Centrifuging, Storing, and Quantitative Dispensing of Micro Samples; An Improved Technic

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In an adaptation of standard technics and apparatus, aqueous homogenates from single rat lenses are introduced into capillary tubes, soluble and insoluble constituents are separated using a micro hematocrit centrifuge, and samples are frozen and stored in the capillary tubes. To allow quantitative transfer of sample aliquots from these capillaries for chemical and immunologic determinations, commercial micro titrator was modified.

The development of more and more sensitive methods for chemical and immunologic analysis of biological material goes hand in hand with the demand for a considerable decrease in the size of the sample to be analyzed or the volume of solutions to be used. Frequently, however, the advantage of using micro methods is jeopardized by a loss in accuracy—e.g., the slightest mistake in the handling of microliter pipets may send the experimental error soaring.

During our studies on galactose-induced rat cataracts various quantitative chemical, immunologic, and electrophoretic determinations on water-soluble components of individual baby rat lenses were necessary. Since a lens from a young rat yields only 50 \( \mu \)l. of homogenate, on the average, and since the material involved, especially the protein portion, is labile and easily affected by repeated freezing and thawing or by prolonged exposure to room temperature, the following adaptation of technics and available equipment proved to be very helpful to us.

Procedure

Lens homogenates are introduced into nonheparinized micro hematocrit capillary tubes, 75 mm. long. These tubes are available for use with

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the micro hematocrit centrifuge which was developed a number of years ago by Guest (1). The capillaries, which have a total capacity of 40-50 μl, are filled to about three quarters with the tissue homogenate, sealed with a sealing compound, and centrifuged 4 or 5 min. in a precooled micro hematocrit centrifuge. The same technic is used to centrifuge deproteinated tissue extracts and blood samples. The ratio of insoluble matter to total volume is determined, if required, with the help of a hematocrit reading chart (1). The capillaries are then stored in a freezer until further use. There is no waste because very small quantities can be retained in the capillary tubes. Individual capillary samples are removed from the freezer as needed. The damaging effect of repeated freezing and thawing of protein material is reduced since only those capillaries to be used are removed from the freezer. Furthermore, the content of the tubes freezes and thaws rapidly; lens material, therefore, is not exposed to room temperature for any significant length of time before being ready for use. The frozen capillaries can easily be stored in micro slide folders and stacked in the freezer.

After the portion of the glass capillary which contains seal and precipitate is broken off, aliquots of the supernatant are transferred and delivered quantitatively from the capillaries in amounts of 1 μl or more, using a modified Beckman Spinco microtitrator* (Fig. 1). The delivery tip of the titrator is replaced by a glass adapter ground to accept a standard No. 18 hypodermic needle which, in turn, is attached to a short length of polyethylene tubing, No. 190. The entire unit is filled with water, leaving an air space of approximately 3 mm. at the distal end of the

*Beckman Instruments, Inc., Fullerton, Calif.
polyethylene tubing before the sample capillary is connected. The air-space prevents the sample from contacting the water and becoming diluted.

Greater accuracy in pipetting is obtained when samples are first transferred from the storage tubes into capillaries, the tips of which have been drawn out in the flame. This method was used successfully for transferring and pipetting aliquots as small as 1 μl. with an error of less than 4% (2).

References