moved lenses. Such partial reconstitution of lenses, which is not a true regeneration, has often been reported (*13*). Törö's contrary results should be evaluated in the light of this knowledge.

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* Present address: Biology Department, Wesleyan University, Middletown, Conn.

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Ultraviolet Polymerization of Monomeric Methacrylates for Electron Microscopy

Stanley Weinreb

Department of Pathology, University of Wisconsin, Madison

Specimens to be sectioned for electron microscopic techniques are usually imbedded in monomeric methacrylate mixtures that are subsequently polymerized by the addition of a suitable chemical catalyst and the application of heat (*1*). However, because of the small quantities of monomer used, it is often inconvenient and/or difficult to dissolve the catalyst. In addition, chemically catalyzed samples have a tendency to develop undesirable bubbles during polymerization.

Massey (*2*) reported that tissue imbedded in chemically catalyzed monomeric methacrylates often suffers from a "popcorn reaction." To minimize the disadvantages of chemical polymerization, Massey suggested the use of ultraviolet polymerization of uncatalyzed monomers from which the inhibitor had been removed. Her ultraviolet source appears to have been a type RS-40 sun lamp designed for home use. However, this sun lamp also emits large amounts of infrared radiation, and its operating temperature is so high that it will char paper on contact. This necessitates special precautions against fire hazard during the long periods of continuous operation needed for photopolymerization. In addition, forced draft ventilation is usually necessary to avoid overheating of the specimen, and the effective irradiated area at the recommended distance is small and not uniform.

Because of the advantages of using photopolymerization, it was decided to develop a modified method. There are many commercially available radiation sources that can supply the near ultraviolet needed for the photopolymerization of monomeric methacrylates. Most of these, however, require special electric equipment or have such high emission values that the small samples used may boil. It was, therefore, decided to employ a lamp of relatively low emission value that would still result in photopolymerization within a convenient period of time.

The lamp chosen was a Westinghouse FS20T12 fluorescent sun lamp (*3*). This lamp is physically and electrically similar to a standard 20-w fluorescent lamp and operates in standard 20-w fluorescent-lamp fixtures. It has approximately the same emission range as the RS-40 sun lamp, an effective life of about 4000 hr, a normal operating temperature of about 100°F, and very little infrared radiation. No forced draft ventilation is necessary.

The sun lamp was utilized in conjunction with a standard 20-w fluorescent channel-strip fixture containing lamp holders, starter, and ballast (Fig. 1). A polished aluminum reflector, *R*, with a 1⅛-in. radius of curvature was attached to the unit. The reflector was designed so that it also served as a mount for the specimen holders, *H*, and backing reflectors, *BR*.

Specimens were imbedded in No. 4 gelatin capsules using a mixture of 1 part ethyl methacrylate and 3 parts *N*-butyl methacrylate (*4*) from which the inhibitor had been removed. The filled capsules, *S*, were then attached in a vertical position to a strip of cellulose tape, *T*, that had been fastened to a specimen holder, *H*. The specimen holder was then clipped

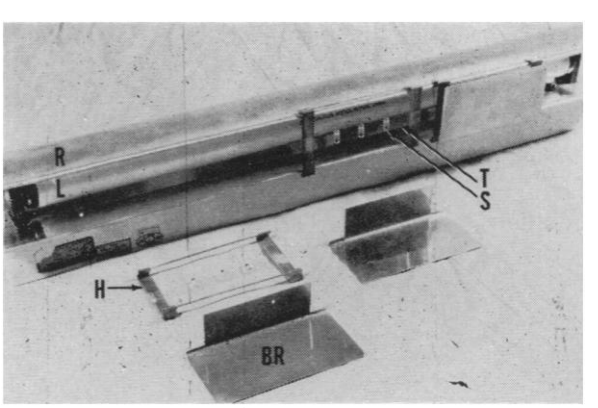

Fig. 1. Ultraviolet sun lamp assembly for the photopolymerization of monomeric methacrylates. *BR*, backing reflector; *H*, specimen holder; *L*, fluorescent sun lamp; *R*, reflector; *S*, specimen capsule; *T*, cellophane tape.

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.

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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

Norman G. Anderson

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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

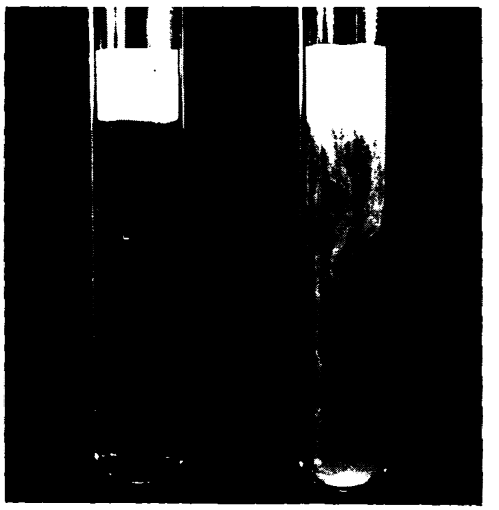

Fig. 1. Streaming effect in a layered system. A 10-percent rat liver brei in 0.25*M* sucrose-0.0018*M* $CaCl_2$ (density 1.038) layered over 0.34*M* sucrose-0.00018*M* $CaCl_2$ (density 1.046). (Left) System immediately after layering. (Right) Streaming after 30 min at 0°C (not centrifuged).

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

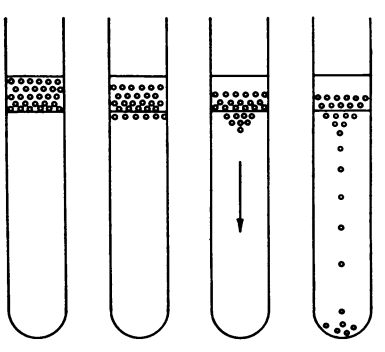

Fig. 2. Turnover effect. A suspension of particles layered over a denser medium (*A*) is centrifuged until some of the particles have passed the interface (*B*). The upper part of the lower layer is then denser by virtue of the particles it now contains. It moves as a body to the bottom of the tube (*C*,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