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# Isolation of Glomeruli and Tubular Fragments from Rabbit Kidney

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Summary. A simple, rapid method of obtaining pure glomeruli and tubular fragments from the renal cortex of the rabbit is described. The morphological examination indicates a high degree of purity of the fractions and good preservation of cell structure.

Key words: Tubules, glomeruli, isolation, morphology.

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Many methods have been described for isolating glomeruli and tubular fragments from the renal cortex of different species. Mc Cann (10), Kissane (5), Mattenheimer et al. (9) isolated by microdissection different, precisely defined regions of the nephron. Greenspon & Krakower (6) prepared glomeruli enriched fractions by pressing renal tissue through a sieve. Richterich & Franz (12) used nylon filters to separate glomeruli from the rest of the tissue, whilst Mathieu & Winand (8) used a stainless steel molecular sieve to obtain crude fractions of glomeruli and tubules from which they isolated the basement membranes. Spiro (14) used a similar technique to isolate basement membrane from a crude glomerular fraction. Recently, von Glocker et al. (17) purified glomeruli by fractionating cortex homogenates on a column made of four sieves of decreasing size. Burg & Orloff (2) were the first to use collagenase to obtain renal cell suspensions. Since then, other authors have used this enzyme to disrupt renal tissue: Van Breda, Vriesmand & Willighaven (16) combined this technique with the method of using sieves. Taylor, Price & Robinson (15) fractionated the suspension obtained after incubation with collagenase by density gradient in a zonal rotor. Several authors (1-3-4-11) used collagenase digestion to obtain a suspension of tubular fragments. However, our observations reveal that this suspension inevitably is contaminated with glomeruli.

We devised a method of obtaining, simultaneously and with a good yield, glomeruli and pure tubular fragments from renal cortex which is simpler and gives purer fractions then most of the published methods.

## Material and Methods

### Fractionating of the renal cortex

A rabbit, weighing 1.5-2 kg is killed by the injection of 0.15 mg Nesdonal per kg. The kidneys are removed and decapsulated, and the renal cortex separated from the medulla. The cortex is pulped by forcing it through 1 mm diameter pores in a tissue press (Havard, U.S.A.). The pulp (5 to 8 g wet weight) is incubated for 20 min at 37°C in 100 ml of medium (13) containing 154 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$, sodium potassium phosphate (3 mM, pH 7.4) and 0.1% collagenase, using a gyratory water bath (130 strokes/min). Then the suspension is decanted and filtered through two layers of nylon tissue. The undigested residue is resuspended in 100 ml of fresh collagenase solution in the same buffer and incubated for another 20 minutes. This procedure is repeated until the kidney pulp is completely dissociated (usually 2 x 20 min incubations). The suspension is centrifuged for one minute at 150 g. The pellet is resuspended in the medium without collagenase, centrifuged once more for the same length of time and at the same speed and the supernatant discarded. This process is repeated once.

The pellet resuspended in medium is placed on a discontinuous sucrose gradient (150-200 mg pro-

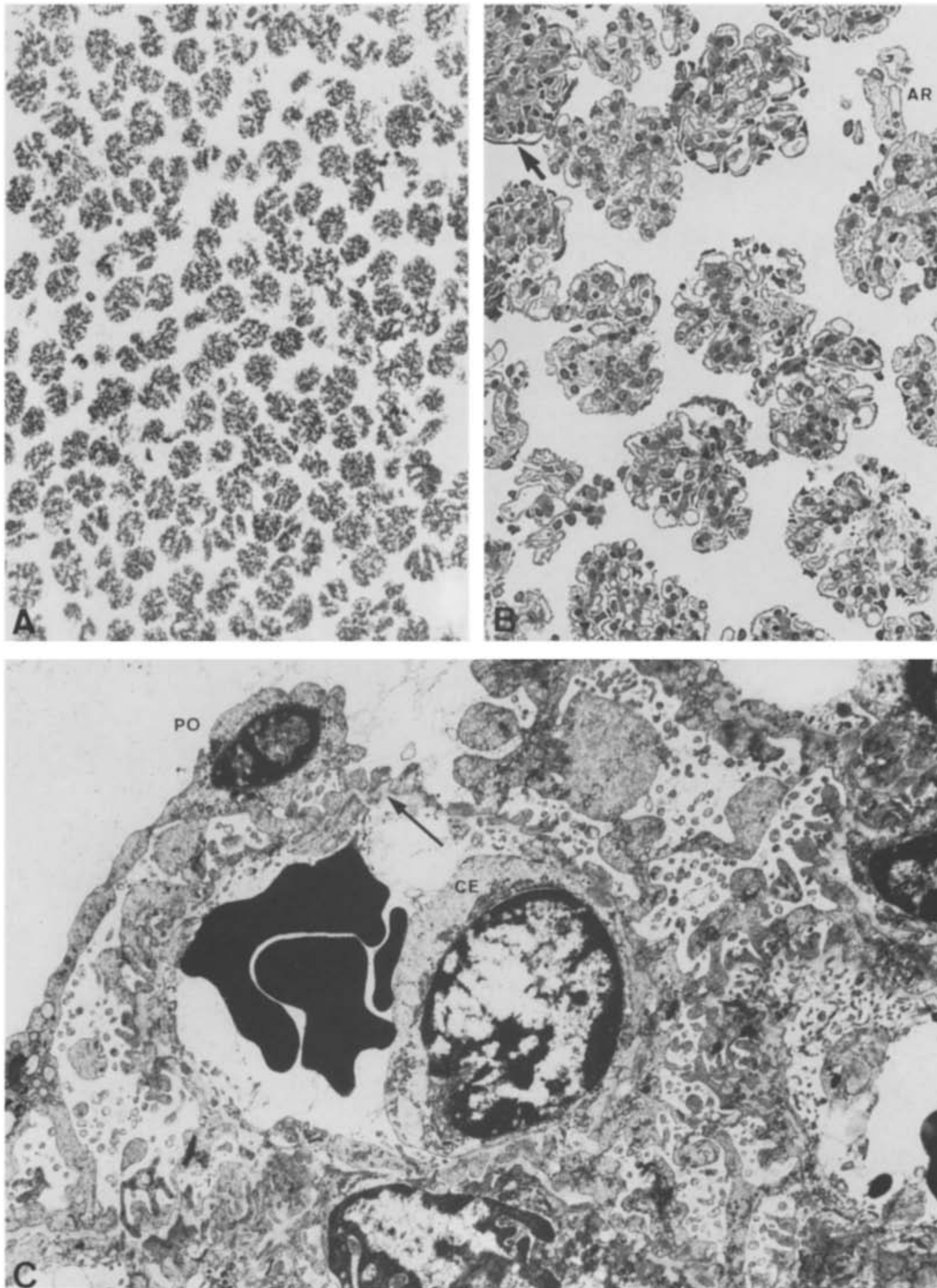


Fig. 1. Glomerular fraction. A) Appearance of the preparation after the material has been spread out and fixed between two glass slides; stained by Glychemalin-eosin. Magnification = x 150. B) Semi-thin slice. Ar : arteriole in relation to a glomeruli. Arrow : fragment of Bowmann's capsule. Stained by Hematoxylin of Harris-Eosin. Magnification = x 1060. C) Ultra-thin slice. Note the presence of the basement membrane (arrow). P0 : podocyte. CE : endothelial cell. Magnification = x 6000

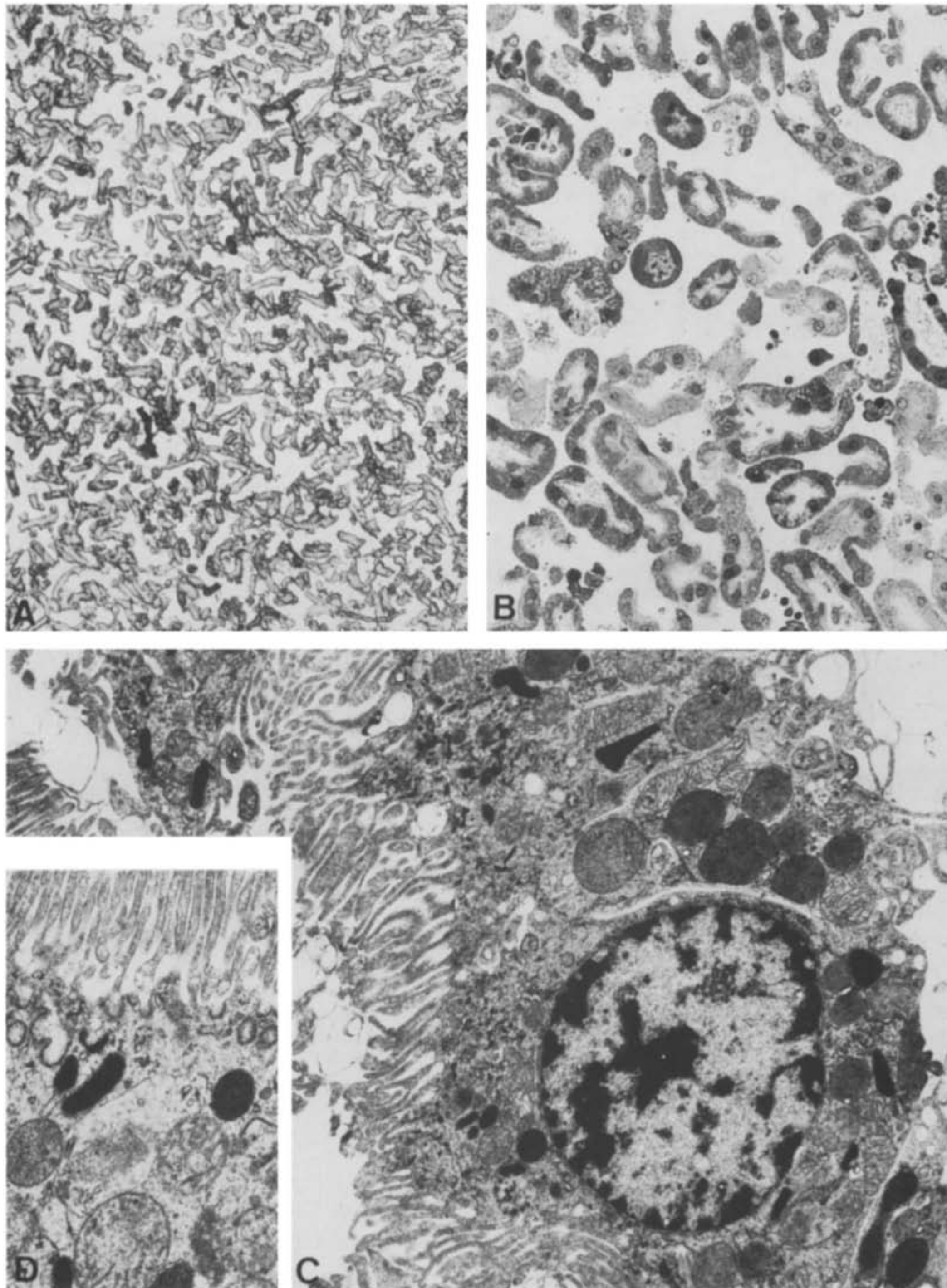


Fig. 2. Tubular fraction. A) Appearance of the preparation after the material has been spread out and fixed between glass slides; stained by Glychemalin-eosin. Magnification = x 90. B) Semi-thin slice. Stained by Hematoxylin of Harris-Eosin. Magnification = x 1060. C) Ultra-thin slice. Ultrastructural appearance of a proximal convoluted tubule fragment. Fine structure identical to that of the "in situ" tubule. Magnification = x 10,000. D) Detail of the apical of a proximal convoluted tubule cell. Magnification = x 18,000

tein per gradient) made up of three sucrose layers : 8 ml 75%, 30 ml 58.6%, 15 ml 8.56%. After being centrifuged for 16 minutes at 5000 g in a MSE Mistral 6 L centrifuge (4500 rpm), the purified glomeruli are localized on the interface of the 75- and 58.6% layers whilst the purified tubular fragments are stopped on the interface of the 58.6- and 8.56% layers. The fragments are collected by piercing the tubes from the bottom. A few rare glomeruli could still be present at the level of the tubular fraction. Therefore, the tubules are collected together with the layer of 58.6% sucrose and recentrifuged under the same conditions. The purified tubular fraction is then collected with the help of a pipette whilst the residual glomeruli coat the bottom of the tube.

Proteins are determined by the method of Lowry et al. (7).

## Morphology

The tubular and glomerular fractions are collected and fixed with 5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4). This suspension is centrifuged at 20,000 rpm for 15 minutes. The dense pellets obtained are fragmentated and postfixed in 1% osmic acid. Inclusion is made in "Araldite"; the ultra-thin slices are contrasted with lead uranyl-citrate acetate and observed under a Siemens Elmiskop E 1 microscope. The semi-thin slices obtained from the same material are stained with Hematoxylin of Harris-Eosin.

## Results and Discussion

Our procedure of time-fractioned collagenase digestion permits a considerable increase in yield. On the average, 300 mg of tubular protein and 10 mg of glomerular protein are recovered for one gramme of protein present in the cortical homogenate - an overall yield of 30% to 35%. The yield is only 8% when one does a single 40- to 60 min treatment with collagenase. This low yield is accounted for by the presence, in high proportion, of isolated cells in the suspension which do not sediment with the tubular or glomerular fractions. Timefractioned incubation allows the recovery of glomeruli and tubules progressively as the tissue is dissociated and therefore limits the production of isolated cells.

Observation of the fractions under the optic microscope shows that the very simple discontinuous gradient we used permitted us to obtain pure material devoid of contamination of one fraction by the other (fig. 1A and 2A). Moreover, these fractions contain only a very few isolated cells.

The glomerular fraction is essentially made up of tuft capillaries (Fig. 1B). Numerous glomeruli have kept a more or less long fragment of their pre-glomerular arterioles. Bowmann's capsule, rarely present, is found only in a residual state around some glomeruli. Ultrastructural study reveals the presence of podocytes, endothelial cells and mesangial cells. The spatial organization of these three types of cells conforms with that of the "in situ" glomeruli, especially as far as podocyte-capillary relations are concerned (Fig. 1C). The basement membrane appears normal in spite of collagenase treatment. The podocyte digitations probably assure effective protection of the basement membrane. We observed however, that few endothelial cells were not well preserved after passage in hypertonic medium.

The tubular fraction is essentially made up of fragments of the proximal convoluted tubule that are distinguished from other regions of the nephron by their brush border (Fig. 2B). The proximal convoluted tubule constitutes, in effect, approximately 70% of the cortex mass in the rabbit (1). The distal tube is only barely represented and may be recognized on the transversal semi-fine slices by its diameter which is smaller than that of the proximal convoluted tubule and by the absence of a brush border. Here again, the structural integrity is well preserved if one considers the treatment undergone during isolation. Ultrastructural study confirms the predominant presence of the proximal convoluted tubule. From a cytological point of view, it is suitable to note that the base pole of the cells has a digitated appearance, probably due to the loss of basement membrance after treatment with collagenase (Fig. 2C). The wealth in vesicules and tubules of pinocytotic origin of the apical part of the cells indicates the still functional nature of the fraction (Fig. 2D). We may nevertheless point out the presence of cell debris probably produced during the passage of the renal cortex in the "Tissue Press".

The simplicity of the technique put into operation and the purity of the tubular and glomerular fractions that we obtain allows us to take up the study of numerous physiological phenomena, localizing them with certitude, such as the metabolic functions of the tubules and glomeruli and problems of antigenicity. Moreover, certain enzymes can be established as biochemical markers for the fractions - for example, alkaline phosphatase or 5'-mononucleotidase for the tubules and guanylcyclase for the glomeruli (in preparation).

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