APPENDIX

A Simply Constructed Tissue Homogenizer

BY H. KAMPHAUSEN AND R. K. MORTON
Department of Biochemistry, University of Melbourne

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Preliminary experience in the preparation of nuclei from mouse mammary gland was very unsatisfactory with a Potter & Elvehjem (1936) type of ground-glass homogenizer (Branster & Morton, unpublished). There was some improvement with a smooth pestle of Perspex operating in a sand-blasted mortar. However, consistently satisfactory results have been obtained in the preparation of nuclei from a variety of mouse tissues with an homogenizer constructed as described. It is a modification of that described by Potter & Elvehjem (1936) and consists essentially of a smooth polyethylene (Alkathene, Imperial Chemical Industries Ltd.) pestle operating in a smooth-walled glass mortar. It is simply constructed in the laboratory from readily available, inexpensive materials. This has particular advantage when a number of homogenizers are required.

The mortar is formed by sealing and rounding one end of a uniform Pyrex-glass tube, approx. 8 cm. long, diameters 13.0 mm. (external) and 10.9 mm. (internal), and the insert for the pestle is made from either sealed-glass tube or solid rod 6.6 mm. external diameter and about 20 cm. long. 'Nyathene'-brand tube (Moulded Products of Australasia), diameters about 10.4 mm. (external) and 5.9 mm. (internal), is expanded and softened by immersion in boiling water and then slipped over one end of the pestle insert, previously lubricated with glycerol. The Nyathene is pushed home to form a sleeve about 3.5 cm. long, and then cut, leaving 2-3 mm. projecting beyond the end of the glass insert. When cooled, the sleeve binds securely to the glass insert, forming a pestle.

The inside of the mortar is lubricated with a few drops of glycerol and the base is immersed for about 1 cm. into a paraffin bath kept at about 160-170°.

Alternatively, with a little experience the base of the mortar may be heated to this temperature in a flame. The pestle is now inserted into the mortar, forced home and rotated by hand. The end of the Nyathene sleeve softens sufficiently to seal over the glass insert and is moulded perfectly to the shape of the base of the mortar. The glycerol lubricant facilitates a mirror-finish to the plastic and prevents any sticking or charring. Grooving the surface of the plastic has not been found to be of any particular advantage.

The measurements given above refer to the homogenizer used for preparation of nuclei. In this case, the clearance between pestle and mortar is about 0.1 mm. Glass tubing usually varies slightly and continuously throughout its length. For any mortar, pestles may be constructed which vary in fit from no clearance to about 0.3 mm. clearance by using inserts cut from the one length of glass tubing. There is, of course, a limit to the expansion of the Nyathene sleeve. In practice, a number of homogenizers are constructed and the most satisfactory selected for any required purpose, by trial. The simple mortar described here is of advantage for some work in that it may be centrifuged, but it may be fitted with the usual type of cup at the top if this is required.

The same principle of construction has been used to make smaller and larger homogenizers [up to 75 ml. capacity, pestle diameter 3.2 cm. (external)] for other purposes. For such instruments, of course, Nyathene tubing of suitable diameter is used.

REFERENCE

- Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.