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Relationship between energy requirements for Na^+ reabsorption and other renal functions

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In the intact organism, while the kidney is only ~1% of total body weight, it utilizes approximately 10% of the whole body O_2 consumption ($\dot{Q}$-O_2). Because there is a linear relationship between change in Na^+ reabsorption and suprabasal renal O_2 consumption, the high suprabasal renal $\dot{Q}$-O_2 has been attributed principally to the energy requirements for reabsorption of the filtered load of Na^+ [1–3]. The basal metabolism is the energy which is required for the maintenance of the renal tissue integrity and turnover of its constituents without measurable external (net transport or net synthetic) work being done. However, there is now considerable evidence that there are other suprabasal functions of the kidney which change in parallel with Na^+ reabsorption and which require an energy input separate from that used for Na^+ transport.

Thus, while there is little doubt that the rate of glomerular filtration initiates and determines the major portion of the renal suprabasal $\dot{Q}O_2$, this paper focuses on two questions: *(1)* Is the renal suprabasal O_2 uptake related only to Na^+ reabsorption? *(2)* Is the suprabasal O_2 uptake also related to other renal functions which are proportional similarly to the rate of glomerular filtration and which also contribute to the regulation of the volume and composition of the body fluids?

Does the ratio, Δnet $\dot{T}$-$Na^+/\Delta Q$-O_2, provide an accurate estimate of the cost for renal Na^+ reabsorption?

Separate roles of other energy-requiring transport mechanisms along the nephron which are independent of Na^+ transport and of $NaHCO_3$ reabsorption in the proximal tubule in modulating the ratio, $\Delta net\ \dot{T}$-$Na^+/\Delta\dot{Q}$-O_2. The use of the ratio, ΔNet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, to estimate the mean energy requirement for net Na^+ transport in the intact kidney [1] is based on the studies of Zerahn [4] done with a "tight" epithelium of the isolated frog skin. A linear correlation was observed between changes in net Na^+ transport (Δnet $\dot{T}$-Na^+) and suprabasal O_2 uptake rates ($\Delta\dot{Q}O_2$). From the slope of this relationship, the mean ratio, $\Delta\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, was found to be ~18. Similar observations were made with the toad bladder [5, 6]. These anuran epithelia are similar in function to the distal nephron (that is, the portion of the renal tubular epithelium beyond the thick ascending limb of the loop of Henle) of the mammalian kidney in that: *(1)* Na^+ transport is inhibited by amiloride [7, 8] and *(2)* only a small fraction (5 to 10%) of the Na^+ transported from the mucosal to serosal side leaks back to the mucosal side [7, 8]. In such a tight epithelium, net Na^+ flux is therefore a close approximation of the unidirectional active Na^+ flux. If no other energy-requiring functions are changed when Na^+ transport is varied, then a reasonable estimate of the energy requirement for both net and unidirectional active Na^+ transport may be obtained from measurements of the ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2.

Assuming that 3 moles of ADP are phosphorylated to form ATP per atom of O_2 reduced in the aerobic oxidation of 1 mole of NADH by the mitochondrial electron transport chain (that is, P:O = 3), then in a tight epithelium with a $\Delta\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2 of 18, the $\Delta\dot{T}$-$Na^+/\Delta\dot{Q}$-ATP would be ~3. This calculated stoichiometry between Na^+ transport and ATP utilized in the anuran skin or urinary bladder is similar to the 3:1 stoichiometry observed between ATP hydrolysis and Na^+ transport by the Na,K-ATPase, when Na^+ is extruded from the cell or when Na^+ is taken up by lipid vesicles into which the Mg^{++} Na,K-ATPase has been incorporated [9]. Importantly, these observations and calculations have been used with the assumption that Na^+ transport is the only major suprabasal function occurring in a tight epithelium such as the toad bladder or frog skin. However, for this approach of estimating the energy requirement for Na^+ transport to be valid, it must be shown that no other *independent* function is changed when Na^+ transport is changed.

That this assumption may not always apply is indicated by the presence of an energy-requiring, ouabain-insensitive, H^+-secretory mechanism in the urinary bladder obtained from certain toad species [10], and fresh water turtles [11, 12, 13] and in mammalian collecting tubules [14]. However, changes in H^+ secretion have not been measured at the same time Na^+ transport was changed. If both Na^+ and H^+ transport rates increased in parallel, the cost of Na^+ transport would be overestimated from measurements only of the ratio, $\Delta\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2. On the other hand, if Na^+ transport rate increased, while H^+ secretion decreased simultaneously, the value of Na^+ transport alone would be underestimated. For example, reciprocal changes in the transport rates of Na^+ and H^+ have been observed in the turtle bladder in the presence of spironolactone [13]. Thus, in epithelia where changes in the rates of independent, energy-requiring transport of other solutes occur when the rate of Na^+ transport is changed, errors in estimating the value of Na^+ transport will result. Nevertheless, the measure-

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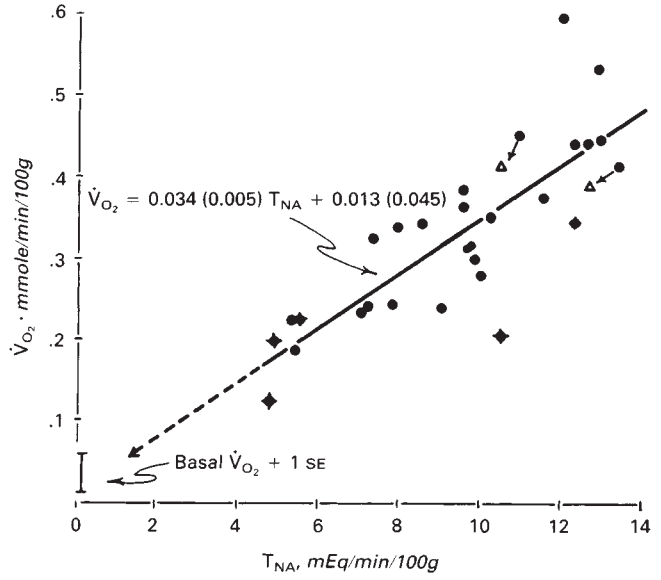

Fig. 1. *Correlation between $\dot{Q}$-O_2 and T-Na^+. The reductions in net Na^+ reabsorption were induced by raising ureteral pressure and decreasing GFR.* Symbols are: during water diuresis (○); water diuresis + elevated ureteral pressure (◆); and following Pitressin infusion (△) sufficient to inhibit water diuresis without reducing blood flow. Figure 1 reproduced with permission from [15].

ment of $\Delta\dot{Q}$-O_2 due to changes in overall function of an epithelium or of the intact kidney are qualitatively important for initially characterizing the changes in total metabolism which a change in transport rate causes. Simultaneous changes in the magnitude of energy-requiring transport other than Na^+ transport must then be identified and quantified to determine what fraction of the total $\Delta\dot{Q}$-O_2 each transport mechanism requires.

As a contrast, the observations made in tight anuran epithelia show that, the mean ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, in the intact mammalian (dog) kidney (Fig. 1) is much higher: Ratios as low as 22 and as high as 48 [2, 3] have been reported, although the ratio is usually ~30 [2, 15]. Thus, the mean molar energy requirement for net Na^+ reabsorption in the intact mammalian kidney is considerably less than in anuran epithelia. Because the major fraction (~75%) of the filtered Na^+ is reabsorbed along the proximal tubule, this higher ratio must reflect primarily a lower mean molar energy requirement for the net proximal reabsorption of Na^+.

In all likelihood the basis for the lower energy requirement for proximal Na^+ reabsorption relates to the high ionic conductance and high hydraulic conductivity of this region of the nephron. Thus, unlike the "tight" anuran or the distal renal tubular epithelium, the proximal tubule is a "leaky" epithelium [16, 17]: This is manifested by the considerable reabsorptive flux of Na^+ and Cl^- through the proximal paracellular pathway [16–19]. This paracellular pathway also permits variable degrees of bidirectional fluxes of Na^+ and Cl^- to occur. As a result, the unidirectional flux of Na^+ from the proximal tubular lumen is several-fold greater than the net Na^+ flux. The high hydraulic conductivity and the relatively low reflection coefficient for Cl^- (compared to HCO_3^-) in the paracellular pathway of the proximal tubule permit a net efflux from the lumen of Cl^-, with Na^+, to occur when small concentration gradients for Cl^- are established [20–24]. Indeed, it has been estimated that approximately two-thirds [22] of the net proximal reabsorption of Na^+ occurs by these passive mechanisms. Thus, the O_2-requiring direct active transport of a small fraction of the filtered load of Na^+ from the proximal lumen produces forces resulting in an additional large net efflux of solute and water from the lumen which is not directly O_2-requiring. This is consistent with the high ratios (ranging from 22 to 48) for Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2 observed for the whole kidney [2, 3]. It is apparent that mechanisms which produce osmotic gradients across the proximal tubular epithelium can have profound effects on the calculations of the mean molar energy requirement for net Na^+ reabsorption [2, 15, 24, 25].

Ingenious, carefully designed experiments have been performed to obtain an estimate of the energy requirement, in vivo, for the moiety of Na^+ reabsorption which is active in the proximal tubule. For example, Mathisen, Montclair, and Kiil [3] reported that in the proximal tubule in dog kidney in vivo *all* NaCl reabsorption is passive; they suggest that it is only the reabsorption of $NaHCO_3$ which is directly energy-requiring. In this hypothesis, all the proximal $NaHCO_3$ reabsorption is considered to be unidirectional and occur as a result of the Na^+-H^+ antiport mechanism at the brushborder membrane. The favorable electrochemical gradient for continued Na^+ entry across the luminal cell membrane in exchange for H^+ is maintained by active Na^+ extrusion across the basolateral membrane by the Na,K-ATPase. As a result of this Na^+-H^+ antiport, there is initially a preferential reabsorption of $NaHCO_3$ from the glomerular filtrate. As this $NaHCO_3$ reabsorption occurs, the Cl^- concentration in the proximal tubular lumenal fluid rises above that in plasma. The development of reciprocal transtubular concentration gradients for HCO_3^- and Cl^-, in concert with a higher reflection coefficient for HCO_3^- than for Cl^- [20, 22, 24], then provides the driving forces [24] for the paracellular efflux of Cl^- (with Na^+) from the lumen into the intercellular and interstitial spaces and then into the peritubular capillary.

However, that all HCO_3^- reabsorption from the proximal tubule is unidirectional may not always be the rule. The Mathisen, Montclair, and Kiil [3] experiments were done in markedly volume-expanded dogs during metabolic alkalosis; Na^+ reabsorption in the thick ascending limb was blocked by ethacrynic acid; net $NaHCO_3$ reabsorption was varied by administering acetazolamide or by changing $PaCO_2$. Under these conditions the mean ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, for the proximal reabsorbate was ~48. The calculated ratio for $[Cl^-]$:$[HCO_3^-]$ in the reabsorbate was 2:1, and the entire $\Delta\dot{T}$-Cl was assumed to be passive, that is, it was due to the chemical gradient for Cl^- resulting from the selective reabsorption of HCO_3^- via the Na^+ - H^+ antiport mechanism. From these calculations, it was concluded that only one-third of the change in net Na^+ reabsorption was mediated by the energy-requiring Na,K-ATPase. With these assumptions, the calculated ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, for only the HCO_3^--associated moiety of Na^+ reabsorbed was ~16. This latter value is similar to the mean ratio observed in tight anuran epithelia [4–6], where net Na^+ transport has been shown to closely approximate unidirectional Na^+ transport. Mathisen, Montclair, and Kiil [3] concluded that the value for active unidirectional flux of Na^+, with HCO_3^-, out of the proximal tubule is the same as in the

tight anuran epithelium and lipid vesicles into which the purified Na,K-ATPase had been incorporated [9].

These well-designed and executed studies [3] show how the characteristics of the "leaky" proximal tubular paracellular pathway may account for the high $\Delta\dot{T}\text{-}Na^+/\Delta\dot{Q}\text{-}O_2$ in the intact kidney. Nevertheless, that the calculated ratio [3] is accurate requires some important qualification. Although the reflection coefficient for HCO_3^- in the proximal tubule is high [20, 24], paracellular backflux of HCO_3^- and Na^+ does occur, particularly during volume expansion [18, 22, 26]; marked volume expansion was present in the studies done by Mathisen, Montclair, and Kiil [3]. While the exact magnitude of the HCO_3^- backflux during volume expansion is unknown, the presence of a significant HCO_3^- backflux would require higher simultaneous rates of unidirectional HCO_3^- and Na^+ movement out of the lumen for a given net rate of $NaHCO_3$ reabsorption. The molar energy cost for HCO_3^--associated, unidirectional Na^+ reabsorption would then be less than it was estimated to be [3] from the observed ratio, Δnet $\dot{T}\text{-}NaHCO_3/\Delta\dot{Q}\text{-}O_2$.

The assumption that all of the Cl^- reabsorption in the proximal tubule is passive is also questionable. In the pars recta of the rabbit proximal tubule, active transport of NaCl probably occurs [24]. How this moiety of active Na^+ with Cl^- transport changes when HCO_3^- reabsorption is altered is not yet known. Nevertheless, some of the $\Delta\dot{Q}\text{-}O_2$ may, in fact, relate to Δ active NaCl reabsorption.

Another significant fraction of active Na^+ reabsorption from the proximal tubule is the Na^+ which is co-transported from the lumen into the cell with filtered substrates such as lactate, glucose, glutamine, other amino acids, citrate, other substrates, as well as with PO_4 and SO_4 [27–30]. If either glomerular filtration rate (GFR) or the substrate composition of the arterial plasma increases this portion of proximal Na^+ reabsorption could also be affected by the maneuvers used by Mathison, Montclair, and Kiil [3], and an additional $\Delta\dot{Q}O_2$ would then be coupled to the Na^+ co-transported with these substances.

Overall, unless backflux of HCO_3^- were not present and no changes in active NaCl transport or in co-transport of substrates with Na^+ occurred when HCO_3^- reabsorption was changed, it appears that the unidirectional proximal reabsorption of Na^+ linked to the H^+-Na^+ antiport in the intact dog kidney during volume expansion may have a lower molar energy requirement than 3 $NaHCO_3$:1 ATP.

Estimates have also been made for the energy cost of K^+ transport across the basolateral cell membrane in an in vitro preparation of rabbit proximal tubules. Harris, Balabon, and Mandel [31] used isolated (collagenase treated) rabbit proximal tubules which had been depleted of K^+ and then measured the stoichiometry between the stimultaneous changes in total net K^+ uptake and the increment of O_2 uptake. In these experiments, the K^+ content of the proximal tubules was first decreased to a low steady-state K^+ content by incubation in a K^+-free medium. Then, a bolus of K^+ was added to the medium, and both the total net disappearance of K^+ from the medium and the simultaneous increment in O_2 uptake were compared during a short transient. The mean Δnet K^+ uptake/$\Delta Q\text{-}O_2$ was ~11.8. Neither a net disappearance from the medium or an increment in $\dot{Q}\text{-}O_2$ occurred in tubules pre-treated with 60 μM ouabain which were still losing K^+ when the bolus of K^+ was added to the medium. Harris, Balabon, and Mandel [31] concluded that their observations show that all of the net K^+ uptake was due to active transport of K^+ and was mediated by Na,K-ATPase. The calculated stoichiometry of 2 K^+ transported/mole of ATP hydrolyzed was thus consistent with the theoretical stoichiometry for a P/O ratio of 3.

There are, however, certain reservations about these conclusions: In view of the high K^+-permeability of the basolateral cell membrane of the mammalian proximal tubule [19], it is unlikely that all of the net K^+ uptake by the K^+-depleted tubules occurred only via a Na,K-ATPase-mediated pathway. No measurements of the unidirectional fluxes of K^+ were made and also no estimates of the driving forces for K^+ entry were made. Thus, it seems likely that a substantial portion of the K^+ which entered the K^+-depleted cells may not have required ATP. If this is the case, the observations can be interpreted to indicate that the stoichiometry between net K^+ entry and Δ total O_2 uptake does not provide an accurate estimate of the value only of K^+ uptake via Na,K-ATPase. It is apparent that measurements of the driving forces for the K^+ movements into K^+-depleted tubules in the presence and absence of ouabain are needed. Also, as indicated next, the addition of K^+ to the medium, which contained lactate, glucose, and alanine, may also have resulted in significant simultaneous changes in synthetic mechanisms due to the alterations in the intracellular ion composition. Such increases in suprabasal proximal tubular synthetic functions would also have utilized a portion of the observed increment in $\dot{Q}\text{-}O_2$, with a resulting reduction in the ratio, net K^+ uptake: $\Delta\dot{Q}O_2$. No estimates either of gluconeogenesis or other syntheses by these rabbit tubules were made; hence, that all the $\Delta Q\text{-}O_2$ was related only to the net transport of K^+ [31] into the cells remains to be established.

Along the same lines, and as indicated earlier, if, in the distal nephron, other energy-requiring solute transport rates which are independent of Na^+ transport, such as H^+ secretion, change in a parallel or reciprocal manner when changes in Na^+ transport occur, the estimated energy requirements for distal Na^+ transport alone will be either too high or too low, respectively. Thus, under all circumstances, the simultaneous changes in the utilization of energy for active transport functions other than for Na^+ and for other functions, such as synthetic mechanisms, must be estimated to better approximate the energy requirement for $\dot{T}\text{-}Na^+$ alone. Indeed, to my knowledge, the changes in renal synthetic mechanisms which are coupled to changes in Na^+ reabsorption have been largely ignored in studies designed to estimate the energy cost for Na^+ transport in kidney.

Effect of net renal synthesis of new substances on the estimates of the energy requirement for Na^+ reabsorption. As indicated earlier, in addition to the transport work done in the conservation (reabsorption) of the filtered solutes and water and in the tubular secretion of solutes [1], the kidney, and the proximal tubule in particular, also performs external work in the energy-requiring, net syntheses of new substances, such as glucose [32–35], serine [36], creatine [37], and other substances [1, 38–40] which leave the kidney either in renal venous blood or urine. The kidney's role as a major site of detoxification of endogenous and exogenous (xenobiotics) substances is also manifest in the energy-requiring syntheses and excretion of glucuronides [41, 42], SO_4 esters [41], methyl esters [43, 44],

mercapturic acid derivatives [45–47] and conjugates formed with glycine [48, 49] and acetate [50]. New substances are also produced in exergonic reactions which are not coupled to ATP utilization, but should contribute to renal heat production, for example, the renal decarboxylation of dihydroxy-phenylalanine (DOPA) to dopamine [51], or the oxidative deamination of catecholamines [44]. Thus, the kidney, like the liver, is an important site for detoxication and the interconversion of substrates.

The potential for renal interconversions is highlighted by the fact that ~20% of the resting cardiac output enters the kidney; another 20% of the cardiac output enters the liver. As a result, the combined capacity of the kidney plus the liver for rapidly regulating the substrate composition of the blood and removing toxic substances from the blood is substantial. Most of the above syntheses ordinarily occur at a low rate in kidney due to the low concentrations of the precursors in blood. However, no quantitative assessment of the *summed* simultaneous energy requirements for all these net renal syntheses has been made in vivo as yet and whether their rates change when $\dot{T}$-Na^+ is changed. Because the production of these substances represents an external synthetic mechanism, the energy requirements are part of the suprabasal renal $\dot{Q}$-O_2 and are not part of the basal renal $\dot{Q}$-O_2.

That the renal substrate-interconversion phenomena are always occurring in the functioning kidney becomes apparent when one sums the rates at which all the major substrates are taken up by the kidney in vivo, while simultaneously, renal O_2 uptake rate is measured [1, 52, 53]: As much as one-third to one-half more substrate is utilized by the kidney than can be oxidized completely to CO_2 and H_2O. Such an "excess" of renal substrate uptake can be explained either by incomplete oxidation of some of the substrates or by their utilization in energy-requiring syntheses [35]. From the studies showing that net renal syntheses of glucose, creatine, serine, and of several conjugates do occur, the excess substrate utilization by the kidney is thus related primarily to its capacity to perform these types of energy-requiring interconversions of substrate. As implied above, this substrate-interconversion function of the kidney is another manifestation of the renal regulation of the composition of the body fluids [1, 2] and complements similar non-excretory, energy-requiring phenomena which also occur in the liver. For example, during starvation, when triglycerides are hydrolyzed at a high rate and an excess of glycerol is produced in other tissues, the kidney becomes a major source of glucose [32]. The glucose leaving the kidney is then used to maintain the metabolism of tissues such as muscle and brain.

Similarly, when the concentration of lactate in blood is increased, the rate of renal glucose production [33, 35] also increases. Therefore the excess lactate which accumulates in blood during exercise is converted to glucose, in all probability by the kidney as well as by the liver, thus completing the Cori cycle. In effect, the renal and hepatic conversion of lactate to glucose is an example of how these two organs together can contribute to the regulation of the substrate composition of blood.

The renal conversion of Na-lactate to glucose also results in regeneration of the [HCO_3^-] in blood which was lowered by the production of lactic acid from glucose in tissues such as muscle. Thus, the renal substrate-interconversion of lactate to glucose also performs a pH-regulatory function which is analogous to ammonium and titratable acid excretion in that HCO_3^- is also regenerated. However, such a metabolic pH-regulatory function of the kidney (and liver) cannot be detected by measurement of the urinary excretion of titratable acid or NH_4^+; separate measurements of the net metabolism of these precursors and products across the kidney must be made. In addition, the increased rate of formation of glucose from lactate gradually decreases the filtered load of lactate, reducing the possibility for renal excretion of lactate and hence effecting the conservation of the potential energy contained in the lactate molecule. Overall, the substrate interconversion function of the kidney regulates the substrate composition of blood, contributes to pH regulation, and conserves energy.

Because increases in the concentrations in blood of the precursors for the syntheses can change the rates of production of new substances [32, 33, 35] by kidney, a portion of the rate of the suprabasal ATP produced from renal oxidative metabolism must be used to support the additional synthetic work being done. However, it has been suggested that the energy available to the kidney is limited so that this suprabasal O_2 uptake can only be used either for Na^+ reabsorption, or, for glucose synthesis, but not for both functions simultaneously [34]. This suggestion was based on observations made in the isolated rat kidney, perfused with pyruvate as the only exogenous substrate present: Silva, Ross, and Spokes [34] reported that when the rates of glomerular filtration and net total Na^+ reabsorption were reduced by raising the albumin concentration (and hence the colloid osmotic pressure) of the perfusate, there was a concomitant increase in net renal glucose production. These observations were interpreted to indicate that the total renal energy production is fixed; by reducing the mechanism of Na^+ reabsorption, more energy became available for gluconeogenesis.

However, by contrast with the observations made with the isolated rat kidney perfused with pyruvate as the only substrate available [27], observations made in vivo do not indicate that there is such a readily approached limit to the availability of energy for simultaneous support of both Na^+ reabsorption and an increase in gluconeogenesis [33]. In the dog, increases in net glucose production due to isohydric increases in blood lactate concentration did not impair the relatively higher (compared to the isolated rat kidney) rate of net Na^+ reabsorption [33]. There must be a limiting rate of energy production for support of the several renal functions. However, it appears that when there is an ample supply of both O_2 and oxidizable substrates in the arterial blood delivered to the kidney, and over the relatively wide range of increasing lactate delivery rates studied in vivo [33], the extra energy required for the measured increased rate of gluconeogenesis is produced readily, and no impairment of net Na^+ reabsorption occurs. Thus, the additional substrate delivery rate (lactate) alone will drive gluconeogenesis, and the increased ATP requirement becomes available while the ATP required for Na^+ reabsorption continues to be produced.

Similar observations have been made in the isolated kidney, perfused with substrates other than pyruvate: When GFR was increased, no limit to the rate of renal O_2 consumption was apparent; $\dot{Q}$-O_2 increased to as high as ~14 μmoles $g^{-1} \cdot min^{-1}$ [54]. This latter rate of renal O_2 uptake is at least fourfold greater than the rate reported in the studies by Silva, Ross, and

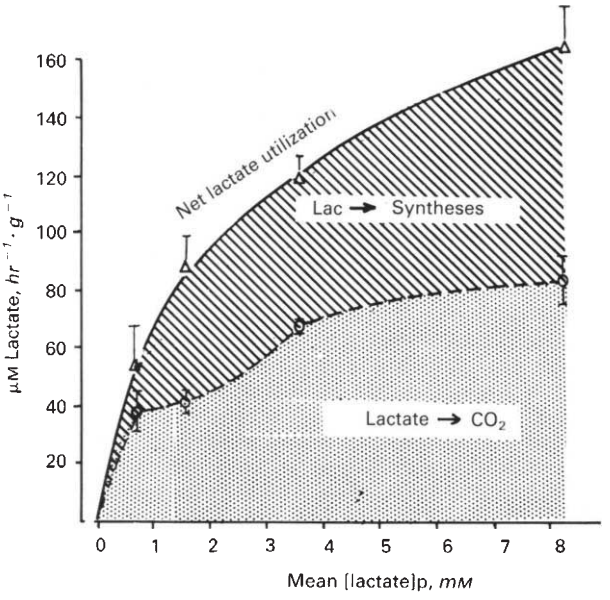

Fig. 2. *Gross metabolic rates of lactate in the isolated perfused rat kidney as lactate concentration in the perfusate increases. More lactate is utilized than is oxidized as lactate concentration rises. Approximately 30% of the lactate entering into syntheses was accounted for by accumulation of glucose and glutamate.* Reproduced with permission from [53].

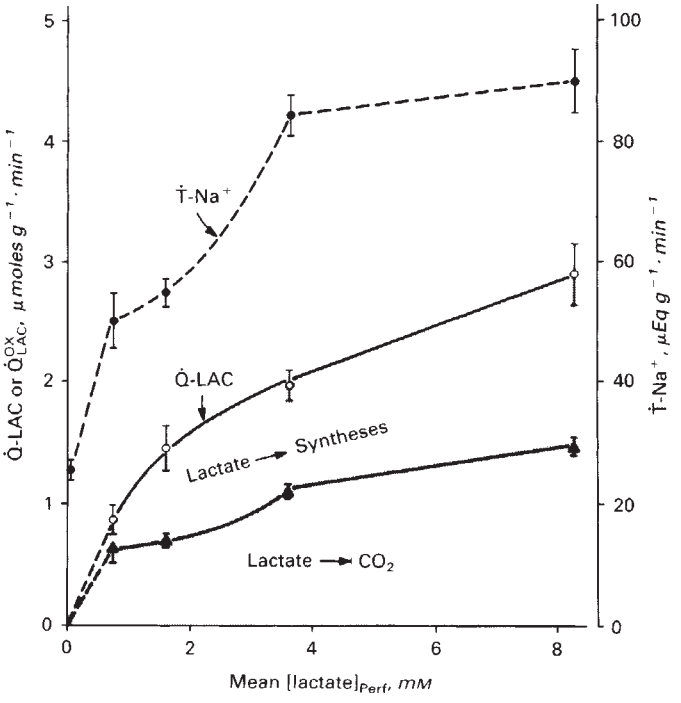

Fig. 3. *Mean rates of net lactate utilization and Na^+ reabsorption in the isolated perfused rat kidney. Both net Na^+ reabsorption and net lactate utilization increase. Although some of the lactate utilized enters into energy requiring syntheses (see Fig. 2), net Na^+ reabsorption is not decreased.* Reproduced with permission from [53].

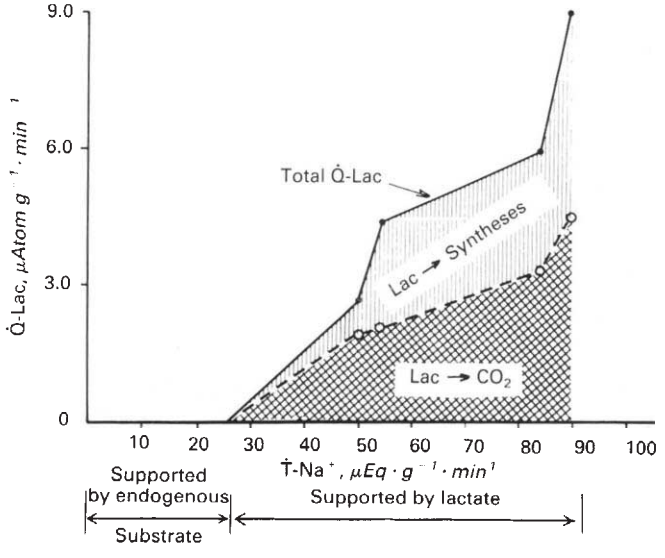

Fig. 4. *Rates of Na^+ reabsorption, lactate entry into syntheses, and lactate decarbosylation all increase as lactate concentration in the perfusate is raised.* Reproduced with permission from [53].

Spokes [34] where O_2 uptake apparently could not be increased to support both Na^+ reabsorption and an increase in glucose production.

Also consistent with the ability of the kidney to provide ATP for the energy requirements both for net Na^+ reabsorption and simultaneously, for net syntheses, are other studies with the isolated perfused rat kidney [53]: Renal function and metabolism were increased simultaneously by progressively raising the lactate concentration in the perfusate. Lactate was the only exogenous substrate present in the perfusate. As lactate concentration was increased, there was an "excess" of lactate utilized, that is, more lactate was utilized than was decarboxylated (Fig. 2) as occurs in vivo [1] and in vitro [35]. At these high rates of lactate utilization approximately 30% of the excess lactate utilized was accounted for by the glucose and glutamate produced which accumulated in the perfusate. While there were increasing rates of synthesis of glucose and of other products as well (not all the products were identified), net Na^+ reabsorption was not reduced (Fig. 3); indeed Na^+ reabsorption was increased. These latter studies [53] are consistent with the in vivo observations [33] that renal synthetic functions can increase without decreasing Na^+ reabsorption: Both the rate of synthesis and the rate of Na^+ reabsorption can increase at the same time (Fig. 4).

The several observations summarized here show that changes in suprabasal O_2 uptake are unrelated only to changes in $\dot{T}$-Na^+. When the rates of both Na^+ reabsorption and net syntheses increase, the increase in renal O_2 uptake cannot be attributed only to the work of Na^+ reabsorption: a portion of the suprabasal O_2 uptake is also being used for net synthetic work. Similar conclusions were drawn [55] from studies with isolated rabbit proximal tubules, although the measurements of glucose production and Na^+ reabsorption rates were not done simultaneously or under the exact same conditions. It is apparent that in the presence of concurrent changes in $\dot{T}$-Na^+ and in net syntheses, the use of the ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}_2$ to approximate the energy requirement for net Na^+ reabsorption in these studies would have resulted in an overestimation of this cost.

Also consistent with the concept that total renal energy

production can increase without affecting net Na^+ reabsorption are the observations for the effects of glycine on renal heat production in the dog, observed by Johannesen, Lie, and Kiil [56]. When glycine was infused into the renal artery, there was a large increase in renal heat production. Presumably, renal O_2 uptake increased as well [57]. However, there was only a small increase in net Na^+ reabsorption; importantly, no decrease in Na^+ reabsorption occurred. Therefore, it is likely that the increased delivery of glycine to the kidney [56] stimulated endergonic synthetic work functions as manifested by the increased renal heat production. Indeed, glycine is a substrate known to enter several energy-requiring synthetic pathways: It is utilized in glutathione synthesis, in forming acyl-glycines, in the renal synthesis of creatine, in the synthesis of serine, and because it enters the pyruvate pool, it may also enter into the syntheses of fatty acids and glucose. Thus, with an excess of glycine available, if the cost of Na^+ reabsorption had been calculated using the ratio, $\Delta net\ \dot{T}\text{-}Na^+/\Delta\dot{Q}\text{-}O_2$, it would have been overestimated because renal heat production parallels O_2 utilization [57]. These observations again show that if the ratio, $\Delta\dot{T}\text{-}Na^+/\Delta\dot{Q}\text{-}O_2$ is measured under circumstances when net synthetic functions are also changing, spurious estimates for the mean cost of net Na^+ reabsorption will result.

Role of reabsorptive co-transport of substrate with Na^+ from the proximal tubule lumen in initiating renal synthetic function

The virtually complete conservation of filtered substrates occurs almost entirely along the proximal tubule by their being co-transported into the tubular cells with Na^+ [27–30]. In this context, it is pertinent to recognize that in most of the studies performed to determined the energy cost of net Na^+ reabsorption, Na^+ reabsorption was varied by changing the filtration rate in normal animals [1, 2, 15]. A change in filtration rate not only alters the filtered (and reabsorbed) load of Na^+, but also the filtered (and reabsorbed) load of metabolizable substrates such as lactate, glutamine, and citrate. Thus, the rates of entry both of Na^+ and of substrates into the proximal tubular cells are proportional to the filtration rate. Because most studies were done with normal animals, substrate concentrations in blood were probably similar among the several studies and hence the ratio of $[Na^+]$:[substrate] in plasma was also similar. Thus, changes in the filtered and reabsorbed quantities of Na^+ and substrate should parallel one another. While the fates of each of the substrates co-transported into the proximal tubular cells with Na^+ have not been identified or quantified in vivo, the sum of the several substrates reabsorbed is greater than can be oxidized by the simultaneous renal O_2 uptake [1, 2]. A substrate which enters the proximal tubular cell by co-transport with Na^+ has three possible fates:

- The substrate may be oxidized. The ATP produced can then be utilized for the extrusion of the cotransported Na^+ across the basolateral cell membrane via Na,K-ATPase.
- The substrate may be used in syntheses. Indeed, the ATP produced by the oxidation of a portion of the substrate entering the cell with Na^+ may also be used for support of the synthetic function, initiated by the increased availability of the cotransported substrate.

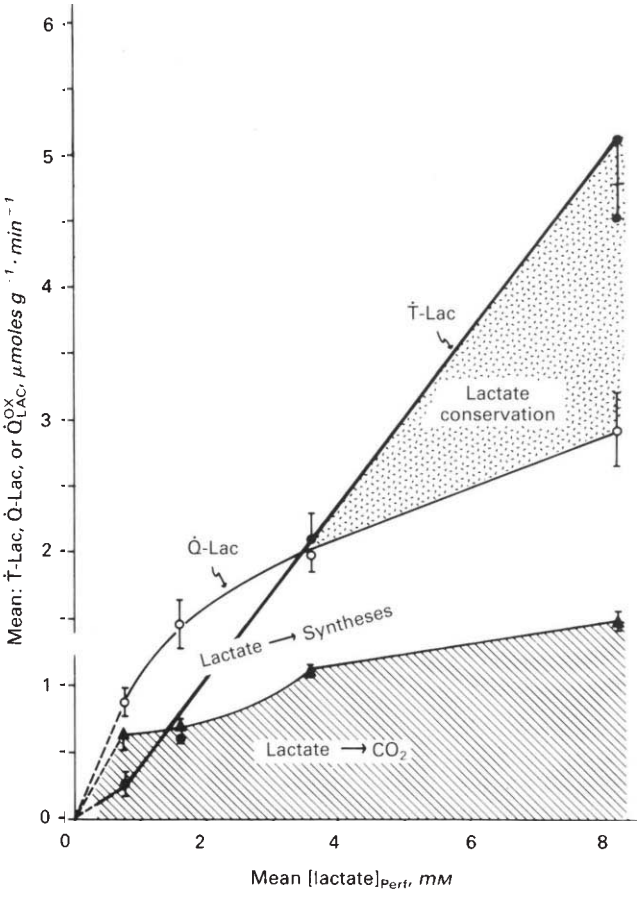

Fig. 5. *Relationship between lactate entering the tubular cells from the lumen and the fates of lactate.* At the lower concentrations of lactate in the perfusate most of the "reabsorbed" lactate can be accounted for by either decarboxylation or utilization in syntheses. Only at the higher concentrations of lactate is the lactate "reabsorbed" in fact, conserved. Reproduced with permission from [53].

- The reabsorbed substrate may be conserved (that is, undergo net reabsorption). By leaving the cell across the basolateral membrane and entering the peritubular capillaries, the substrate thus leaves the kidney in the renal venous blood.

The relationship among these three possible fates of lactate are illustrated by the observations summarized in Figure 5. The studies shown were done with the perfused rat kidney [53] in which lactate was the only exogenous substrate available to the kidney. At the lower concentrations of lactate in the perfusate (< 2.0 mM), all the reabsorbed (Ṫ-LAC) lactate could be accounted for by decarboxylation. Indeed, at these lower concentrations of lactate, additional lactate was also utilized from the peritubular capillaries, because the rate of lactate decarboxylation was greater than the net rate of lactate entry across the luminal membrane ("reabsorption"). At higher lactate concentrations (above ~2.0 mM), when lactate decarboxylation rate was approaching a maximum, some of the "reabsorbed" lactate entered into energy-requiring synthetic pathways (net glucose and glutamate production accounted for ~30% of the lactate entering into syntheses); lactate for the syntheses was also taken up across the basolateral cell mem-

brane. It was only when the synthetic reactions were approaching a maximum that a fraction of the lactate entering the cells from the lumen could be accounted for by conservation, that is, "true" net reabsorption of lactate occurred, in which some of the filtered lactate crossed the proximal tubular epithelium, entered the peritubular capillary, and left the kidney in the renal venous blood.

The data in Figure 5 can also be used to illustrate how, if GFR was reduced, a decrease in $\dot{Q}O_2$ would have occurred due to decreases in both $\dot{T}Na^+$ and in synthetic functions: At lactate concentrations above ~1.6 mM, lactate entered the proximal tubular cells and was used for both oxidation and syntheses. If GFR had been reduced, one would then expect that both Na^+ reabsorption and the rate at which lactate entered into synthetic pathways from the lumen would have been decreased also. Thus, a reduction in O_2 uptake would have resulted from the simultaneous decreases both in $\dot{T}$-Na^+ and in synthetic functions and not from a decrease in $\dot{T}$-Na^+ alone.

These relationships for lactate in the isolated perfused kidney are analogous to the situation in vivo where the sum of the endogenous substrates reabsorbed is greater than can be oxidized. Therefore, as indicated above, the energy requirement for $\Delta\dot{T}$-Na^+ calculated from $\Delta\dot{T}Na^+/\Delta\dot{Q}$-$O_2$ under such circumstances is overestimated, the $\Delta\dot{Q}$-O_2 provides an estimate of the total increment in energy required for *both* the increment in transport functions *and* the increment in substrate-interconversion functions. These relationships are summarized in the schema shown in Figure 6.

It is apparent that more experimental data are required to determine the O_2 requirements of these multiple renal synthetic functions which occur and change in parallel with Na^+ reabsorption. Because virtually all the measurements of the ratio, Δnet $\dot{T}Na^+/\Delta\dot{Q}$-$O_2$, have been recorded from animals whose substrate concentrations in arterial blood were probably in the normal range, the ratio between cotransported Na^+ and substrate was similar in all instances. However, if changes in the concentrations of substrates in blood occur, as may have been the case in the studies of Mathison, Montclair, and Kiil [3], then the effects of changes in synthetic function rates would also have affected the ratio, $\Delta\dot{T}Na^+/\Delta\dot{Q}$-$O_2$. Studies of the ratio, $\Delta\dot{T}Na^+/\Delta\dot{Q}$-$O_2$, are needed under conditions where the concentrations in the arterial blood of those substrates which are used readily in syntheses are elevated, as was done in the perfused rat kidney for lactate [53] (and could have been done in vivo with the dog with increased blood levels of glycine [56] or lactate [33] so that both synthetic work and $\dot{T}$-Na^+ would be changed. Depending on which substrate concentrations were increased, one would then expect the ratio, Δnet $\dot{T}Na^+/\Delta\dot{Q}$-$O_2$, to be lower (Fig. 6).

This aspect of the discussion has dealt primarily with the substrate metabolism of the proximal tubule where virtually all the filtered substrates are reabsorbed. Because the tubular fluid entering the loop is ordinarily substrate-free, substrates utilized by the distal nephron must be delivered to these cells exclusively from the peritubular capillaries. This is in contrast to the proximal convoluted tubule, where filterable substrates can enter these cells across both the luminal and basolateral membranes (Fig. 5). However, from in vivo studies, the uptake of free fatty acids (FFA) which are bound to albumin, occurs only across the basolateral membranes [58]. Whether this uptake of

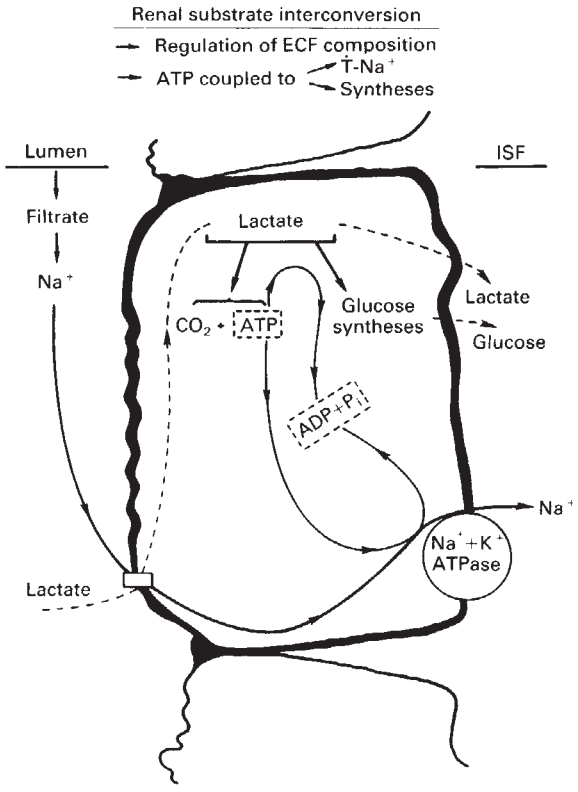

Fig. 6. *Schema showing the possible metabolic fates of lactate entering the proximal tubular cell by cotransport with Na^+, based on observations shown in Figures 2–5.* Lactate oxidation supports both Na^+ extrusion (reabsorption) into the interstitial fluid and synthesis of glucose from the excess lactate entering the cells. When more lactate enters the cell than can be oxidized or utilized in syntheses, it enters the interstitial fluid and is accounted for by reabsorption.

FFA is limited to the proximal tubule or occurs along the more distal nephron segments in unknown. The uptake of citrate [59] and malate [60] across the basolateral membrane vesicles isolated from cortex has been shown to be coupled to Na^+. Whether these basolateral vesicles were all derived from the proximal tubule or from other more distal nephron segments present in the cortex was not determined. Thus, no definitive information is available about either the mechanisms for the uptake of substrate across the basolateral membranes or the metabolic fates of substrates taken up by the distal nephron [61]. In particular, to my knowledge, whether significant rates of energy-requiring syntheses of new substrates occur in the distal nephron has not been determined. If net synthetic function rates are found to be relatively high in the distal nephron, then there will be a further limitation in estimating the energy requirement for distal $\Delta\dot{T}$-Na^+, as is the case in the proximal tubule.

Summary

In the mammalian kidney, the use of the ratio, Δnet $\dot{T}$-$Na^+/\Delta\dot{Q}$-O_2, provides an overestimate of the energy requirements for unidirectional active Na^+ transport. In the proximal tubule, the overestimate of the energy cost for $\dot{T}$-Na^+ is due to these phenomena: *(1)* The "leaky" characteristics of the proximal tubule does not permit an accurate estimate to be made of

the active fraction of the unidirectional flux of Na^+. Thus, the net Na^+ or net HCO_3^- reabsorption rate alone cannot be used to determine the stoichiometry for unidirectional extrusion of Na^+ (with HCO_3^-) by the Na,K-ATPase, since backflux of HCO_3^- into the lumen occurs. *(2)* There is a moiety of active Na^+ with Cl^- along the pars recta. Whether this reabsorptive rate is altered and O_2 uptake also changed when GFR or $NaHCO_3$ reabsorption is varied is not yet known. *(3)* The occurrence of energy-requiring synthetic functions (substrate-interconversions) in the proximal tubule, coupled, in part, to the rate of Na^+ entry into the proximal tubule cells, results in changes in renal O_2 uptake proportional to some (undetermined) fraction of the change in Na^+ reabsorption. The utilization of a portion of these reabsorbed substrates in endergonic syntheses must account for a portion of the Na^+-stimulated suprabasal O_2 uptake rate. Hence, the presence of synthetic functions in the proximal tubule also contributes to the overestimation of the energy value of net Na^+ reabsorption when the ratio, $\Delta\text{net } \dot{T}Na^+/\Delta\dot{Q}\text{-}O_2$, is used. By quantifying the rate of synthetic function and the ATP requirement for it, one can estimate more accurately the moiety of O_2 uptake related only to Na^+ reabsorption.

While the value of unidirectional active Na^+ transport due to Na^+-H^+ antiport must be less than present estimates suggest, this estimate may be the closest approximation available at this time. Importantly, this approach accounts for the high ratio, $\Delta\text{net } \dot{T}Na^+/\Delta\dot{Q}\text{-}O_2$, observed in the intact kidney. In the distal nephron (beyond the thick ascending limb of the loop of Henle), the "tightness" of the epithelium results in net Na^+ reabsorption rates which are close to the unidirectional Na^+ transport rates. If there are no other major, independent (of Na^+) transport or synthetic phenomena occurring in the distal nephron, a measurement of $\Delta\text{net } \dot{T}\text{-}Na^+/\Delta\dot{Q}\text{-}O_2$ provides a close approximation of the energy cost for unidirectional distal Na^+ transport. However, change in simultaneous active H^+ secretion (and active K^+ transport) require sources of energy separate from and in addition to that required for Na^+ reabsorption. If such changes also occur, there also will result an overestimation of the energy requirement for distal Na^+ reabsorption when $\Delta\dot{T}\text{-}Na^+/\Delta\dot{Q}\text{-}O_2$ is used.

Whether quantitatively (that is, relative to the total renal $\dot{Q}\text{-}O_2$) significant rates of synthetic function occur in the distal nephron cells or in medullary interstitial cells is unknown.

Quantitative information is needed concerning the specific metabolic fates of substrates along all regions of the nephron relating to transport and synthetic functions. From such data a more complete understanding of the energy requirements for the several renal functions may be obtained.

The multiple transport and synthetic renal functions which are supported by the renal O_2 uptake indicate the several mechanisms by which the metabolism of the kidney contributes to the regulation of the volume and composition of the body fluids and solutes.

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