scure points in our knowledge of sex differentiation, especially as this involves the early phases controlled by the so-called asexual sporophyte. The slit panel technique, combined with various types of experimental defoliation and exfloration, reveals striking species differences in photoperiodic response.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETERMINATION OF SEDIMENTATION RATE AND EQUILIBRIUM IN CENTRIFUGES AND OPAQUE ULTRACENTRIFUGES

THE great importance of the ultracentrifuge as developed by Svedberg and his collaborators at Uppsala is universally recognized, whether for applications in colloid science, in biology, in medicine or in industry. Such equipment has, in spite of its extreme costliness, been installed in a number of laboratories outside Sweden; and similar transparent rotors, run *in vacuo*, although still distinctly expensive, are being used at several other centers. A less expensive but smaller transparent ultracentrifuge has been developed in the author's laboratory.[1]

It may not be generally realized[2] that there now exists for every laboratory a choice of methods using either commercial centrifuges or still less expensive air-driven ultracentrifuges. With these, quantitative measurements may be made of particle size or molecular weight for every kind of solution or of suspension. They possess the great advantage that the substances or materials measured are withdrawn for direct chemical or physical analysis, or for estimation by biological inoculation, etc.

The descriptions of these various methods are scattered through journals in quite different fields of science, and it is worth while to list them here. There are three differing groups of procedures. The first permits or encourages convection of the whole or of a large part of the liquid. In the second, the sedimenting liquid is immobilized within a jelly or gel, and in the third the sedimentation takes place within narrow spaces mechanically shielded from convection. These methods have yielded quantitative measurements of the rate of sedimentation, sedimentation equilibrium and actual density of suspended or dissolved particles.[3] The results are in good agreement with each other and with measurements made with the transparent ultracentrifuge including that of Elford using scattered light, and also with the less accurate but definite results given by the method of ultrafiltration.

The first method, the Bechhold-Schlesinger convective procedure, was originated in 1931,[4] and during the following years various qualitative and semi-quantitative observations of its occurrence were made in the author's laboratory at Stanford, in that of Beams at Virginia, and also by Gratia in Belgium, using the simplest form of one piece hollow rotor of Henriot and Huguenard.[5] This simple equipment is unsurpassed for centrifugal force and costs only a few dollars. Although admitting of quantitative results, in this form it is not an ultracentrifuge, for it is an essential in the latter that convection be eliminated in the liquid actually studied. A simple modification has yielded quantitative results, in the Middlesex Hospital, for sedimentation velocity[6] of bacteria, viruses, phages and oxy-hemoglobin, and for their specific gravity. For example, McIntosh and Selbie obtained a diameter of 56 Å for oxy-hemoglobin, identical with that quoted from Svedberg.

The method of immobilization by a jelly was introduced by McBain and Stuewer[7] and was first applied to the measurement of rate of sedimentation of the jelly structure itself. With 0.3 per cent. agar jelly, it gave the same sedimentation rate (65×10^{-13}) as was given (63×10^{-13}) by the transparent ultracentrifuge of McBain and O'Sullivan. Swelling pressures of the jelly were also measured. Soap curd has been used in the measurement of sedimentation equilibrium of sucrose.[8] We found that the theoretical sedimentation equilibrium is attained. On the other hand, the rate of sedimentation of hemoglobin is retarded. Dilute agar jelly has been used in the National Institute for Medical Research, London,[9] to convert the Sharples Super-Centrifuge into a convectionless ultracentrifuge. Five cc of virus solution gelatinized with dilute agar lines the closed bowl to a depth of 0.18 mm. Another 5 cc is then added and

[1] J. W. McBain and C. O'Sullivan, *Jour. Am. Chem. Soc.*, 57: 780, 1935; *ibid.*, 2631–41; and J. W. McBain, *ibid.*, 58: 2652, 1936.

[2] *Cf.* reference 11.

[3] Such densities are measured by altering that of the medium and observing the effect upon sedimentation; J. W. McBain, *Jour. Am. Chem. Soc.*, 58: 315–17, 1936; many examples in later references, for example, McIntosh and Selbie, 1937.

[4] H. Bechhold and M. Schlesinger, *Biochem. Zeit.*, 236: 392, 1931; *Zeit. Hygiene*, 112: 668, 1931; *ibid.*, 115: 342 and 354, 1933; *Phytopath. Zeit.*, 6: 627, 1933; M. Schlesinger, *Zeit. Hygiene*, 114: 161, 1932; *Biochem. Zeit.*, 264: 6–12, 1933; *Kolloid-Zeit.*, 67: 135, 1934; *Biodynamica*, 1935, 1.

[5] *C. R. Acad. Sci.*, 180: 1389, 1925; *Jour. Phys. Radium*, 8: 433, 1927.

[6] J. McIntosh, *Jour. Path. and Bact.*, 41: 215, 1935; J. McIntosh and F. R. Selbie, *Brit. Jour. Exp. Path.*, 18: 162–174, 1937.

[7] J. W. McBain and R. F. Stuewer, *Kolloid-Zeit.*, 74: 10–16, 1936.

[8] J. W. McBain and C. Alvarez-Tostado, *Nature*, 139: 1066, June, 1937, and *Jour. Am. Chem. Soc.*, 59: 2489, 1937.

[9] M. Schlesinger, *Nature*, 138: 549, 1936; M. Schlesinger and I. A. Galloway, *Jour. Hygiene*, 37: 445 and 463, 1937.

the film is so thin that convection does not occur, thus allowing both rate and equilibrium to be measured. Virus of foot-and-mouth disease is measured after three minutes. An antibody required only 30 minutes for sedimentation equilibrium. It is necessarily assumed that the agar jelly is of such concentration that it neither swells nor sediments. This, however, can be verified by direct experiment and adjusting the concentration of agar to the requisite value. Any influence of the agar on the absolute rate has to be tested by comparison in some other ultracentrifuge. Sedimentation equilibrium is of course unaffected.

The third method is to prevent convection by simple mechanical design. It is most general because the solution is uncontaminated in any way, and it is equally good for aqueous and non-aqueous systems. It also ranges equally well from the smallest molecules to the largest particles. Elford[10] described such a method for comparatively large particles such as phage or virus, avoiding convection by using an inverted glass, silica or metal tube, 1, 2 or 3 mm in internal diameter, immersed in a commercial or Henriot and Huguenard centrifuge. The particles settle within the tube with an undisturbed boundary, and before this reaches the outer opening of the tube the contents are analyzed. The position of the boundary may sometimes be followed by eye, using scattered or fluorescent light, and the results are fairly accurate. Many parallel holes in one block may be used to give larger volumes.

The author with Alvarez-Tostado has developed completely general methods for the quantitative study of sedimentation equilibrium and of sedimentation rate in mono-disperse or poly-disperse systems of any sort. The sedimentation equilibrium of sucrose[8] gave a molecular weight of 341 in exact agreement with the theoretical value, 342. The essential feature of these extremely simple air-driven ultracentrifuges is the use of a pile or piles of very thin plane horizontal annular rings or washers, with or without spacing pieces, to immobilize the whole or a part of the solution. For sedimentation equilibrium of any mono-disperse system, one pile is sufficient. For rate or for poly-disperse systems a number of concentric piles of these loose washers, each set held together by vertical pins fastened only to the top and bottom of the pile, are placed in the rotor so that after stopping the rotor each set can be lifted out and the contents analyzed to determine rate, equilibrium, true density and number of particle sizes. Another method which we had previously developed was to use piles of sectorial baffles built up horizontally like brick work, in the simplest one-piece metal rotor, obtaining successive portions of the liquid from between them by displacement with a heavier liquid put through a distributing plate while the rotor is running.

Lastly, Tiselius, Pedersen and Svedberg[11] have now put a partition of filter paper in the middle of their transparent ultracentrifuge cell so that an analysis is possible for determining the position of a single mono-disperse boundary.

A complete generalization of the mechanical method for rate of sedimentation is suggested by the author. It consists merely of a pile of horizontal circular solid disks, alternately wide and narrow, placed in the axis of a simple two-piece air-driven rotor. The small disks serve as spacing pieces for the larger ones between which the liquid is wholly immobilized, permitting ideal radial undisturbed sedimentation.

Particles or molecules of different sizes in the same fluid are detected and measured by varying the rate and extent of sedimentation in successive experiments.

In conclusion, it may be noted that even the Bechhold method may be used for distinguishing between mono-disperse and multi-disperse systems. It is easy to ascertain whether or not two substances are combined or associated with each other or sediment separately (as was done, for example, by Gratia in 1934).

It is evident that the problem of obtaining exact quantitative data on one or all of the quantities mentioned has been completely solved by simple means within the reach of every scientific laboratory.

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[10] W. J. Elford, *Brit. Jour. Exp. Path.*, 17: 399, 1936; Elford and C. H. Andrewes, *ibid.*, 422; Elford and I. A. Galloway, *ibid.*, 18: 155, 1937; F. F. Tang, Elford and Galloway, *ibid.*, 269.

[11] A. Tiselius, K. O. Pedersen and T. Svedberg, *Nature*, 140: 848–49, 1937.

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