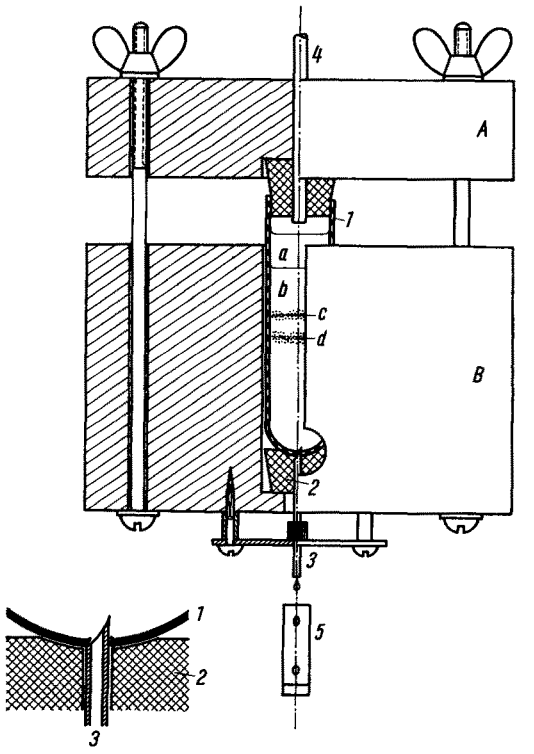
PRO LABORATORIO

Sampling of Virus Particles and Macromolecules Sedimented in an Equilibrium Density Gradient[1]

Separation of large molecules on the basis of differences in density has been accomplished by high-speed centrifugation in CsCl equilibrium density gradients[2]. This procedure, originally developed for analytical purposes, was subsequently applied to preparative fractionation of whole viable phage particles[3] and isolated DNA preparations. For the latter purpose, model L of the refrigerated Spinco centrifuge, equipped with the SW-39L swinging-bucket rotor and temperature sensing device PS003, was employed. The position of the phage or DNA layers (*c*, *d*), formed at their neutral buoyancy levels, was determined by punching a small hole in the bottom of the siliconized centrifuge tube and collecting the effluent droplets individually in a large series of small test tubes pending assay of their content. Neither this fractioncollecting operation nor others reviewed by DEDUVE *et al.*[4] were found to be entirely satisfactory until the simple instrument depicted in the Figure was devised. The operation, as shown in the Figure, involves collecting the effluent samples through a needle, which penetrates the centrifuge tube from below, in such a manner that (1) stratification of the layers is not disturbed; (2) the tip of the needle barely penetrates the tube, avoiding formation of a 'dead' volume of liquid at the bottom; and (3) a seal is formed around the needle at the point of its introduction. The latter requirement was satisfied by compressing the tube between two blocks of wood or metal (*A* and *B*), which step, while forming a tight seal between the bottom of the centrifuge tube *1* and the soft-rubber stopper *2*, caused the emergence of the tip of the needle *3* from the stopper *2* into the centrifuge tube. The block *B* was so designed that the whole piercing operation could be carefully observed. The rate of drip depended on the air pressure in the tube *1*, which in turn was regulated by manipulating the difference between the water levels in the two vessels connected to the tubing *4*. For most DNA centrifugations, Lusteroid or polypropylene tubes *1* (5 ml; Spinco No. 5050 or 3682), containing 3 ml of CsCl[5] solution *b* overlayered with 1·5 ml of parafin oil *a* to prevent collapse of the tube, were used. With the proper adaptor (Spinco No. 5527), 0·8-ml tubes (Spinco No. 5528) can be employed. The volume of each drop, using a silicon-treated needle (N.Y. Board of Health needle 19 G, No. 496 NR, Becton, Dickinson & Co., Rutherford, N. J.), was approximately 0·01 ml. The drops were collected in 40 to 120 vials *5* (9 × 30 mm, 1/4-dram shell vials) which were arranged serially in a long supporting block sliding on a track under the needle *3*. The vials were sealed at once with a strip of adhesive tape. For ideal separation of the fractions centrifugation and all operations were performed at 0°C, using ice-water-cooled block *B*, and the outflow rate was kept below 60 drops/min. Higher flow rates caused dispersion of the distribution peaks. With this arrangement, sampling and analysis of the effluent can be either discontinuous or continuous. Information may be obtained concerning the CsCl density gradient (refractive index measurements) and about the distribution of several high molecular weight materials, including viable phage particles[3] (plaque assay) and DNA (ultraviolet light absorption and/or radioactivity determination).



Fraction collector for determining the distribution of macromolecular substances in the equilibrium density gradient established by high-speed centrifugation

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Résumé

La distribution de composés macromoléculaires, stratifiés dans un gradient de densité de CsCl établi par une ultracentrifugation prolongée, est déterminée quantitativement par le recoupage du contenu du tube. Une courte aiguille hypodermique est tout simplement insérée dans le fond du tube. Le contenu du tube s'épanche goutte à goutte. Les fractions de recoupage peuvent être ensuite analysées une à une.

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[2] M. MESELSON, F. W. STAHL, and J. VINOGRAD, Proc. nat. Acad. Sci., Wash. *43*, 581 (1957).

[3] A. W. KOZINSKI and W. SZYBALSKI, Virology *9*, 260 (1959).

[4] C. DEDUVE, J. BERTHET, and H. BEAUFAY, Progr. Biophys. *9*, 325 (1959).

[5] For DNA sedimentation, a saturated solution of CsCl (0–4°C) in water or in 0·01 *M tris* (hydroxymethyl) aminomethane buffer (pH 8) was prepared, and subsequently slightly diluted with water, to obtain a refractive index $n_D = 1{\cdot}4120$. A 2·5 ml quantity of this solution was mixed with 0·5 ml DNA dissolved in 1 *M* NaCl. After 72 h of centrifugation at 35000 r.p.m., the density gradient extended from 1·65 to 1·82 g/cm³. The CsCl was purified by repeated recrystallization from boiling water after filtration of a hot solution through activated carbon, until the O. D. at 260 mμ became less than 0·05 as measured against a water blank.