

but the residue was dissolved at room temperature in 500 mls of distilled water by adjusting the reaction to pH 8.0 with a 0.1 per cent. aqueous suspension of calcium oxide. The celite was removed and recrystallization brought about as in procedure A. Procedure B was followed for the third crystallization.

Procedure C. Centrifugation at 1,500 times gravity for 10 minutes with treatment of sediment (supernatant liquid decanted) as in procedure A. Procedure C was followed for the third crystallization.

Assay of virus infectivity by the local-lesion method on primary leaves of Scotia beans was made by Mr. H. H. McKinney. Primary leaves of 20 bean plants at a susceptible age were inoculated with a 10^{-4} dilution (in 0.1 molar phosphate buffer at pH 7.0) of each sample previously adjusted to 1 mgm of protein per 1 ml of solution (nitrogen analysis by microkjeldahl of the material precipitated by 5 per cent. trichloroacetic acid). The average number of lesions per leaf was 4.2; 8.1; 4.4; 4.9; and 4.3 for the cheesecloth filtrate; celite filtrate of the crude juice; and the dissolved material from the third crystallization by procedures A, B and C, respectively. Infectivity of the material of the first or second crystallization was not determined.

These infectivity data based upon the protein content of each sample show no marked change in activity upon crystallizing the protein three times by either of the three procedures. The increase in infectivity of the celite filtrate, if significant, may have been due to particles of the celite in the liquid aiding infection or to filtration increasing the infectivity possibly by increasing the dispersion of the virus or removing some inhibitor. The increase is in agreement with that reported⁴ from filtering crude juice in 0.1 molar phosphate buffer at pH 8.5 through Berkefeld "W" candles.

Tobacco juice from healthy plants when treated in a similar manner failed to develop any evidence of crystalline protein. The precipitate that formed was composed of amorphous material as far as could be determined microscopically.

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PHOTOPERIODIC STIMULUS TRANSFER IN PLANTS

IN experiments to determine the perceptive locus and mode of transfer of photoperiodic stimuli in plants, investigators have commonly used grafts of flowering scions upon vegetative stocks or *vice versa*.^{1,2,3} Such entire grafted plants under a vege-

tative photoperiod have shown transfer of the residual flowering stimulus across the graft union, presumably in the form of a hormone, tentatively designated florigen.

These techniques are open to the objections that they introduce the extraneous stimulus of severe traumatism, result in considerable mortality, necessitate prolonged post-operative care, and interfere with nutrition. They are tedious, entail delay with its attendant hazards and inconvenience, and to a great degree they impose such severe numerical limitations as to preclude statistical evaluation of results. The photoperiodic responses also vary with the type of graft employed.

In order to minimize these difficulties, while at the same time differentially illuminating contiguous parts of the same plant, the writer has employed a thin, opaque panel with an adjustable, horizontal slit through which the tops of potted plants are trained from both sides as they grow under a vegetative photoperiod. At the desired time, short-day lighting is initiated on one side and long day on the other, thereby keeping the bases of one set vegetative and inducing flowering of the tops of the same plants, while the reciprocal responses are simultaneously induced in the other set on the opposite side of the panel. During the spring and summer, the daylight period can be shortened by use of a curtain suspended from a wire frame on the panel. Simpler, however, is the performance of the experiment under natural conditions of short day with extension of illumination for several hours by use of automatically timed, adjustable electric lights on the long-day side of the panel. Undesired reflection on the short-day side can readily be prevented by sufficient height and length of the panel. The slit panel technique is especially convenient when an interchange of lighting on opposite sides is desired, as it can be reversed without moving the plants.

The panel procedure yields more consistent results and hence makes interpretations simpler which, in combination with larger experimental populations, adds an important element of reliability. In an investigation on dioecious plants the writer has found the functional and structural responses of staminate parts to be distinctly different from those of pistillate parts to a given photoperiod, when top and base of the same plant are subjected to contrasted length of day. In other words, the reproductive photoperiod both in long- and short-day species exerts a different effect upon the "male" and "female" processes, a response which may permit the elucidation of many as yet ob-

⁴ H. H. Thornberry, *Phytopath.*, 25: 618-627, 1935.

¹ M. C. Cajlachjan and L. M. Yarkovaja, *C. R. Acad. Sci. URSS*, 15: pp. 215-217, 1937. Also pp. 85-88; 3: pp. 443-447; and 4: pp. 77-81, 1936.

² B. S. Moskov, *C. R. Acad. Sci. URSS*, 15: pp. 211-213, 1937.

³ J. Kuijper and L. K. Wiersum, *Proc. Acad. Sci. Amsterdam*, 39: pp. 1114-1122, 1936.

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 experimen-

tal 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 realized² 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 convec-

tive 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 velocity⁶ 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 Stuewer⁷ 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

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.

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.