Technical methods

A simplified rapid method for purification of glomeruli

J. L. PORTIS, S. K. WIKEL, AND F. J. MCATEE *US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840, USA*

Isolated renal glomeruli have been used in various immunological, metabolic, and morphological studies. Several techniques have been described for purification of glomeruli usually involving graded sieving or differential density gradient centrifugation and often requiring renal perfusion (reviewed by Nørgaard, 1976). In the current report, we present a simple technique for purifying glomeruli, which requires no special equipment other than a routine laboratory centrifuge. The technique is rapid (less than 30 minutes) and results in ultrastructurally intact glomeruli without renal tubular or vascular contamination.

Material and methods

ANIMALS

Sapphire mink were obtained from the closed colony maintained at the Rocky Mountain Laboratory (RML). New Zealand white rabbits were obtained from a local source. Hartley guinea pigs, outbred Syrian hamsters, C57BL/10 mice, and Lewis rats were raised at the RML.

SOLUTIONS

Seventy-four percent w/v sucrose, specific gravity 1·29 g/ml, was prepared in 0·85% saline and brought to pH 8·3 to 8·6 with 1 M NaOH. The final dilution of sucrose to yield a specific gravity of 1·21 to 1·24 g/ml was made with 1/15 M phosphate buffered saline (PBS), pH 7·2 (34 ml sucrose + 10 ml PBS).

KIDNEYS

Kidneys from laboratory animals were obtained fresh at necropsy after exsanguination. The human kidney used in this study was from a patient with a hypernephroma of the superior pole of the kidney. The inferior pole appeared grossly and microscopically normal and was frozen and stored at −90°C. Portions were thawed and used in the glomerular purification procedure.

ISOLATION OF GLOMERULI

Whole kidneys were minced in cold PBS with two scalpel blades drawn across one another, and the tissue was pressed through a 40 mesh stainless steel screen with the plunger from a 5 ml disposable syringe. The material was then suspended in 30 ml cold PBS and allowed to settle for 5 minutes on ice. The supernate was discarded, and the sediment was resuspended in PBS and centrifuged at 250 *g* for 2 minutes. After the supernate had been aspirated, the sucrose solution (specific gravity 1·21 to 1·24 g/ml) was added, the pellet dispersed by shaking vigorously, and the suspension centrifuged at 2000 *g* for 5 minutes. The floating debris was then removed with an aspirator, and the pellet consisting of the purified glomeruli was suspended in PBS and passed through a 40 mesh stainless steel screen to remove any remaining tissue fragments.

ELECTRON MICROSCOPY

Isolated glomeruli and material before purification were fixed in 2% paraformaldehyde, 2·5% glutaraldehyde, and 0·025% $CaCl_2$ in 0·1 ml cacodylate buffer (pH 7·4) at 4°C for 8 hours. The material was washed with several changes of 0·1 M cacodylate buffer (pH 7·4) at 4°C for 18 hours. Post-fixation was carried out in 1% osmium-0·1 M cacodylate (pH 7·4) for 2 hours at room temperature. Preparations were dehydrated in a graded ethanol series and embedded in Epon via toluene. Thin sections were cut on a Reichert OmU-2 ultramicrotome with a diamond knife, picked up on bare 300 mesh copper grids, stained in uranyl acetate, and counter-stained in lead citrate (Reynolds, 1963). Sections were viewed in an Hitachi HU11E-1 electron microscope operating at 75 kV. One-micron sections used to orientate the specimen were stained with toluidine blue.

Results and discussion

Because of our interest in Aleutian disease of mink, the procedure was initially defined for this species. However, the technique was subsequently found to be applicable to most common laboratory animals. Figure 1 illustrates the degree of purification achieved

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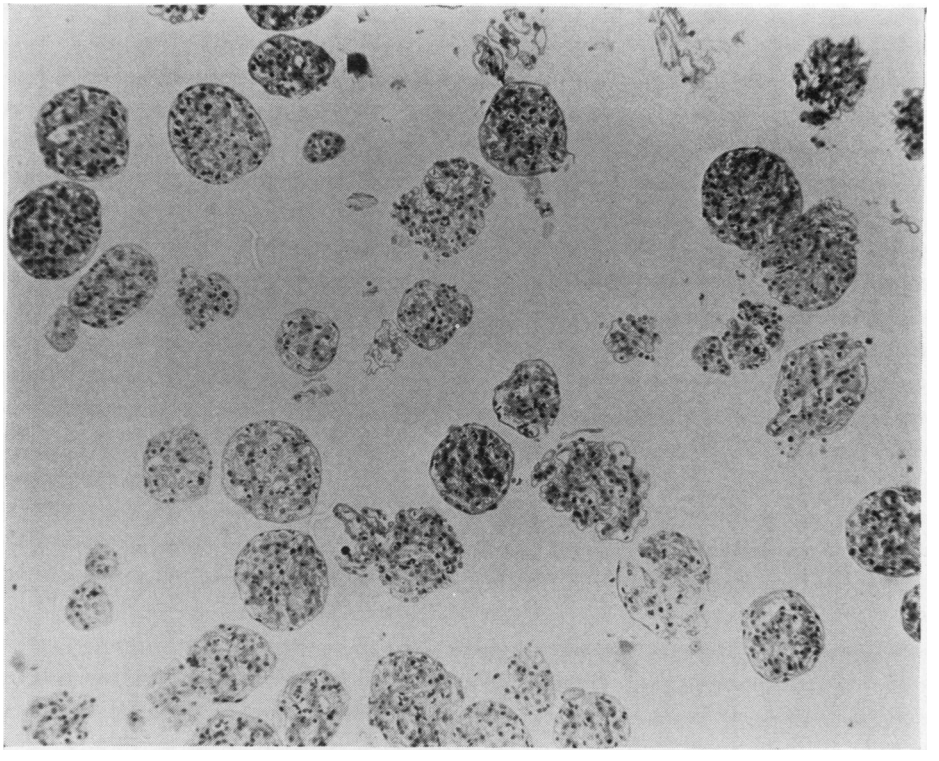

Fig. 1 *One-micron section of Epon-embedded isolated mink glomeruli. Most glomeruli have intact Bowman's capsules, and there is minimal extraneous debris.* (× *25 toluidine blue*)

by pelleting the kidney preparation through sucrose. The abundant cellular debris, along with tubular and vascular fragments seen before centrifugation, are in sharp contrast to the virtually pure glomeruli with intact Bowman's capsules present in the pellet.

The yield of purified mink glomeruli was 40·8 ± 6·0% (Table). Optimal recovery was achieved with 25 to 50 mg wet kidney per ml of sucrose. Overloading the system decreased the yield and increased contamination of the pellet with non-glomerular debris.

Table *Yield of glomeruli*

Kidney	*Tube No.*†	*No. of glomeruli* × 10^3*		*% Yield*
		Before	*After*	
Mink	1	77	18	23
	2	89	42	47
	3	87	43	49
	4	101	44	44
Human	1	9·6	5·9	73
	2	3·6	3·12	87
	3	1·16	0·87	75

*Glomeruli were counted in the crude kidney suspension (before) and in the suspended pellet after centrifugation through sucrose (after).
†Separate aliquots of the same kidney suspension were run through the purification procedure.

Isolated mink glomeruli were found by ultrastructural criteria to be generally normal in appearance (Jørgensen, 1967; Latta, 1970). Visceral epithelial cells were well preserved in all sections examined (Fig. 2), and the filtration slit membrane was evident between foot processes of these cells (Insert, Fig. 2). The basement membrane was well defined into the dense inner layer and the less dense outer layers and was never found to be disrupted. The only evidence of cellular damage was found when endothelial cells along the peripheral areas of the isolated glomeruli were examined. These cells displayed some disruption of the limiting plasma membrane and the formation of small cytoplasmic vacuoles and had a slightly swollen appearance. These changes were not evident when endothelial cells were examined in deeper regions of the glomerulus. Alterations in the appearance of peripheral endothelial cells might be attributed to

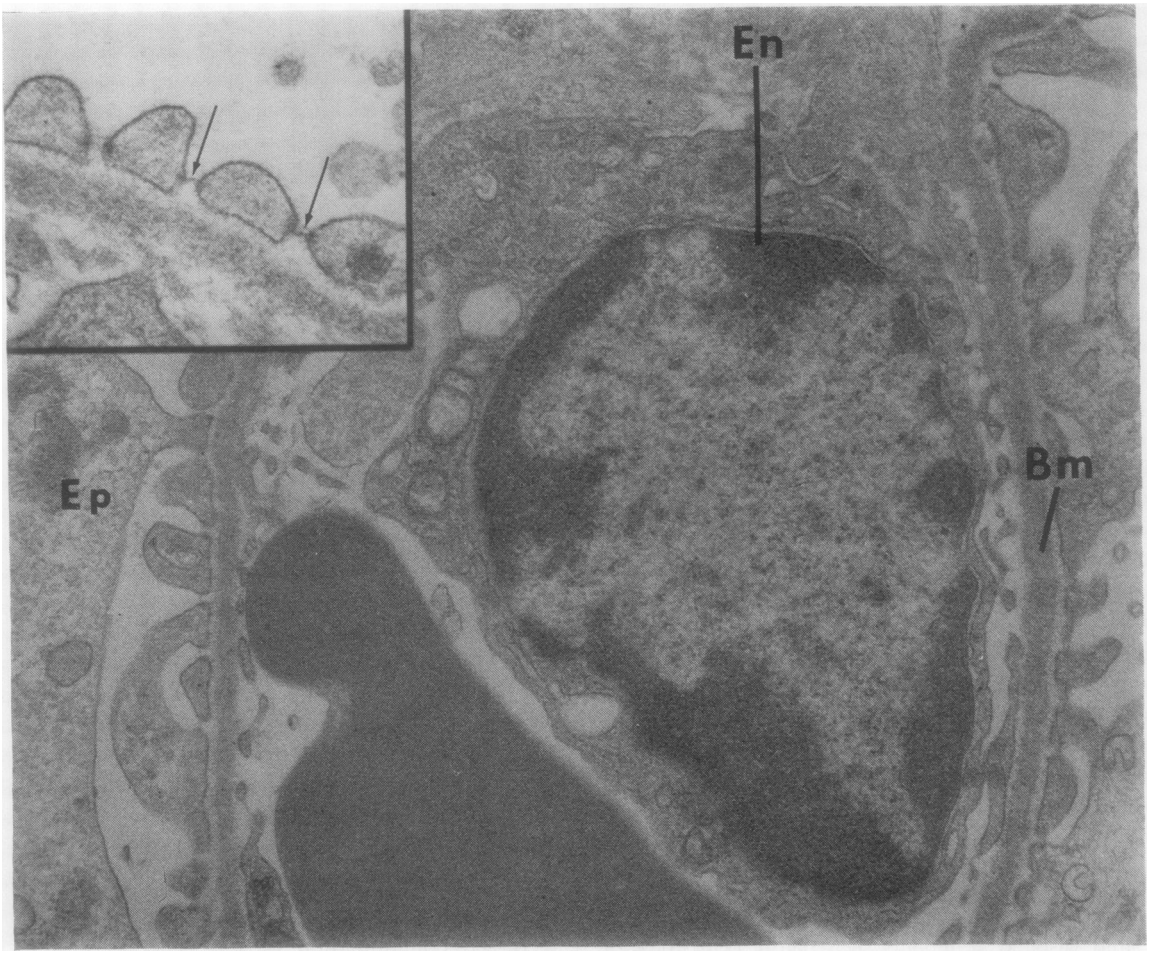

Fig. 2 *Electron microscopic appearance of a purified mink glomerulus. Endothelial cell (En), basement membrane (Bm), and visceral epithelial cell (Ep) show good ultrastructural preservation. Insert: foot processes from a visceral epithelial cell; arrows point to filtration slit membrane.* (× *24 750; insert* × *49 500*)

osmotic changes when the isolated glomeruli were placed into fixative. Electron microscopic examination of glomeruli before purification revealed similar changes in peripheral endothelial cells; however, the changes observed in purified glomeruli were slightly more evident.

This technique has been used with similar results on kidneys from rabbits, guinea pigs, rats, and hamsters. The only laboratory animal for which this procedure did not appear to be suitable was the mouse; yields were low and contamination was high. We tested a wide range of specific gravities but never achieved satisfactory glomerular purification.

The purification procedure was modified slightly for use with human kidney. The initial settling period was found to be unecessary. By eliminating this step, yields were increased without a significant effect on purity. Otherwise the conditions for optimal yield and purity were identical with those for kidneys of laboratory animals. The yield of human glomeruli is shown in the Table. The majority of purified glomeruli were without Bowman's capsules. This was not the result of the sedimentation procedure since glomeruli had a similar appearance before centrifugation through sucrose.

This procedure takes advantage of the high density of glomeruli, which is probably due to their high ratio of blood per gram of tissue. Other techniques that have been described require initial renal perfusion to evacuate the glomeruli of blood and

repeated centrifugation and/or a series of sieving steps (Krakower and Greenspon, 1951; Spiro, 1967; Fong and Drummond, 1968; Gang, 1970; Misra, 1972). Nørgaard described a technique utilizing density gradient centrifugation. Although high purity and ultrastructural preservation were seen, the procedure required renal perfusion with a special medium, and yields of pure glomeruli were relatively low (10 to 20%). The current procedure is simple and rapid and requires neither renal perfusion nor special media or equipment. Because of the ultrastructural preservation and relatively high yield, we think this technique should have wide application in the study of glomerular metabolism and is suitable for both immunological and virological studies.

References

Fong, J. S. C., and Drummond, K. N. (1968). Method for preparation of glomeruli for metabolic studies. *Journal of Laboratory and Clinical Medicine*, **71**, 1034-1039.

Gang, N. F. (1970). A rapid method for the isolation of glomeruli from the human kidney. *American Journal of Clinical Pathology*, **53**, 267-269.

Jørgensen, F. (1967). Electron microscopic studies of normal visceral epithelial cells. *Laboratory Investigation*, **17**, 225-242.

Krakower, C. A., and Greenspon, S. A. (1951). Localization of the nephrotoxic antigen within the isolated renal glomerulus. *Archives of Pathology*, **51**, 629-639.

Latta, H. (1970). The glomerular capillary wall. *Journal of Ultrastructural Research*, **32**, 526-544.

Misra, R. P. (1972). Isolation of glomeruli from mammalian kidneys by graded sieving. *American Journal of Clinical Pathology*, **58**, 135-139.

Nørgaard, J. O. R. (1976). A new method for the isolation of ultrastructurally preserved glomeruli. *Kidney International*, **9**, 278-285.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 208-212.

Spiro, R. G. (1967). Studies on the renal glomerular basement membrane. *Journal of Biological Chemistry*, **242**, 1915-1922.

Requests for reprints to: Dr John L. Portis, Department of Health, Education, and Welfare, Public Health Service, National Institute of Allergy and Infecting Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840, USA.

A modified device for replica plating

KENT CROSSLEY AND KAREN MEAD *Department of Internal Medicine, St Paul-Ramsey Hospital and Medical Center, St Paul, Minnesota, USA*

Replica plating, described 26 years ago by Lederberg and Lederberg (1952), is a standard technique for the selection of bacterial mutants. The method utilises a template covered with sterile cotton velveteen. By pressing the surface of a solid medium on which bacterial colonies are growing against the velveteen and then pressing the velveteen against an uninoculated medium, bacteria may be transferred from surface to surface in a way that retains the spatial orientation of the original colonies.

The principal difficulty with this technique is smudging and loss of colonial identity, which occurs as the velveteen collects moisture from the agar surface (May and Houghton, 1965). Although predrying the plates and increasing the concentration of agar in the medium decreases this problem to some extent, it is difficult to entirely eliminate smudging.

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In the course of some studies designed to determine the nature of aminoglycoside resistance in strains of *Staphylococcus aureus* we encountered significant difficulties with smudging during attempts to replica plate with velveteen. It is likely that modifications in the manufacture of this material during the past quarter century were responsible for these problems. Sources in the fabric industry indicate that the pile thickness, fabric finish, and chemical treatment of velveteens have changed appreciably in recent years.

As a result of the difficulties we encountered with velveteen, we attempted to find a fabric that would be suitable for replica plating but would not become saturated with moisture during the procedure. By replacing velveteen with the 'hook-half' of Velcro