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Newer micropuncture studies on sodium transport[1]

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The study of the tubular transport of sodium in single mammalian nephrons yields information on the contribution of various tubular segments to the urinary excretion pattern under a wide variety of experimental conditions. Furthermore, these studies permit a direct evaluation of some cellular mechanisms of transtubular sodium movement, particularly with regard to the electrochemical driving forces involved in the transfer across the tubular cell membranes. Also, the simultaneous measurement of both sodium and potassium concentrations along the nephron, and the topography of the net movement of each has provided some insight into their mutual interaction with respect to transtubular net movements.

Some general features of tubular sodium transport and some differences in the behavior of various nephron segments will be considered first. Fig. 1 summarizes the data of a micropuncture study in non-diuretic rats [1]. The concentration of sodium was measured by ultramicroflame-photometry, and compared with that in plasma water. Tubular fluid/plasma concentration ratios (TF/P ratios) are plotted as function of proximal and distal tubular length. Ureteral urine/plasma ratios (U/P ratios) are also included. Inspection of proximal data indicates that no concentration gradients are established across this nephron segment, an observation in agreement with the isotonic nature of proximal tubular fluid. At the beginning of the distal tubule the concentration of sodium is generally variable but significantly less than that of plasma, as indicated here by TF/P ratios of less than unity. There is a trend for the sodium concentration in distal tubular fluid to fall as the distal nephron segment is traversed, and it is the later part of the distal tubular epithelium which establishes the steepest gradients within this nephron part. The behavior of the collecting duct appears variable under these experimental conditions: the concentration of sodium may be either somewhat higher or lower than that in late distal tubular samples, depending on the relative rates of water movement. Also, some caution

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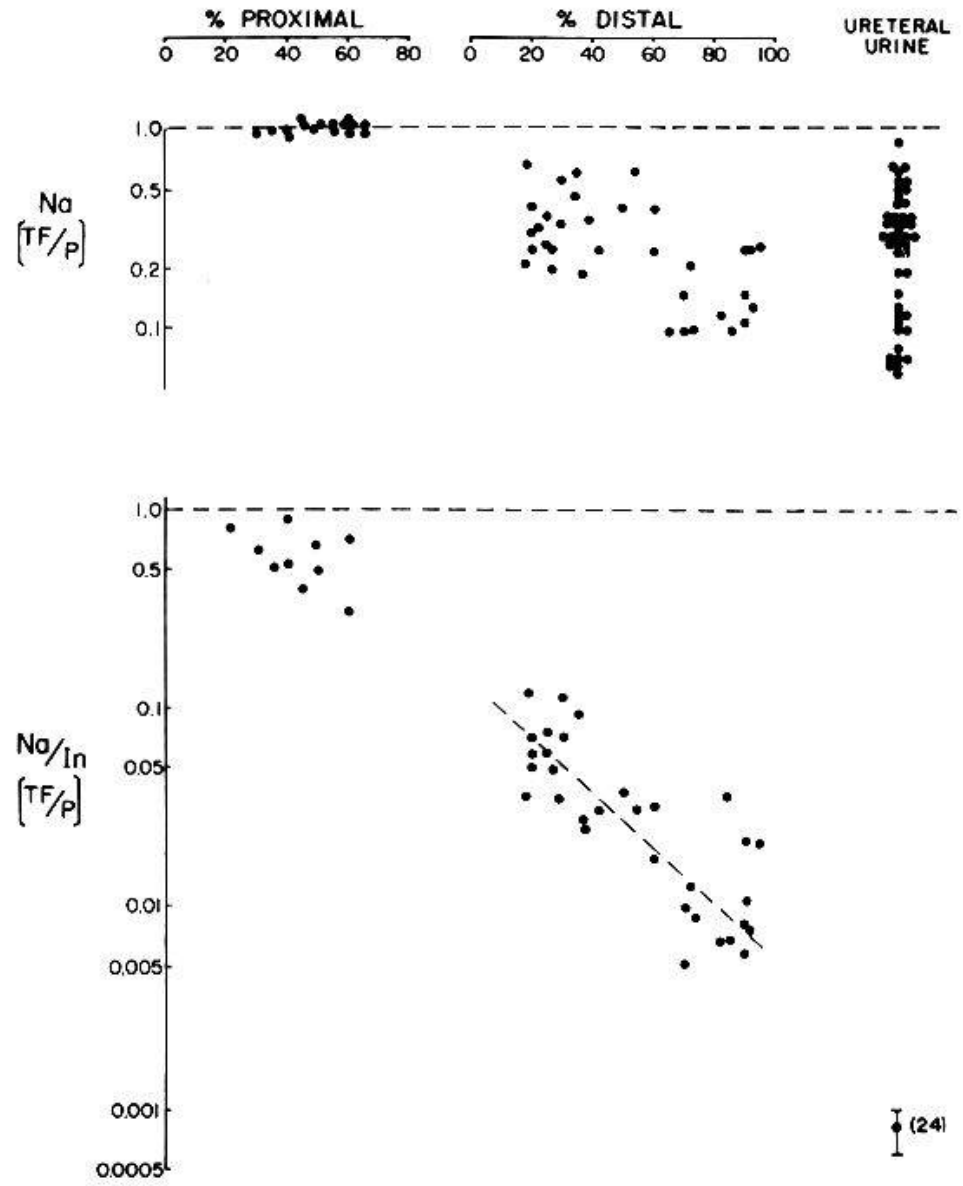

Fig. 1. Summary of sodium and sodium-to-inulin concentration ratios from rats on a control diet. – Upper: Tubular fluid-to-plasma concentration ratios of sodium as function of tubular length. – Lower: Sodium-to-inulin concentration ratios as function of tubular length. – From Amer. J. Physiol. *211*, 532 (1966).

must be exercised when comparing late distal tubular samples of superficial distal tubules with ureteral urine, the latter being a confluent of long *and* short nephrons.

In the lower section of Fig. 1, the magnitude of the segmental reabsorption of sodium is summarized. This quantity is obtained by dividing sodium tubular fluid/plasma ratios by the corresponding inulin ratios, obtained in the same experiments. Thus, the fraction of filtered sodium remaining in the tubular lumen is plotted as function of nephron length, a downward slope representing net reabsorption. Inspection of the data obtained under normal antidiuretic conditions shows a large fraction, some two-thirds, of the filtered sodium being reabsorbed along the proximal convoluted tubule. A comparison of late proximal with early distal data indicates the reabsorption

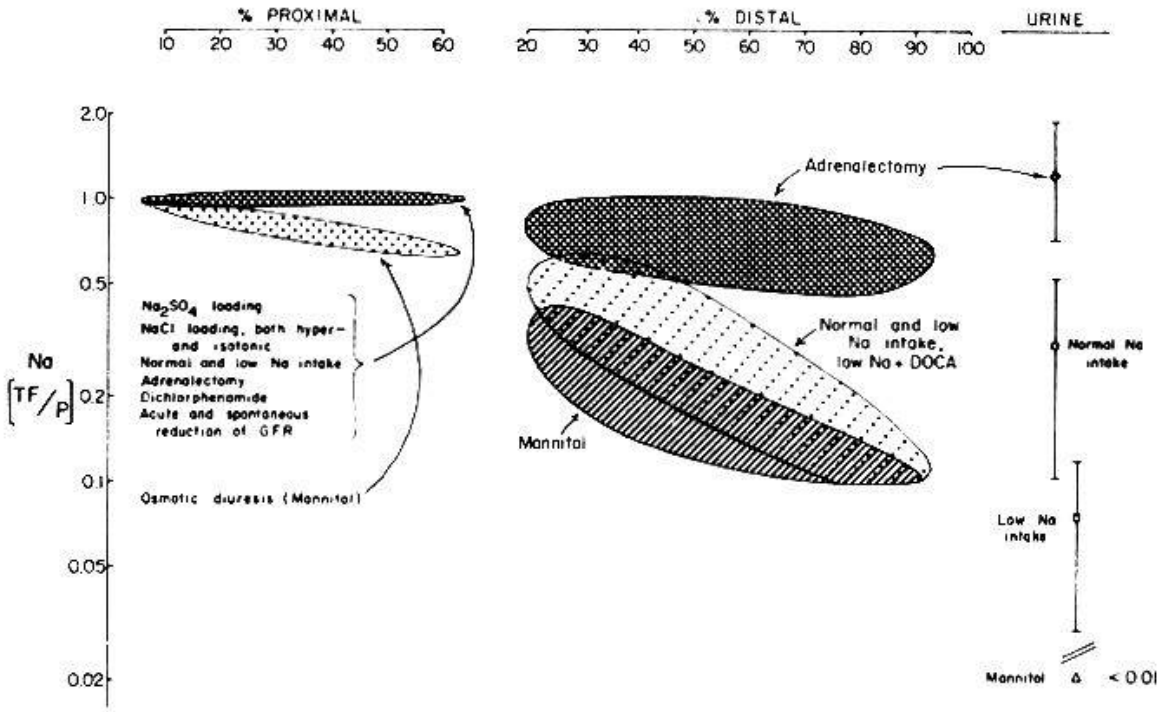

Fig. 2. Comparison of tubular fluid-to-plasma and urine-to-plasma concentration ratios of sodium under various experimental conditions. – From Jap. Soc. Nephrol. *8*, 2 (1966).

of additional, and significant quantities of sodium along the loop of Henle, since only some 10% of the filtered sodium is present when tubular fluid enters the distal convoluted tubule. It is also apparent that, in terms of fractional reabsorption, the collecting duct reabsorbs very little, the fractional amounts being in the order of 1%.

In Fig. 2, a number of additional micropuncture experiments are presented which summarize transtubular concentration gradients and indicate some modification of the control pattern [2]. It is apparent that with the exception of intravenous mannitol administration, none of the procedures indicated here lead to a change in proximal tubular sodium concentration with respect to plasma water. The fall in sodium concentration subsequent to mannitol administration is due to the fact that this substance remains within the tubular lumen and that the reabsorptive process continues isosmotically. Isotonic reabsorption of sodium chloride from a tubular solution which contains both sodium and mannitol, gives rise to a reabsorbate having a higher sodium concentration than that from which it originates, leading to the development of modest but significant concentration gradients for sodium.

The distal tubular epithelium shows a more variable pattern. Under control conditions there is a tendency of the sodium concentration to decline along the distal tubule. The shaded area is indicative of the range of concentration gradients observed. After mannitol loading, there is a trend for a lower sodium concentration to obtain in the earlier parts of the distal tubule, an effect no doubt related to the retention, in the tubular fluid, of a poorly reabsorbable solute. At the end of this part of the nephron, however,

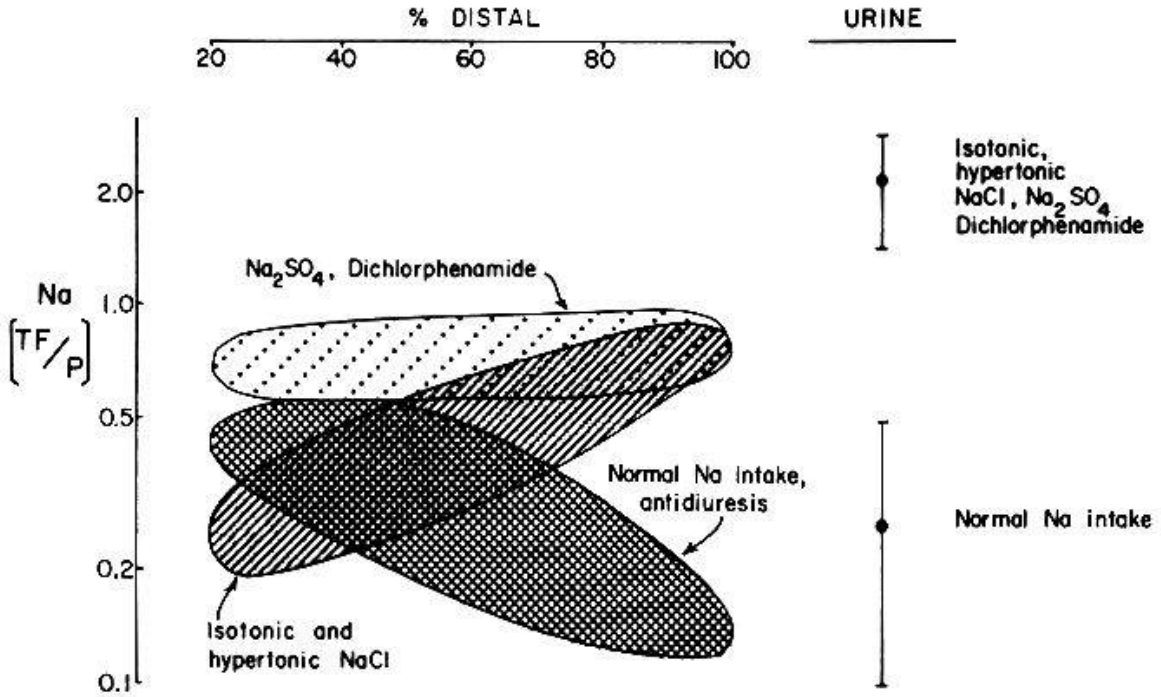

Fig. 3. Comparison of distal tubular fluid-to-plasma and urine-to-plasma concentration ratios under different experimental conditions. – From Jap. Soc. Nephrol. *8*, 4 (1966).

the maximally achieved gradients are not steeper. The presence of such a poorly reabsorbable solute appears necessary to permit a maximal decline in sodium concentration along the distal tubule. This is a consequence of the tendency of late distal tubular fluid to reattain isosmoticity. It should be noted that neither sodium depletion, with or without the superimposition of DOCA, nor the acute reduction of filtration rate by unilateral renal arterial clamping leads to a reduction of the sodium concentration below control values [1, 3]. It is, in the rat at least, the collecting duct which lowers the sodium concentration to its final levels. It is the collecting duct where the ultimate removal of sodium leads to the low final urine concentration typical for mannitol-loading, for sodium depletion, and lowering the filtration rate. Also shown in this figure is one experimental condition in which the ability of the distal tubular epithelium to establish concentration gradients for sodium is diminished. HIERHOLZER and his associates have shown that the concentration of sodium fails to decline along this tubular segment after adrenalectomy. This failure is completely reversible by the administration of aldosterone and indicates that one of the sites of action, not the sole one, of steroids with mineralocorticoid action is the distal tubule [4].

In Fig. 3, distal tubular data obtained in a series of additional experimental conditions are shown [2]. Here, emphasis is placed upon a comparison of control data obtained during antidiuresis with a number of situations characterized by a conspicuous failure of the distal tubular sodium concentration to decline. Loading with either hypertonic or isotonic sodium chloride solution is associated with normally low sodium concentration at the beginning of the distal tubule (there is no statistically significant difference

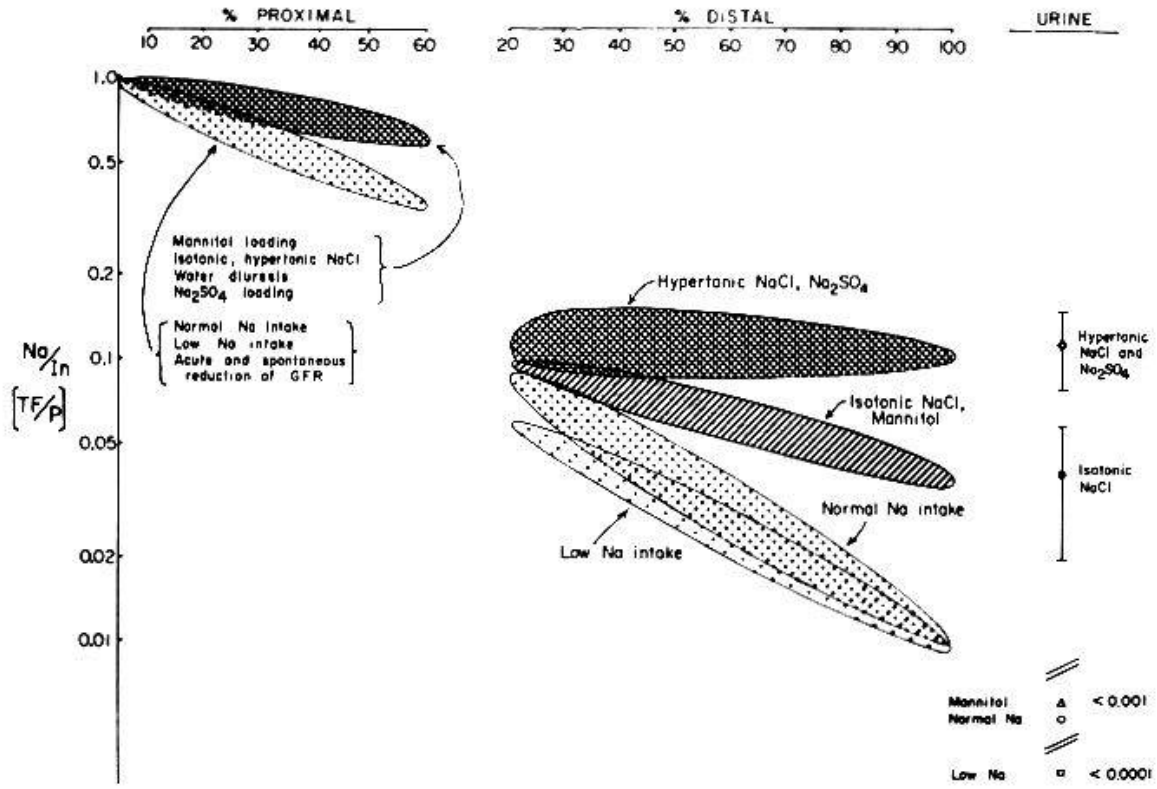

Fig. 4. Segmental contribution of various tubular structures to sodium reabsorption. – From Jap. Soc. Nephrol. *8*, 6 (1966).

between control and sodium-loaded rats), but a failure to decline further downstream. Actually, we have frequently observed an increase in the concentration of sodium when diuresis is induced by sodium chloride infusions. High distal tubular sodium concentrations are also seen after sodium sulfate loading and after the administration of some diuretics, notably dichlorphenamide. It is noteworthy that, during sulfate diuresis, a contributing factor opposing reabsorption could be the higher than normal level of the transtubular potential difference. In stationary microperfusion experiments, a limiting maximal transtubular concentration gradient for sodium obtains which, when expressed in electrical terms, approaches the value observed during sulfate diuresis: consequently, it is understandable that sodium reabsorption, and the establishment of a concentration gradient declines whenever the opposing electrical potential difference approaches this limiting energy barrier. It should be noted that, quite generally, the failure of the distal tubular epithelium to lower the sodium concentration is always associated with a similar functional pattern along the collecting ducts, illustrated by the continued rise in sodium concentration [5].

In Fig. 4, the magnitude of the segmental reabsorption of sodium and its modification under some experimental conditions are summarized [2]. Hence, the fraction of filtered sodium remaining in the tubular lumen is plotted as function of nephron length. The contribution of various nephron segments to the overall process of sodium reabsorption has been discussed during control nondiuretic conditions. There are a number of situations

leading to diminished fractional proximal tubular sodium reabsorption: mannitol diuresis, sulfate diuresis, dichlorphenamide administration, the infusion of large amounts of isotonic, and sometimes, that of hypertonic sodium chloride solution leads to a significant depression of fractional, and depending on the changes of glomerular filtration rate, also to depression of the absolute amounts of sodium reabsorption. This is indicated by the darkly-shaded area, and signifies that by the end of the proximal convolution, in contrast to some 65% (lightly shaded area), only some 30 to 40% of the filtered sodium load have been reabsorbed. It is significant to note that in the rat, only a relatively small fraction of this amount escaping proximal reabsorption appears in the urine. Most of the increment delivered to the loop of Henle is reabsorbed there, and attests to the very significant adaptive properties of this nephron segment in accommodating the increased load of sodium. We conclude this from the fact that despite some rather dramatic changes in proximal tubular sodium transport, the fraction of sodium at the early distal tubular level is brought back to within a fairly narrow range. On the other hand, both the distal tubular and the collecting duct epithelium conspicuously lack such adaptive power. In contrast to more proximally located nephron segment, the increased delivery of sodium does not result in an increased reabsorptive rate. This is evidence of a limited capacity of the terminal nephron segments to reabsorb increments of sodium as they escape more proximal reabsorption. Again, the fact should be stressed that the almost complete removal of sodium from the final urine, typical for various sodium-conserving states, is a function not of the distal tubule but of the collecting ducts.

With regard to proximal tubular reabsorption, it can be shown that there exists often but not always a remarkable constancy of fractional sodium reabsorption in the presence of variations in glomerular load. Pertinent examples are micropuncture studies in which glomerular filtration rate varies spontaneously, is artificially changed or experiments in which hypertonic sodium chloride is administered. This implies that the overall proximal reabsorptive mechanism exhibits kinetics in which net transfer of sodium is not only dependent upon concentration but also on the amount at the reabsorptive site. This situation is sometimes referred to as glomerulotubular balance, a phenomenon on which there presently exists conspicuous disagreement as to the factors involved. Data from two series of experiments from our laboratory will be presented, one dealing with an experimental situation demonstrating the absence of such balance, the other dealing with one of the factors of possible significance in this phenomenon.

The administration of large amounts of isotonic sodium chloride to rats leads to the excretion of some 8 to 10% of the filtered sodium load, to a copious diuresis and a significant increase in glomerular filtration rate [6]. Under these conditions, proximal transtubular concentration gradients of inulin are diminished, and the same is true for the distal tubule. Obviously then, so-called glomerulo-tubular balance with respect to proximal reab-

sorption does not hold under these conditions. Some sort of glomerulotubular balance in the wider sense is still present since the excretion increments are much smaller than the fraction of sodium escaping proximal reabsorption. Clearly, this is not a consequence of proximal tubular adaptation, but a consequence of the adaptive behavior of the loop of Henle. There are two factors which could be responsible for diminished proximal tubular sodium reabsorption during isotonic sodium chloride loading: the intrinsic reabsorptive properties of the tubular epithelium could be altered, that is, diminished, or the increase in glomerular filtration rate could lead to an increased linear flow velocity and the reabsorption of a smaller than normal fraction of filtrate due to inadequate time of exposure. This problem can be experimentally approached. The intrinsic transport capacity of the proximal tubular epithelium has been measured, in collaboration with LANDWEHR and KLOSE, by the split-drop method of GERTZ, as well as tubular passage-time by a modification of STEINHAUSEN's Lissamin-green method [6]. The split-drop method consists in the deposition of isotonic saline between colored mineral-oil and the subsequent measurement of the time of disappearance of the isolated saline. The tubular passage time is an estimate of the time of passage of a color-front of Lissamin-green from the beginning to the end of the proximal convolution. GERTZ has presented evidence that the extent of fractional reabsorption in the proximal tubule can be predicted from the knowledge of these two parameters. Essentially, the results can be summarized by stating that the half-time of disappearance of saline was prolonged, and the tubular passage-time unchanged, during saline-loading. The calculated free-flow inulin concentration ratios, or fractional reabsorption of sodium, approaches closely that directly found. Thus, all available evidence is consistent with the view that the primary effect of isotonic saline loading on proximal tubular reabsorption is to reduce the intrinsic transport capacity of the tubular epithelium for sodium ions. Changes in the level of a circulating humoral agent have been implied by others, but we have no data bearing on this. Changes in tubular flow kinetics can, in addition, modify and accentuate the changed pattern, but are not an obligatory prerequisite.

An interesting series of experiments was carried out in collaboration with HIERHOLZER, WINDHAGER and WIEDERHOLT [7]. Single proximal tubules were perfused at different rates, using a micropump, with Ringer's solution. Changes in luminal diameter of proximal tubules, estimated photographically, and calculated linear velocity were related to fluid reabsorption. Several series of perfusion experiments were carried out, and the most essential points were: first, inulin concentrations increase as a function of tubular perfusion length. Secondly, doubling the perfusion rate does not lead to a changed relationship between inulin concentration ratios and distance along the tubule. This is in agreement with the concept of glomerulotubular balance. Tubular diameters increase with an increase in perfusion rate, and it was found that glomerulotubular balance, that is, constancy of

inulin TF/P ratios as function of tubular distance, breaks down only then when additional flow increments are not accompanied by increases in diameter. A good relationship obtains over the whole range of flow rates between amount reabsorbed and tubular cross-sectional area, but not between reabsorption and linear velocity of flow. It was concluded from these experiments that proximal tubular geometry is one factor which should be considered when discussing glomerulo-tubular balance. This is not thought to be the whole explanation. One key issue on which opinions are divided is whether tubular diameter obligatorily increases with elevation of glomerular filtration rate. Some clarification of these issues may be expected.

Summary

Some general properties of renal tubular sodium transport have been studied by estimating the contribution of various tubular segments to the urinary excretion pattern under different experimental conditions. Along the proximal tubule, and along the loop of Henle, by far the largest fraction of filtered sodium is reabsorbed. A number of factors modifying the reabsorptive pattern along the proximal and distal tubule are discussed. Quite generally, total urinary sodium excretion varies significantly less than would correspond to the depression of proximal reabsorption. It can be shown that it is the loop of Henle which importantly contributes to conserving sodium when proximal reabsorptive capacity is diminished. Evidence is presented to suggest that tubular geometry can affect the rate of sodium reabsorption: during perfusion of single proximal tubules, a good relationship obtains between amount reabsorbed and tubular cross-sectional area. This relationship may contribute to the proportional variation in tubular sodium reabsorption subsequent to changes in glomerular filtration rate (glomerulo-tubular balance).

Zusammenfassung

Unter verschiedenen Versuchsbedingungen wird die Bedeutung einzelner Segmente der Nierenkanälchen beim Natriumtransport bestimmt. Der Großteil des filtrierten Natriums wird auf der Höhe des proximalen Tubulus und längs der Henleschen Schleife reabsorbiert. Im allgemeinen verändert sich die Gesamtausscheidung des Urinnatriums bei einer Verringerung der proximalen Natriumreabsorption weniger, als man annehmen könnte, und die Konservierung dieses Ions geschieht erwiesenermaßen hauptsächlich in der Henleschen Schleife. Es wird gezeigt, daß die tubuläre Geometrie die Natriumreabsorption beeinflussen kann: In der Tat bemerkt man bei der Perfusion von isolierten proximalen Kanälchen eine gute Korrelation zwischen der reabsorbierten Quantität und dem Querschnitt des tubulären Segmentes. Diese Beziehungen können bis zu einem gewissen Grade die Veränderungen der tubulären Natriumreabsorption erklären, welche bei einem Wechsel der glomerulären Filtration festgestellt worden sind (glomerulo-tubuläres Gleichgewicht).

Résumé

Des expérimentations diverses permettent de définir l'importance des différents segments du tubule rénal dans le transport du sodium. C'est à la hauteur du tube proximal et le long de l'anse de Henle qu'est réabsorbée la majeure partie du sodium filtré. De façon générale, lors d'une diminution de la réabsorption proximale du sodium, l'excrétion totale du sodium urinaire varie moins qu'on pourrait le supposer et l'on peut montrer que c'est principalement à l'anse de Henle que l'on doit la conservation de ce ion. On démontre que la géométrie tubulaire peut influencer la réabsorption du sodium: on observe en effet, lors de la perfusion de tubules proximaux isolés, une bonne corrélation entre la quantité réabsorbée et la section tubulaire transversale. Cette relation peut expliquer dans une certaine mesure les variations de la réabsorption tubulaire du sodium constatées lors de changements de la filtration glomérulaire (équilibre glomérulo-tubulaire).

Riassunto

Diversi esperimenti permettono di precisare l'importanza dei differenti segmenti del tubolo renale per il trasporto del sodio. La maggior parte del sodio filtrato viene riassorbita all'altezza del tubo prossimale e dell'ansa di Henle. Nel caso di una diminuzione del riassorbimento prossimale, l'escrezione totale del sodio nell'urina varia in generale meno di quello che si potrebbe supporre e si può dimostrare che è principalmente l'ansa di Henle che provvede alla conservazione di questo ione. Nel presente lavoro si dimostra che la geometria tubolare può influenzare il riassorbimento del sodio: mediante perfusione di tuboli prossimali isolati si osserva infatti una buona correlazione fra la quantità riassorbita e la sezione tubolare trasversale. Tale relazione può spiegare almeno in parte le variazioni del riassorbimento tubolare del sodio che si osservano nel caso di cambiamenti della filtrazione glomerulare (equilibrio glomerulo-tubolare).

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