Further Information on the Cortical Countercurrent System in Rat Kidney

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Recently we reported the existence of a cortical countercurrent system on the surface of the rat kidney(1). The countercurrent system is arranged in such a way that proximal tubular fluid and efferent peritubular blood flow in opposite directions. The following findings may be cited in support of this thesis.

1. The superficial nephrons of the rat kidney are characterized by an efferent arteriole which generally runs at a right angle to the surface of the kidney and branches only after having reached the renal surface. Usually this vessel separates into three to four branches. Pictures of casts of such long efferent arterioles were published as early as 1955 by Wirz(2). We term this branching point of the efferent arteriole the welling point because it is possible to observe by means of high-frequency microcinematography the appearance of the flow of erythrocytes from deeper regions of the kidney. Beginning with the three to four branches of the welling point the more peripheral capillary bed branches repeatedly and finally is collected into the renal venous tree in close proximity of the efferent arteriole. The morphological situation is described by means of a typical cast shown in Fig. 1.

2. We could demonstrate by intravital microphotography and the use of lissamine green that proximal tubular urine (from glomerulus to pars recta) frequently flows in the opposite direction from the capillary blood flow which originates from the welling point. Stated differently, proximal tubular loops situated close to the glomerulus are generally found at a greater distance from the welling point. Inversely, tubular loops at a greater distance from the glomerulus are generally close to the welling point.

We are reporting two new techniques which have been found useful for the examination of this countercurrent system:

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Fig. 1. Afferent arteriole, efferent arteriole, and proximal convolution of a superficial rat nephron. The drawing is based on a cast preparation (from Ref. 1). The tubular flow direction is shown by arrows and marked alphabetically. Venous vessel is not shown here. It runs parallel to the afferent arteriole and with tributaries joining close to the glomerulus.

1. High-frequency microcinematography of the renal surface, a method which has made it possible to measure the directional flow and the volume flow rate in peritubular capillaries, and

2. Direct fluorescence microscopy which makes it possible to study a functional relationship between vascular and renal tubular system.

METHODS

For our studies Wistar rats of both sexes weighing about 300 g were used. After inducing anesthesia by Inactin, the trachea, jugular vein, carotide artery, and the ureter was cannulated, the left kidney was exposed by a flank incision(1,3). The kidney was bathed with warm Tyrode’s solution or liquid parafin. High-frequency cinematography was carried out using an Ultropak system, vertical illuminators or the Fluopak system with xenon illumination (Leitz). A HYCAM-16-mm camera was used. The speed of the camera was 400–1000 frames/sec. A number of representative photo micrographs have been published(4). For

1 This movie was shown during the presentation at the Workshop. It can be obtained from the “Institut für den Wissenschaftlichen Film, 34 Göttingen, Nonnenstieg 72, Germany” under the number E 1679.
a second series of experiments fluorescence microscopy of the renal surface was carried out using the Fluopak system and immersion objectives. Again illumination was achieved by xenon light. Filters were used that were appropriate for the excitation and emission maxima of the fluorescent dyes used. For acriflavin the excitation maximum is close to 450 m\(\mu\) and the emission maximum close to 510 m\(\mu\). This dye was used in a dilution of 1:10,000 in saline and administered at a rate of 0.2 ml/min into the jugular vein. Single-frame microphotographs were taken by means of a robot camera with exposure times of several seconds. For slow-motion cinematography frequencies of one frame/sec were used.\(^2\)

RESULTS AND DISCUSSION

Table 1 provides a summary of vascular diameters, velocities of perfusion, and calculated volume flow rates as obtained by high-frequency microcinematography of the branching points on the renal surface. For technical reasons only the first four branches of each welling point have been studied but in principle similar calculations may be applied to more peripheral branching. We have previously shown by means of casts that each nephron on the renal surface of rats is supplied preferentially by its own efferent arteriole. This finding has been recently confirmed by Faarup(5). Similar observations on superficial nephrons of the dog kidney have been made by Beeuwkes(6).

If the calculated volume flow rate in the efferent arteriole is multiplied by the number of nephrons (32,800)(3) the total renal blood flow can be obtained. Values thus derived are quite similar to blood flow values calculated from PAH-clearances and hematocrits.

Observation of the renal surface during the continuous infusion of acriflavin shows that the dye, as judged by the fluorescence, moves slowly from the welling point toward the tubular system. The fluorescence is first seen around those tubular loops which are situated closest to the branching points. Figure 2 shows the distribution of fluorescence during acriflavin infusion. If the infusion is continued, fluorescent patches increase in size but nevertheless, as shown in Fig. 2, one continuously observes a concentration gradient of fluorescence from the welling points toward the periphery. Similar results are generally obtained after the use of other basic acridine dyes but these findings are modified by the additional staining of nuclei, etc.

It is also possible to localize the relative position of tubular segments by the injection of lissamine green(1,3). In such experiments it is possible to ascertain that tubular segments that show fluorescence first are the last to fill with lissamine green. Accordingly they may safely be identified as terminal proximal tubular loops.

It is reasonable to conclude from these experiments that substances that leave the efferent arteriole will be handled in a similar way and maintain similar con-

\(^2\)During the presentation of these lectures the fluorescence microscopic movie of the renal surface was demonstrated.
Fig. 2. Fluorescence microphotograph of the renal surface of a rat 7 min after starting the intravenous infusion of a solution of acriflavine (1:10,000, 0.2 ml/min). The light areas show the fluorescence of the dye surrounding the black welling point. The latter is not stained.

### TABLE 1

**SUMMARY OF FLOW VELOCITIES AND VOLUME FLOW RATES**

| Vascular segment   | Diameter (μ) | Flow velocity (mm·sec⁻¹) | Volume flow rate (nl·min⁻¹) | Number of Animals | Vasc. segments | Measurements |
|--------------------|--------------|---------------------------|----------------------------|-------------------|----------------|--------------|
| Welling point      | 23.00 ± 1.73 | 4.11 ± 0.59a              | 102.53 ± 14.74b             | 9                 | 12             | 12c          |
| First branching point | 15.91 ± 0.92 | 2.32 ± 0.24               | 28.80 ± 4.14                | 9                 | 27             | 133d         |
| Second branching point | 14.48 ± 0.64 | 1.61 ± 0.20               | 16.62 ± 1.14                | 6                 | 10             | 53d          |
| Third branching point | 12.78 ± 0.25 | 1.51 ± 0.26               | 11.64 ± 2.16                | 4                 | 7              | 20d          |

* Calculated from volume flow rate and diameter.
* Calculated by multiplication of the volume flow rates at the first branching point with the mean branching factor at the welling point (3.56±0, 19; n=9).
* Vessel diameter.
* Flow velocity.
* *= P < 0.001.  ** *= P < 0.01.  *** *= P < 0.05.
concentration gradients as acriflavine. This is shown in a schematic simplification in Fig. 3, where the distribution of concentration gradients are shown within the countercurrent flow system. On the left side of the diagram the glomerulus at the beginning of the efferent arteriole is shown whereas on the right side the branching point (welling point) of the capillaries is indicated. Based on the distribution of fluorescent dyes it is likely that substances secreted by the kidney reach the late proximal part of the nephron in higher concentrations than the early tubular segments located close to the glomerulus. This is shown schemati-

**Model of Proximal Tubular Secretion**

![Model of Proximal Tubular Secretion](image)

**Model of Proximal Tubular Reabsorption**

![Model of Proximal Tubular Reabsorption](image)

**Fig. 3.** Model of a possible arrangement of the cortical countercurrent flows including possible concentration gradients along the tubule and the capillaries.
cally by the broken line extending from the welling point to the glomerulus. Some of the secreted substances are also filtered and carried downstream within the tubule. Accordingly, their concentration within the lumen may increase toward the branching point on the kidney surface (solid line). For certain secretory mechanisms such an arrangement may be useful because it assures that the concentration gradient between vascular and tubular system will be maintained at optimal levels. Thus, if back diffusion of a secreted substance were to occur, the driving force would be minimized by the intratubular concentration increase. If the process of secretion is modulated by concentration gradients the described countercurrent system may be useful. On the other hand, close to the glomerulus both the concentration within the tubule and within the capillaries would be low, again assuring the maintenance of a minimal concentration difference between the tubular and vascular system.

As shown in the lower part of Fig. 3 with respect to reabsorptive transport processes, opposite conditions would be maintained. Again, the countercurrent system would assure an optimal efficiency of the reabsorptive process provided that its efficiency depends on back diffusion. The latter would be minimized by the establishment of increasingly steeper transepithelial concentration differences as the fluid passes along the proximal tubule.

In summary, two new methods, high-frequency microcinematography of the peritubular blood flow and intravital fluorescence microcinematography have been used to study the cortical countercurrent system in the rat kidney. The possible physiological role of this unique arrangement of flow between tubular and vascular system has been discussed.

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