Separation of Cell Particles and Molecules
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The first edition of this book was my PhD thesis in biochemistry at the University of Uppsala, Sweden. In 1954 I began my graduate studies with Arne Tiselius, my supervisor was Håkan Leyon, an electron microscopist interested in the chloroplast. My first task was to isolate pyrenoids, characteristic structures of chloroplasts from green algae. Besides centrifugation I also used chromatography on columns of hydroxyapatite, which had been introduced by Tiselius for separation of proteins. It was of great methodological interest to see whether these columns could be applied also to cell particles.

My experiments failed, however, due to irreversible adsorption. In a typical chromatographic experiment with hydroxyapatite, a substance is first adsorbed at low concentrations of phosphate buffer and then eluted at higher concentrations. The chloroplast particles were easily adsorbed on the column but could not be desorbed. The green band on top of the column did not budge upon elution with various buffers. Could it be that the relatively large particles were mechanically trapped between the hydroxyapatite grains of the column? To eliminate this possibility, I switched to batch experiments wherein mixtures of hydroxyapatite and chloroplasts suspensions were shaken and the settling of the green particles was observed. Again the chloroplasts were firmly adsorbed even in the presence of 1-2 molar (M) phosphate.

I decided to use a detergent to facilitate desorption. From a book on detergents, I remembered the name polyethylene glycol (PEG). Since this happened to be in a bottle on the shelf, I tried it. An aqueous solution of PEG was mixed with an hydroxyapatite sediment containing the firmly adsorbed chloroplast particles in about 1 M potassium phosphate. The result was most spectacular. The intense green color of the chloroplasts, earlier so strongly associated with hydroxyapatite, was now present in a liquid layer on top of the phosphate buffer; the hydroxyapatite turned white and was completely purged of chloroplasts. Owing to the high phosphate concentration, a two-phase system was formed and the upper, PEG-rich phase apparently had a stronger affinity for the chloroplasts than did the hydroxyapatite. Later I found that PEG is not a detergent; it is used for the manufacture of certain detergents. Thus, my experiment was a rewarding “mistake.” Due to the intense color of the chloroplasts, the phenomenon was very impressive, and this helped me to realize that partitioning might be used for the separation of cell particles.

Since PEG is a polymer and somewhat hydrophobic compared to potassium phosphate buffer, the latter was replaced by another hydrophilic polymer, dextran. In this way an aqueous polymer-polymer two-phase system was obtained. The dextran PEG phase system, which is very mild towards biological material, has since been used for many different separation purposes. It has, e.g., been successfully used for purification of plasma membranes and for separation of inside out from right side out thylakoid or plasma membrane vesicles. The method is highly versatile and can be applied to both a small and on a large industrial scale involving several thousands of liters. The Japanese translation of my book was published in 1971; a Russian, in 1974.

However, my original problem as a PhD student in 1954, to isolate pyrenoids, still remains to be solved.