond length of 50 Å or a ten-fold length of the monomer. This conclusion was supported by results from diffusion experiments, and the 100-unit particle was suggested as a rod-random coil transition region. Solvent interaction of cellulose trinitrate suggested a ribbon-shaped molecule with polar groups along the edges and with non-polar faces. The extent of the heterogeneity of the higher molecular weight fractions was shown by the ratio M_w/M_n in the fractions of cellulose trinitrate investigated by Holtzer, Benoit and Doty[177], who were able to confirm the unusual stiffness of the cellulose trinitrate chain. Their molecular weight results showed an average error for M_w by light-scattering of approximately 10 per cent and for M_n by osmotic pressure of ± 5 per cent.

The physical characteristics of potato[178] and corn[179] amylopectin and carrageenin[180] have also been elucidated by light-scattering.

In contrast to the high molecular weights previously discussed, the molecular weight of 338 ± 6 for sucrose was determined by Maron and Lou[181]. The versatility of the light-scattering method for molecular weight determination is clearly shown.

Penicillin

Investigations on the physico-chemical states of penicillin in aqueous systems is controversial. Hauser and colleagues[182,184] reported that, on the basis of surface tension and ultracentrifuge results, both penicillin and streptomycin salts in aqueous solution were present as colloidal micelles. They found that particle sizes varied from 200 to 5,000 Å at concentrations above 20,000 units/ml.

Conductivity and surface tension results of Kumpler and Alpen[185] showed that both potassium and sodium salts of benzylpenicillin are only monodispersed in water.

McBain, Huff and Brady[186] conducted several experiments from which they concluded that benzylpenicillin above 0·25M is a colloidal electrolyte but below this concentration is an ordinary electrolyte.

Using the light-scattering method Hocking[187] found that potassium benzylpenicillin in a concentration range 3 to 30,000 units/ml. showed less scattering intensity than would be expected if large micelles had been present at that concentration as had previously been reported. In the presence of electrolytes which increase micelle formation, the angular scattering exhibited perfect symmetry and Hocking deduced that the maximum dimension of the particles present did not exceed 300 Å. The system appeared to show small scattering, indicating a maximum molecular weight of 3,000. Although benzylpenicillin is surface active the bulk phase showed very little probability of large aggregates even in conditions favouring micelle formation.

Antigen-Antibody Reactions

Despite the large quantity of literature on immunology, studies of the reactions between antigens and antibodies by physico-chemical methods appear to be few. The light-scattering method has been employed to investigate the kinetics of some antigen-antibody reactions, but the mechanisms have proved difficult to follow on account of the high initial rate of reaction and solubility relations.

Singer and Campbell[188] studied the reaction between bovine plasma albumin and its rabbit antibody at neutral pH by ultracentrifugation and obtained strong evidence for bivalency of the antibodies; the antigen was known to be multivalent. Most antigen-antibody reactions occur in the framework of these combining powers according to the hypothesis formulated by Goldberg[189].

The reaction between bovine serum albumin and its purified homologous rat antibody has been investigated by light-scattering by Goldberg and Campbell[190]. The reduced intensity of scattering as a function of time showed that, for equivalent or excess amounts of antibody, complex formation and subsequent precipitation was rapid. Conversely, maintaining antigen in excess, in which the antigen-antibody complex was soluble, the reduced intensity: time relationship was unchanged.

Gitlin and Edelhoch[191] followed the turbidity changes (measured in terms of R_{90}) in the reaction between human serum albumin and its homologous equine antibody and found that equilibrium was reached more rapidly in the region of antigen excess than antibody excess, but the turbidity for a definite antigen-antibody ratio appeared to be the same in the equivalence region from either side of approach to the final state. Large soluble asymmetric complexes were found to exist for all ratios of antigen-antibody. In contrast with the results of Goldberg and Campbell the precipitation point was reached more rapidly when antigen was in excess.

In further work, Gitlin[192] suggested that formation of antibodies was a two-phase process; that its first phase is the formation of long species-specific peptide chains, and that these chains in a second phase are folded to form globular molecules. The nature of determinant groups of antigens, mode of entry into the reticulo-endothelial cells, and liberation of antibody, were also discussed.

The interaction between diphtheria toxin and antitoxin followed by Pope and Healey[193] showed a similar precipitation curve to the human serum albumin : homologous equine antibody system, with little or no precipitation of a complex when either component was markedly in excess. Using a low salt concentration Johnson and Ottewill[194] were able to show that diphtheria toxin-antitoxin combination was rapid, forming a maximum size of particle at the equivalence point. The overall reaction was found to be approximately second order and at a low ionic strength of solution small additions of salt accelerated the reaction rate and promoted larger particle formation. Flocculation occurred when the equivalence zone was reached. The presence of urea diminished the overall effect, and dispersed the toxin-antitoxin aggregates already formed. These workers clearly demonstrated the presence of stable antitoxin-rich aggregates and were able to suggest a mechanism for the Danysz phenomenon in which the resultant toxicity of the mixture is dependent on the method of mixing the toxin and antitoxin (Fig. 11).

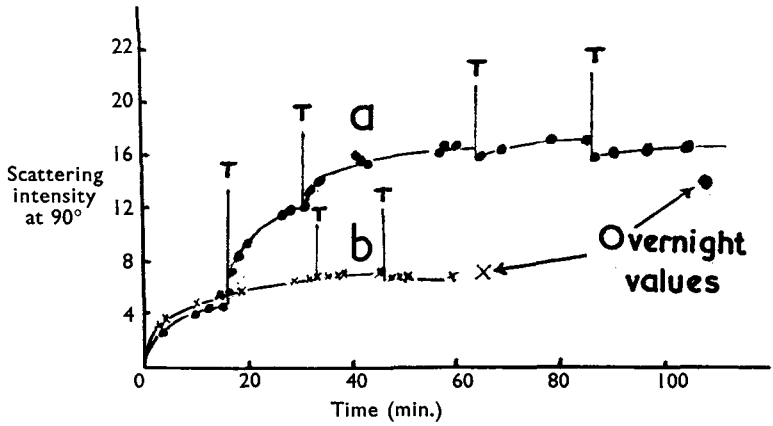

FIG. 11. Scatter from toxin-antitoxin as a function of time. Johnson and Ottewill's light-scattering counterpart of the Danysz phenomenon[194] (*Disc. Farad. Soc.*, 1954, **18**, 327, with permission).

T = Addition of toxin.

Stepwise addition of toxin to antitoxin up to equivalent amounts resulted in large particle size formation (higher values of I_{90} in curve *a*) than a single addition of toxin (curve *b*), indicating solution of large antitoxin-rich aggregates; further stepwise addition of toxin combined only slowly, leaving the solution active in toxin.

A multicomponent system was prepared by Steiner[198] to study the association of human serum albumin and rabbit antibodies. Results from the experimental work were therefore interpreted in terms of the special treatment derived by Stockmayer[195], also Kirkwood and Goldberg[196] and Edsall[197]. Steiner[198] used a lightly iodinated human serum albumin antigen which appeared homogeneous in the ultracentrifuge and had a

light-scattering molecular weight of 79,000. Reciprocal reduced turbidity : concentration curves for the antisera suggested a typical reversibly associating sytems. In general, between pH 6 and 9 the complexes were stable but outside this range a rapid fall in molecular weight, that is to say, rapid dissociation, was apparent. Dissociation was also effected by an increase in ionic strength, addition of urea, or the substitution of sodium iodide for sodium chloride.

While most of the work has been semi-qualitative, the quantitative aspect has also been studied. Goldberg[189] suggested that the reaction sites of an antigen and antibody combined to form a "bond" and on the assumption that this association was a reversible one governed by a single equilibrium constant, Goldberg was able to calculate the distribution of species for a system of uni- and bivalent antibodies and multivalent antigens. Providing the valencies were known, the distribution of the species could be calculated and the average ratios of antibody to antigen, for a critical and maximum extent of reaction between the products, thereby obtained. At these extents Goldberg considered that the aggregates were so large that their ratios approximated to the ratios for precipitation. This theory prompted several investigators to evaluate the equilibrium constant for the reversible reaction and other thermodynamic properties of antigen-antibody associations.

Epstein, Doty and Boyd[199] considered that the multivalency of an antigen, even if this is known, gives rise to diverse reaction products, making the extent of reaction difficult to determine. By replacing the multivalent antigen by a hapten, association with divalent antibody was followed by light-scattering; the molecular weight, equilibrium constant and other thermodynamic properties were subsequently evaluated.

Systems of General Interest

With increasing knowledge of the interpretation of results relating to the behaviour of substances in solution, more complicated systems further removed from ideality are being studied by the light-scattering technique. Some of these have already been reviewed, a few other examples of more general chemical interest will be mentioned.

Emulsions

The scope of investigations on the structure of emulsions by the light-scattering method is limited by particle size and turbidity. Workers in India have concentrated on the property of oil-in-water emulsions to polarise scattered light. Another important contribution has been made by Schulman and others, who used light-scattering to supplement and complete results obtained on emulsion studies by other physical methods.

From the results of X-ray and surface measurements, Schulman and others[200,202] postulated that in oil-water systems the disperse phase was present in spherical oil or water droplets stabilised by an interfacial mixed monolayer of the soap and alcohol molecules used. It was suggested that droplets were arranged in uniform close-packing; the diameters of the droplets and their distances apart were examined by

X-ray analysis. Light-scattering studies subsequently confirmed the model postulated and the dimensions of the particles. Since there is a high degree of orderliness in the liquid lattice of the emulsion, concentration fluctuations would be very small. Consequently, application of the Einstein-Smoluchowski equations would give a low value. Furthermore, the diameters of the particles were known to be too large in the normal conditions to apply the Rayleigh equation, which does not account for interference effects associated with large particles.

Schulman and Friend[202] attempted to achieve a degree of randomness which would give a particle dimension approaching the theoretical value, by increasing the ratio continuous phase: disperse phase of the system. Results calculated from the Rayleigh equation gave diameters of about 100 to 400 Å; these were in good agreement with X-ray and surface chemistry measurements for particles with an apparent molecular weight of under 30 million.

Van der Waarden[203] obtained values for particle diameters by the light-scattering method which he subsequently employed to study the viscosity and electroviscous effect of emulsions. From particle diameter, emulsifier content and viscosity data he calculated an apparent increase in particle diameter during flow which was ascribed to a surface charge originating from the emulsifier. Since the apparent increase in diameter of the particles of oil during flow was peculiarly independent of particle size, van der Waarden suggested that the oil droplets behaved as if they were enveloped by a rigid layer, the charged emulsifier molecules residing in the interfacial layer causing the electroviscous effect. The high electric field at the interface may have influenced adsorption of water molecules, resulting in the apparent increase in radii of the particles.

Sol-Gel Transformation

The process of gelation of agar and gelatin sols has been followed by light-scattering. Katti[204] reported a similarity between agar and gelatin in terms of hydration, increasing scattering and increasing particle size. His results indicated that the particles grew more symmetrical with increasing size up to concentrations of 0·5 per cent but they became more asymmetrical as the concentration was further increased.

An interesting analysis of the gelatin transformation process was reported by Boedtker and Doty[205], who were able to show that, in high salt concentrations at 25° gelatin had a molecular weight of 96,000 $\pm$ 3 per cent, the undissociated molecules present having the random coil structure. On dilution of the gel a critical concentration value was evident when the state became one of aggregation rather than gelation. From the values for molecular weight (M) and radius of gyration (ρ) Boedtker and Doty reasoned that, if the ratio $\frac{M}{\rho}$ was constant, aggregation would have been end-to-end whilst a constant ratio $\frac{M}{\rho^3}$ would have indicated spheres of constant density; in fact, the ratio $\frac{M}{\rho^2}$ was nearly constant,

indicating that an intermediate form ("brush heap model") of aggregation resembling cross-linking was most likely. This was supported by nearly constant values for the ratios $\frac{[\eta]}{M^{\frac{1}{2}}}$ and $BM^{\frac{1}{2}}$ and led to the probability that the distribution of mass within the aggregates was Gaussian. Aggregation of gelatin molecules in water at the isoelectric point was complicated by electrostatic attractive forces. In gelatin gels at 18° the intensity of scatter was greater than that of corresponding solutions at 40°, indicating a greater randomness in the gels. Figure 12 shows that the scattering

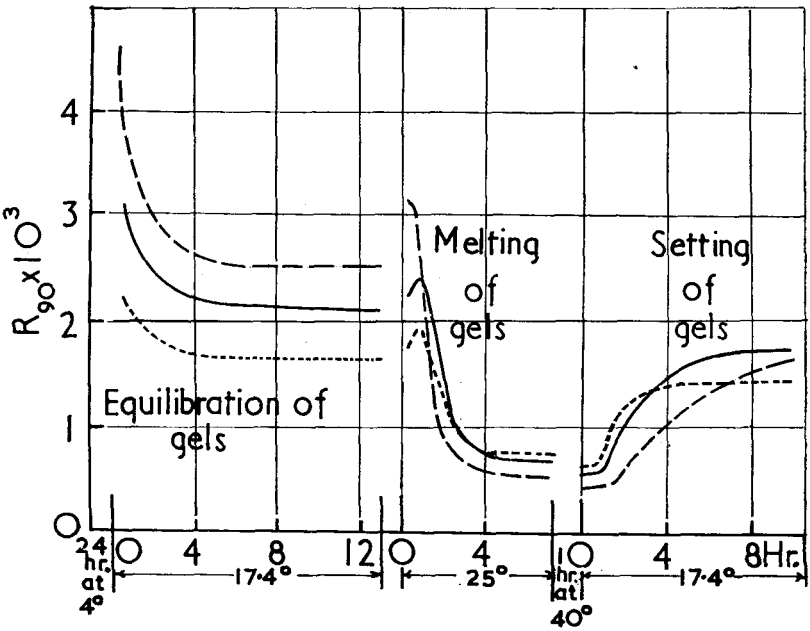

FIG. 12. Variation of scattering intensity with time for gelatin gels in 0·15M sodium chloride at pH 6·5 (after Boedtker and Doty[205], *J. phys. Chem.*, 1954, **58**, 968, with permission).

——— 0·0090 g./ml.
——— 0·0201 g./ml.
. . . . . 0·0284 g./ml.

of light from the gels decreased with increasing concentration in contrast to the behaviour of the sols. It also shows that gel formation is a slower process than melting, the most concentrated solutions showing the least change in scattering during the transformation. Boedtker and Doty suggest that gelation of gelatin is a process of cross-linking of aggregates which are held together by the formation of crystallites (evidenced by the melting and gelling behaviour and the crystalline structure observed in X-ray diagrams).

Workers in India[206,207] have made an important contribution to the study of sol-gel transformation by investigating hydrophilic (and also a considerable number of hydrophobic) sols by the light-scattering method.

They found that hydrophobic sols showed a general trend; in the sol state, scattering intensities due to particle size and anisotropy were independent of concentration and temperature but transformation to the gel state increased the size of the particles and also their asymmetry, but the anisotropy varied according to the system. Prasad and others[208] have found that shaking during the thixotropic gelation of aluminium molybdate and thorium molybdate did not affect the size and shape of the particles which were isotropic.

Soaps

Light-scattering studies on soaps and detergents have proved valuable in elucidating the structure of micelles, the critical micelle concentration and the effect of different salts on these properties.

Micelles of *n*-hexadecyltrimethylammonium bromide in 0·178M and 0·233M potassium bromide which might perhaps be thought to be spherical were shown by Debye and Anacker[209] to have angular dissymmetry and a rod-like structure. Another investigation by Debye[210] showed which ion of an added electrolyte was the determining factor in its effect on the molecular weight of the micelle of dodecylamine hydrochloride.

Phillips and Mysels[211] found that the effective charge residing on sodium lauryl sulphate molecules in pure water ($M_w = 23{,}000$) and in increasing concentrations of electrolyte ($M_w = 35{,}000$) remained constant at 14 units. The values for the critical micelle concentration were found to agree with conductivity and dye stabilisation methods.

Ludlom[212] was able to show by the light-scattering method that an increase in the hydrophobic portion of a typical detergent (sodium dodecylbenzene sulphonates) increased the size of micelles but lowered the critical micelle concentration.

High Polymers

Some of the more recent advances in the theory and practice of light-scattering have developed mainly through their application to problems in high polymer chemistry.

The volume of researches is extensive and has been of value in solving problems on polymer-solvent interaction. The dependence of the diameter of coiled high polymer molecules on the solvent and temperature has been amply confirmed by light-scattering. The structural changes and degradations arising from chemical reaction, irradiation by electrons and various polymerisation conditions have been reported (see Peterlin[213]).

The weight-average molecular weight obtained by light-scattering is complementary to the number-average molecular weight from osmotic pressure. Stacey[24] has given a good account of the thermodynamics of polymers from light-scattering data and points out that molecular weights from 2,000 to 600 million have been determined by this method. Recently Benoit[214] investigated selected samples of polyoxyethylene glycols within the molecular weight range of 300 to 8000 and obtained good agreement with the values determined by titration of terminal hydroxyl groups. The light-scattering method certainly extends the range of many other methods.

Acknowledgements. The writer would like to thank the authors and publishers who kindly gave permission to reproduce the figures and also Dr. L. Saunders for his interest.

REFERENCES

75. Mommaerts, *J. biol. Chem.*, 1948, **188**, 553.
76. Portzehl, *Z. Naturforsch.*, 1949, **5b**, 61, 75.
77. Haltzer and Lowery, *J. Amer. chem. Soc.*, 1956, **78**, 5954.
78. Rupp and Mommaerts, *J. biol. Chem.*, 1957, **224**, 277.
79. Blum, *Fed. Proc.*, 1952, **11**, 14.
80. Morales and Botts, *Disc. Faraday Soc.*, 1953, **13**, 125.
81. Johnson and Landolt, *ibid.*, 1951, **11**, 179.
82. Snellman, Erdos and Tenow, *Proc. 6th Int. Cong. Expt. Cytol.*, 1947, Suppl. 1, 147.
83. Tsao, *Biochim. Biophys. Acta*, 1952, **11**, 227.
84. Johnson and Landolt, *Disc. Faraday Soc.*, 1951, **11**, 227.
85. Steiner, Laki and Spicer, *J. Polymer Sci.*, 1952, **8**, 23.
86. Engehardt, Lyubimova and Meitina, *C.R. Acad. Sci., U.S.S.R.*, 1941, **30**, 644.
87. Szent-Gyorgyi, *Chem. of Muscular Contraction*, N.Y. Acad. Press, N.Y., 1947.
88. Blum and Morales, *Arch. Biochem. Biophys.*, 1953, **43**, 208.
89. Dainty, Kleinzeller, Lawrence, Miall, Needham and Shen, *J. gen. Physiol.*, 1944, **37**, 355; *Nature, Lond.*, 1942, **150**, 46.
90. Mommaerts, *Expt. Cell Research*, 1951, **2**, 133.
91. Weber, *Proc. Roy. Soc.*, 1950, **B137**, 50.
92. Jordan and Oster, *Science*, 1948, **108**, 188.
93. Tonomura, Watanabe and Yagi, *J. Biochem. Japan*, 1953, **40**, 27; *Nature, Lond.*, 1952, **169**, 112.
94. Watanabe, Tonomura and Shiokawa, *J. Biochem. Japan*, 1953, **40**, 387.
95. Veis and Eggenberger, *J. Amer. chem. Soc.*, 1954, **76**, 1560.
96. Oakley, *Trans. Faraday Soc.*, 1935, **31**, 136.
97. Flory and Fox, *J. Polymer Sci.*, 1950, **5**, 745.
98. Oster, *J. gen. Physiol.*, 1947, **31**, 89.
99. Oster, Doty and Zimm, *J. Amer. chem. Soc.*, 1947, **69**, 1193.
100. Bawden and Pirie, *Proc. Roy. Soc.*, 1937, **B123**, 274.
101. Oster, *J. gen. Physiol.*, 1950, **33**, 445.
102. Onsager *Ann. N.Y. Acad. Sci.*, 1949, **51**, 627.
103. Oster, *Science*, 1946, **103**, 306.
104. Oster, *Chem. Rev.*, 1948, **43**, 339.
105. Neurath and Cooper, *J. biol. Chem.*, 1940, **135**, 455.
106. Stanley and Lauffer, *ibid.*, 1940, **135**, 463.
107. Goring and Johnson, *Trans. Faraday Soc.*, 1952, **48**, 367.
108. Van Slyke and Bosworth, *J. biol. Chem.*, 1913, **14**, 203, 228.
109. Pauli and Matula, *Biochem. Z.*, 1919, **99**, 219.
110. Yamakami, *Biochem. J.*, 1920, **14**, 522.
111. Cohn, Hendry and Prentiss, *J. biol. Chem.*, 1925, **63**, 721.
112. Svedberg, Carpenter and Carpenter, *J. Amer. chem. Soc.*, 1930, **52**, 241, 701.
113. Burk and Greenberg, *J. biol. Chem.*, 1930, **87**, 197.
114. Halwer, *Arch. Biochem. Biophys.*, 1954, **51**, 79.
115. D'Yachenko and Vlodavets, *Colloid J. U.S.S.R.*, 1952, **14**, 367.
116. D'Yachenko and Vlodavets, *ibid.*, 1954, **16**, 105.
117. Fuoss, *Disc. Faraday Soc.*, 1951, **11**, 125.
118. Doty and Steiner, *J. chem. Phys.*, 1952, **20**, 85.
119. Gordon, Semmett, Cable and Morris, *J. Amer. chem. Soc.*, 1949, **71**, 3293.
120. Scheraga and Laskowski, *Advances Protein Chem.*, 1957, **12**, 1.
121. Katz, Gutfreund, Schulman and Ferry, *J. Amer. chem. Soc.*, 1952, **74**, 775.
122. Ferry, Schulman, Gutfreund and Katz, *ibid.*, 1952, **74**, 5709.
123. Schulman, Ferry and Tinoco, *Arch. Biochem. Biophys.*, 1953, **42**, 245.
124. Scheraga, Carroll, Nims, Sutton, Backus and Saunders, *J. Polymer Sci.*, 1954, **14**, 427.
125. Oncley, Scatchard and Brown, *J. phys. Chem.*, 1947, **51**, 184.
126. Nanninga, *Arch. neerl physiol.*, 1946, **28**, 241.
127. Hall, *J. Amer. chem. Soc.*, 1949, **71**, 1138.
128. Hall, *J. biol. Chem.*, 1949, **179**, 857.
129. Casassa, *J. phys. Chem.*, 1956, **60**, 926.

130. Ehrlich, Schulman and Ferry, *J. Amer. chem. Soc.*, 1952, **74**, 2258.
131. Steiner and Laki, *Arch. Biochem. Biophys.*, 1951, **34**, 24.
132. Casassa, *J. chem. Phys.*, 1955, **23**, 596.
133. Casassa and Billick, *J. Amer. chem. Soc.*, 1957, **79**, 1376.
134. Franklin and Gosling, *Acta Cryst.*, 1953, **6**, 673; *Nature, Lond.*, 1953, **171**, 740.
135. Shooter and Butler, *Nature, Lond.*, 1955, **175**, 500.
136. Goldstein and Reichmann, *J. Amer. chem. Soc.*, 1954, **76**, 3337.
137. James, Ph.D. Thesis, 1954, through reference 141.
138. Goodgall, Rupert and Herriot, *Trans. Faraday Soc.*, 1957, **53**, 257, through reference 141.
139. Fluke, Drew and Pollard, *Proc. Nat. Acad. Sci., Wash.*, 1952, **38**, 180.
140. Doty, *Proc. 3rd Int. Cong. Biochem.*, Brussels, 1955, p. 135.
141. Brown, M'Ewen and Pratt, *Nature, Lond.*, 1955, **176**, 161.
142. Sadron, *Proc. 3rd Int. Cong. Biochem.*, Brussels, 1955, p. 210.
143. Reichmann, Rice, Thomas and Doty, *J. Amer. chem. Soc.*, 1954, **76**, 3047.
144. Oster, *Trans. Faraday Soc.*, 1950, **46**, 794.
145. Rowan, Eden and Kahler, *Biochim. Biophys. Acta*, 1953, **10**, 89.
146. Peterlin, *Macromol. Chem.*, 1953, **9**, 244.
147. Smith and Sheffer, *Amer. J. Res.*, 1950, **28**, 96.
148. Doty and Bunce, *J. Amer. chem. Soc.*, 1952, **74**, 5029.
149. Shooter, *Prog. in Biophys.*, 1957, 8.
150. Pouyet, Hermans and Vendrely, *Trans. Faraday Soc.*, 1957, **53**, 247.
151. Guiduschek and Gray, *J. Amer. chem. Soc.*, 1956, **78**, 879.
152. Peterlin, *Nature, Lond.*, 1953, **171**, 259; *J. Polym. Sci.*, 1953, **10**, 425.
153. Doty, *Trans. Faraday Soc.*, 1957, **53**, 251.
154. Reichmann, Bunce and Doty, *J. Polymer Sci.*, 1953, **10**, 109.
155. Rowan, *Biochem. Biophys. Acta*, 1953, **10**, 391.
156. Hermans and Overbeek, *Recueil*, 1948, **67**, 761.
157. Rowan and Norman, *Arch. Biochem. Biophys.*, 1954, **51**, 524.
158. Alexander and Stacey, *Biochem. J.*, 1955, **60**, 194.
159. Thomas and Doty, *J. Amer. chem. Soc.*, 1956, **78**, 1854.
160. Watson and Crick, *Nature, Lond.*, 1953, **171**, 737.
161. Doty and Rice, *Biochim. Biophys. Acta*, 1955, **16**, 446.
162. Alexander and Stacey, *Nature, Lond.*, 1955, **176**, 162.
163. Geiduschek and Doty, *Biochim. Biophys. Acta*, 1952, **9**, 609.
164. Alexander and Stacey, *Prog. in Radiobiology*, 1956, 105.
165. Harrap and Manners, *Nature, Lond.*, 1952, **170**, 419.
166. Foster and Sterman, *J. Polymer Sci.*, 1956, **21**, 91.
167. Paschall and Foster, *ibid.*, 1952, **9**, 73, 85.
168. Pauling, Corey and Branson, *Proc. Nat. Acad. Sci.*, 1951, **37**, 205.
169. Arond and Frank, *J. phys. Chem.*, 1954, **58**, 953.
170. Tsuchiya, *J. Amer. chem. Soc.*, 1955, **77**, 2412.
171. Price, Bellamy and Lawton, *J. phys. Chem.*, 1954, **58**, 821.
172. Fishman and Mastrangelo, Abst. 124th Meeting Chicago A.C.S., Sept., 1953, 2D.
173. Mastrangelo, Clay, Fishman, Hagan, Lazrus and Zagar, *Analyt. Chem.*, 1955, **27**, 262.
174. Graham, *Canad. J. Technol.*, 1956, **34**, 83.
175. Stacey, Foster and Erlander, *Makromol. Chem.*, 1956, **17**, 181.
176. Badger and Blaker, *J. phys. Chem.*, 1949, **53**, 1056; *J. Amer. chem. Soc.*, 1950, **72**, 3129.
177. Holtzer, Benoit and Doty, *J. phys. Chem.*, 1954, **58**, 624.
178. Witnauer, Senti and Stern, *J. chem. Phys.*, 1952, **20**, 1978.
179. Stacey and Foster, *J. Polymer Sci.*, 1956, **20**, 57.
180. Goring, *Canad. J. Chem.*, 1953, **31**, 1078.
181. Maron and Lou, *J. phys. Chem.*, 1955, **59**, 231.
182. Hauser, *Kolloid Z.*, 1948, **111**, 103.
183. Hauser and Marlowe, *J. phys. Chem.*, 1950, **54**, 1077.
184. Hauser, Phillips and Phillips, *Science*, 1947, **106**, 616.
185. Kumpler and Alpen, *ibid.*, 1948, **107**, 567.
186. McBain, Huff and Brady, *J. Amer. chem. Soc.*, 1949, **71**, 373.
187. Hocking, *Nature, Lond.*, 1951, **168**, 423.
188. Singer and Campbell, *J. Amer. chem. Soc.*, 1952, **74**, 1794; 1953, **75**, 5577.
189. Goldberg, *ibid.*, 1952, **74**, 5715, 5716.
190. Goldberg and Campbell, *J. Immunol.*, 1951, **66**, 79.
191. Gitlin and Edelhoch, *ibid.*, 1951, **66**, 67.

192. Gitlin, *Rutgers Univ. Bur. Res. Ann. Conf. Prot. Metab.*, 1954, 23, through *Chem. Abstr.*, 49, 4854.
193. Pope and Healey, *Brit. J. exp. Path.*, 1938, **19**, 397.
194. Johnson and Ottewill, *Disc. Faraday Soc.*, 1954, **18**, 327.
195. Stockmayer, *J. chem. Phys.*, 1950, **18**, 58.
196. Kirkwood and Goldberg, *ibid.*, 1950, **18**, 54.
197. Edsall, *J. Amer. chem. Soc.*, 1950, 4641.
198. Steiner, *Arch. Biochem. Biophys.*, 1955, **55**, 235.
199. Epstein, Doty and Boyd, *J. Amer. chem. Soc.*, 1956, **78**, 3306.
200. Schulman and McRoberts, *Trans. Faraday Soc.*, 1946, **42B**, 165.
201. Schulman and Riley, *J. Colloid Sci.*, 1948, **3**, 383.
202. Schulman and Friend, *ibid.*, 1949, **4**, 497.
203. Van der Waarden, *ibid.*, 1954, **9**, 215.
204. Katti, *Proc. Ind. Acad. Sci.*, 1948, **28A**, 216; 1949, **30A**, 35.
205. Boedtker and Doty, *J. phys. Chem.*, 1954, **58**, 968.
206. Desai and Sundaram, *J. Univ. Bombay*, 1953, **22**, 3, 8, 24; 1954, **23**, 3, 10.
207. Prasad and Guruswamy, *Proc. Ind. Acad. Sci.*, 1944, **19A**, 47, 66, 77.
208. Prasad, Sumbramanian, Desai and Kanekar, *J. Colloid Sci.*, 1952, **7**, 178.
209. Debye and Anacker, *J. Phys. Colloid Chem.*, 1951, **55**, 644.
210. Debye, *J. Colloid Sci.*, 1948, **3**, 407.
211. Phillips and Mysels, *J. phys. Chem.*, 1955, **59**, 325.
212. Ludlom, *J. phys. Chem.*, 1956, **60**, 1240.
213. Peterlin, *Prog. in Biophys.*, 1959, **9**, 175.
214. Benoit, *J. Polymer Sci.*, 1957, **24**, 155.