Brei Fractionation
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over the open end of the reflector, and a polished aluminum backing reflector, *BR*, was placed in position.

It is preferable to utilize capsules that contain prepolymerized plugs at the bottom (*2*). These are prepared in advance by photopolymerizing 2 drops of the methacrylate mixture in No. 4 gelatin capsules.

When the photopolymerization unit described in this paper (*5*) is used, the specimens are positioned approximately 1 in. from the surface of the fluorescent lamp. Up to three horizontal rows of specimen capsules can be accommodated over the full available length, approximately 22 in. Thus, more than 100 specimens can be photopolymerized at one time. Full capsules are polymerized by an overnight exposure to the ultraviolet radiation. However, preparation of the methacrylate plugs may require exposures of 24 to 48 hr because of air inhibition.

References and Notes

1. S. B. Newman, E. Borysko, M. Swerdlow, *Science* **110**, 66 (1949).
2. B. W. Massey, *Stain Technol.* **28**, 19 (1953).
3. An equivalent Westinghouse fluorescent sun lamp in a 40-w size, FS40T12, is available if a larger photopolymerization capacity is desired.
4. Obtained through the courtesy of Rohm & Haas Co., Philadelphia, Pa.
5. This investigation was supported by a grant from the Life Insurance Medical Research Fund.

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Brei Fractionation

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It does not appear to have been generally realized that the centrifugal procedures used for brei fractionation have a much lower resolution than would be expected from a simple uncritical application of Stokes' law. Techniques for fractionating breis have therefore been investigated in this laboratory during the past 3 years with the aim of developing an analytic medium-speed centrifuge (*1*). The equipment presently in use incorporates a shatterproof glass observation port on the centrifuge, a stroboscopic system providing both high- and low-intensity illumination, circuitry for constant and variable flashing rates, a contactor on the centrifuge shaft that allows a stable image to be observed during sharp changes in speed, a speed-measuring system for indicating speeds down to 25 rev/min (accuracy > 0.02 percent), and a system for recording data for speed-versus-time curves. With the latter, the total effective centrifugation may be integrated. Cut-out centrifuge tube shields allow the behavior of breis and layered systems to be observed directly during centrifugation.

Layered systems, which are necessary for high-resolution work, are subject to several artifacts. The first, shown in Fig. 1, occurs in a matter of minutes

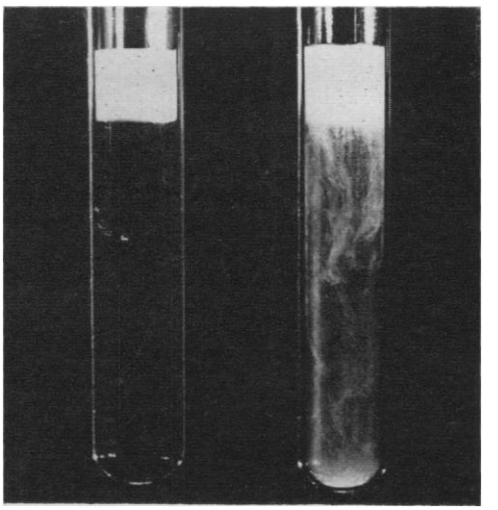

Fig. 1. Streaming effect in a layered system. A 10-percent rat liver brei in 0.25*M* sucrose-0.0018*M* $CaCl_2$ (density 1.038) layered over 0.34*M* sucrose-0.00018*M* $CaCl_2$ (density 1.046). (Left) System immediately after layering. (Right) Streaming after 30 min at 0°C (not centrifuged).

without any centrifugation in a layered system such as the one advocated by Hogeboom *et al.* (*2*) for nuclear isolation. Nuclei, whole cells, and mitochondria are all carried down indiscriminately. This *streaming effect* involves the movement of droplets and does not appear to depend on the sedimentation of individual particles. The second effect is introduced by the use of centrifugal fields sufficient to move particles across the density interface. The upper portion of the lower, denser layer increases in density by virtue of the particles entering it from above and moves to the bottom of the tube, resulting in a *turnover effect* (Fig. 2). This accounts for the poor resolution of most layered systems. The use of a number of discrete layers of different densities does not solve

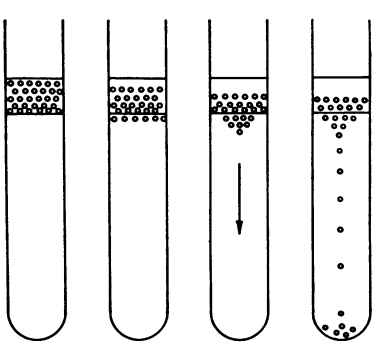

Fig. 2. Turnover effect. A suspension of particles layered over a denser medium (*A*) is centrifuged until some of the particles have passed the interface (*B*). The upper part of the lower layer is then denser by virtue of the particles it now contains. It moves as a body to the bottom of the tube (*C*, *D*).

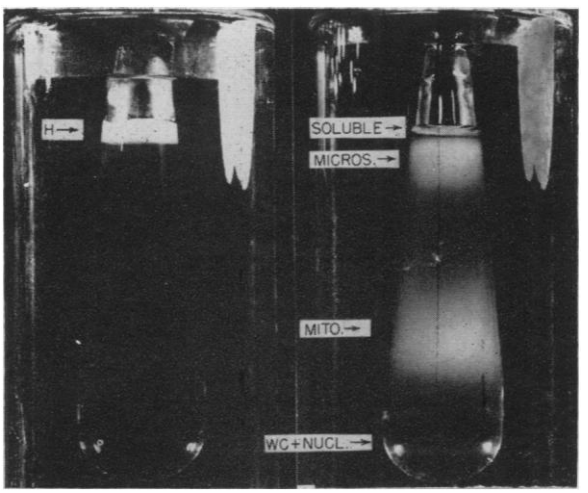

Fig. 3. Separation achieved in a continuous sucrose gradient. (Left) Before centrifuging; (right) after centrifuging. Two milliliters of 5-percent rat liver homogenate was used.

the problem, because the same effect occurs at each interface. This results in the accumulation of particles above each interface with little difference in the microscopic appearance of the various layers (*3*).

With cylindrical centrifuge tubes an appreciable portion (in some instances as much as 50 percent) of the sedimented material hits the side of the tube before it reaches the bottom. This difficulty is met by the use of modified sector-shaped tubes, which are used routinely in this laboratory.

Other difficulties arise during centrifugation because of changes in tube orientation and uneven acceleration and deceleration. These have been eliminated by the use of a reproducible system for slow, controlled acceleration and deceleration.

The difficulties described have been further minimized by use of a *continuous density gradient* produced by differentially driven pistons. Other methods for producing gradients have been examined and found unsatisfactory for this purpose. The characteristics of the gradient in terms of density, viscosity, and composition are accurately known. The results obtained with a dilute homogenate layered over a continuous gradient having a very steep increase in density near the bottom are shown in Fig. 3. No cross-contamination of nuclei, mitochondria, or microsomes has been observed microscopically. A considerable difference in the appearance of particles at the top and bottom of the mitochondrial layer is seen. With longer centrifugation the microsomes have been completely separated from the soluble layer.

All fractions may be quantitatively recovered by introducing a very dense solution to the bottom of the tube, floating the undisturbed gradient into either a constricted portion of the tube or into a sliding system for shearing the liquid similar to that used in the Tiselius cell.

It is evident from Fig. 3 that the continuous spectrum of particles ranging from mitochondria to microsomes, which had been postulated on the basis of previous centrifugal techniques (*4*), does not exist. It is now feasible to make quantitative studies of changes in the ratios and total amounts of brei fractions in response to experimental conditions. The techniques are of interest in connection with virus purification, platelet isolation, and the separation of small particles generally. Since the smaller particles need be moved only a relatively short distance, high gravitational fields are not required.

Continuous gradients were first employed by Behrens (*5*) for the isopycnic separation of nuclei and cytoplasm in nonaqueous media. Steep short gradients of sucrose were introduced by the Harveys (*6*) for the stratification and separation of marine eggs into nucleate and nonnucleate halves. I employed starch gradients in 1949 (unpublished) in attempts to separate cytoplasmic fractions. The techniques described here are an extension of that work. Sucrose gradients for the isolation of plant viruses were introduced by Brakke (*7*).

References and Notes

1. Work performed under contract No. W-7405-eng-26 for the U.S. Atomic Energy Commission.
2. G. H. Hogeboom, W. C. Schneider, M. J. Striebich, *Cancer Research* **13**, 617 (1953).
3. E. L. Kuff and W. C. Schneider, *J. Biol. Chem.* **206**, 677 (1954).
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5. M. Behrens, *Handbuch der Biologischen Arbeitsmethoden*, E. Abderhalden, Ed. (Urban and Schwarzenberg, Berlin, 1938), vol. 5, pt. 10–II, p. 1363.
6. E. N. Harvey, *Biol. Bull.* **61**, 273 (1931); E. B. Harvey, *Biol. Bull.* **62**, 155 (1932).
7. M. K. Brakke, *J. Am. Chem. Soc.* **73**, 1847 (1951).

16 February 1955.

It is certain that a serious attention to the sciences and liberal arts softens and humanizes the temper, and cherishes those fine emotions of which true virtue and honor consist. It rarely, very rarely happens that a man of taste and learning is not, at least, an honest man, whatever frailties may attend him.—DAVID HUME.