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Formation and Excretion of $NH_3 \leftrightarrow NH_4^+$. New Aspects of an Old Problem

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Summary. The proximal tubule cell is the major site of renal ammoniagenesis. Glutamine is the major substrate. Deamidation by mitochondrial glutaminase yields glutamate$^-$ and NH_4^+ (not NH_3, as traditionally taught). A second NH_4^+ ion is obtained by deamination of glutamate$^-$ to 2-oxo-glutarate^{2-}. NH_4^+ preferentially enters the tubule lumen primarily, but probably not exclusively, by non-ionic diffusion of NH_3. For each NH_3 formed in the cell one H^+ ion is left behind. H^+ and NH_3 are secreted on separate routes, but recombine in the lumen to NH_4^+ and reach the final urine in this form. This process *per se* does not net-remove H^+ from the organism. For this purpose, the anionic products of ammoniagenesis (2-oxo-glutarate^{2-} and others) have to be converted into neutral compounds (CO_2, glucose). This metabolism again takes place usually in the tubule cell. For each negative charge one HCO_3^- is formed which enters the peritubular blood. Luminal γ-glutamyl transferase-mediated ammoniagenesis contributes to NH_4^+ accumulation in the proximal tubule to a small extent. The endproximal NH_4^+ delivery exceeds the filtered load by a factor of 9. Only 1/3 of it reaches the distal convoluted tubule mainly because NH_4^+ as such is reabsorbed from the thick ascending limb of Henle's loop by secondary active transport or electrodiffusion. Both processes are energized by the active Na^+ transport in this segment. Thereby $NH_3 \leftrightarrow NH_4^+$ is accumulated in the medullary interstitium, which establishes the chemical gradient for non-ionic diffusion of NH_3 into the lumen of the collecting ducts. This is favoured by the acidic disequilibrium pH in the lumen of this segment. Secretion of $NH_3 \leftrightarrow NH_4^+$, probably by non-ionic diffusion, also into the descending limb of the loop is hypothesized to maintain (together with the NH_4^+ reabsorption in the thick ascending limb) the high interstitial $NH_3 \leftrightarrow NH_4^+$ concentration increasing towards to inner medulla. Thus, the principle of counter current multiplication seems to be involved also in NH_4^+ excretion.

Renale Bildung und Ausscheidung von $NH_3 \leftrightarrow NH_4^+$. Neue Aspekte eines alten Problems

Zusammenfassung. Hauptort der renalen $NH_3 \leftrightarrow NH_4^+$-Bildung ist die proximale Tubuluszelle. Glutamin ist das wesentliche Substrat dafür. Die Desamidierung durch die mitochondriale Glutaminase ergibt Glutamat$^-$ und NH_4^+ (und nicht NH_3, wie oft behauptet). Ein zweites NH_4^+-Ion wird aus der Desaminierung von Glutamat$^-$ zu 2-Oxo-Glutarat^{2-} gewonnen. NH_4^+ gelangt in das Tubuluslumen großteils (aber nicht ausschließlich) in der Form von NH_3 (nicht-ionische Diffusion). Für jedes sezernierte NH_3 bleibt ein H^+-Ion in der Zelle zurück, das separat ins Lumen transportiert wird. Im Lumen wird aus beiden wieder NH_4^+ gebildet, das dann im Urin ausgeschieden wird. Dieser Prozeß *per se* entfernt keine H^+-Ionen aus dem Körper. Zu diesem Zwecke müssen erst die anionischen Metaboliten, die bei der Ammoniagenese entstehen (2-Oxo-Glutarat^{2-} u.a.), in neutrale Stoffe umgewandelt werden (CO_2, Glukose). Dies geschieht ebenfalls, wenn auch nicht notwendigerweise, in der Tubuluszelle. Für jede negative Ladung des Substrats entsteht dabei ein HCO_3^--Ion, das die Zelle auf der Blutseite verläßt. Eine luminale NH_4^+-Bildung, katalysiert durch γ-Glutamyltransferase, trägt in geringem Ausmaß zur NH_4^+-Anhäufung im Lumen bei. Am Ende des proximalen Konvoluts findet sich 9mal mehr $NH_3 \leftrightarrow NH_4^+$ als im Glomerulusfiltrat. Nur ein Drittel davon erreicht das distale Konvolut, vor allem weil NH_4^+ als solches im dicken, aufsteigenden Teil der Henleschen Schleife durch Co-Transport oder Elektrodiffusion resorbiert wird. Beide Prozesse erhalten ihre Energie durch den aktiven Na^+-Transport in diesem

Nephronteil. Dadurch wird $NH_3 \leftrightarrow NH_4^+$ u.a. im medullären Interstitium akkumuliert, so daß ein chemischer Gradient für die nicht-ionische Diffusion von NH_3 ins Lumen des Sammelrohrs aufgebaut wird. Begünstigt wird dies auch durch den sauren Disäquilibriums-pH-Wert im Lumen dieses Segments. Es wurde vorgeschlagen, daß $NH_3 \leftrightarrow NH_4^+$, wohl via nicht-ionische Diffusion, auch in den absteigenden Teil der Henleschen Schleife sezerniert wird, um, zusammen mit der NH_4^+-Resorption im aufsteigenden Schleifenteil, die hohe interstitielle $NH_3 \leftrightarrow NH_4^+$-Konzentration, die markwärts zunimmt, aufrechtzuerhalten. Das Prinzip der Gegenstrom-Multiplikation scheint daher auch bei der NH_4^+-Ausscheidung eine wesentliche Rolle zu spielen.

Key words: Kidney – Ammoniagenesis – Glutamine metabolism – Ammonium excretion – Ammonium transport – Nonvolatile acids

The Traditional Approach

In the traditional view, metabolism of certain dietary amino acids yields strong nonvolatile acids (H_2SO_4, HCl). The kidney excretes this acid load in three forms, i.e. as titrable acid (mainly phosphate), as NH_4^+ and as free protons, of which the last form is quantitatively negligible. Beside respiratory influences, the renal ammoniagenesis is thought to be the central metabolic pathway in the regulation of acid-base balance because NH_4^+ excretion rises in chronic metabolic acidosis severalfold.

The tubule cell is thought to form NH_3 from glutamine. The uncharged base NH_3 then enters the tubule lumen solely by non-ionic diffusion. At the same time, protons are secreted into the lumen and titrate the NH_3 to NH_4^+, for which the tubule wall is thought to be virtually impermeable. Therefore, the NH_4^+ trapped in this way in the lumen is excreted in the final urine. For each proton secreted and excreted this way, one HCO_3^- ion is formed according to

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+. \quad (1)$$

The HCO_3^- leaves the tubule cell on the peritubular side. Although this approach is only partially correct, and, in some respects misleading [2, 20], it is taught that way in most textbooks (see Fig. 1a).

This article mainly deals with a revised view of these processes and reviews some of the most recent experimental data on the renal production, transport, and excretion of $NH_3 \leftrightarrow NH_4^+$.

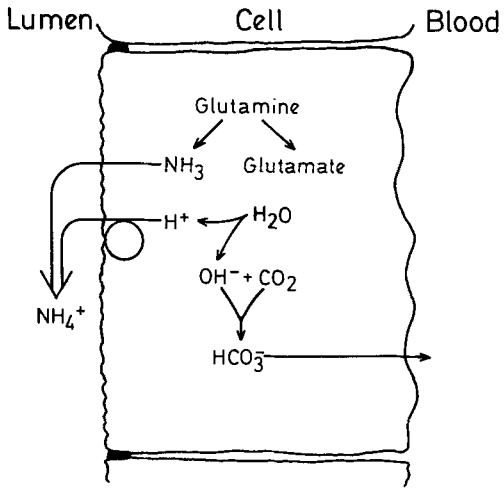

Fig. 1a. Traditional view of proximal tubular ammoniagenesis. It is wrong because at the physiological pH of the tubule cell (around 7.3), NH_4^+ (not NH_3) and glutamate$^-$ are formed. Secretion of NH_3 and H^+ *per se*, therefore, does not represent net acid removal

Production and Consumption of NH_4^+ and Nonvolatile Acids

Although sometimes named this way, NH_4^+ is neither a strong acid nor is the pair $NH_3 \leftrightarrow NH_4^+$ (pK about 9) a significant buffer within the physiological pH range. Thus, if during oxidation of a neutral amino acid like alanine

$$CH_3-\underset{\displaystyle NH_3}{\underset{|}{CH}}-COO^- + 3O_2 \rightarrow 2CO_2 + HCO_3^- + NH_4^+ + H_2O \quad (2)$$

equal amounts of HCO_3^- and NH_4^+ are formed, the bicarbonate cannot be directly buffered by NH_4^+ [2]. Gill-breathing vertebrates can eliminate the two products and the CO_2 into the water surrounding the gills. Air-breathing animals can get rid of the CO_2 by expiration only. From the usual dietary intake of 100 g protein in humans, roughly 1 mol HCO_3^- and 1 mol NH_4^+ are formed. Because there is no way of excreting these big amounts of the two compounds as such in the urine or in the feces, the liver combines both substances to a neutral compound, i.e. urea, by utilizing high amounts of metabolic energy. The net reaction of this biosynthesis is:

$$2NH_4^+ + 2HCO_3^- \xrightarrow{4ATP} Urea + CO_2 + 3H_2O. \quad (3)$$

The energy supply is needed because NH_4^+ is a much weaker acid than carbonic acid and, therefore, the direct or indirect transfer of protons from NH_4^+ to HCO_3^- cannot occur spontaneously. Atkinson and Camien [2] even called this H^+ transfer a kind of "ATPdriven proton pump".

Thus, the bulk of the protein metabolites are excreted in the urine as urea and the CO_2 is blown off by respiration. However, degradation of certain amino acids yields products carrying a net charge. In general, metabolism of dietary constituents liberates H^+ when a neutral nutrient is converted into a metabolite which contains a net anionic charge [21]. This applies for the neutral (superscript: ^{0}) amino acids cysteine or methionine.

$$\text{Met}^0 \text{ (or Cys}^0) \rightarrow \text{glucose}^0 \text{ (or triglyceride}^0) + \text{urea}^0 + SO_3^{2-} + 2H^+: \quad (4)$$

Because a usual diet contains 12 mmol cysteine and 24 mmol methionine per day [21], 2 times $(12+24) = 72$ mmol H^+ are formed this way every day.

In a further metabolic process, positively charged amino acids (arginine, lysine and part of the histidine residues) are converted into neutral metabolites. Here H^+ ions are formed again:

$$\text{Arg}^+ \cdot Cl^- \rightarrow \text{glucose}^0 \text{ (or triglycerides}^0) + \text{urea}^0 + H^+ + Cl^-. \quad (5)$$

From this degradation, usually about 138 mmol H^+ are formed per day [21].

In contrast to these proton-generating processes, metabolism of anionic compounds, i.e. the salts of the acidic amino acids, glutamate and aspartate (100 mmol/day), or other organic anions like lactate, acetate, malate, citrate, etc. (roughly 60 mmol/day), to neutral metabolites will *remove* H^+ ions:

$$\text{lactate}^- + H^+ \rightarrow \text{glucose}^0 \text{ (or triglyceride}^0) + CO_2. \quad (6)$$

Summing up the production and consumption of H^+ by all these processes, the net formation of H^+ by the liver will be about $73+138-100-60=50$ mmol/day. As these protons are buffered, an equimolar amount of H^+ have to be removed from the body in order to restore the body's HCO_3^-.

How Does the Kidney Remove Strong Nonvolatile acids?

Protons are excreted by the kidney "in the form of" titratable acid and NH_4^+. Whereas the first process is a real intraluminal titration mainly of filtered HPO_4^{2-} to $H_2PO_4^-$ by H^+ ions secreted into the lumen, this is not the case with $NH_3 \leftrightarrow NH_4^+$.

If, as usually drawn in textbooks, NH_3^0 were the product of, for instance, glutamine deamidation, glutamic acid^0 rather than glutamate$^-$ would be formed. At the physiological tubular cell pH of about 7.3, however, NH_4^+ and glutamate$^-$ are the products of the glutaminase reaction. Assuming non-ionic diffusion of ammonia, for each NH_3 leaving the cell, one H^+ is formed in the cell according to:

$$NH_4^+ \leftrightarrow H^+ + NH_3. \quad (7)$$

In the tubule lumen, both, the NH_3 and the H^+, secreted by separate mechanisms, combine again and are excreted in the form of NH_4^+ (see Fig. 1 b). Thus, this process *per se* clearly does not net-remove H^+ ions from the body. What is needed here, is a *second process* which consumes H^+ by converting the anionic metabolite glutamate$^-$ to a neutral compound (CO_2^0 or glucose^0). However, this second step does not necessarily occur within the kidney. Nevertheless as has recently been reviewed by Welbourne and Phromphetcharat [55], incorporation of glutamine carbon into neutral metabolites within the kidney has been shown for instance with rat kidney cortical slices [17] as well as in dogs in vivo [27]. Moreover, the glutamate content of the tuble cell decreased during metabolic acidosis, and glutamate even inhibits ammoniagenesis under these conditions [12]. Thus, not only deamidation of glutamine but also the removal of the 2-amino group of glutamate is followed by tubular consumption of one proton for each NH_4^+ formed. Therefore, the total conversion of the glutamine^0 to CO_2^0 and/or glucose^0 in the tubule results in the formation of $2HCO_3^-$ (entering the blood) if both nitrogens are excreted by the kidney as NH_4^+. In conclusion, NH_4^+ excretion is normally a *measure* of HCO_3^- formation (or H^+ loss), although excretion of NH_4^+ *per se* is not identical with H^+ excretion (see above).

In alkalosis, however, the anionic citric acid cycle intermediates citrate and 2-oxo-glutarate are hyperexcreted [4, 8, 23, 44]. In this case, renal HCO_3^- formation may become smaller than NH_4^+ production because anionic metabolites of glutamine leave the kidney and even the body what indirectly helps to reduce the HCO_3^- content of the organism. Quantitatively, however, renal excretion of HCO_3^- as such is more important under such conditions.

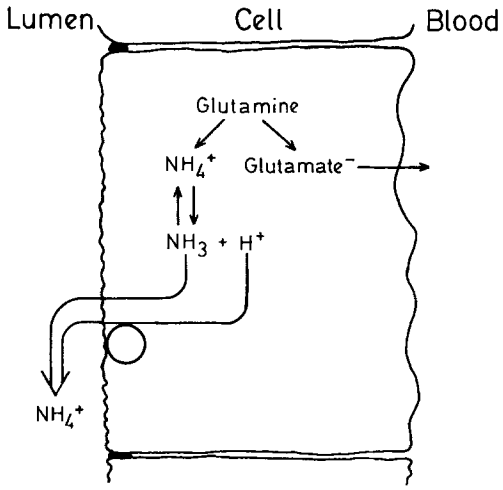

Fig. 1b. Proximal tubular ammoniagenesis as revised by Atkinson and Camien [2]. It is chemically right, but physiologically wrong because glutamate$^-$ (and also other anionic metabolites of tubular ammoniagenesis) does not leave the kidney to any greater extent

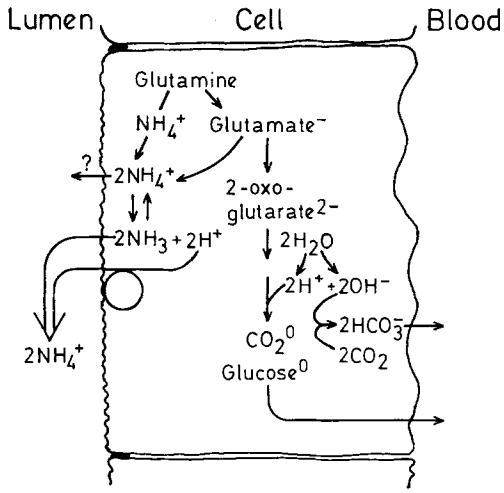

Fig. 1c. Proximal tubular ammoniagenesis as seen today. The main substrate glutamine enters the cell from both sides. The mitochondrial glutaminase produces NH_4^+ + glutamate$^-$. NH_4^+ is secreted as NH_3 and H^+ by separate mechanisms forming NH_4^+ in the lumen. Glutamate$^-$ is further metabolized to a second NH_4^+ and 2-oxo-glutarate^{2-}. The conversion of this twofold negatively charged metabolite to the neutral substances CO_2 and glucose utilizes two H^+. The remaining OH^- combines with CO_2 (carbonic anhydrase-catalyzed) yielding HCO_3^- which enters the peritubular blood. This latter process represents net removal of H^+ from the body if the NH_4^+ formed is excreted in the urine. A small amount of NH_4^+ is also formed from filtered glutamine within the lumen (see text)

Is H^+ Excretion Regulated in the Kidney or in the Liver?

It is well-known that renal ammoniagenesis highly increases in chronic metabolic acidosis, although the signal governing this change is still only partly understood [33, 46]. More recently, a major role of the liver in pH-homeostasis has been proposed [1, 2, 29]. The authors [1, 29] emphasize that the urea cycle not only removes $NH_3 \leftrightarrow NH_4^+$ and converts it to an easily excretable compound but also disposes an equimolar amount of HCO_3^- generated in metabolism (about 1 mmol/day; see above). They state that "These considerations demonstrate that the concentration of HCO_3^- in the blood is regulated primarily by the liver; they are incompatible with the textbook statement that the kidney regulates the blood bicarbonate level" [2]. They only agree with the traditional view in as far as the kidney can have a direct effect on the level of HCO_3^- in the blood, if, under extreme conditions, the metabolic production of HCO_3^- exceeds the NH_4^+ formation thus far that it is leading to HCO_3^- concentrations in the blood above 30 mmol/l. These statements are partly based on the assumption that all glutamine-derived glutamate$^-$ leaves the kidney (see Fig. 1b). However, no reference is given for this. In contrast, renal glutamate release in normal human subjects has been found to be only about 12% of extracted glutamine. In acidosis, glutamate is even taken up by the kidney in humans as well as in dogs [30, 32]. Also in alkalotic dogs, glutamate release is less than 5% of the glutamine extracted [32]. As outlined above, the glutamine carbon nearly completely goes into neutral metabolites within the tubule cell (see Fig. 1c). If gluconeogenesis, i.e. one pathway leading to a neutral endproduct of glutamine metabolism, is inhibited, ammoniagenesis is even lowered to 50% [34]. For these and other [51] reasons, the kidney is clearly involved in the regulation of pH-homeostasis.

Nevertheless, it seems to be feasible that, beside the kidney, the liver might take part in this regulation. Indeed, it has been found [22] that a low pH value in the portal vein decreases urea formation and glutamine deamidation in the periportal hepatocytes and at the same time favours glutamine synthesis in perivenous hepatocytes. From these results it has been suggested that the pH dependence of these processes represents a tool for hepatic pH control in the organism [22]. By these means, NH_4^+ would be diverted from ureagenesis to hepatic synthesis of glutamine. Consequently, more of it is exported to the kidney in metabolic acidosis. Here, it enters the glutaminase pathway resulting in an increased NH_4^+ excretion. The HCO_3^- saved by the decreased urea formation (see formula 3) would be able to restore the decreased HCO_3^- levels in acidosis. Thus, the question arises, whether the urea synthesis is regulated primarily

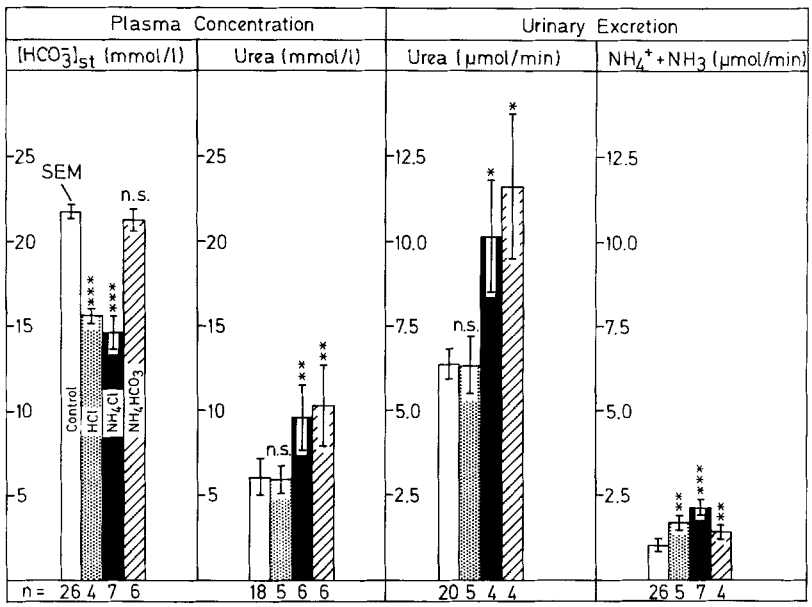

Fig. 2. The effect of HCl (1 mmol) and NH_4Cl and NH_4HCO_3 (2.5 mmol each) given by stomach tube to rats (270 g B.W.) on standard HCO_3^-, on the plasma level of urea, and on the excretion of urea and ammonia. The values are obtained 2 hours after application. GFR was constant in all cases (Scheller and Silbernagl, published in abstract form; Ref. [36])

by the needs of NH_4^+ disposal, as traditionally seen, or of pH-homeostasis, as suggested by the authors quoted above [1, 2, 22, 29].

We tested these alternative hypotheses by loading rats with NH_4Cl, or HCl by a stomach tube. If the ureagenesis is regulated by the needs of pH-homeostasis, urea formation should decrease during loading of HCl (because standard HCO_3^- is lowered), but should be unchanged after loading of NH_4Cl. According to the traditional view, i.e. urea synthesis is primarily governed by the needs of NH_4^+ disposal, ureagenesis should increase with NH_4Cl but not with HCl. The experimental results obtained are shown in Fig. 2 where the values after two hours are given. The GFR was constant in all cases. The results [36] clearly show that all predictions favouring the traditional view are matched by the results. Similarly, Halperin et al. [19] showed that ureagenesis did not change during a severe acidosis induced by HCl applicated intravenously. Thus, these results argue strongly against a primary role of hepatic urea formation in controlling pH-homeostasis. This conclusion is at variance with data [29] obtained after chronic HCl- or NH_4Cl loading (earliest measurements after 24 hours). In contrast to the above mentioned acute study, secondary metabolic changes might have come into play in the chronic study.

How Does $NH_3 \leftrightarrow NH_4^+$ Cross Tubular Cell Membranes?

According to the 'classic' view [review: 13], $NH_3 \leftrightarrow NH_4^+$ is transported through cell membranes at least primarily by non-ionic diffusion, i.e. in the form of the uncharged base NH_3. Accordingly, the total ammonium concentration, $[NH_4^+ + NH_3]$, in all compartments in NH_3 equilibrium can be predicted, if their pH and $[NH_4^+ + NH_3]$ in one compartment is known. Thus, if the readily diffusible form NH_3 would be in diffusion equilibrium throughout the whole kidney, urinary $[NH_3 + NH_4^+]$ could be only elevated above renal venous $[NH_3 + NH_4^+]$ by the factor (urinary $[H^+]$/ renal venous $[H^+]$).

$$[NH_4^+]_U = \frac{[H^+]_U}{[H^+]_{ren.venous}} \cdot [NH_4^+]_{ren.venous}. \quad (8)$$

(In this simplified equation the very small NH_3 content, if compared to NH_4^+, is neglected.) Taking experimental data of Pitts et al. [32] obtained from acidotic dogs, the measured urinary $[NH_4^+ + NH_3]$, however, is 2 to 4 times higher than the value calculated from the above equation (see also Ref. [20]). These and other considerations already suggest that renal handling of $NH_4^+ \leftrightarrow NH_3$ involves other major transport processes than non-ionic diffusion.

In order to better understand these processes, the technique of perfusing isolated nephron segments has recently begin to be applied to the investigation of renal ammonium transport (review: [15]). Nagami and Kurokawa [28], for instance microperfused isolated proximal tubules which are considered to be the major site of ammonium secretion [11, 14, 35, 43]. No $NH_3 \leftrightarrow NH_4^+$ was initially present in the bath and in the perfusate. The total ammonia concentration in the fluid exiting

the lumen turned out to be 300-fold greater than the concentration in the bath. Assuming intracellular (not luminal) ammoniagenesis as the primary source, and non-ionic diffusion of NH_3 alone across these tubules, the ΔpH between lumen and bath must have been nearly 2.5. The maximum ΔpH experimentally measured across the wall of proximal tubules, however, lies somewhere between 0.7 and 0.9 [26, 50]. Even assuming a very high maximum ΔpH of 1, luminal NH_4^+ should not exceed a value 10-fold higher than found in the bath. Thus, the luminal NH_4^+ accumulation observed is not compatible with non-ionic diffusion of NH_3 alone followed by NH_4^+ trapping in the lumen. Similarily, the $NH_3 \leftrightarrow NH_4^+$ concentration found endproximally in rats in vivo [11, 35], can be explained only with difficulties by non-ionic diffusion of NH_3 alone. We, therefore, have to consider other possibilities. One would be, that NH_4^+ is formed from glutamine within the lumen. Another explanation would be that the charged NH_4^+ as such is secreted into the lumen. These two possibilities are considered in the two following sections.

Luminal Ammoniagenesis?

The main substrate for renal ammoniagenesis is glutamine [32, 49]. Filtered glutamine enters the cells of the early proximal tubule from the lumen [28] but peritubular uptake also occurs [42]. Potentially, there are three renal pathways by which NH_4^+ can be split from glutamine [46]. The amido group can be hydrolyzed by two enzymes [24], (a) by the mitochondrial phosphate-dependent glutaminase (PDG) or (b) by the phosphate-independent glutaminase (PIG), which is identical with the brushborder γ-glutamyl-transferase or γ-GT [9, 47]. Pathways (a) and (b) yield glutamate$^-$ and NH_4^+. (c) In the so-called glutaminase II pathway, initially, the 2-amino group of glutamine is transaminated to a ketoacid by cytosolic glutamine ketoacid aminotransferase. The products are the respective amino acid and 2-oxoglutaramate which in turn is deamidated by ω-amidase, yielding NH_4^+ and 2-oxoglutarate^{2-}.

Today, there is little doubt that the mitochondrial PDG is quantitatively most important for renal ammoniagenesis at least during chronic metabolic acidosis [46, 10]. There is no convincing evidence to suggest that the glutaminase II pathway plays an important role in renal ammonia production [46]. As suggested by Welbourne and coworker [52, 53], this may be not true for PIG, i.e. γ-GT.

γ-GT is located in the tubular brushborder where its catalytic activity faces the tubule lumen [40, 41]. The glutaminase activity of γ-GT can be highly increased by the toxic xenobiotic maleate and, more interestingly, by the non-toxic hippurate [48] which is secreted into the tubule lumen under physiological conditions. Thus, the question arises, whether and to what extent the γ-GT can contribute to renal ammoniagenesis [52] if hippurate is present in the lumen.

It has been shown most recently in microperfusion experiments performed with rats in vivo that a high luminal glutamate formation from glutamine can be demonstrated in the proximal tubule [39] if 10 mmol/l hippurate are present in the perfusate. However, it has been shown in the same study, that endogenous hippurate concentrations in the lumen of the late proximal convoluted tubule and in the early distal convolution reach only levels of 0.16, or 0.6 mmol/l, respectively. From these values, it can be estimated that luminal γ-GT-mediated glutamine hydrolysis constributes 5–10% to the normal renal ammonium excretion [39]. This number may increase during treatment of children suffering from an enzymatic defect in the urea cycle with the hippurate precursor benzoate [3]. An important role of luminal ammoniagenesis during metabolic acidosis, at least in rats, is, however, questionable [37]. Confirming this view, it has been also disproved very recently (Silbernagl, unpublished results) that luminal hippurate concentrations in the rat proximal tubule rise significantly during chronic metabolic acidosis as suggested earlier [53].

In contrast to the small contribution of γ-GT-mediated luminal ammoniagenesis to the total kidney excretion of NH_4^+ in the rat as found in vivo [37, 39], a high rate of luminal glutamine hydrolysis was found in the isolated perfused rabbit proximal tubule in vitro, even in absence of exogenous hippurate [54]. Whether this means a considerable species difference or an artifact of the in-vitro preparation, remains to be elucidated. Such an artifact is likely because any impairment of the tubule cell function in vitro would primarily affect mitochondrial- and brushborder uptake of the substrate glutamine rather than luminal γ-GT activity. Under such conditions, the relative contribution of luminal ammoniagenesis would be favoured to an extent not seen under normal in-vivo conditions.

Is NH_4^+ also Transported as such through Tubule Cell Membranes?

There is no doubt that the permeability of lipid bilayers for NH_3 is much higher than for NH_4^+

[15]. According to Fick's Law, however, diffusional transport is also determined by the concentration of the diffusing species. Within the kidney, the concentration of NH_4^+ is 100 to 10,000 times higher than that of NH_3. From this point of view, NH_4^+ may be transported to a considerable extent, even if its permeability coefficient is relatively low. Moreover, if NH_4^+ could cross the cell membrane through pores or carriers, NH_4^+ could considerably contribute to net $NH_3 \leftrightarrow NH_4^+$ transport because backdiffusion of NH_3 is not indefinitely high. (The permeability coefficient of NH_3 is lower than that of CO_2.)

As excellently reviewed by Good and Knepper [15], the hydrated ionic radius and the ionic conductance of NH_4^+ is virtually the same as that of K^+. It is also well established that NH_4^+ can substitute for K^+ in supporting the hydrolysis of ATP by the Na-K-ATPase in nerves as well as in rat kidney [review: 15]. Thus, specific segments of the nephrons were investigated in vitro in order to determine to what extent NH_4^+ as such may contribute to transcellular movement of $NH_3 \leftrightarrow NH_4^+$.

Although a favourable NH_3 gradient directed into the lumen of the *proximal tubule* certainly exists, the $NH_3 \leftrightarrow NH_4^+$ concentration found in the lumen of the isolated perfused proximal tubule cannot be fully explained by NH_3 diffusion [28: see above]. If an unphysiological high γ-GT-mediated luminal ammoniagenesis, as discussed above for the data of Welbourne and Park [54], can be excluded in this case, secretion of NH_4^+ as such has to be postulated.

A reabsorption of NH_4^+ as such has been clearly established in the *thick ascending limb* of Henle's loop [16]. Here, $NH_3 \leftrightarrow NH_4^+$ resorption takes place against a NH_3 gradient. Furosemide inhibits this NH_4^+ reabsorption. As this diuretic reduces the lumen-positive transepithelial potential normally found in this nephron section to zero [6], and at the same time blocks the reabsorptive $Na^+ - 2Cl^- - K^+$ co-transport [18], NH_4^+ could be reabsorbed either through the paracellular shunt by electrodiffusion or by the co-transporter where NH_4^+ replaces K^+ [15].

In the isolated perfused cortical *collecting duct* of rabbits, $NH_3 \leftrightarrow NH_4^+$ secretion was found despite a simultaneous HCO_3^- secretion [25] leading to a higher HCO_3^- in the lumen as compared to the bath. This result seemingly was incompatible with NH_3 diffusion. However, addition of carbonic anhydrase to the lumen converted $NH_3 \leftrightarrow NH_4^+$ secretion to net absorption without affecting HCO_3^- secretion [25]. This indicates an acid disequilibrium pH in the lumen which can passively drive NH_3 in the secretory direction (see Ref. [15] for further discussion). If such an acid disequilibrium pH also exists in vivo, it may be an important component for luminal trapping of the NH_3 which enters the colllecting duct from the medullary interstitium by diffusion.

The Route of $NH_3 \leftrightarrow NH_4^+$ through the Kidney

The proximal tubule cell is the most important site of NH_4^+ production. Although part of it enters the peritubular capillaries, the major part is secreted into the lumen (see Fig. 3) at least partly by non-ionic diffusion of NH_3. Whether NH_4^+ as such enters here too, remains to be demonstrated in vivo.

By the end of the superficial proximal convoluted tubule, the fractional delivery amounts to about 900% of the filtered load [43] whereby a small fraction of the late-proximal delivery stems from intraluminal γ-GT-mediated ammoniagenesis [39]. In the early distal superficial tubule only 265% of the filtered is found [43] demonstrating that about 2/3 of the late proximal $NH_3 \leftrightarrow NH_4^+$ is reabsorbed from the loop of Henle. In the thick ascending limb of Henle's loop, NH_4^+ as such is reabsorbed [16]. It is unknown whether electrodiffusion and/or Na^+-cotransport is the underlaying mechanism of luminal uptake. In any case, the active basolateral Na^+ extrusion in this segment is the energizing event [6, 18]. Basolateral exit probably occurs by non-ionic diffusion. Consequently, as hypothesized by Good and Knepper [15], the interstitial concentration of $NH_3 \leftrightarrow NH_4^+$ is increased towards the papillary tip by counter current multiplication. This implies that $NH_3 \leftrightarrow NH_4^+$ is secreted into the descending limb of Henle's loop probably in the form of NH_3. Thus, the collecting ducts are surrounded by an interstitium of a high concentration of $NH_3 \leftrightarrow NH_4^+$. This and the fact, that a very low luminal pH may be present in the lumen of the collecting duct (acidic disequilibrium pH) favour non-ionic diffusion of NH_3 from the medullary interstitium into the urine of collecting ducts [25].

Final urinary $NH_3 \leftrightarrow NH_4^+$ excretion normally is only a little smaller than the late proximal delivery. The fact that urinary excretion of $NH_3 \leftrightarrow NH_4^+$ often (not always) correlates with the urinary H^+-concentration to some extent, most probably reflects the fact that the important final step in renal $NH_3 \leftrightarrow NH_4^+$ transport localized in the collecting duct is favoured by an acidic urine. The short-cut of $NH_3 \leftrightarrow NH_4^+$ transport form the loop of Henle to the collecting duct needs an interstitial concentration gradient increasing from the cortex to the medulla. Consequently, if, e.g. in diuresis,

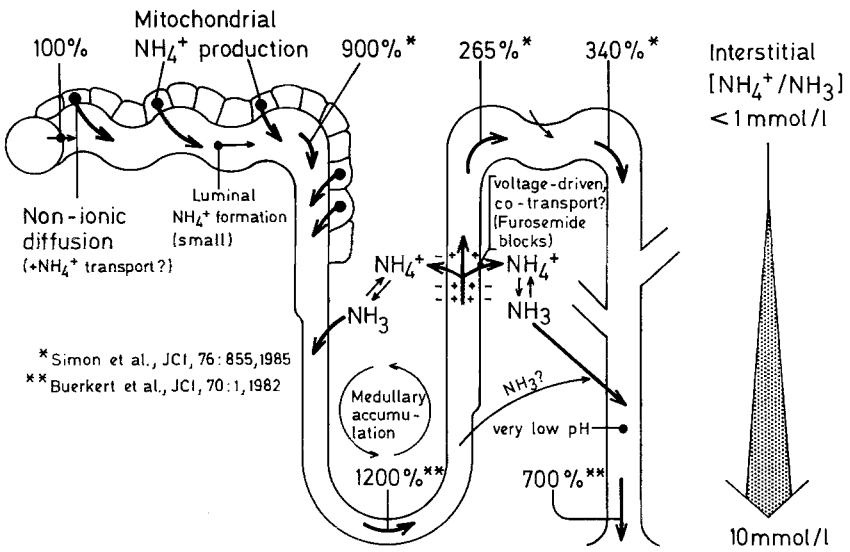

Fig. 3. Renal formation and transport of $NH_3 \leftrightarrow NH_4^+$. For details see text (Data from Refs. [5 and 43])

medullary $NH_3 \leftrightarrow NH_4^+$ is washed out, ammonium excretion transiently should increase and should become subnormal later on. Loop diuretics in addition block NH_4^+ reabsorption in the thick ascending limb whereby ammonium excretion should decrease to some extent. It has to be emphasized, however, that the way of $NH_3 \leftrightarrow NH_4^+$ through the kidney is not understood in every detail. The sequence outlined above is partly hypothetical and needs to substantiated in the future.

The General Significance of Renal Ammoniogenesis

The urinary excretion of the expendable cation NH_4^+ (NH_3 is quantitatively negligible), the production and excretion of which rises manyfold in chronic metabolic acidosis, facilitates the excretion of the anionic titratable "acids" and other anions without obligatorily depleting the body's content of Na^+ and K^+. Tubular ammoniagenesis is usually coupled to renal HCO_3^- formation which makes the kidney an important organ for controlling pH-homeostasis. Renal excretion of NH_4^+ (+titrable acid) quantitatively matches the correction for metabolic acidosis [26a]. The signals for the increase of renal ammoniagenesis which has to be paralleled also by a readjustment of the metabolism in other organs (liver, gut, muscle) during metabolic acidosis, however, are only partly understood [33]. Renal degradation of glutamine also provides the precursor needed for the increased gluconeogenesis associated with metabolic acidosis [10]. It has been estimated [7] that following adaptation to a prolonged fast, about 50% of the total gluconeogenesis in humans occurs in the kidney.

References

1. Atkinson DE, Bourke E (1984) The role of ureagenesis in pH homeostasis. TIBS 7:297–298
2. Atkinson DE, Camien MN (1982) The role of urea synthesis in the removal of metabolic bicarbonate and the regulation of blood pH. In: Current topics in cellular regulation, Vol 21. Academic Press, New York, p 261–302
3. Bachmann C (1984) Treatment of congenital hyperammonemias. Enzyme 32:56–64
4. Balagura S, Stone WJ (1967) Renal tubular secretion of alpha ketoglutarate in dog. Am J Physiol 212:1319–1326
5. Buerkert J, Martin D, Trigg D (1982) Ammonium handling by superficial and juxtamedullary nephrons in the rat. J Clin Invest 70:1–12
6. Burg MB, Green N (1973) Function of the thick ascending limb of Henle's loop. Am J Physiol 224:659–668
7. Cahill GF Jr (1970) Starvation in man. New Engl J Med 282:668–675
8. Cohen J, Wittmann E (1963) Renal utilization and excretion of alpha ketoglutarate in dog: effect of alkalosis. Am J Physiol 204:795–811
9. Curthoys NP, Kuhlenschmidt T (1975) Phosphate-independent glutaminase from rat kidney. Partial purification and idendity with gamma-glutamyltranspeptidase. J Biol Chem 250:2099–2105
10. Curthoys NP, Shapiro RA, Haser WG (1984) Enzymes of renal glutamine metabolism. In: (Ed.) Häusinger D, and Sies H, Glutamine metabolism in mammalian tissues. Springer, Berlin Heidelberg New York, p 16–31
11. Glabman S, Klose RM, Giebisch G (1963) Micropuncture study of ammonia excretion in the rat. Am J Physiol 205:127–132
12. Goldstein L (1966) Relation of glutamate to ammonia production in the rat kidney Am J Physiol 210:661–666
13. Goldstein L (1976) Ammonia production and excretion in the mammalian kidney. Int Rev Physiol 11:284–315
14. Good DW, Burg MB (1984) Ammonia production by individual segments of the rat nephron. J Clin Invest 73:602–610
15. Good DW, Knepper MA (1985) Ammonia transport in the mammalian kidney. Am J Physiol 248:F459–F471
16. Good DW, Knepper MA, Burg MB (1984) Ammonia and

bicarbonate transport by thick ascending limb of rat kidney. Am J Physiol 247:F35–F44
17. Goodman AD, Fuizy RE, Cahill GF (1966) Renal gluconeogenesis in acidosis, alkalosis and potassium deficiency: Its possible role in regulation of renal ammonia production. J Clin Invest 45:612–619
18. Greger R (1981) Chloride reabsorption in the rabbit cortical thick ascending limb of the loop of Henle – a sodium dependent process. Pflügers Arch 390:38–43
19. Halperin ML, Cheema-Dhadli S, Chen CB, West ML, Jungas RL (1986) Is urea formation regulated primarily by acid-base balance? J Clin Chem Clin Biochem 24:267
20. Halperin ML, Goldstein MB, Stinebaugh BJ, Jungas RL (1985) Biochemistry and Physiology of Ammonium Excretion. In: (Ed.) Seldin DW, Giebisch G, The Kidney: Physiology and Pathophysiology Raven Press, New York, p 1471–1490
21. Halperin ML, Jungas RL (1983) Metabolic production and renal disposal of hydrogen ions. Kidney Int 24:709–713
22. Häussinger D, Gerok W, Sies H (1984) Hepatic role in pH regulation: role of the intercellular glutamine cycle. TIBS 7:300–302
23. Jenkins AD, Dousa TP, Smith LH (1985) Transport of citrate across renal brush border membrane: effects of dietary acid and alkali loading. Am J Physiol 249:F590–F595
24. Katunuma N, Tomino I, Nishino H (1966) Glutaminase isozymes in rat kidney. Biochem Biophys Res Comm 22:321–328
25. Knepper MA, Good DW, Burg MB (1984) Mechanism of ammonia secretion by cortical collecting ducts of rabbits. Am J Physiol 247:F729–F738
26. Lang F, Silbernagl S, Quehenberger P (1980) Hydrogen ion pump bicarbonate leak in the proximal convoluted tubule. In: (Ed.) Schulz I, Sachs G, Forte JG, Ullrich KJ, Hydrogen Ion Transport in Epithelia Elsevier, Amsterdam, p. 303–314
26a. Maren TH, Wadsworth BC, Yale EK, Alonso LG (1954) Carbonic anhydrase inhibition. III. Effects of Diamox on electrolyte metabolism. Bull Johns Hopk Hosp 95: 277–321
27. McGilvery RW (1979) Biochemistry: A functional approach. Saunders, Philadelphia, p 764–767
28. Nagami GT, Kurokawa K (1985) Regulation of ammonia production by mouse proximal tubules perfused in vitro. Effect of luminal perfusion. J Clin Invest 75:844–849
29. Oliver J, Bourke E (1975) Adaptations in urea ammonium excretion in metabolic acidosis in the rat: a reinterpretation. Clin Sci Mol Med 48:515–520
30. Owen EE, Robinson RR (1963) Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. J Clin Invest 42:263–276
31. Pitts RF (1972) Control of renal production of ammonia. Kidney Int 1:297–305
32. Pitts RF, DeHaas J, Klein J (1963) Relation of renal amino and amide nitrogen extraction to ammonia production. Am J Physiol 204:187–191
33. Ross B, Lowry M (1981) Recent developments in renal handling of glutamine and ammonia. In: (Ed.) Greger R, Lang F, Silbernagl S, Renal transport of organic substances. Springer, Berlin Heidelberg New York, p 78–92
34. Ross BD (1976) Effect of inhibition of gluconeogenesis on ammonia production in the perfused rat kidney. Clin Sci Mol Med 50:493–498
35. Sajo IM, Goldstein MB, Sonnenberg H, Stinebaugh BJ, Wilson DR, Halperin ML (1981) Sites of ammonia addition to tubular fluid in rats with chronic metabolic acidosis. Kidney Int 20:353–358
36. Scheller D, Silbernagl S (1986) Do HCl or NH_4Cl induce metabolic acidosis by different mechanisms? J Clin Chem Clin Biochem 24:267
37. Shapiro R, Curthoys P (1981) Differential effect of AT-125 on rat renal glutaminase activities. FEBS LETT 133:131–134
38. Silbernagl S (1980) Tubular reabsorption of L-glutamine studied by free-flow micropuncture and microperfusion of rat kidney. Int J Biochem 12:9–16
39. Silbernagl S (1986) Ammoniagenesis catalyzed by hippurate-activated gamma-glutamyltranferase in the lumen of the proximal tubule. A microperfusion study in rat kidney in vivo. Pflügers Arch: in press
40. Silbernagl S, Curthoys NP, Shapiro R (1982) The role of renal tubular brushborder enzymes in metabolism and absorption of glutathione and cysteinyl-glycine. In: Biochemistry of Kidney Functions. (Ed) Morel F, INSERM Symposium No. 21. Elsevier Biomedical Press, Amsterdam New York Oxford, p 303–314
41. Silbernagl S, Pfaller W, Heinle H, Wendel A (1978) Topology and function of renal gamma-glutamyl transpeptidase. In: (Ed.) Sies H, Wendel A, Functions of glutathione in liver and kidney. Springer, Berlin Heidelberg New York, p 60–69
42. Silverman M, Vinay P, Shinobu L, Gougoux A, Lemieux G (1981) Luminal and antiluminal transport of glutamine in dog kidney: Effect of metabolic acidosis. Kidney Int 20:359–365
43. Simon E, Martin D, Buerkert J (1985) Contribution of individual nephron segments to ammonium handling in chronic metabolic acidosis in the rat. Evidence for ammonia disequilibrium in the renal cortex. J Clin Invest 76:855–864
44. Simpson DP (1983) Citrate excretion: a window on renal metabolism. AM J Physiol 13:F223–F234
45. Stern L, Backman KA, Hayslett JP (1985) Effect of corticalmedullary gradient for ammonia on urinary excretion of ammonia. Kidney Int 27:652–661
46. Tannen R (1978) Ammonia metabolism. Am J Physiol 235:F265–F277
47. Tate SS, Meister A (1974) Interaction of gamma-glutamyltranspeptidase with amino acids, dipeptides, and derivatives and analogs of glutathione. J Biol Chem 249:7593–7602
48. Thompson GA, Meister A (1980) Modulation of gamma-glutamyl transpeptidase activities by hippurate and related compounds. J Biol Chem 255:2109–2113
49. Van Slyke DD, Phillips RA, Hamilton PB, Archibald RM, Futcher Ph, Hiller A (1943) Glutamine as source material of urinary ammonia. J Biol Chem 150:481–482
50. Vieira FL, Malnic G (1968) Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony microelectrode. Am J Physiol 214:710–718
51. Walser M (1986) Roles of urea production, ammonium excretion, and amino acid oxidation in acid-base balance. Am J Physiol 19:F181–F188
52. Welbourne TC (1974) Ammonia production and pathways of glutamine metabolism in the isolated perfused rat kidney. Am J Physiol 226:544–548
53. Welbourne TC, Dass PD (1981) Role of hippurate in acidosis-induced adaptation in renal gamma-glutamyltransferase. Life Sciences 29:253–258
54. Welbourne TC, Park PH (1986) Fate of intraluminal glutamine in the perfused renal tubule. Life Sciences 38:941–946
55. Welbourne TC, Phromphetcharat V (1984) Renal Glutamine Metabolism and Hydrogen Ion Homeostasis. In: (Ed) Häussinger D, Sies H, Glutamine Metabolism in Mammalian Tissues. Springer, Berlin Heidelberg New York Tokyo, pp. 161–177