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TECHNICAL NOTE

Preparation of renal papillary collecting duct cells for study *in vitro*

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The mammalian kidney is comprised of a heterogeneous array of tubules and blood vessels. Over the years the function of the individual nephron segments in the production of urine has been reasonably well defined by micropuncture and *in vitro* microperfusion techniques. Beyond this descriptive step, however, to clarify the mechanisms of solute and water transport, it is necessary to study cellular compartments, specifically. Several techniques are used currently for cell studies, but in each the usefulness is limited. For example, thin slices of kidney tissue contain a variety of cell types as do suspensions of kidney cortex [1]. By dissection *in vitro* it is possible to isolate specific segments of nephron, but the mass of tissue is insufficient for the limits of resolution of many analytical techniques [2]. The purpose of this report is to describe a method for collecting milligram quantities of viable cells from the papillary collecting duct of the rabbit kidney.

Methods. Female New Zealand white rabbits (1.5 to 2.5 kg) were fed standard laboratory chow and water prior to the experiment. Each animal was anesthetized with pentobarbital and the kidneys were removed through a midline abdominal incision. A slice was taken along the medial surface with a sharp scalpel, thereby exposing the papilla. The papilla was transected at its base to yield a cone of tissue. The outer layer of epithelium was removed from the papilla by sharp dissection with the scalpel. The papilla was diced into small pieces (about 1 mm square) and placed in 10 ml of incubation medium in a 30-ml siliconized centrifuge tube. The composition of the medium in mmoles/liter was as follows: 150, NaCl; 5, KCl; 10, Na acetate; 1.2, NaH_2PO_4; 25, $NaHCO_3$; 1.0, $CaCl_2$; 1.2, $MgSO_4$; 5, glucose, and 300, urea; to which was added calf serum (Microbiological Associates, Bethesda, MD), 5% by volume, and collagenase (Worthington Biochemical Corp., Freehold, NJ), 2 mg/ml. The solution was agitated gently by bubbling with 5% CO_2 and 95% O_2. All experiments were conducted at room temperature (23 to 26°C).

After 60 min of incubation, the container was centrifuged at low speed and the supernatant was removed and replaced with fresh collagenase medium. The incubation was continued for an additional 120 min on the average. The collagenase caused disruption of the papilla freeing up large collecting ducts, loops of Henle and blood vessels (Fig. 1). The collecting ducts underwent progressive fragmentation with release of clumps of tubule cells which assumed a cup-shaped configuration with the apical (urinary) surface on the convex side (Fig. 2). The suspension was centrifuged at low speed, the supernatant was removed and fresh medium containing no collagenase was added. At this stage individual clumps of papillary collecting duct cells could be removed for selective study. Such a study involving measurement of the deformability of the apical surface has been described previously [3, 4].

To gather relatively large numbers of the collecting duct cells selectively, we transferred aliquots of the crude suspension into a hemispherical well in a glass serological plate. The diameter of the well was 22, and the depth, 8 mm. A jet of CO_2-O_2 was directed parallel to the surface of the solution in a manner to cause the medium to swirl in a vortex. The heavy collections of papillary cells migrated to the bottom of the well in the center, whereas the lighter loops of Henle, blood vessels and interstitial debris remained suspended in the supernatant. The supernatant was removed periodically and replaced with more crude suspension to permit collection of large numbers of papillary cells at the bottom. The cells were harvested and suspended in fresh medium and the vortexing procedure was repeated several times to purify the collection further. In this manner approximately 50

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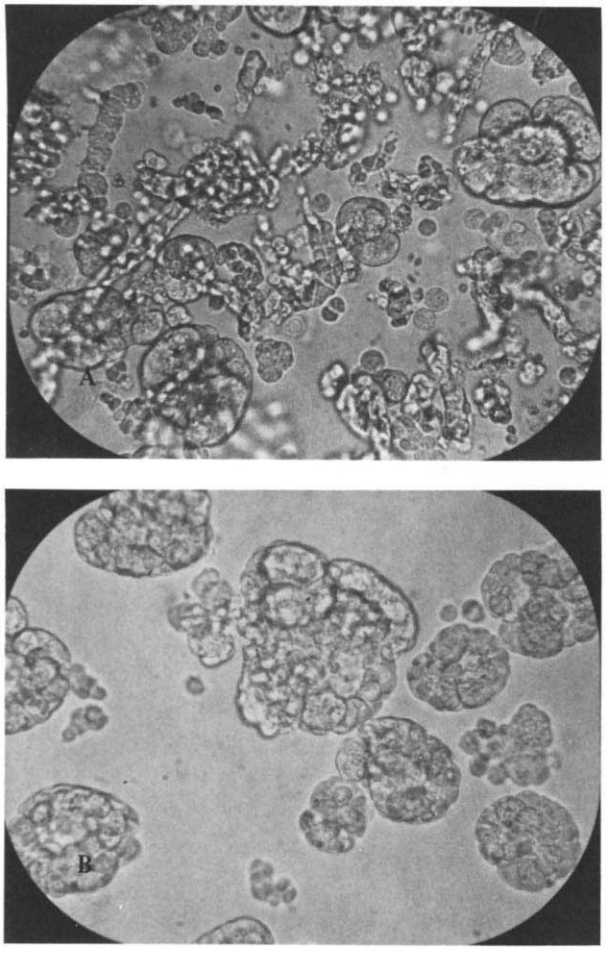

Fig. 1. *Appearance of papilla after incubation in collagenase.* A, The crude suspension is shown, including large clumps of collecting duct cells, segments of Henle's loop and isolated cells. B, Appearance of the preparation after purification by differential vortexing. Large clumps of papillary collecting duct cells are visible. The cells in the clumps are readily identified at higher power (×1000, not shown here) as originating from collecting duct due to their larger size, the basilar location of the nuclei and the paucity of large organelles (mitochondria) in the apical cytoplasm.

to 100 mg of cells was prepared from 200 to 400 mg of fresh papillae of two kidneys. Electron micrographs (kindly performed by Dr. Charles Ganote, Department of Pathology, Northwestern University School of Medicine, Chicago, Illinois) confirmed that *a*) the cells were from the papillary collecting duct, *b*) the cells appeared morphologically normal and *c*) adherent capillaries and loops of Henle were not encapsulated in the cell clumps to a significant extent. In photomicrographs of "clean" suspension of papillary cells at high magnification (× 1000), we estimated from measurements of plain surface area that greater than 90% of the final cellular material was collecting duct epithelium.

In the light microscope the cells appeared healthy (Fig. 1). The nuclei were oriented at the base and the cells maintained their volume and shape for several hours at room temperature. We have not made an extensive study of the effect of different media, or of higher temperatures on the morphologic aspects or function of the cells. It is reasonable to assume, however, that the cells could withstand higher temperatures provided that appropriate nutrients were placed in the medium to support accelerated rates of metabolism.

We measured the cell content of water, sodium and potassium, and the response to ouabain as an indicator of the viability under these conditions of study. To measure water content we added tritiated water (International Chemical and Nuclear Corp., Irvine, CA, 80 μCi/ml) and ^{131}I-albumin (Abbott Laboratories, North Chicago, IL, 10 μCi/ml) to the pure suspension of cells. After equilibration for 15 min, the suspension was sucked into a precision glass capillary (I.D., 1 mm). One end of the capillary was

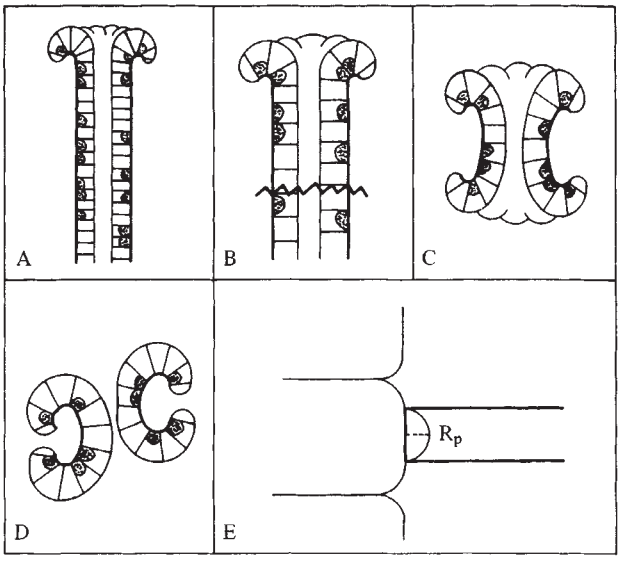

Fig. 2. *Fragmentation of papillary collecting ducts in collagenase.* Early in the course of enzymatic fragmentation in collagenase, relatively long segments of collecting duct are released (A). The ends of the individual ducts roll back (B) exposing the apical surface of the cells. Upon further agitation the ducts break into smaller pieces (C) so that finally the ducts are fragmented into cup-shaped collections of cells with the urinary plasma membrane on the convex surface (D). The apical surface is readily accessible to direct study. As shown diagrammatically (E), the apical membrane can be sucked into a pipet. R_p is the radius of the hemispherical bulge of cell in the deforming pipet (see also [3] and [4]).

Table 1. Electrolyte and water content of isolated papillary collecting duct cells[a]

	EW μl	CV μl	IW $\mu l/\mu l$ *of cells*	K *mEq/liter of cells*	Na *mEq/liter of cells*	K_i *mEq/liter of IW*	Na_i *mEq/liter of IW*
Mean	0.24	0.76	0.59	69	40	98	71
SEM	0.03	0.06	0.03	6	5	11	18
N	(13)	(13)	(3)	(13)	(12)	(3)	(3)

[a]EW, extracellular water; CV, cell volume; IW, intracellular water; K_i and Na_i, intracellular potassium and sodium; *N*, number of different papillae analyzed.

sealed in a flame. The cells were centrifuged in a clinical microhematocrit centrifuge at high speed for ten minutes. The supernatant in the capillary was removed with a fine needle; the capillary was transected 1 mm above the column of cells and the packed cells were extruded into a 50 μl droplet of extracting medium under mineral oil. The extracting fluid contained 0.1M HNO_3, 0.03M $CsNO_3$ and 0.005M NH_4PO_4.

To analyze the total water content of the cells, we determined the tritium activity in aliquots of the extract and the supernatant fluid by methods reported previously [5]. The extracellular fluid contamination of the packed cells was assessed by counting the entire sample of tissue and an aliquot of the supernatant in a gamma detector (Baird-Atomic). Thus total water in the plug of cells (TW) was equal to the following:

$$\text{TW } (\mu l) = \frac{^3H_2O \text{ total (cpm)}}{^3H_2O \text{ supernatant (cpm}/\mu l)}. \quad (1)$$

The volume of extracellular fluid in the plug of cells (EW) was as follows:

$$\text{EW } (\mu l) = \frac{^{131}I \text{ total (cpm)}}{^{131}I \text{ supernatant (cpm}/\mu l)}. \quad (2)$$

Intracellular water (IW) was equal to TW − EW.

The total packed cell volume (TPCV) was computed from the length of the column of cells in the precision glass capillary. The cell volume (CV) of the TPCV was equal to

$$\text{CV} = \text{TPCV} - \text{EW} \quad (3)$$

and IW as a fraction of CV was IW/CV.

The sodium and potassium content was determined in the tissue extract using a helium glow photometer [2]. Intracellular cations were calculated by subtracting from the total amount that which was "trapped" in the extracellular fluid.

Results and discussion. The technique described in this report provides a method for collecting significant quantities of papillary collecting duct cells for detailed study *in vitro*. The composition of the packed cells is given in Table 1. On the average, the packed cell volume of each sample was 1.00 μl, of which 24% was trapped extracellular fluid. In three studies in which intracellular water was measured, the water content was 0.59 $\mu l/\mu l$ of cells. The concentration of potassium in cell water consistently exceeded that of the bath. The intracellular K concentration in three studies was 98 mEq/liter, corresponding to a cell:bath concentration ratio of 19.6:1. The sodium concentration of intracellular water in three studies was 71 mEq/liter, corresponding to a cell:bath concentration of 0.38:1. In three experiments the incubation in control media was extended more than 150 min with maintenance of stable intracellular K and Na concentrations. Thus, the large differences in Na and K concentrations between the intracellular and extracellular fluid compartments indicate that the cells were viable. Moreover, the values compare favorably with those obtained previously in isolated renal tissues studied *in vitro* at room temperature [2].

As a further test of viability, in three studies a portion of the cells was incubated in ouabain (10^{-4}M) for one hour. In these cells the sodium content increased from 29 ± 16 to 87 ± 18 mEq/liter cells and the potassium content decreased from 98 ± 10 to 27 ± 4 mEq/liter cells, lending additional support for the view that in the control state the differences in concentration between the cells and the bath were the consequence of active electrolyte transport.

In the previous studies in which single fragments of collecting ducts from the crude papilla suspension were used, we observed that vasopressin administration increased the deformability of the apical surface of the cells [3]. Thus, the cells are responsive to hormonal stimulation, another reflection of viability.

In summary, significant quantities of collecting duct cells are relatively simple to obtain. This preparation should be a useful adjunct for studies of the mechanisms of action of hormones, specifically vasopressin, and for experiments in which it is important to examine metabolic events in specific types of renal cells.

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