Chapter 3

pH Homeostasis in Terrestrial Vertebrates: A Comparison of Traditional and New Concepts

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Contents

1 Introduction

The view of how metabolism influences acid–base status has been changed and questioned several times during the past few years. For aerobic steady state metabolism a major stimulus arose from a paper by Atkinson and Camien (1982), which by the application of stoichiometric principles led to a reconsideration of the importance of protein/amino acid catabolism and of urea synthesis in acid–base regulation. At the same time using stoichiometric principles also led to a quantification of the influences of non-steady state (anaerobic, exercise) metabolism on the acid–base status (Pörtner et al. 1984; Pörtner 1987, 1989). All of these considerations provide a basis for a general and quantitative understanding of the relationships between metabolic and acid–base regulation.

Much experimental work has been stimulated by the view that urea synthesis contributes to acid–base regulation (reviewed by Atkinson and Bourke, this vol.) and much of this evidence is in favour of a central role of the urea cycle in terrestrial vertebrate acid–base regulation. Since traditional concepts of

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acid–base regulation in vertebrates did not recognize that production of both base and ammonium (not just ammonia) occurs during protein and amino acid catabolism, it was not anticipated that a fine control of urea synthesis could occur via acid–base parameters (pH, HCO_3^-, CO_2). Thus, urea synthesis may also be a metabolic means to regulate the systemic acid–base status.

The present chapter is not intended to question this modified view of the role of urea synthesis but rather to examine whether the traditional concepts of acid–base regulation can be modified accordingly. A second major focus of this contribution is to ask whether these modifications must lead to any revision of the established methods of acid–base physiology. Such a revision, focussing on the physico-chemical characteristics of the metabolites involved in acid–base regulation (especially of ammonium, NH^+_4), has been requested by Atkinson and his colleagues (see Atkinson and Camien 1982; Atkinson 1992; Atkinson and Bourke, this Vol.). If such a revision is accepted traditional and new concepts would yield different results for the analysis of net proton equivalent ion exchange between the terrestrial animal and its environment (i.e. net acid excretion). However, if a different perspective is adopted taking the generation and fate of all ammonium, acid and base in metabolism and their transfer across epithelia into account (Pörtner 1989), both the traditional and the new approach will lead to identical conclusions in quantitative terms. The methodological modifications proposed by Atkinson and Bourke would lead to fatal errors in the analysis of metabolic influences on acid–base status and, thus, net acid excretion. Therefore, this brief chapter is meant to address these discrepancies and to present a counter-argument to the conclusions drawn by Atkinson and Bourke.

2 Nutrients and Acid–Base Status

The composition of the diet determines the net amount of protons formed or consumed in steady state metabolism. The major components are usually fat, carbohydrates, protein and, to a minor extent, nucleic acid, nucleotides, phospholipids, and other organic phosphates, and possibly free organic acids. For an estimate of the quantitative influence on acid–base regulation it must be considered that metabolism of these substrates is complex and occurs in different body compartments. Acid–base relevant ion (and gas) exchange between the body and the environment occurs across various epithelia: For a clear understanding, Pörtner (1989) proposed that the metabolic processes occurring in the lumen of the digestive system can be considered as taking place in the environment of the animals. Substances will then become effective as they cross the gut epithelium and enter the body. However, net ion exchange processes by which the organism influences or regulates the acid–base status in the digestive system have to be taken into account in an overall balance of acid–base events.

Table 1. Proton balance of aerobic catabolism in the post-absorptive organism. (Modified after Pörtner 1989)

Substrate	Chemical reaction	Net stoichiometric turnover (H^+, NH_4^+)
Carbohydrates	$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$	(0,0)
Fatty acids	$CH_3(CH_2)_{n-2}COO^- + [(3n-2]/2]O_2 + H^+ \rightarrow nCO_2 + nH_2O$	(–1, 0)
Fat[a]	$R–COO–R–H_2O \rightarrow R–COO^- + ROH + H^+$	(+1,0)
Glycerol	$C_3H_8O_3 + 3.5O_2 – 3CO_2 + 4H_2O$	(0,0)
Ketobodies	β-hydroxybutyrate$^-$ + H^+ + $4.5O_2 \rightarrow 4CO_2 + 4H_2O$	(–1,0)
Ketogenesis	Palmitate$^-$ + H^+ + $5O_2 \Rightarrow$ 4 β-hydroxybutyrate$^-$ + $4H^+$	(+3,0)
Amino acids:		
General	$R–CH(NH_3^+)–COO^- + O_2 + H^+ \rightarrow R–H + 2CO_2 + NH_4^+$	(–1,+1)
Neutral	Alanine + $3O_2$ + $H^+ \rightarrow 3CO_2 + 2H_2O + NH_4^+$	
Acidifying	Methionine + $7.5O_2$ + $H^+ \rightarrow$ 5 CO_2 + $3H_2O$ + NH_4^+ + SO_4^{2-} + $2H^+$	(–1, + 1)
Alkalizing	Glutamate – + $6O^2$ + $2H^+ \rightarrow$ 5 CO_2 + $3H_2O$ + NH_4^+	(–2, +1)
Organic phosphates:		
Monoesters	$R–PO_4^{2-} + H_2O \rightarrow R–OH + HPO_4^{2-}$	
Phosphagens	$R–NH–PO_3^{2-} + H_2O \rightarrow R–NH_2 + HPO_4^{2-}$	
Oligoesters[b]	$R_1–PO_4–R_2^- + 2H_2O \rightarrow R_1OH + R_2OH + HPO_4^{2-} + H^+$	
All[c]	$HPO_4^{2-} + xH^+ \rightarrow xH_2PO_4^- + (1 - X)\,HPO_4^{2-}$	

Note that phosphate monoesters (and phosphagens, i.e. guanidino-phosphates like phosphocreatine) cause net H^+ consumption, whereas phosphate oligoesters like ATP cause H^+ release.
[a] Fatty acid-glycerol erster.
[b] E.g. R_1, $R_2 = –PO_3^{2-}$
[c] pK around 6.8 (cf. Pörtner 1990 for exact values), $x = 1/(10^{pH-pK} + 1)$.

These processes and the metabolism of absorbed nutrients inside the body determine the net contribution of the kidney to acid–base regulation.

2.1 Sources of Acid or Base in Metabolism

Table 1 summarizes the proton balance of the oxidative catabolism of nutrients and body stores in the resting, fully aerobic post-absorptive organism and reveals to what extent acid–base regulation may be influenced by the different classes of substances. Homeostatic maintenance of body stores (e.g. gluconeogenesis in the liver, cf. Jungas et al. 1992) is neglected in this approach since by catabolism of the synthesized substrate any net effect of this process on systemic acid–base status will be eliminated.

The equations focus on the actual dissociation equilibria of substrates and products and, therefore, the actual generation or consumption of molecules directly and indirectly relevant for the acid–base status (in this case: H^+, NH_4^+). Generally, the oxidative catabolism of amino and other organic acids involves the oxidative decarboxylation of carboxylic acid anions. Net proton consumption during this process will lead, via a contribution of carbonic anhydrase, to the net formation of bicarbonate, which equals the amount of protons removed during oxidative decarboxylation (Pörtner 1987, 1989). Thus, in a more simplified view it may be stated that protein and amino acid catabolism yields ammonium and bicarbonate ions (Atkinson and Camien 1982; protein hydrolysis by itself has no effect on the acid–base status, it can be seen to replenish the pool of free amino acids).

Since most diets include a mixture of proteins and/or amino acids, ammonium ions and bicarbonate may be seen to form the bulk of acid–base relevant substances. However, only "neutral" amino acids like alanine would yield an equimolar quantity of ammonium and bicarbonate. This equimolarity originates from the fundamental chemical structure of any amino acid which is $R–CH(NH_3^+)–COO^-$ at physiological pH (in principle valid for proline, too, cf. Pörtner 1989). During catabolism of –R, the ratio between ammonium and bicarbonate quantities is varied according to the chemical nature of the respective residue. For example, catabolism of dicarboxylic-monoamino acids like glutamate would yield 2 mol bicarbonate per mole of ammonium, whereas lysine yields 2 mol ammonium per mole of bicarbonate. In addition, the oxidation of functional groups like the SH-group in methionine causes net proton formation (Table 1). These protons may be seen to titrate and reduce the amount of bicarbonate. Overall, catabolism of a typical mixture of protein and amino acids yields less bicarbonate than ammonium.

Any additional net consumption or generation of protons would also form or titrate bicarbonate (CO_2 being retained or released by respiratory gas exchange, such that Pco_2 remains constant and respiratory changes in the acid–base status do not occur; Pörtner, 1987). With a fixed amount and composition of the amino acid mixture such processes would not affect the generated quantity of ammonium but rather vary the quantity of bicarbonate. In a simplified view metabolic effects on acid–base status in addition to those exerted by the respective mixture of amino acids modify the overall ratio between bicarbonate and ammonium quantities even further although direct compensation of such disturbances via Na^+/H^+ exchange or bicarbonate excretion is certainly possible. Since the discrepancy between traditional and new concepts arises only when it comes to a discussion of the importance of amino acid catabolism in acid–base balance, such a simplified view, which does not change the quantitative conclusions, provides a clearer basis for the following sections of this chapter.

2.2 Ureagenesis and Acid–Base Turnover

Urea formation removes both the excess base and ammonium formed during amino acid catabolism (Atkinson and Camien 1982; Atkinson 1992; Atkinson and Bourke,

this Vol.) This strategy mostly used by terrestrial animals is certainly more costly than the one water-breathing animals can use where diffusive release of ammonia (NH_3) via the body surface or gills (Cameron and Heisler 1983; Heisler 1989) leaves a proton behind which neutralizes accumulating bicarbonate. In water breathers this mechanism fulfils two goals which are tightly coupled: removal of a toxic waste product and elimination of a base formed in amino acid catabolism (cf. Pörtner 1989). An imbalance between ammonium and bicarbonate quantities as caused by additional metabolic disturbances of the acid–base status (see above) is compensated by net H^+ equivalent ion exchange between animals and water (including the potential removal of excess ammonium; for review of the respective mechanisms, see Heisler 1986, 1989).

In air-breathing vertebrates and invertebrates these two coupled functions are taken over by the urea cycle or by urate and guanine synthesis (cf. Pörtner 1989). Evidence is strong that removal of base is an important function of urea synthesis in air breathers, and in water breathers when the removal of an incoming base load may take precedence over the diffusive elimination of ammonia (e.g. in alkaline lakes; Randall et al. 1989). However, the large quantities of base removed by urea formation should not mislead in that, in accordance with the traditional view, the detoxification of excess ammonium is tightly coupled to this process and may have been one additional driving force for the use of the urea cycle by terrestrial vertebrates (for a review of ammonium toxicity, see Meijer et al. 1990). The need to eliminate the ammonium is also reflected by the use of glutamine (and glycine) as an ammonium shuttle to the kidney which allows ammonium levels to be kept low when urea synthesis is impaired by acidosis (see below).

If the biochemical reality is considered, there is not just a net release of H^+ in urea synthesis but a net consumption of bicarbonate and of NH_3 withdrawn from the dissociation equilibrium of NH_4^+ (at high mitochondrial pH, reviewed by Pörtner 1989).

Thus :

$$NH_4^+ \longrightarrow NH_3 + H^+; \quad (1)$$

$$HCO_3^- + NH_3 + \text{aspartate}^- \longrightarrow \text{urea} + \text{fumarate}^{2-}. \quad (2)$$

These details might become important when the molecular mechanisms regulating urea synthesis are analysed. Aspartate is formed during transamination from another amino acid. When the consumption of one additional proton during oxidative decarboxylation of the respective amino acid carboxyl group after transamination is considered, it is clear that urea synthesis removes base and ammonium in equal quantities (cf. Pörtner 1989; Atkinson and Bourke, this Vol.)

The focus of this chapter is to understand the effects of urea synthesis on acid–base regulation from a quantitative point of view and to see whether the quantitative conclusions must differ between traditional and new concepts as requested by Atkinson and Bourke. First, the question arises what an amino

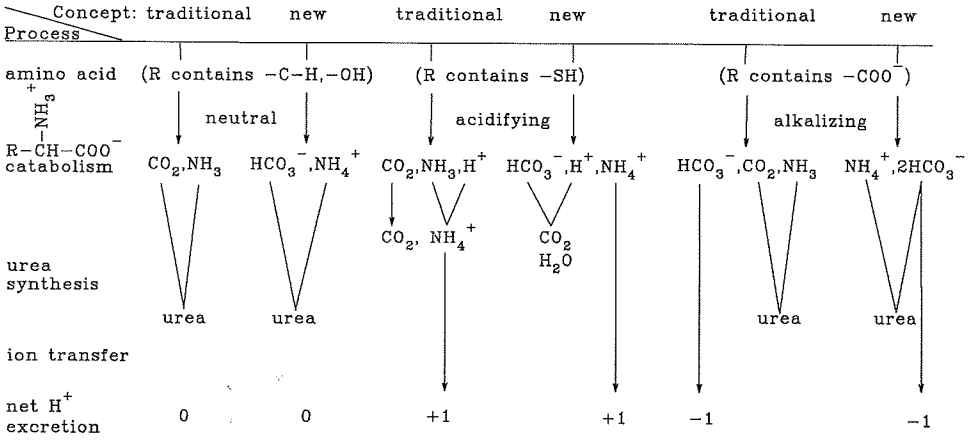

Fig. 1. Simplified, schematic presentation of the quantitative equivalence of traditional and new concepts of how the catabolism of various amino acids influences acid–base regulation in terrestrial vertebrates. In quantitative terms the graph focusses on the equimolar generation of bicarbonate and ammonium from the fundamental amino acid skeleton (Table 1) and the modulation of this ratio by the metabolism of the residues R considering an equimolar consumption of base and ammonium in urea synthesis. Values of net acid excretion are identical for traditional and new concepts. For further explanations, see text and Table 1

acid metabolism neutral for the acid–base status would mean in the two concepts (Fig. 1). In the traditional view, the formation of neutral urea from neutral amino acids like alanine would have been neglected; no net acid excretion would occur. It turns out that the same quantitative conclusions hold true for the new concept as well: Neutrality requires that all base and ammonium are taken up by urea synthesis without influencing the acid–base status. This condition is only fulfilled when ammonium and bicarbonate are generated in equal quantities, for example from "neutral" amino acids like alanine, and are removed in equal quantities during urea formation. In principle, the 1:1 generation of bicarbonate and ammonium from the characteristic carboxyl and α-amino groups of any amino acid is mirrored by the 1:1 stoichiometry of urea synthesis.

However, urea synthesis in vivo occurs based on a ratio of ammonium and bicarbonate quantities which has specifically been modified during oxidation of amino acid residues and has also been modified by other metabolic processes (see Table 1 and Fig.1). Generation of excess bicarbonate from organic acids and amino acids like glutamate would, therefore, cause an alkalosis with the result that both urea formation and ionic base release occur. This result is identical in both concepts. On the other hand, acidifying sulphate generation in methionine catabolism would lead to the titration of bicarbonate yielding excess ammonium. Complete removal of ammonium by urea formation would then lead to the development of metabolic (non-respiratory) acidosis, since

more base would be removed than is being generated. Therefore, excess ammonium is eliminated by ionic exchange. Again, this view is quantitatively equivalent to the traditional conclusion that aerobic metabolism of protein is linked to net proton production and causes net acid excretion to avoid systemic acidosis. However, this conclusion is obviously not shared by Atkinson and Bourke.

2.3 The Role of Glutamine

Based on the regulatory mechanisms reviewed by Atkinson and Bourke an acidosis would lead to a reduced formation of urea, with the effect of protecting the bicarbonate pool of the body. Since this reduction reflects an acidotic threat imposed by metabolism the question arises, how can it adequately be quantified? It is important in this context that compensation of an acidotic trend also means an increased export of ammonium to the kidney proportional to the reduced rate of urea synthesis (Fig. 1). In the whole system it is predominantly glutamine (and to a minor extent glycine) which serves as a vehicle for the net transport

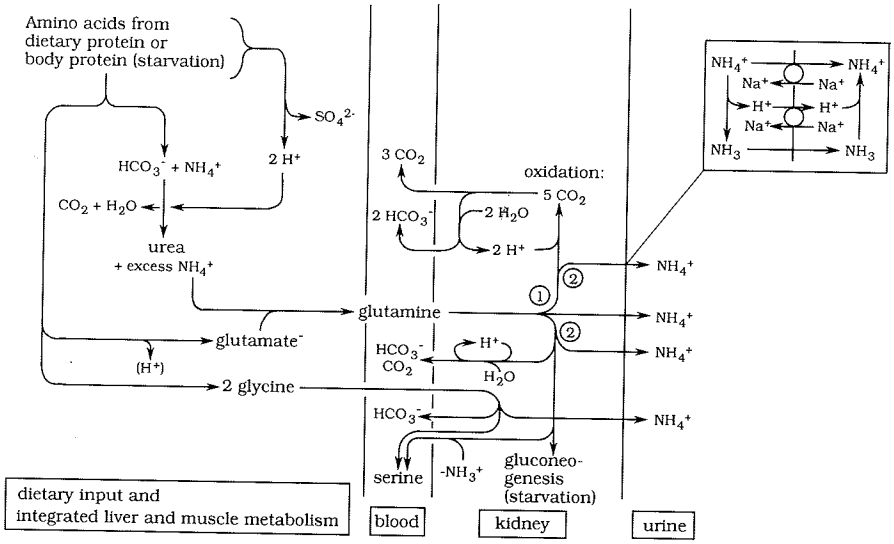

Fig. 2. Schematic presentation of the role of glutamine (and glycine) as an ammonium shuttle between muscle, liver and kidney. In acidosis, an increase in glutamine catabolism in the kidney is balanced by an increased export from muscle and liver. Ammonium is excreted and the remaining base is used to avoid and compensate for metabolic acidification (modified after Pörtner 1989). The basic conclusions remain unchanged when glycine is used as a shuttle metabolite and when serine is formed from glutamine and glycine and released into the circulation (Jungas et al. 1992). Net synthesis of non-dietary glutamate (as a monoamino-dicarboxylate anion) in liver or muscle involes proton production (H^+) when it is synthesized from monoamino-monocarboxylate precursors (like alanine). ① Glutaminase; ② glutamate dehydrogenase; $-NH_3^+$ indicates occurrence of transamination

of ammonium (Pörtner 1989, for a more recent analysis see Jungas et al. 1992). Glutamine synthesis and export from the liver (cf. Häussinger et al. 1988; Häussinger 1990; Almond et al. 1991) and from muscle (see Pörtner 1989) increase in acidosis. pH and hormonal changes stimulate glutamine uptake and oxidation in the kidney (Bogusky and Dietrich 1989; Welbourne 1989; Sastrasinh and Sastrasinh 1990), emphasizing the crucial role of glutamine and the kidney in acid–base homeostasis. Glutamine is deaminated to yield α-ketoglutarate and part of it is completely oxidized or used in gluconeogenesis (Fig. 2). Oxidation of this dicarboxylic acid involves a local net generation of base which has been seen to provide new base for systemic acid–base regulation when ammonium is released into the urine. However, as outlined in more detail by Pörtner (1989) and in accordance with the more recent treatment of Jungas et al. (1992), non-dietary glutamate/glutamine is resynthesized in other organs (e.g. muscle, implying release of protons or consumption of base, Fig. 2) such that, from a whole organism perspective, new or net base generation can be excluded as a special function of glutamine metabolism in the kidney. This view is more to the point than focussing on that the catabolism of glutamine may either occur in the kidney or in other organs (Atkinson and Bourke, this Vol.).

In summary, glutamine synthesis in muscle and liver and its export to the kidney are equivalent to an ammonium shuttle system to the kidney (Pörtner 1989). Excess ammonium not used by urea synthesis is excreted into the urine. The base generated from glutamine (or glycine) catabolism in the kidney represents base originating from overall amino acid catabolism which is protected from being consumed in urea synthesis and can, thus, contribute to maintain systemic acid–base homeostastis. That the kidney plays an active role in the metabolic control of acid–base parameters is emphasized by the observation that acidosis may cause an increase in kidney glutamine catabolism in some species like humans, rats and sheep to an extent that plasma glutamine levels decrease (Welbourne 1987). Thus, the rate of glutamine catabolism in the kidney does not just depend upon the supply by the liver and peripheral tissues but may influence the release of glutamine from other tissues by modulating the plasma concentration. Obviously, liver and kidney cooperate in the fine control of acid–base regulation.

Traditional and new concepts come together when it is appreciated that any excess ammonium not excreted into the urine would have to be used in urea synthesis and would thereby cause metabolic acidification (see below). Only when excess ammonium is generated from diamino-monocarboxylic acids, the contention of Atkinson and Bourke will hold true saying that ammonium production and release will not reflect a direct acidifying influence (titration of base) by metabolism. It would, nonetheless, cause acidosis when NH_4^+ was consumed in ureagenesis instead. Contrary to the emphasis of Atkinson and Bourke, such a threat of acidosis is also imposed when NH_4Cl is added to the system although, by itself, this salt does not represent an acid. Glutamine synthesis and its export to the kidney are thus part of a regulatory fine tuning for the export of excess ammonium to the kidney and prevent both acidosis and also large fluctuations in plasma ammonium levels (Pörtner 1989).

3 Renal Ammonium and Net Acid Excretion

For an evaluation of net acid excretion by the kidney both titratable acidity of the urine and ammonium excretion have traditionally been measured. Thus,

$$\dot{V}u\ ([NH_4^+] + [TA] - [HCO_3^-]) = \text{net acid excretion (mmol kg}^{-1}\ \text{h}^{-1}), \quad (3)$$

where $\dot{V}u$ is the urinary flow rate, $[NH_4^+]$ and $[HCO_3^-]$ are the ammonium and bicarbonate concentrations in the urine and [TA] is the titratable acidity determined by titrating urine samples back to plasma pH. Titratable acidity measurements evaluate the proton quantities transferred by the sodium/proton exchanger (cf. Fig. 2) and take into account to what extent phosphate and other buffers determine the final urine pH. Urinary acidification causes ammonium trapping and supports ammonium excretion (which otherwise occurs by Na^+/NH_4^+ exchange) as well as "bicarbonate reabsorption" (bicarbonate "reabsorption" means the titration of bicarbonate in the acidified urine. Accumulating CO_2 diffuses back into the plasma and is rehydrated forming bicarbonate when the sodium/proton exchanger removes the protons from the respective dissociation equilibrium of carbonic acid; Valtin and Gennari 1987). Atkinson and Bourke have concluded from their point of view that ammonium excretion cannot represent acid excretion, because ammonium is not an acid in a chemical sense. It should, thus, not be considered or included in the term for net acid excretion. How important it is to come to a valid conclusion is reflected by the fact that, according to traditional analyses, changes in net acid excretion during acid or base loads are predominantly due to the respective changes in ammonium excretion (cf. Knepper et al. 1989).

Although Atkinson and Bourke's statement reflects the physicochemical properties of the ammonium ion it is not important in this context whether ammonium functions as an acid but rather what ammonium excretion into the urine means for the acid–base status of the animal: since neutrality requires a strict 1:1 removal of base and ammonium in urea synthesis to match a catabolic 1:1 generation of base and ammonium in metabolism (Fig. 1), ammonium release does certainly not cause an acidosis in the urine by itself, but quantifies bicarbonate which is not used in urea synthesis. It has been stated above that this bicarbonate is (a) consumed by other, acidifying metabolic processes or (b) is not available for the removal of excess ammonium generated from diamino-monocarboxylic acid. In addition (c), it might be bicarbonate which had been lost as base from the body (released into the urine and considered by Eq. (3), or released into the intestinal lumen, see below). In an overall balance of metabolic proton production and the responses of the mechanisms of acid–base regulation this summed amount of bicarbonate must be considered. It is most adequately quantified by analyzing urinary ammonium excretion since, based on the smaller background levels of ammonia in the urine, changes in ammonia excretion are much more easily monitored than small reductions relative to the large background of urea excretion (via kidney and gut). As a corollary, quantities

of excreted ammonium must be taken into account even when accepting the modified concept of urea formation. For a quantification of metabolic influences on acid–base regulation they must be included in the numbers evaluated as net acid excretion. Such a procedure is also in line with the established procedures of measuring net acid excretion in aquatic animals (cf. Heisler 1986, for review).

These conclusions are not changed under conditions when the diet causes an excess of base over the amount of available ammonium (cf. Table 1; Fig. 1). As outlined by Atkinson (1992) and Atkinson and Bourke (this Vol.), urea may be recycled through the gut and ammonium be reabsorbed. It should be emphasized that bicarbonate originally consumed in urea synthesis is then regenerated in the gut and may be disposed of that way, the reabsorbed ammonium being available for amino acid and protein synthesis, and also for urea synthesis, if the additional removal of systemic bicarbonate is required. Should the reabsorbed ammonium be excreted via the kidney instead, it will again reflect the fate of one bicarbonate in the body somewhere else (released via the gut) than in urea synthesis.

4 Conclusions and Summary

In accordance with traditional concepts of pH homeostasis in terrestrial (and aquatic) vertebrates the reabsorbed components of the diet and their metabolism determine the net proton outcome of metabolism. For the analysis of the resulting net acid excretion via the kidney Atkinson and Bourke concluded, based on a new view of the importance of urea formation in acid–base regulation, that ammonium excretion should no longer be considered as acid excretion. A different conclusion arises from a previous and the present analysis (Pörtner 1989). In a comparison and synthesis of traditional and new concepts of pH homeostasis in terrestrial vertebrates the interpretation of the role of renal ammonium excretion needs to be modified: The traditional concept looked at NH_4^+ as a proton vehicle being formed from ammonia in the urine and removing protons from the body. The new, more realistic concept should rather consider the ratio between ammonium and bicarbonate quantities formed in metabolism. Based on the 1:1 generation of base and ammonium from the α-amino and carboxyl groups characteristic for any amino acid and on the 1:1 consumption of base and ammonium in urea synthesis, any ammonium not being used by urea synthesis and excreted into the urine quantifies a loss or deficit of base in excess of what is measured as $[TA]-[HCO_3^-]$ in the urine. Metabolic influences on aerobic acid–base parameters can be understood as causing a deviation from the 1:1 generation of base and ammonium in "neutral" amino acid catabolism. Such a deviation may also be caused by net H^+ equivalent ion exchange across the gut epithelia. Accepting these preconditions, both traditional and new concepts will come to the conclusion, that a protein diet causes net acid excretion, which is reflected by ammonium formation and excretion being in excess of the

amount of bicarbonate removed during urea synthesis. To quantify the base deficit, the analysis of "net acid excretion" must still include a measurement of ammonium excretion. Although the modified view of the importance of urea synthesis in acid–base regulation is the more realistic concept, the new and the traditional concepts of acid–base regulation can adequately be brought together in their identical quantitative conclusions. The methodology for investigating acid–base regulation is still accurate and does not need to be modified (Pörtner 1989).

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