Separation of Plant Particles

Although much of what we know about plant (or animal) metabolism has been generated from studies with isolated subcellular structures such as chloroplasts, mitochondria, and ribosomes, most investigators have had to accept a choice of either "preparative" amounts of crude suspensions or vanishing by small quantities of pure particles. Moreover, the intactness of isolated organelles has been a doubtful quality; most "chloroplast preparations" have been "class II" chloroplasts, a euphemism for stripped and swollen lamellae, devoid of stroma and limiting membrane. The discovery of peroxisomes has glaringly illuminated this unhappy state; for we suddenly find that peroxisomes, not chloroplasts, contain the catalase of green leaves, and that these same bodies, not mitochondria, are the sites of fatty acid oxidation in fatty seedlings.

No one has been content with conventional methods of particle separation and, with the new possibilities for relatively large-scale density gradient centrifugation in zonal rotors, we need no longer be limited by them. It is now possible, at least theoretically, to prepare biochemically useful quantities of all kinds of subcellular particles with defined sedimentation coefficients and equilibrium densities. In the future we should be able to specify that the particles in a chloroplast preparation have *S*-values of between 500 and 550 kilosvedbergs and equilibrium densities between 1.100 and 1.105 g/cm^3. Naturally, we should also demand ultrastructural intactness.

Toward this end a Microsymposium on the Separation of Plant Particles was held at Oak Ridge, Tennessee, 22–24 January 1970. The purpose of the meeting was to exchange practical and theoretical ideas on the separation of cellular, subcellular, and macromolecular particles from plants. In order to describe the current state of the art in selected areas of particle separation independent of the immediate application, a number of the invited speakers were concerned with animal or bacterial systems. The bias of the organizers—N. G. Anderson, R. C. Fuller, and myself—was revealed by the heavy emphasis on density gradient centrifugation in zonal rotors.

On the theoretical level, there was a recurrent theme—the relatively large size and sectorial geometry of zonal rotors have increased the feasibility of and thus stimulated interest in the direct testing of physical and mathematical models of density gradient centrifugation. S. P. Spragg (Birmingham, England) considered the design of gradients for optimizing resolution in which the volume (rather than the radial width) of a particle zone remains constant during sedimentation. Spragg asked if the diffusive flow of solute and solvent induced by a gradient must not set a lower and time-dependent limit on the volume of a particle zone. V. N. Schumaker and B. Halsall (Los Angeles) described a simple model system for measuring zone broadening due to diffusion of the sample particles. Droplet sedimentation was eliminated by incorporating a counter macromolecule in the underlying solution. Their procedure provides simple means of measuring diffusion coefficients and for evaluating additional factors that might influence zone broadening during sedimentation. H. W. Hsu (Oak Ridge and Knoxville) presented equations which predict particle behavior (position and instantaneous velocity) from the radial functions of gradient density and viscosity expressed as polynomials. His calculations should greatly facilitate the calculation of apparent sedimentation coefficients in gradients of known composition.

In the area of centrifuge techniques and hardware, G. B. Cline (Birmingham, Alabama) described recent developments with the K-series of high-speed continuous-flow rotors. Cline's proposals for increasing resolution through the use of step gradients generated considerable discussion. C. R. McEwen, E. T. Juhos, and R. W. Stallard (Palo Alto) discussed the principles and possibilities for continuous-flow fractionation with their elutriation rotor. Although the rotor is still in a developmental stage, its application to the fractionation of whole cell populations will be watched very closely. D. A. Waters (Oak Ridge), speaking of the physical and metallurgical problems of rotor design, illustrated problems of stress limits of different rotor materials by cheerful references to "catastrophic self-disassembly."

The criteria for intactness and the special problems of membrane-bound particles were emphasized by three speakers. W. Laetch (Berkeley) described the different characteristics of chloroplasts in the parenchyma as con-

trasted with those in bundle sheaths of tropical grasses and other "Hatch-Slack" plants. N. E. Tolbert (East Lansing) outlined the metabolic activities of leaf peroxisomes and some of the problems in their isolation. W. D. Bonner (Philadelphia) reported highly intact mitochondria from white potato, but concluded that "God in his infinite wisdom meant that roots were not to be ground up." The consensus from numerous informal discussions was that difficult problems remained in the recovery of pure suspensions of completely intact chloroplasts, peroxisomes, and mitochondria.

The application of zonal centrifugation to the separation of specific particles was discussed by a number of speakers: bacterial "minicells" (W. Fisher, Oak Ridge), animal nuclei of different ploidy (C. A. Albrecht, Oak Ridge), continuous-flow harvesting and separation of intact from stripped chloroplasts (D. H. Brown, Oak Ridge), separation of mitochondria from derepressed and repressed yeast (C. A. Price, New Brunswick), preparation of homogeneous viruses for vaccine production (J. L. Gerin, Bethesda; H. E. Bond, Bethesda), one-step fractionation of serum lipoproteins by density gradient flotation (M. Heimberg, Nashville), and separation of undegraded chromosomal DNA by reorienting gradient techniques (J. Lett, Fort Collins).

Among the most imaginative applications of particle separations was the proposed control of the Douglas fir tussock moth caterpillar through large-scale purification of the specific polyhedrosis virus in a K-type rotor (J. P. Breillatt, Oak Ridge).

The microsymposium and two associated workshops were sponsored by the American Society of Plant Physiologists, the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, and the Molecular Anatomy Program of the Oak Ridge National Laboratory. Support was provided by these organizations plus the Division of Biology and Medicine of the U.S. Atomic Energy Commission, the International Equipment Company, and the Spinco Division of Beckman Instruments. The published proceedings of this microsymposium will be available from the MAN Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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Courses

Experimental and Theoretical Analysis of Modern Characterization Methods Applied to Electronic Materials, Cambridge, Mass., 27 July–7 August. This course will deal with the available methods and techniques (their potential and limitations) for the chemical and physical characterization of materials (for electronic applications, semiconductors, magnetics, dielectrics, and others). The principles of the various characterization techniques will be discussed as they relate to the determination of trace impurities, impurity heterogeneities, crystalline structure, lattice defects, electrical carriers, and surface configuration of specific classes of electronic materials. (Director of the Summer Session, Room E19-356, Massachusetts Institute of Technology, Cambridge 02139)

Anthropology for College Teachers, Boulder, Colo., 15 June–21 August. This summer institute is being offered for the 10th year and has been awarded a grant by the National Science Foundation. Is intended for 30 college and junior college teachers of anthropology whose formal training in the subject is weak. (Dr. A. J. Kelso, Director, Department of Anthropology, University of Colorado, Boulder 80302)

Theory and Practice of the Analytical Ultracentrifuge; Advances in Macromolecular Characterization, Woods Hole, Mass., 11–22 May. Material will include basic information, sedimentation velocity; boundary analysis; zonal and active enzyme sedimentation velocity, sedimentation equilibrium of enzymes, including paucidisperse systems; density gradient sedimentation equilibrium; and optical techniques. *Tuition*: $400. (Dr. David Teller, Department of Biochemisty, University of Washington, Seattle 98105)

Anatomy, Physiology, and Patient Care, Charleston, S.C., 13 July–7 August. The course is designed to familiarize the engineer with the problems involved in the delivery of medical care. The opportunity to observe the activities of the emergency room, operating room, intensive care unit, and other areas of the hospital is provided. Limited to 40 participants. *Tuition*: $500. (Mr. Thomas S. Hargest, Director, Engineering Development Section, Department of Surgery, Medical University of South Carolina, Charleston 29401)

Polymers (Characterization, Morphology, and Structure-Property Relations), Houston, Tex., 4–8 May. *Fee*: $300. (Mary B. Appleton, Office of Continuing Studies, P.O. Box 1892, Rice University, Houston, Tex. 77001)

Practicum in Histology, Boston, Mass., 24 May–5 June. An intensive program in histological techniques, including fixation, embedding, microtomy, staining, and autoradiography, is designed for doctoral level investigators. Highly recommended laboratory assistants will be considered. Limited to 12 students in order to insure maximum practical laboratory experience. (Dr. Clifford F. Youse, Director of Programs in Applied Science, Center for Continuing Education, Northeastern University, Boston, Mass. 02115)