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APPENDIX

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SIMULATION OF MICROSOME SEDIMENTATION

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Smooth- and rough-surfaced microsomes have been separated by zonal centrifugation in a B-XXIX rotor[1]. The RNA concentration in the smooth membrane fraction was much higher than that found by Dallner[2]. One hypothesis that would account for this finding is that free, non-membrane-bound polyribosomes contaminated the smooth vesicle fraction isolated in the zonal rotor, but were absent from fractions prepared by Dallner's method[2].

I have approached the problem by simulating the sedimentation of rough- and smooth-surfaced microsomes and ribosomes under the conditions of the two separation methods. A computer program, code-named DIFSED, recently has been evolved and tested by members of the MAN Program staff (refs 3 and 4; and W. K. Sartory, H. B. Halsall, and J. P. Breillatt, *Separation Method Design by Simulation Techniques. III. Macromolecule Zone Concentration Profiles*, in preparation). This program simulates the movement of sedimenting and diffusing macromolecule zones in a sedimenting and diffusing gradient, with graphic output of results. These calculations have been in precise agreement with experimental results in zonal rotor cavities. In addition, sedimentation in fixed-angle rotors can be approximated by such a mathematical treatment if the results are evaluated with respect to the initial assumptions[5–10].

The smooth-surfaced vesicle population was represented by spheres of 80, 140, and 200 nm diameter, with a particle density of 1.167 g/cm^3. These values, which typified the two extremes and the mean of the population, were derived from electron micrographs and sucrose concentrations of fractions from the zonal centrifuge[1]. The rough-surfaced membrane population was represented as particles of the same size, but of density 1.206 g/cm^3. The ribosomes and polysomes were taken to have particle densities of 1.52 g/cm^3, and the following sedimentation coefficients: monomer, 80 S; tetramer, 180 S; octamer, 265 S (ref. 11). The increase in density differential between the two membrane fractions caused by Cs^+ binding was taken into account; however, it was not included in the ribosome density. This causes the simulated ribosome density to be less than the actual density and gives a conservative estimate of ribosome contamination of the smooth vesicle fraction. The gradient was assumed to consist of a two-component solution of sucrose and water. Density and viscosity increments due to small buffer ions were negligible with respect to those of sucrose.

The simulated separation in a B-XXIX rotor is shown in Fig. 1. The smooth-membrane fraction that was analyzed for RNA[1] was shown to contain the majority of the free polyribosomes and only small quantities of rough-surfaced vesicles. A similar treatment applied to Dallner's preparative method[2] showed that after 1 h

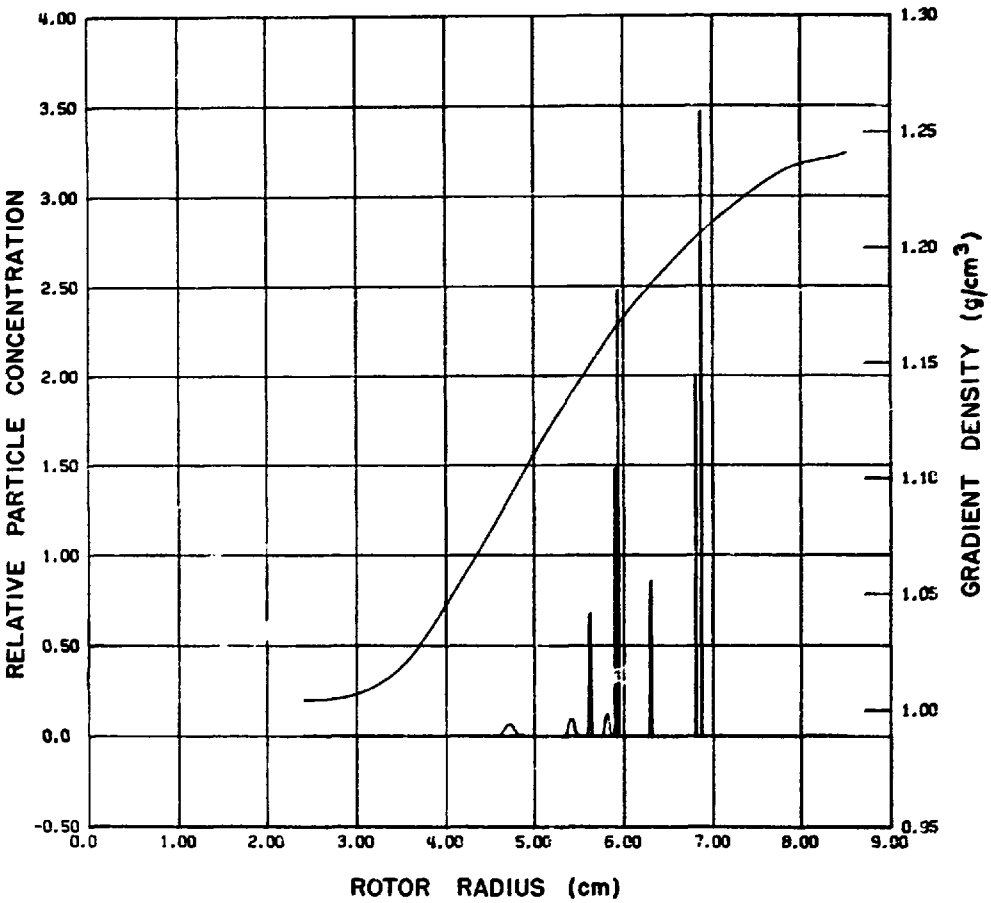

Fig. 1. Simulated separation of ribosomes, polysomes, and smooth- and rough-surfaced microsomal vesicles in a B-XXIX rotor. Centrifugation at 25000 rev./min for 2.18 h at 5 °C. The sedimenting peaks represent (left to right, in order of increasing sedimentation coefficient): monomeric ribosomes, ribosome tetramer, smooth vesicle (80 nm diameter), ribosome octamer, smooth-surfaced vesicle (140 and 200 nm), and rough-surfaced vesicles (80, 140 and 200 nm). The smooth membrane fraction collected by Lee *et al.*[1] included the volume between 4.96 and 6.51 cm radius; the rough-surfaced membranes included the volume between 6.64 and 7.26 cm.

at 60000 rev./min, all rough-surfaced membranes were at the tube bottom, all smooth-surfaced membranes were at the density interface, and the monomeric ribosomes had sedimented past the interface. Thus the smooth vesicle fraction could be collected without ribosomal contamination. The radii attained by the ribosomes are conservative estimates, since at 60000 rev./min, due to wall effects, the ribosomes may tend to move to the bottom more rapidly than calculated for pure sedimentation. The occurrence of enhanced sedimentation due to wall effects in fixed-angle rotors appears to be linked both to the velocity with which the particles strike the wall and to the nature of their interaction with the wall (stickiness). Very large virus particles can sediment in fixed-angle rotors in an apparently ideal manner if suspended in a viscous medium or centrifuged at low rotor velocities (J. N. Brantley and J. P. Breillatt, unpublished observations).

REFERENCES

1 Lee, T-C., Swartzendruber, D. C. and Snyder, F. (1969) *Biochem. Biophys. Res. Commun.* 36, 748
2 Dallner, G. (1963) *Acta Pathol. Microbiol. Scand., Suppl.* 166, p. 1
3 Sartory, W. K. and Breillatt, J. P. (1972), in preparation

4 Breillatt, J. P., Sartory, W. K. and Brantley, J. N. (1971) *Separation Method Design by Simulation Techniques. II. CsCl Gradients in the K-III Rotor*, U.S.A.E.C. Report ORNL-TM-3185
5 Charlwood, P. A. (1963) *Anal. Biochem.* 5, 226
6 Fisher, W. D., Cline, G. B. and Anderson, N. G. (1964) *Anal. Biochem.* 9, 477
7 Brentani, R., Brentani, M. and Raw, I. (1967) *Anal. Biochem.* 20, 361
8 Anderson, N. G. (1968) *Anal. Biochem.* 23, 72
9 Vedel, F. and D'Aoust, M. J. (1970) *Anal. Biochem.* 35, 54
10 Castaneda, M., Sanchez, R. and Santiago, R. (1971) *Anal. Biochem.* 44, 381
11 Breillatt, J. P. and Dickman, S. R. (1966) *J. Mol. Biol.* 19, 227