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Countercurrent System in the Renal Cortex of Rats

Abstract. *Tubules on the surface of the renal cortex are intertwined with the capillaries. Micropuncture experiments on rats show that generally the flow of tubular fluid is against the flow of blood in the peritubular capillaries. A countercurrent flow system, therefore, exists not only in the renal papilla but also in the superficial cortex.*

Although little doubt remains today about the existence of the countercurrent system in the renal papilla (*1*), its physiological mechanisms are still under discussion. Recent work indicates a countercurrent system also exists in the superficial renal cortex of rats (*2*). Here, in contrast to the papilla, the countercurrent system involves tubular (urine) flow and peritubular (blood) flow. We report our results of studies concerning that system. In order to demonstrate it, we combined incident-light photomicrography, micropuncture techniques, and the Lissamine green (LG) method as previously described (*3*). We used male Wistar rats (body weight, 250 to 300 g) which we anesthetized with Inactin (sodium pentobarbital). The decapsulated kidney was rinsed with physiological saline or mineral oil at body temperature.

In an arbitrary field viewed under the microscope (see Fig. 1) we first photo-

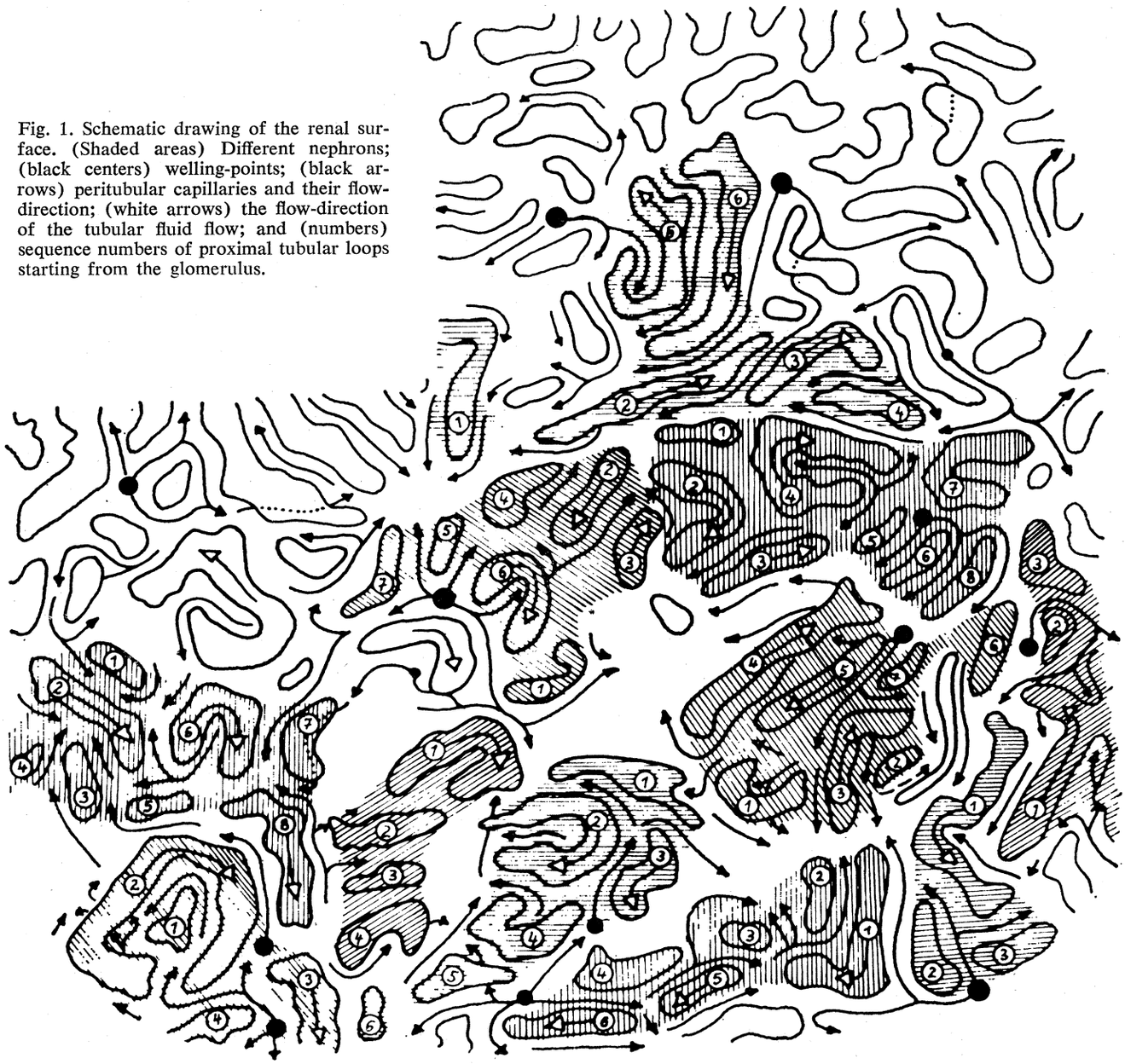

Fig. 1. Schematic drawing of the renal surface. (Shaded areas) Different nephrons; (black centers) welling-points; (black arrows) peritubular capillaries and their flow-direction; (white arrows) the flow-direction of the tubular fluid flow; and (numbers) sequence numbers of proximal tubular loops starting from the glomerulus.

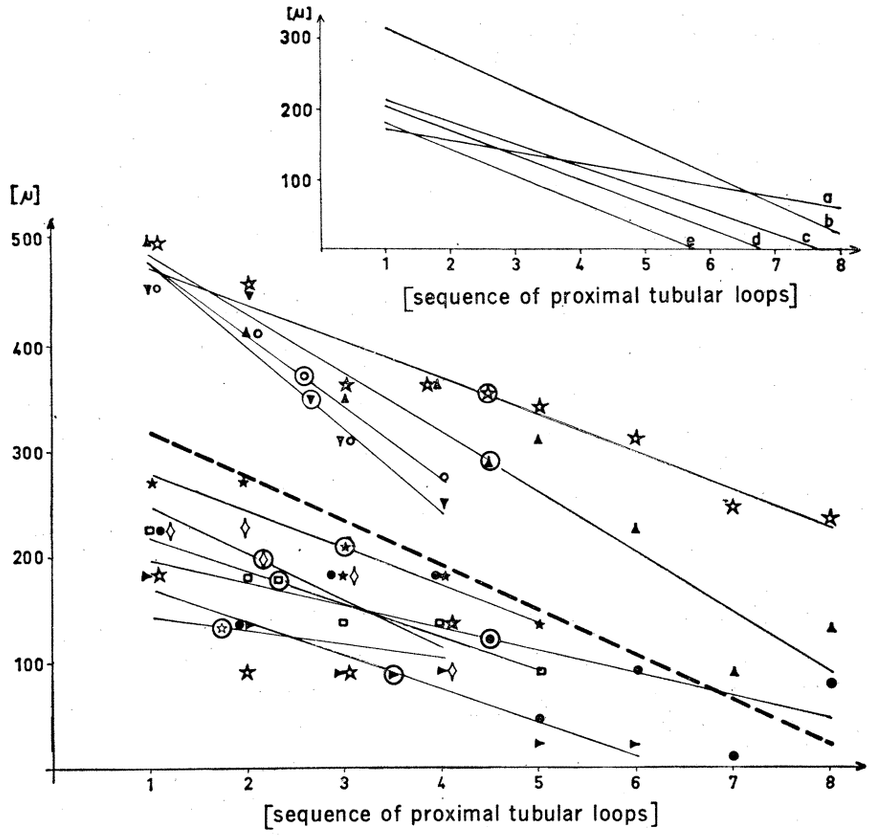

Fig. 2. (Lower part) Distance of tubular loops to their welling-points measured in an arbitrary field viewed under the microscope (compare Fig. 1) on the renal surface of a rat weighing 300 g. (Ordinate) Average distance, in microns, of each tubular loop from its welling-point. (Abscissa) Sequence number of proximal tubular loops. Each linear correlation line represents the data obtained from a single nephron. The encircled symbols mark the relation of the corresponding symbols to their correlation line. The broken line represents the average of the slopes of all correlation lines obtained from one animal. (Upper part) Lines of slope-averages obtained from correlation lines of five animals (*a* to *e*). These straight lines correspond to the broken line in the lower part of the figure. Line *b* is identical with the broken line. The average linear coefficient of correlation of these five average slope lines is 0.81 ± 0.05. The data were obtained from 43 nephrons from five animals.

graphed the passage of LG through all proximal and distal tubules with a sequence of 1 second. In the same field, each nephron was then photographed after it was filled by micropuncture with castor oil stained with Sudan black or with a 2 percent solution of LG. Thus, the sequence of the proximal tubular loops of each nephron was determined by following the passage of LG. The results are shown schematically in Fig. 1.

A proximal tubular loop numbered "1" means that this loop was located closest to its glomerulus, whereas a higher number indicates a more distal part of the proximal convolution. The different nephrons of Fig. 1 were marked by shaded areas. We determined how each nephron was spatially related to branching points of the vasa efferentia (*3*), which we called "welling-points" ("Quellpunkt"). In addition, we drew on the photomicrographs the direction of blood flow in the peritubular capillaries. With a 45° illuminator (incident light directed from the side), the direction of blood flow was determined by following the movement of bright uplighting particles corresponding to leukocytes in peritubular capillaries. Initially, we thought one welling-point supplies several nephrons (*2*) but further investigations indicated that each nephron has its own welling-point.

Measurements of the distance between the different loops and their welling-point disclosed that the loops with low numbers (the most proximal segments) were farthest from their welling-point, and vice versa (see Fig. 2). From these results we concluded that the tubular fluid flowed in a direction opposite to that of the peritubular blood. This is in agreement with in vivo observations of the passage of LG and results obtained by microdissections, in which it was shown that the proximal convolutions converge in groups before they descend below the surface of the kidney and that 52 to 65 percent of the total proximal tubular length surrounded efferent arterioles in 95 percent of the cases (*4*, *5*).

We confirmed these results by determining the direction of flow through each tubular loop, and we compared the directions obtained with the flow-direction of its peritubular capillary. Among 131 observations we found a countercurrent flow in 72.5 percent, whereas only in 10.5 percent the direction of tubular urine flow and peritubular blood flow was the same. In 17.0 percent no predominant direction of flow could be detected.

In order to trace the course of the vasa efferentia in the superficial renal cortex, we filled the renal arterial system with Latex injection compound by tubing the renal artery with a polyethylene catheter. The single nephrons on the cortex were filled also with Latex injection compound by micropuncture. Then we macerated the kidney. From direct observations and photomicrographs of the casts made before and after the casting and during maceration we were able to devise Fig. 3. As a rule, we found that (i) each convolution is supplied by its own vas efferens and represents, therefore, an independent unit, and (ii) each vas efferens passes to the loop of the proximal convolution farthest from the glomerulus. There it branches in such a way that the current of the capillary blood flow is directed against the flow of the tubular fluid.

We should stress here, however, that communications exist between the "vascular units." That is, single loops of a nephron may be supplied by peritubular capillaries from other welling-points, even under physiological conditions.

The physiological purpose of such a cortical countercurrent system would seem to be to maintain a gradient of pressure between tubular and vascular systems along the proximal convolution. This model will be discussed elsewhere (*6*), showing the interaction between intracapillary hydrostatic pressure, interstitial hydrostatic pressure, intracapillary colloidal osmotic pressure, and interstitial colloidal osmotic pressure. If the proximal tubular fluid reabsorption is peritubularly controlled (*5*, *7*), such a

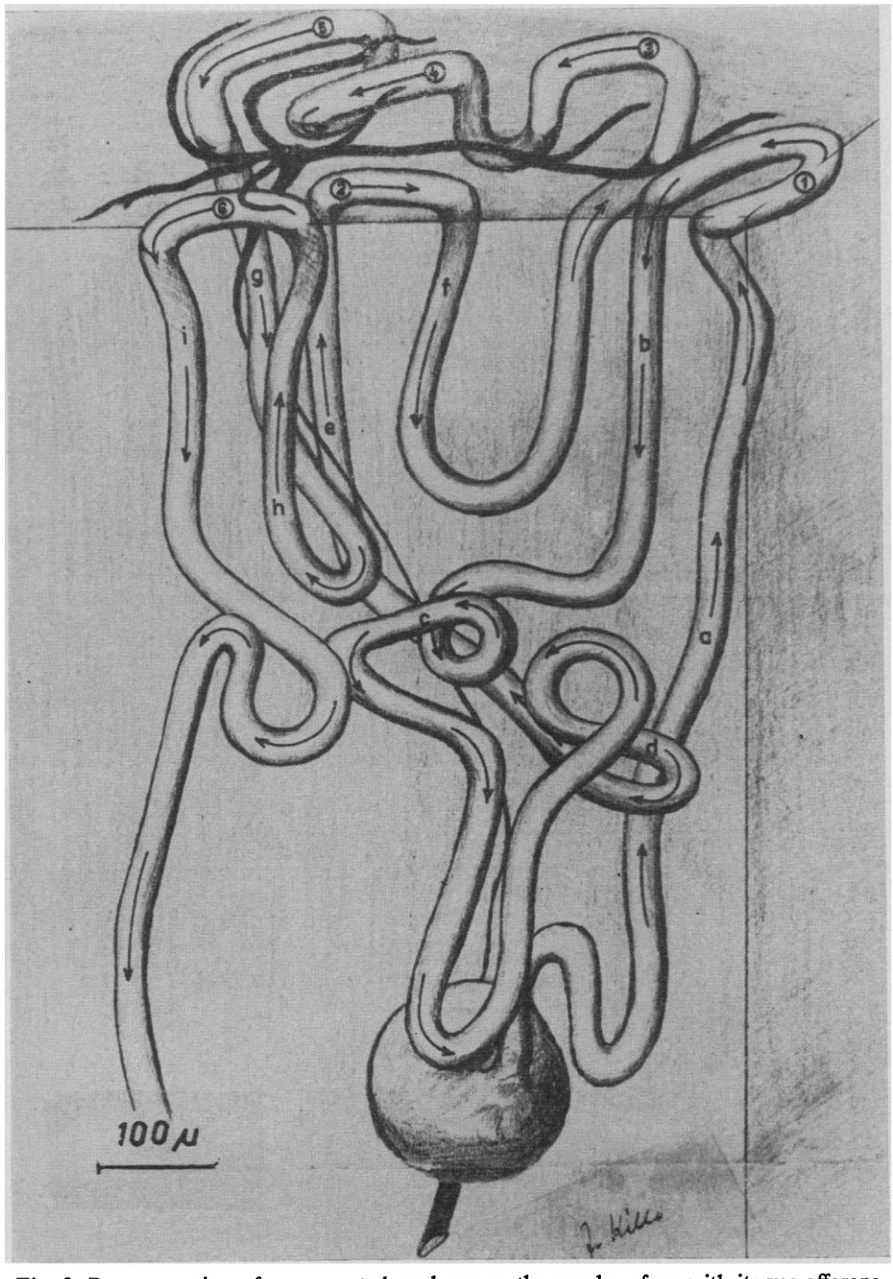

Fig. 3. Reconstruction of a macerated nephron on the renal surface with its vas afferens, glomerulus, and vas efferens after filling the arterial system and the proximal convolution with Latex. (Numbers) Number sequence of proximal loops; (letters) sequence of proximal tubular loops of the whole nephron; and (arrows) flow-direction of tubular fluid flow.

model might be useful in explaining the mechanism of the glomerular tubular balance, or, in a more detailed view, the interaction of glomerular filtration rate and the intracapillary reabsorptive capacity for the reabsorbate actively transported by proximal tubules.

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Plant-Pathogenic Mycoplasma-Like Organism: Maintenance in vitro and Transmission to Zea mays L.

Abstract. *Mycoplasma-like bodies, isolated from corn plants that were infected with Rio Grande strain of corn stunt, were maintained in an artificial cell-free medium. The agent apparently multiplied. Leafhopper vectors that were injected with the pure cultures transmitted the agent to healthy corn plants and induced the corn stunt disease.*

Japanese workers (*1*) recently proposed that yellow diseases of plants may be caused by mycoplasma-like organisms. Their evidence was based on (i) the presence of mycoplasma-like bodies in the phloem elements of infected plants and their absence in healthy plants, (ii) the therapeutic effect of tetracycline antibiotics on infected plants, and (iii) the disappearance of the mycoplasma-like bodies from the phloem of plants treated with tetracyclines. In the past 2 years, mycoplasma-like organisms have been reported as the probable etiological agents of at least 24 plant diseases (*2*, *3*).

Corn stunt, a yellows-type disease affecting corn *Zea mays* L. and teosinte *Euchlaena mexicana* L., is transmitted by several species of leafhoppers (*4*). Before 1968 the causal agent of corn stunt was considered to be a virus (*5*), but mycoplasma-like bodies have been found in both diseased corn plants and in inoculative insect vectors (*2*, *6*). Further evidence supporting the mycoplasma hypothesis of etiology came from results of chemotherapy of diseased corn plants and from blockage of vector transmission by the application of tetracycline antibiotics (*7*).

Maintenance of the agent in pure culture must be achieved before the hypothesis can be fully tested by Koch's postulates. Hampton *et al.* have reported the purification and growth in artificial medium of a mycoplasma isolated from infected pea plants *Pisum sativum*, but their results did not clearly state how Koch's postulates were fulfilled (*8*). Evidently, the tests of infectivity were conducted with a purified preparation from infected plants and not with the pure mycoplasma cultures.

We now report maintenance in vitro and evidence of growth in cell-free medium of a plant pathogenic mycoplasma-like organism, the corn stunt agent. Its original isolation, cultivation