The importance of metabolism in acid–base regulation and acid–base methodology

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Metabolism not only affects the acid–base status of an animal by means of proton stoichiometries but, by feedback regulation, acid–base parameters (pH, P_{CO_2}, bicarbonate) influence metabolic rates and the pathways used. This leads to a significant contribution of metabolism to acid–base regulation under both aerobic and anaerobic conditions. The relationship between amino acid metabolism, urea synthesis, and NH_4^+ excretion is discussed as an example important for steady-state metabolic acid–base regulation during aerobiosis. Generally, acid–base relevant metabolism may be regulated through the effect of acid–base disturbances on hormonal mediation, allosteric modulation of enzyme proteins, pH optima, and the levels of substrates or products, some of these being acid–base relevant substances like bicarbonate, CO_2, inorganic phosphate, and NH_3. During functional or environmental anaerobiosis the same relationships prevail. Metabolic proton accumulation is counterbalanced by phosphagen depletion and ammonia accumulation in adenylate catabolism. In addition, in integrated control of metabolic and acid–base status, long-term (mitochondrial) anaerobiosis leads to reductions in metabolic rate and increased removal of acidic groups. The importance of metabolic processes in acid–base methodology is discussed in terms of traditional concepts and recent developments.

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Le métabolisme n'affecte pas seulement l'équilibre acide–base d'un animal par stoïchiométrie des protons; en effet, par contrôle et rétro-contrôle, les facteurs responsables de l'équilibre acide–base (pH, P_{CO_2}, bicarbonate) influencent les taux de métabolisme et les voies empruntées. Ces propos soulignent l'importante contribution du métabolisme dans le contrôle de l'équilibre acide–base dans des conditions aérobies aussi bien que dans des conditions anaérobies. L'interaction entre le métabolisme des acides aminés, la synthése de l'urée et l'excrétion de NH_4^+ constitue un exemple important du contrôle métabolique de l'équilibre acide–base durant l'aérobie. Généralement, la partie du métabolisme qui affecte l'équilibre acide–base est contrôlée par l'intermédiaire de l'effet des perturbations acide–base sur la médiation hormonale, la modulation allostérique des protéines enzymatiques, les pH optimaux et les concentrations de substrat ou de produits dont certains sont des substances qui influencent l'équilibre acide–base (les bicarbonates, le CO_2, le phosphate inorganique ou le NH_3). Durant une anaérobie fonctionnelle ou ambiante, le mêmes interactions prévalent. L'accumulation de protons métaboliques est compensée par la diminution des phosphagènes et l'accumulation d'ammoniac au cours du catabolisme des adénylates. De plus, une anaérobie prolongée (mitochondriale), dans un système de contrôle intégré de l'équilibre métabolique et de l'équilibre acide–base, entraîne des réductions du taux de métabolisme et une disparition accélérée des groupements acides. L'importance des processus métaboliques dans la méthodologie acide–base est examinée à la lumière des concepts traditionnels et des découvertes récentes.

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Introduction

Metabolism has long been considered essential for the modification of acid–base parameters in animal tissues and body fluids. Even earlier workers like Meyerhof and Lohmann (1928*a*, 1928*b*) and Lipmann and Meyerhof (1930) focused on the influence of anaerobic processes like lactate formation or phosphagen depletion on pH regulation. Steady-state aerobic metabolism, however, may also influence acid–base regulation by yielding a net amount of protons. An overview of the net proton outcome of human metabolism has recently been given by Halperin and Jungas (1983).

Ion-exchange mechanisms are traditionally seen as the predominant mechanisms responsible for the regulation of acid–base parameters. During recent years this view has been questioned, in acid–base regulation in mammals and other air-breathing vertebrates (Atkinson and Camien 1982; Atkinson and Bourke 1987), for example, and, accordingly, the whole concept of steady-state acid–base regulation in the mammalian (human) organism has been reviewed and modified (Guder *et al.* 1987). During disturbance of aerobic steady-state metabolism, metabolic pathways come into play that, during anaerobiosis, for example, may also constitute mechanisms by which changes in acid–base status are minimized (Pörtner 1987*a*). This review is therefore intended to summarize the importance of metabolism in acid–base regulation, and to examine to what extent metabolic processes have to be considered in the conception of physiological techniques for measuring acid–base parameters. Another important aspect of the integration of metabolic processes and acid–base regulation, i.e., the importance of ionic regulation in metabolic events, is reviewed by Walsh and Milligan (1989).

Aerobic metabolism

In any animal the net amount of protons formed in steady-state metabolism of the whole organism depends upon the composition of the diet. The major components are usually carbohydrates, fat, protein, and, to a minor extent, nucleic acids, nucleotides, phospholipids, and other organic phosphates, and possibly free organic acids. If interest focuses on the acid–base homeostasis of tissues and blood (or other body fluids of similar function in animals), the metabolic processes taking place in the lumen of the digestive system can be considered to occur in part of the "environment" of the animal. Nevertheless, ion exchange, by which the organism determines and regulates the pH in different parts of the digestive system, has to be taken into account. The sum of these pro-

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cesses and the metabolic events in the tissues determine the contribution of the kidney (and gills or other exchange surfaces in water-breathing animals) to acid–base regulation, so that, for overall balance, (*i*) the pH of the nutrient mixture and its composition, (*ii*) changes in composition, volume, and acid–base parameters during digestion and feces formation in relation to the respective parameters of the urine, and (*iii*) the fate of the absorbed susbtances in the organism yield exact values for the metabolic background to the kidney's contribution to acid–base regulation (cf. Halperin and Jungas 1983).

Nutrients affect the acid–base parameters of blood and tissues as they enter the body across epithelia. The balance of final oxidation of important nutrients entering the internal milieu is shown in Table 1 (for the metabolism of organic phosphates see also Fig. 2). It is also valid for the consumption of internal substrate stores like glycogen, fat, or body protein during starvation.

If the basic difference between volatile and nonvolatile acid production is considered, and the production and release of CO_2 is seen as a mechanism to remove metabolic protons (cf. Pörtner 1987*a*; Table 4), no net H^+ release occurs in the oxidation of lipids and carbohydrates. The fatty acids, which are absorbed in dissociated form, will cause some H^+ consumption during oxidation or gluconeogenesis, as outlined later for carbonic acids. In the aerobic oxidation of amino acids, production of bicarbonate and of ammonium ions occurs, which is linked to proton consumption during oxidative decarboxylation of the carboxylate anions. For simplicity this is interpreted as causing equimolar production of ammonium and bicarbonate from "neutral" amino acids (i.e., amino acids that carry no net charge (Atkinson and Camien 1982; Walser 1986). Anionic amino acids like glutamate or aspartate lead to the formation of an excess of bicarbonate over ammonium ions, whereas cationic amino acids like arginine or lysine deliver more ammonium ions than bicarbonate. Bicarbonate, however, is not the direct end product of oxidative decarboxylation, which releases CO_2 during H^+ consumption (Fig. 1; cf. Pörtner 1987*a*). Carbonic anhydrase activity is required to allow for concomitant bicarbonate formation. As bicarbonate is the reactant in biotin-dependent carboxylation reactions (important in gluconeogenesis and fatty acid synthesis, but also in urea synthesis (Walsh 1979)), inhibition of carbonic anhydrase activity in hepatic mitochondria can be demonstrated to exert a strong inhibitory effect on urea synthesis (Dodgson *et al.* 1984; Häussinger and Gerok 1985), and very likely also on pyruvate carboxylase and acetyl-CoA carboxylase activity in gluconeogenesis and fatty acid synthesis (Dodgson *et al.* 1984; Herbert and Coulson 1984; Fig. 1). Generally, the dissociation equilibria of substrates and the proton stoichiometries of enzymatic reactions are likely to be important in the integration of metabolic and acid–base regulation (Table 2).

Proton formation in protein metabolism is linked to the *de novo* synthesis of sulfate in methionine or cysteine oxidation. Phosphate, the other strong acid anion, is not formed *de novo* but enters the organism esterified in phospholipids, nucleic acids, nucleotides, or phosphorylated amino acids (phosphoserine), or as free phosphate. Net proton production always occurs when phosphate esters are hydrolyzed, whereas hydrolysis of guanidino phosphate groups in phosphagens leads to proton consumption (Fig. 2; cf. Pörtner 1987*a*). Most of these reactions are finished before phosphate is finally absorbed. Intact phospholipids will lead to some net proton release during hydrolysis in the postabsorptive body. In addition, there may be proton consumption if it is only P_i^{2-}, which is absorbed, or proton production if it is P_i^-, because the first of the two species would take up H^+ and the second would release them to reach dissociation equilibrium ($pK' \sim 6.8$). One would speculatively assume that the predominant species, which is P_i^{2-} at physiological pH values above 6.8, is taken up, leaving some protons behind and reconsuming them when entering the organism. After ultrafiltration in the glomerula, phosphate tends to take up protons as the urine pH falls below the plasma pH and thereby forms the predominant buffer contributing to titratable acidity. Other substances involved in urine buffering are creatinine ($pK \sim 5$) and, mainly in uricotelic animals, urate ($pK \sim 5.8$). The latter two components resist a drastic drop of urine pH below the major buffering range of phosphate. (Precipitation of uric acid from urate represents an additional mechanism to buffer urine pH in uricotelic animals (Long 1982).)

Besides the production of nonvolatile acid, aerobic metabolism also provides the volatile acid CO_2, which during obstruction of gas exchange may cause a drop in pH in respiratory acidosis.

The basic characteristics of aerobic metabolism in invertebrates are the same as in vertebrate organisms. Some special features may arise from the fact that some amino acids are present in high concentrations, in marine invertebrates, for example, but are found in much lower levels in the vertebrates, or that substrate preferences are different in invertebrate and vertebrate organs. One outstanding example is the utilization of proline as a substrate for aerobic mitochondrial metabolism in some insects during flight and in squid mitochondria during swimming activity (Beenakkers *et al.* 1984; Storey and Storey 1983). Figure 3 shows the proposed pathway of proline metabolism in these animals. Proline enters the citric acid cycle via glutamate and α-ketoglutarate, and as indicated in Fig. 3, proton balance depends on the fate of these substances. In final oxidation, a stoichiometric production of ammonium and bicarbonate results, as for any other amino acid.

Nitrogen excretion and acid–base regulation

The huge formation of base in protein catabolism, together with the formation of ammonium, requires a steady-state strategy that allows pH to be maintained and avoids the accumulation of toxic ammonium. For animals living in water, the medium usually provides an infinite sink for ammonium. This may allow for a high contribution of protein catabolism to energy production (e.g., up to 85% in fish (van Waarde 1983)). The removal of ammonia by nonionic diffusion, which has been proposed as the predominant mechanism for ammonia excretion in fish (Cameron and Heisler 1983), would lead to an inexpensive neutralization of the respective bicarbonate because the proton of the ammonium ion stays behind (cf. Heisler 1986*b*; Pörtner 1987*a*; Fig. 4). It has been proposed for rainbow trout that ammonia release may be facilitated by trapping the volatile base in an acid boundary layer on the gill surface (Randall and Wright 1987). Low pH in this layer is claimed to be maintained by CO_2 accumulation and carbonic anhydrase activity. Ionic transport of proton equivalents may also support the steady-state trapping of ammonia. Some of these protons could originate from the excess protons expected from aerobic metabolism. Ion exchange would be minimized if this boundary layer gets rid of both accumulated ammonium and accumulated base by ion diffusion.

TABLE 1. Proton balance of the oxidative metabolism of nutrients and body stores in the postabsorptive organism

		ΔH^+	ΔNH_4^+
Carbohydrates	$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$	—	—
Fatty acids	$CH_3(CH_2)_{n-2}COO^- + [(3n - 2)/2]O_2 + H^+ \longrightarrow nCO_2 + nH_2O$	−1	—
Fat (fatty acid − glycerol ester)	$R - COO - R + H_2O \longrightarrow R - COO^- + ROH + H^+$	+1	
	Glycerol: $C_3H_8O_3 + 3.5O_2 \longrightarrow 3CO_2 + 4H_2O$	—	—
Ketobodies	β-hydroxybutyrate$^-$ + H^+ + $4.5O_2 \longrightarrow 4CO_2 + 4H_2O$	−1	—
Ketogenesis	Palmitate$^-$ + H^+ + $5O_2 \longrightarrow$ 4 β-hydroxybutyrate$^-$ + $4H^+$	+3	—
Amino acids	Alanine + $3O_2$ + $H^+ \longrightarrow 3CO_2 + 2H_2O + NH_4^+$	−1	+1
	Methionine + $7.5O_2$ + $H^+ \longrightarrow 5CO_2 + 3H_2O + NH_4^+ + SO_4^{2-} + 2H^+$	+1	+1

NOTE: Proton consumption results from the metabolism of carbonic acid anions, whereas proton production in aerobic metabolism results from ketogenesis and the formation of sulfate during complete oxidation of sulfur-containing amino acids. (For the metabolism of phosphates and further explanations see Fig. 2 and the text.)

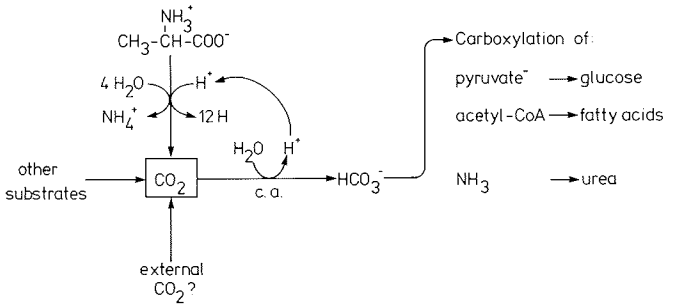

FIG. 1. Relationship between carbonic anhydrase (*c.a.*) function and bicarbonate metabolism in the liver. The fact that oxidative decarboxylation of carboxylic acid anions releases CO_2 and not bicarbonate explains why carbonic anhydrase is essential for hepatic metabolism. Moreover, carbonic anhydrase allows for the use of external CO_2 if endogenous CO_2 production is not sufficient.

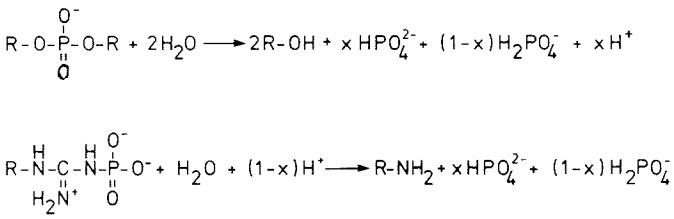

FIG. 2. Hydrolysis of phosphate esters and phosphate bound to guanidino groups. For nucleic acids, nucleotides, and phosphagens these reactions are very likely complete before absorption of phosphate occurs in the gut. The exact proton stoichiometries depend on the pK' of inorganic phosphate in relation to the pK' of phosphate bound to the respective residue. As the latter is below 6 in most cases (with the major exception of nucleoside tri-, di-, and monophosphates not bound to Mg^{2+}, cf. Pörtner *et al.* 1984*a*), proton balance at pH values above 7 is mainly determined by the dissociation equilibrium of free inorganic phosphate (for a detailed analysis of adenylate metabolism see Pörtner 1987*a*).

Although fish rely mostly on gill ion exchange for the removal of excess acid, the kidney may also contribute to net proton release (for reviews see Sullivan 1986; Heisler 1986*b*). It is interesting in this context that renal ammonium excretion in fish relies largely on the metabolization of glutamine (King and Goldstein 1983), as outlined later for air-breathing vertebrates. Some of the excess protons in aerobic metabolism of fishes are neutralized by bicarbonate, which stays behind when ammonium ions are removed by ion exchange or diffusion across the gills. Acid−base regulation, therefore, may play an important role in determining which of the multiple pathways for ammonia removal from the water-breathing animal (Evans and Cameron 1986) finally contributes to the release of this substance to the environment. In contrast to the situation in air-breathing vertebrates, however, acid stress does not necessarily cause an increase in ammonia production by ammonotelic fish, because only the mode of NH_3 release may be affected. If an increase is observed (for review see Heisler 1986*b*), with steady-state ammonia levels in the animal, it is more likely that the metabolic rate is elevated under acid stress, possibly linked to the metabolic cost of ionic acid−base regulation.

In air-breathing vertebrates the synthesis of urea or urate, which are the predominent end products of ammonia metabolism, has been described as the mutual neutralization of bicarbonate and ammonium at the expense of ATP (Atkinson and Camien 1982; Table 3 shows the actual participants in the reaction). The synthesis of urate$^-$ involves removal of more bicarbonate per ammonium (5/4) than for urea (2/2) (Atkinson and Camien 1982), resulting in some excess ammonium in the body and the need for enhanced ammonium release via the kidney. In uricotelic animals proton disposal in the urine is increased via proton uptake by urate$^-$ and uric acid precipitation. The nonstoichiometric disposal of bicarbonate and ammonium is avoided in the synthesis of guanine (e.g., in spiders), which by additional amination leads to the removal of five units of bicarbonate per five units of ammonium utilized. It is still a matter of dispute, however, whether a relationship exists between acid−base regulation and the regulation of urea formation. As the formation of ammonium and the consumption of protons in aerobic protein oxidation are tightly coupled, as are the removal of ammonium and of bicarbonate in urate and urea synthesis, the major influence on acid−base balance remains unchanged, even if urea synthesis is mainly regulated by ammonium accumulation. However, although ammonium accumulation (after injection) triggers urea production and leads to metabolic acidification (Häussinger *et al.* 1986), some evidence has accumulated that urea synthesis is pH dependent by itself (Kashiwagura *et al.* 1985), and that metabolic acidosis reduces the rate of urea formation, leads to a net formation of glutamine, and causes a relative increase in nitrogen excretion via ammonium excretion in the kidney (for review see Guder *et al.* 1987; Häussinger *et al.* 1988). For uric acid synthesis such an elaborate approach to the relationship between urate formation and acid−base regulation is not yet available. The results given by Craan *et al.* (1982) indicate that urate forma-

TABLE 2. Dissociation equilibria of substrates or products may influence the apparent pH optima of enzymes

Location	Enzyme	Pathway	Equilibrium	Substrate (S) or product (P)	ΔH^+	Supporting acid–base condition
Mitochondria	Carbamoyl-phosphate synthetase[a]	Urea synthesis[b]	$NH_3 - NH_4^+$	NH_3 (S)	$+2+x$	Alkalosis
			$HCO_3^- - CO_2$	HCO_3^- (S)		
Mitochondria	Pyruvate carboxylase[a]	Gluconeogenesis[b]	$HCO_3^- - CO_2$	HCO_3^- (S)	$+1+x$	Nonrespiratory alkalosis
Cytosol	Acetyl-CoA carboxylase[a]	Fatty acid synthesis	$HCO_3^- - CO_2$	HCO_3^- (S)	$+1+x$	and (or) (compensated)
Mitochondria	Propionyl-CoA carboxylase	Fatty acid oxidation	$HCO_3^- - CO_2$	HCO_3^- (S)	$+1+x$	respiratory acidosis
		Succinate propionate pathway[b]	$HCO_3^- - CO_2$	HCO_3^- (P)	$-1-x$	Nonrespiratory acidosis
Cytosol	Phosphoenolpyruvate carboxykinase	Gluconeogenesis[b]	$HCO_3^- - CO_2$	CO_2 (P)	—	Low CO_2
		Succinate propionate pathway[b]	$HCO_3^- - CO_2$	CO_2 (S)	—	High CO_2
Mitochondria	Pyruvate decarboxylase	Ethanol synthesis[b]	$HCO_3^- - CO_2$	CO_2 (P)	-1	Acidosis?
Cytosol	Glycogen phosphorylase[a]	Glycogenolysis	$HPO_4^{2-} - H_2PO_4^-$	HPO_4^{2-} (S)	$+1$	Alkalosis

NOTE: Metabolic pathways are likely to be sensitive to acid–base control when respective substrate levels are below $2 \times K_m$ for flux-generating enzymes. Sensitivity to pH may also be reflected by the overall proton stoichiometry of the reaction (ΔH^+, influenced by $MgATP^{2-}$ turnover in some cases, $\Delta H^+ = x$, (see Fig. 2)). Future research must focus on the extent to which pH changes in different cell compartments support the regulation of the respective metabolic reaction and how this form of regulation is integrated with pH optima and hormonal control so that the pathway responds to pH, Pco_2, and bicarbonate changes in a typical manner. (For further discussion and references see Pörtner 1987*a*; Walsh and Milligan 1989.)

[a]Flux generating.

[b]Contributes to minimizing or compensating for acid–base disturbances under certain conditions.

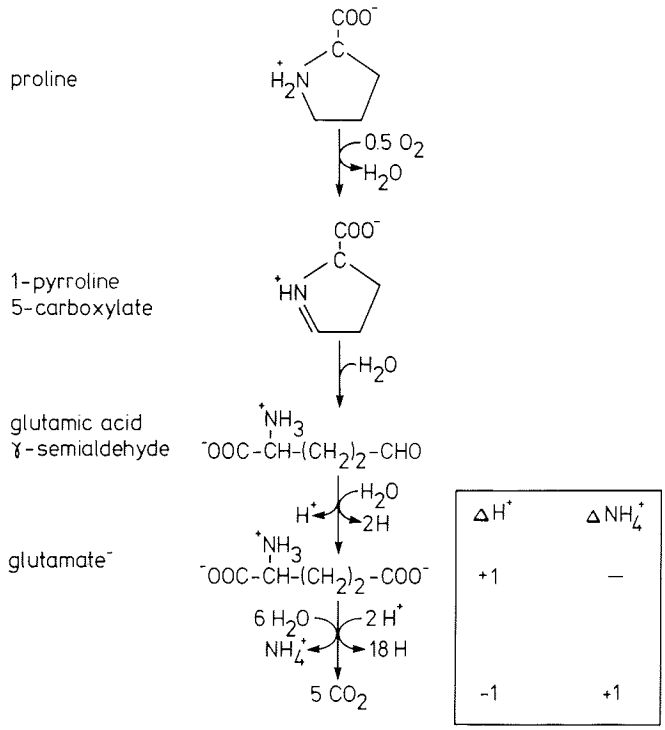

FIG. 3. Proton balance of proline metabolism. This scheme may become important when proline is net metabolized during rest to work transitions in insect and cephalopod muscles (see text).

tion may be decreased and ammonia formation from glutamine increased during chronic metabolic acidosis (cf. Table 3).

Integration of metabolism in muscle, liver, and kidney

Within the animal the influence of metabolism on pH in body and (or) tissue compartments may vary considerably. Muscle tissue mostly relies on fatty acid or glucose supply via the blood. On the other hand, muscle releases most amino acids generated by net protein breakdown in the postabsorptive or fasting state and converts them into glutamine (Goldberg and Chang 1978) and (or) releases alanine after transamination of pyruvate (Felig 1975). During starvation, muscle tissue even becomes the major source of amino acids, which are exported to other tissues mainly in the form of alanine and glutamine. Skeletal muscle may significantly contribute to metabolic acid–base regulation, because glutamine release is enhanced in metabolic acidosis (Schröck and Goldstein 1981; King *et al.* 1983), indicating that muscle supports ammonium release by the kidney under these conditions (for reviews and species differences among mammals, see Welbourne 1987). A large net turnover of base occurs in liver tissue, however, because net metabolization of amino acids (predominantly alanine from muscle tissue), including gluconeogenesis, takes place mainly in this organ. Considering the huge amount of ammonium and bicarbonate mutually neutralized in urea or urate synthesis, the importance of the liver in the removal of base becomes evident. In addition, because acid is formed in aerobic metabolism (which is equivalent to bicarbonate consumption when pH is assumed to be constant, as no nonbicarbonate buffering then occurs), the stoichiometry of amino acid metabolism and of urea synthesis leads to an excess of ammonium over bicarbonate production.

Ion exchange with the blood could provide the liver with

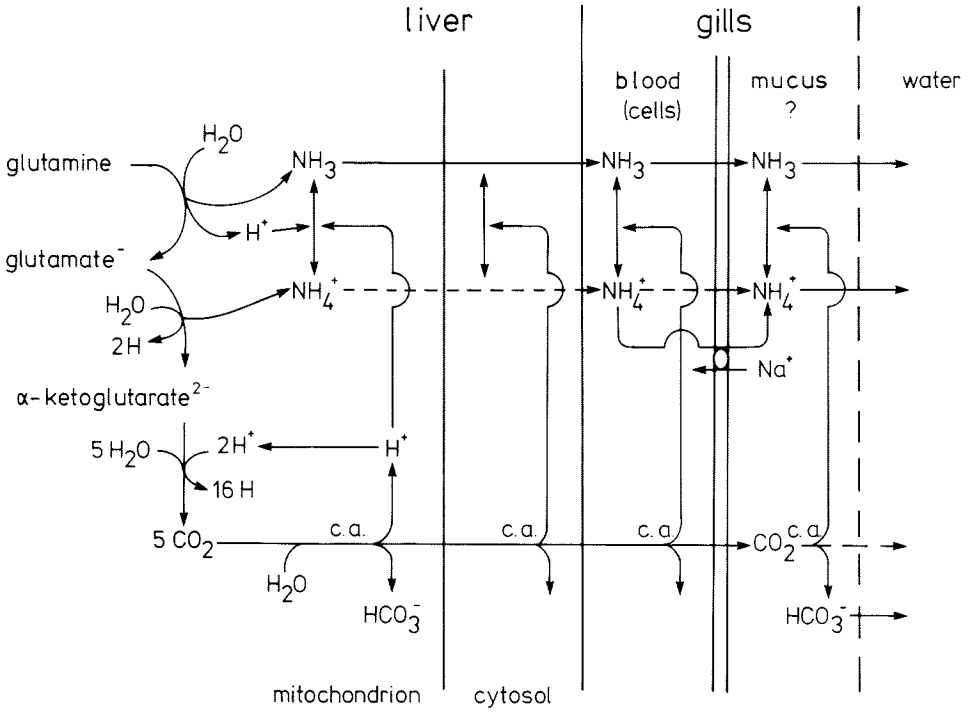

FIG. 4. Amino acid metabolism (based on van Waarde 1983) and mechanisms of ammonia excretion in fish. Carbonic anhydrase (*c.a.*) may help to buffer acid–base disturbances in the pathway of ammonium from the liver to the gills. Ambient water represents an infinite sink for ammonium and bicarbonate ions. This may allow fish to efficiently release ammonia by passive diffusion across gill epithelia. That the ammonium is trapped in a mucous layer acidified by respiratory CO_2 accumulation has been proposed by Randall and Wright (1987). In contrast to the situation in air-breathing animals, it may be the diffusion of NH_4^+ and bicarbonate$^-$ into the ambient water that allows for maintenance of the pH gradient across the gills. ATP turnover is then only involved in the steady-state maintenance of ion gradients and in the release or uptake of proton equivalents that are net produced or consumed by metabolism.

TABLE 3. The net reactions and the participants involved in ammonium metabolism in air-breathing vertebrates

Glutamine – glutamate metabolism:

$$\text{glutamine} + H_2O \longrightarrow \text{glutamate}^- + NH_4^+$$
$$\text{glutamate}^- + H_2O + 0.5\,O_2 \longrightarrow \alpha\text{-ketoglutarate}^{2-} + NH_4^+ + H_2O$$
$$\text{glutamate}^- + \text{oxaloacetate}^{2-} \longrightarrow \alpha\text{-ketoglutarate}^{2-} + \text{aspartate}^-$$
$$NH_4^+ \longrightarrow NH_3 + H^+ \text{ (at mitochondrial pH)}$$

Urea – urate – guanine synthesis:

$$HCO_3^- + NH_3 + \text{aspartate}^- \longrightarrow \textit{urea} + \text{fumarate}^{2-}$$
$$2\,\text{glutamine} + \text{glycine} + 2\,\text{serine} + HCO_3^- + \text{aspartate}^- + 2O_2 + 2H_2O \longrightarrow \textit{urate}^- + 2\,\text{glutamate}^- + \text{glycine} + \text{fumarate}^{2-} + 8H_2O + 3H^+$$
$$3\,\text{glutamine} + \text{glycine} + 2\,\text{serine} + HCO_3^- + \text{aspartate}^- + O_2 + 4H_2O \longrightarrow \textit{guanine} + 3\,\text{glutamate}^- + 2\,\text{glycine} + \text{fumarate}^{2-} + 7H_2O + 3H^+$$

NOTE: Bicarbonate and ammonia are the primary reactants in urea synthesis, whereas in urate and guanine formation, amino groups are included from amino acid precursors only. In all cases, glutamine, glutamate, and bicarbonate (as a substrate of carboxylases) are important participants. Aspartate is regenerated by transamination from glutamate. This explains why the regulation of glutamine metabolism in muscle, liver, and kidney plays an important role in metabolic pH regulation (see text and Fig. 5). In addition, the proton stoichiometries and the use of bicarbonate as a substrate reflect how urea (and possibly also urate and guanine) synthesis depends on the action of carbonic anhydrase and on (cytosolic–mitochondrial) pH (see text and Fig. 1). The final stoichiometries for urea ($\Delta NH_4^+/\Delta Bic = 1$), urate ($\Delta NH_4^+/\Delta Bic = 4/5 = 0.8$), and guanine ($\Delta NH_4^+/\Delta Bic = 5/5 = 1$) result from the additional consideration of proton consumption in oxidative decarboxylation of the carboxyl groups belonging to the amino groups removed. (The imbalance in the case of urate results from proton release by uric acid titrating 1 bicarbonate.)

additional bicarbonate or remove the excess metabolic protons to ensure the availability of equimolar bicarbonate for the synthesis of urea. A metabolic mechanism that allows the excess ammonium to be removed is the formation of glutamine from glutamate and ammonium. An increase in the formation of glutamine in muscle and the onset of net formation in the liver is the general response to systemic metabolic acidosis (Schröck and Goldstein 1981). Häussinger (1986) established an intercellular glutamine cycle in the liver, glutamine production on the perivenous side being compensated by glutamine consumption and urea formation on the periportal side. Metabolic acidosis leads to inhibition of glutamine consumption and urea formation. The effect of low pH on urea formation may include a reduction in the availability of bicarbonate, linked to carbonic anhydrase sensitivity to pH (cf. Fig. 1), and also a reduction in free ammonia levels, ammonia being the substrate

of carbamoyl-phosphate synthetase (Cohen *et al.* 1985; cf. Table 2). This inhibitory effect is described as not prominent during respiratory acidosis, owing to a sufficient supply of bicarbonate under these conditions (Guder *et al.* 1987). Net formation of glutamine from accumulating ammonium leads to its utilization as a transport vehicle for ammonium to the kidney. There, muscle and liver glutamine is deamidated and deaminated, and the resulting oxoglutarate is subjected to gluconeogenesis or, predominantly, final oxidation (cf. Fig. 5). If glutamine is fully oxidized, the formation of ammonium ions is quantitatively equal to renal bicarbonate formation. Any uncharged amino acid, however, would lead to the same result in final oxidation.

Why is alanine, which is the major transport vehicle of α-NH_2 groups from gut and muscle, not utilized for this purpose? The use of glutamine is exceptional in that the anionic amino acid glutamate can be charged with one additional ammonium to yield the 1:1 stoichiometry. Glutamine allows 2 mol of ammonium to be transported, and is subject to direct deamidation or deamination, which would be the case for most other amino acids usually only after transamination reactions. Besides, the carbon skeleton of glutamine is mostly derived from other amino acids, whereas alanine is formed from glycolytic pyruvate (Goldberg and Chang 1978) in the fed animal and mainly from amino acid carbon skeletons during starvation (Snell 1980). Glutamine formation allows for a net export of amino acid carbon chains from the muscle tissue under all conditions. If glutamine (i.e., ammonium + substrate) export is increased in acidosis, this might be explained as follows: protein metabolism is enhanced during acidosis (May *et al.* 1986) because (*i*) metabolization of the respective amino acid carboxylate anions provides base for the metabolic correction of the acidosis, (*ii*) steady-state catabolism of protein provides both the carbon chains for energy production in the kidney and ammonium for NH_4^+ export, and thereby compensates for higher energy needs during increased ammonium excretion in acidosis. (*iii*) In addition, glutamine formation may be the short-term response of muscle tissue to ammonium accumulation during anaerobic exercise.

Renal ammonium excretion

If endogenous glutamate were oxidized in situ, it could yield excess bicarbonate. The question arises as to why the liver releases this amino acid in acidosis and does not produce the excess bicarbonate itself to provide equimolar bicarbonate and ammonium for urea synthesis.

One of the most crucial points in this context is, what does renal ammonium excretion mean under these conditions? Atkinson and Camien (1982) and Atkinson and Bourke (1987) have gone so far as to pretend that it is not to be seen as acid release, and therefore that NH_4^+ has to be omitted from evaluation of acid excretion. By the use of glutamate, however, net ammonium transport reduces the excess of ammonium in metabolic acidosis without exporting base. This view is more to the point than emphasizing net base generation by glutamine oxidation in the kidney (see Halperin and Jungas 1983; Halperin *et al.* 1986), because if no steady-state supply of glutamate exists, base may have to be reconsumed somewhere else for resynthesis of glutamine, involving net formation of the respective carbonic acid anion. In fact, glutamate and aspartate are not released into the blood after protein ingestion, but are consumed in the intestine (Felig 1975). In the muscle tissue net glutamine synthesis occurs from amino acid precursors like glutamate, aspartate, asparagine, valine, and isoleucine (Goldberg and Chang 1978), and only from endogenous glutamate, aspartate, or asparagine does this involve no proton release. However, resynthesis of glutamine in muscle or liver in excess of the metabolism of other dicarboxylic acids (i.e., implying reconsumption of base) would still allow for net ammonium export to the kidney (Fig. 5) and is equivalent to an ammonium shuttle system to the kidney.

The adjustment of systemic bicarbonate levels in pH homeostasis, which is likely to be regulated by liver, muscle, and kidney metabolism (see earlier) requires that the excess ammonium is excreted by the kidney. In metabolic acidosis glutamine export saves bicarbonate, which is needed for the maintenance of pH, from being consumed during urea synthesis. In respiratory acidosis, which does not represent a limitation of bicarbonate availability to urea formation, an increase in glutamine export could still leave an excess of bicarbonate behind. Together with the stimulation of bicarbonate reabsorption under these conditions (Sullivan 1986; Valtin and Gennari 1987), this process may help to compensate for the increase in P_{CO_2}.

As the stoichiometry in final oxidation or gluconeogenesis is the same as for any other uncharged amino acid, glutamine metabolism can be seen as a branch of overall amino acid metabolism transferred to the kidney (see earlier). This analysis answers the question as to why glutamate is saturated with ammonium instead of producing excess bicarbonate in situ. The reason is that with ionic release of the ammonium in the kidney, two protons are net consumed instead of only one during glutamate metabolism and urea formation in the liver (Fig. 5). This amplification helps to efficiently provide base to the body under the precondition that ammonium is dealt with by ion exchange and glutamine is formed from excess dicarboxylic acid anions. Saving bicarbonate in the liver, therefore, may be coordinated with additional net base production in the kidney, and reduces the extent to which ammonium shuttling from the liver would otherwise be necessary.

Net acid excretion

In quantitative terms, this conclusion is equivalent to the classical view of glutamine transport as analogous to net acid export to the kidney. The question arises as to how the kidney is able to remove the acid, i.e., the proton carried by NH_3, or, in other words, is able to remove the ammonium and conserve the associated base. The discussion of the function of urea and glutamine metabolism in acid–base homeostasis should not be misinterpreted: the net effect on the acid–base status of the organism results from the fact that instead of mutual neutralization taking place as it does in the liver, H^+ leaves the body via Na^+-H^+ exchange (including diffusive loss of NH_3). Other contributing mechanisms, which would also be equivalent to net acid release, may involve NH_4^+ exchange (Good and Knepper 1985). Net removal of bicarbonate from the urine into the blood occurs through back-diffusion of CO_2 from the acidified urine and its binding by the action of carbonic anhydrase. The predominant role of Na^+-H^+ exchange was substantiated early by demonstrating that carbonic anhydrase is essential to the proton exchange taking place. This mechanism creates bicarbonate in the blood, the ammonia being reprotonated in the urine. This trapping of ammonia on the acid side would, as discussed for fish, greatly facilitate ammonium release into the urine. In any case, in contrast to the situation in the gills of water breathers, net proton transfer

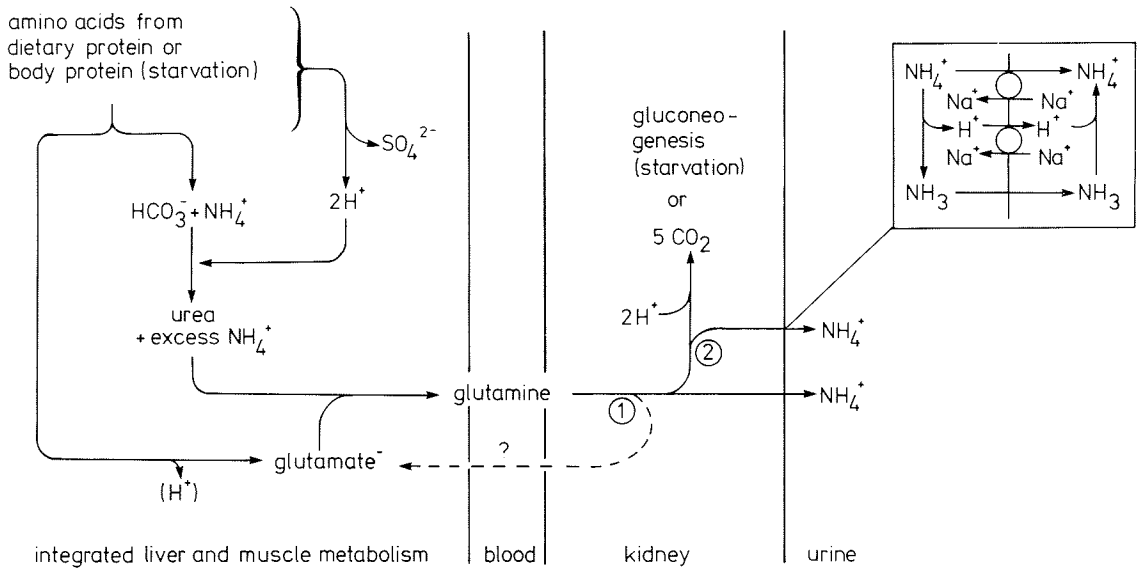

FIG. 5. The role of glutamine in compensating for an imbalance between ammonium and bicarbonate generation with respect to urea formation (see text). In acidosis, liver and muscle contribute to net glutamine export to the kidney for gluconeogenesis, final oxidation, and ammonium release. The basic contribution of glutamine, however, would already be fulfilled in a shuttle system for ammonium ions, as indicated by the hypothetical return of glutamate to muscle or liver. Net proton (H^+) production may be involved in glutamate formation if this process exceeds the availability of dicarboxylate anions as carbon precursors. A discussion of the alternative pathways of NH_4^+ formation from glutamine in the kidney is presented by Brosnan *et al.* (1987). 1, glutaminase; 2, glutamate dehydrogenase.

is very likely required for each ammonia molecule trapped, because of the limited volume of the sink and the fact that ammonium levels in the ultrafiltrate are already as high as in the plasma. This requires a high ATP turnover, as already suggested by Halperin *et al.* (1982), but the energy cost during removal of the net acid formed per mole of consumed substrate should be similar to the cost experienced by ammonotelic fish. It is the ATP consumed in urea and urate synthesis, however, that represents some of the additional expenses involved in living with restricted water availability.

In the quantitative investigation of acid–base regulation in animals the appearance of ammonium outside the organism (in urine or water) has to be taken into account (in contrast to the conclusions drawn by Atkinson and Camien 1982; Atkinson and Bourke 1987), because ammonium release by the kidney indicates that base is accumulated or retained by the body. If the amount of ammonium formed is in excess of the amount of bicarbonate generated, the excess of ammonium (assuming constant acid–base parameters in the tissues and blood) is due to the consumption of bicarbonate or net acid production by metabolism. Even under these conditions, therefore, the release of NH_4^+ is equivalent to the net release of protons. In air-breathing vertebrates this method of disposing of ammonium represents the only possible way of eliminating excess ammonium in acidosis, as the release of bicarbonate via the kidney is the only way to dispose of excess base in alkalosis (if an excess of bicarbonate over ammonium is produced by metabolism). Metabolism can compensate for such disturbances only transiently (see later for non-steady-state conditions), whereas steady-state regulation requires transmembrane ionic regulation. Ammonium accumulation would not be an alternative to steady state; its complete removal in urea formation would lead to acidosis, therefore ammonium excretion is always equivalent to acid excretion or base conservation. Whether base is really retained in the animal (e.g., during NH_4^+ excretion) or is formed in the external medium (e.g., during NH_3 diffusion) is seen from the titratable acidity in urine (TA-HCO_3^-, Hills 1973) or, under conditions of constant P_{CO_2}, from pH (i.e., bicarbonate) changes in the water (Heisler 1986*a*). pH and bicarbonate levels in the animal or in the ambient medium (urine or water) change in accordance with metabolism, and with proton, bicarbonate, and ammonium transfer, and these relationships cannot be disregarded.

As ammonia is already protonated when it arrives at the kidney tubule, and is released by ionic transport or, alternatively, by nonionic diffusion and trapping due to at least stoichiometric proton release, it has no significant effect on urine buffering because of its high pK'. However, a lower pH in the urine than in the plasma helps to support ammonia trapping, and this additional fixed acid flux is determined by titratable acidity measurements. Carbon dioxide accumulated from bicarbonate titration would also help to maintain a low pH (cf. Gennari *et al.* 1986). TA-HCO_3^- can be seen as net acid release by the kidney brought about by ion exchange in excess of ammonium exchange (including the proton movements needed for reprotonation of ammonia). Therefore, TA-HCO_3^-, by itself, affects the ammonium–bicarbonate balance of protein metabolism. The sum of $[\dot{V}(\text{TA-}HCO_3^-) + \dot{V} \times NH_4^+]$, however, includes the amount of base retained in the body and is equivalent to net acid production by the organism. Consequently, although the view of how aerobic metabolism can contribute to acid–base regulation has changed considerably, the methodology for investigating acid–base regulation is still accurate and does not need to be modified.

Non-steady-state metabolism

A deviation from the balance of steady-state metabolism (see Table 1) may occur when, during a change in physiological status, intermediary or end products are formed which accumulate and cause a transient acid–base disturbance. One well-known example is ketogenesis during starvation, which leads

to metabolic acidosis (Table 1). Another deviation from metabolic steady state is observed when final oxidation of substrates is impaired during hypoxia and anaerobiosis (see later). Similar effects are observed when the Embden–Meyerhof pathway is net utilized under aerobic conditions.

In a theoretical discussion of proton formation by anaerobic glycolysis, Gevers (1977) proposed that the adenylates are generally involved in the proton turnover of this pathway, even going so far as to assume that MgATP is the mediator of proton release by metabolic pathways. This view was stressed by Hochachka and Mommsen (1983), and was used as a means of analyzing the H^+ outcome of anaerobic metabolism by Pörtner (1982) and Pörtner *et al.* (1984*a*). This simplified view, however, proved to be not tenable when the proposed mechanism of ATP formation in oxidative phosphorylation (Kagawa, 1984) was taken into account (Pörtner 1987*a*). In every example of substrate-level phosphorylation analyzed, net or transient proton release from the substrate is likely to be involved in the mechanisms of ADP phosphorylation, but pathway protons are very likely not absorbed in the phosphorylation reaction (for a more thorough treatment see Pörtner 1987*a*). Net proton production by metabolic pathways is probably essential in substrate-level phosphorylations, but the proton source is unequivocally the substrate.

Muscular activity

During muscular activity, the accumulation of glycolytic end products indicates the equimolar formation of protons in the Embden–Meyerhof pathway. This is valid, regardless of whether lactate or opines are formed (Pörtner 1982; Hochachka and Mommsen 1983; Pörtner *et al.* 1984*a*), because the only net processes are the production of pyruvate$^-$ and H^+ (Pörtner 1987*a*). Muscle specialized for rapid and high-power emergency responses preferentially relies on anaerobic sources for ATP replenishment, the phosphagen being utilized initially, before stimulation of anaerobic glycolysis is complete. Utilization of ATP, which is rephosphorylated by phosphagen depletion, leads to the net formation of inorganic phosphate. Phosphagen utilization consumes protons by itself (Lipman and Meyerhof 1930), and the accumulated inorganic phosphate, besides triggering the phosphorolysis of glycogen, resists further pH changes by increasing the intracellular nonbicarbonate buffer value (Piiper 1980; Pörtner 1986).

Usually, the time course of phosphagen depletion and the subsequent predominance of anaerobic glycolysis are reflected in an initial intracellular alkalosis followed by an acidosis in the working muscle (Chih and Ellington 1985). The extent to which accumulated protons finally lead to intracellular acidification depends on the size of the phosphagen and glycogen stores and their relative utilization.

Acidification during exercise contributes to the depletion of the phosphagen according to the equilibrium of the phosphagen kinase. Finally, if phosphagen levels are no longer high enough to buffer cellular ATP concentrations (Kushmerick 1983), a depletion of the adenylate pool is observed as an additional mechanism to maintain high ATP/ADP ratios. The removal of AMP from the adenylate kinase equilibrium occurs through direct deamination, or deamination of adenosine after dephosphorylation in the 5′-nucleotidase reaction (Jennings and Steenbergen 1985). Ammonia contributes to proton buffering (Pörtner *et al.* 1984*a*) because it is not released from the tissues under these conditions (Terjung *et al.* 1985). Ammonia

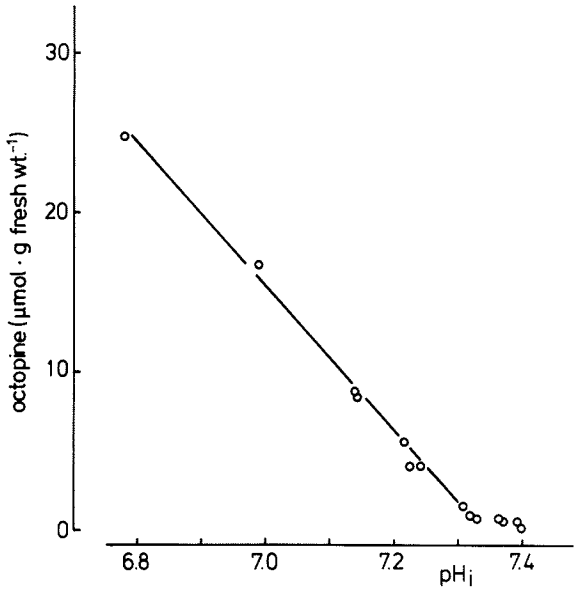

FIG. 6. Octopine levels and intracellular pH values found in the mantle musculature of squid (*Illex illecebrosus*) during fatiguing exercise and subsequent recovery. The linear correlation suggests that proton (and end product) release from the tissues into the blood is minimal. This conclusion is consistent with the observation of high priority given to the function of the noncellular haemocyanin in these animals (see text).

formation may completely compensate for proton accumulation originating from ATP depletion (Pörtner 1987*a*). Accumulated ammonia, however, must be dealt with during aerobic recovery. Increased synthesis of glutamine in acidotic muscle may play an important role (see earlier), whereas accumulated lactate or opines and an equimolar amount of protons are consumed in gluconeogenesis or final oxidation. In addition, transamination of pyruvate originating from glucose or lactate–opines may take over some ammonia originating from increasing amino acid catabolism and contribute to gluconeogenesis in the liver (Snell 1980). Ammonia needed for replenishing the adenylate pool is gained by transamination from aspartate which in turn may be replenished by transamination from glutamate (Dudley and Terjung 1985; for a discussion of the purine nucleotide cycle in fish see Dobson and Hochachka 1987). Evidently, a close relationship may exist between muscle amino acid metabolism during acidosis (see earlier) and adenylate metabolism during and after exercise.

It has been proposed that buffer values are high in anaerobic white muscle tissues to allow for high rates of acidifying anaerobic metabolism (Davey 1960; Lykkeboe and Johansen 1975; Castellini and Somero 1981; Morris and Baldwin 1984; Eberlee and Storey 1984). In a study on fish muscle this has been attributed to higher protein content, high levels of carnosine and anserine, and high levels of inorganic phosphate in white as opposed to red muscle (Abe *et al.* 1985). Ionic release of H^+ to the extracellular compartment and finally to the ambient water may further reduce the proton load of the tissues (see Heisler (1986*b*) for a discussion of the importance of these mechanisms during exhaustive exercise and subsequent recovery in fish). These mechanisms may become especially important during long-term exercise, when H^+ transfer is fast enough to compete with the rate of glycolytic H^+ accumulation. In addition, lactate release reduces the intracellular

TABLE 4. Mechanisms for the reduction of proton accumulation by anaerobic metabolism

Mechanism	Enzymatic reaction involved
Oxidative decarboxylation (CO_2)	Malic enzyme, α-ketoglutarate dehydrogenase, isocitrate dehydrogenase, pyruvate dehydrogenase
Biotin-dependent decarboxylation (HCO_3^-)	Propionyl-CoA carboxylase
Nonoxidative thiamin-dependent decarboxylation (CO_2)	Pyruvate decarboxylase
Oxidative phosphorylation	Fumarate reductase, reduction of double bonds in lipids
Phosphagen hydrolysis, accumulation of inorganic phosphate	Aphosphagen kinase, ATPases
AMP or adenosine deamination	AMP or adenosine deaminase
Further processes	
Reduction in Mg^{2+} binding from ATP over ADP towards AMP	
Metabolization of carboxylic acid anions	
Ammonium accumulation and elimination by ionic transport, amidation, or reductive amination	

NOTE: Those enzymatic reactions are given that will lead to an excess of regenerated ATP over the amount of protons released. An increase in the yield of ATP per mole of degraded substrate and the concomitant reduction of proton accumulation by both, the reduction of metabolic rate, and the elimination of acidic groups are general trends observed during long-term anaerobiosis (for further details see Pörtner 1987*a*). During aerobiosis, oxidative decarboxylation and oxidative phosphorylation are the main mechanisms allowing for a high ATP turnover with minimal net formation of protons (see text). Decarboxylation products are shown in parentheses.

osmotic disturbance. Such a disturbance does not occur when opines are accumulated as glycolytic end products in marine invertebrates (Fields 1983). Accordingly, opines do not usually leave the tissues where they are formed. Depending on the degree of adaptation to the utilization of anaerobic mechanisms, the associated protons may be released into the extracellular space or kept in the muscle tissue: efficient H^+ release was observed in the intertidal worm *Sipunculus nudus* which is adapted to digging in the sand (H.-O. Pörtner, M. K. Grieshaber, and N. Heisler, in preparation). pH_i was calculated to remain essentially constant despite anaerobic H^+ accumulation (see Pörtner 1986). In pelagic squid, however, the protons remain in the muscle tissue, together with the end product (Fig. 6; H.-O. Pörtner, D. M. Webber, R. G. Boutilier, and R. K. O'Dor, in preparation). Exhausted squid, *Illex illecebrosus*, exhibit a severe intracellular acidosis in the mantle musculature, whereas extracellular pH is hardly affected by anaerobic mantle metabolism. Such a contrasting degree of adaptation of proton equivalent ion transport mechanisms to anaerobic metabolic acidification does not become as evident in fish, which show varying degrees of activity and rely on anaerobic metabolism to a different extent depending on their mode of life (Wood and Perry 1985; Milligan and Wood 1987).

It seems, generally, as if highly aerobic swimmers like trout and squid, which only experience anaerobic fatigue under extreme conditions, do not rely on H^+ ion release to the extracellular compartment and ambient water to the same extent as animals that more consistently rely on anaerobic mechanisms during muscular activity. This situation is very common in squid, because a small amount of H^+ released to the blood would interfere with arterial oxygen binding by the noncellular haemocyanin (H.-O. Pörtner, in preparation). Protons accumulated in the tissues must finally be eliminated, however, to allow the phosphorylation status of the phosphagen and adenylate system to be restored (see later). Metabolic H^+ consumption in oxidative decarboxylation of pyruvate (originating from accumulated octopine) or in gluconeogenesis becomes essential during recovery, if H^+ release into the blood has to be avoided. As proton consumption in metabolism is time-consuming, differentiation of muscle in pelagic swimmers like trout and squid could help to maintain swimming via aerobic fibers (cf. Milligan and Wood 1987; Mommsen *et al.* 1981) during periods when the anaerobic white musculature is still recovering from exhaustion.

Environmental hypoxia

Though anaerobic exercise is usually a short-term response, characterized by high power output, long-term hypoxia requires strategies that involve reduced rates of energy turnover (de Zwaan and van den Thillart 1985), in invertebrates living in the intertidal zone, for example. Metabolic acidification and depletion of substrate and high-energy phosphate stores in the body are factors that contribute to setting the limits for survival. Metabolic acidification may be detrimental, the more so because the transition to hypoxia may involve CO_2 accumulation and respiratory acidosis in the natural environment owing to restriction of respiratory gas exchange (for example, see Toulmond 1973). Adaptation to survival under long-term hypoxia includes the reduction of metabolic rate and the selection of pathways that involve a higher energy yield than the Embden–Meyerhof pathway. The proton output per mole of glycosyl units utilized, however, is hardly elevated with this kind of metabolism, and may even be reduced, because anaerobic oxidative decarboxylation reduces the amount of generated carboxyl groups, and because amino acid anions (aspartate) or other organic acid anions (malate in freshwater invertebrates) are utilized as substrates (for a more thorough treatment of this subject see Pörtner *et al.* 1984*a*; Pörtner 1987*a*). Table 4 summarizes the metabolic mechanisms that contribute to the reduction of proton accumulation by anaerobic metabolism. The contribution of aspartate catabolism to the reduction of proton accumulation can be substantiated on the basis of the data of Graham and Ellington (1985) (cf. Pörtner 1987*b*).

It can be demonstrated in the marine worm *Sipunculus nudus* that changes in the acid–base status are qualitatively (Pörtner *et al.* 1984*b*) and quantitatively (Pörtner 1987*b*) related to proton turnover in metabolism, the role of phosphagen being the same as during muscular activity (Pörtner et al. 1984*b*). The

TABLE 5. Release of nonrespiratory (n.r.) protons from the body wall musculature into the coelomic fluid of *Sipunculus nudus* during 24 h or anaerobiosis (for details see Pörtner 1987*b*)

pH_e		pH_i		
Control	24 h	Control	24 h	% $\Delta H^+_{e\ n.r.}$
8.09	8.20	7.27	7.19	30
8.09	8.14	7.37	7.19	41
8.09	7.71	7.32	7.02	49
7.97	7.42	7.27	7.01	57

NOTE: A comparison of different experiments revealed that an increase in anaerobic ATP turnover (M_{ATP}) led to an increase in the percentage of protons ($\Delta H^+_{e\ n.r.}$) found in the extracellular space. This is interpreted as being linked to ionic acid–base regulation, the defended pH_i value being lower during anaerobiosis than during aerobiosis.

efficiency of acid–base regulation is reduced during anaerobiosis, as indicated by the fact that more than 80% of the protons remain in the animal even during long-term (24 h) hypoxia (Pörtner 1987*b*). The remainder are released into the ambient water (H.-O. Pörtner and N. Heisler, in preparation). The reduction in ionic acid–base regulation, however, could be part of a strategy that lowers the defended pH_i value and thereby allows for a contribution of pH to metabolic regulation during anaerobiosis (Table 5; Graham and Ellington 1985; Pörtner 1986, 1987*b*).

The efficiency of ionic acid–base regulation is regained during aerobic recovery. This allows for complete rephosphorylation of the phosphagen, which clearly depends on the restoration of pH_i (Pörtner *et al.* 1986*a*, 1986*b*). As gluconeogenesis or final oxidation of glycolytic end products is delayed in these animals, the rapid phase of pH_i restoration can be seen to be of primary importance in the reconsumption of free inorganic phosphate and the restoration of phosphorylation status. Although the extracellular space is utilized as a transient sink for protons, full pH_i recovery may depend on the restoration of pH_e. Of the accumulated end products, the restoration of propionate levels especially, may be correlated with pH_i recovery (including mitochondrial pH) to some extent, as suggested by the time course of changes and the dependence of propionyl-CoA carboxylase on acid–base parameters (Table 2). Overall, the metabolic contribution to anaerobic acid–base regulation is nicely reflected by the fact that the protons bound during phosphagen depletion are only released when oxygen is available to allow for efficient ionic H^+ transfer.

Acid–base methodology

The investigation of acid–base regulation requires adequate methodology for analyzing acid–base parameters in the compartments involved. It had already been confirmed that the evaluation of TA-HCO_3^- in urine and of bicarbonate levels in water provides estimates of net proton movements between animals and external medium if ammonium levels are included (see earlier). Blood acid–base parameters are best measured in samples withdrawn anaerobically via permanently implanted catheters.

Tissue acid–base parameters are not as easily accessible as the respective body-fluid values. The measurement of these parameters requires sampling of the tissue, which may involve a marked disturbance of the animal. The effect of this disturbance is minimal in the case of pH_i calculations, which are based on the distribution of the weak acid 5,5-dimethyl-2-4-oxazolidinedione (DMO) between intra- and extra-cellular compartments (Waddell and Butler 1959). DMO takes a long time to reach equilibrium and is therefore expected not to follow pH changes that may occur during tissue sampling. This implies, however, that this method is inadequate for following rapid pH changes, e.g., during anaerobic exercise, in most cases.

For this reason pH has frequently been measured in tissue homogenates, mainly of human muscle, to yield approximate data for pH changes during activity (Hermansen and Osnes 1972; Shalin *et al.* 1975). However, metabolic changes in the homogenate, which are mostly due to anaerobic ATP regeneration and ATP depletion, have always been considered to possibly affect the measurements. Sahlin *et al.* (1975) have tried to correct for this influence by graphical extrapolation of the pH drift. This procedure, however, neglects the possibility of rapid nonlinear changes in pH. Sahlin *et al.* (1976) and Costill *et al.* (1982) have tried to chemically arrest glycolysis and, in addition, Spriet *et al.* (1986) have focused on the influence of phosphagen hydrolysis on the measured pH.

Recently, a method was developed that allows the complete arrest of metabolism in the homogenate by binding and precipitation of Mg^{2+} and Ca^{2+} through the action of the ion chelator nitrilotriacetic acid and potassium fluoride (H.-O. Pörtner, R. G. Boutilier, and D. P. Toews, in preparation). The effect of the medium on intracellular pH is minimized by maintaining ionic strength during dilution of intracellular buffers, and as a result of the low buffer capacity of the medium in relation to tissue buffering. The influence of the extracellular fluid on measured pH values depends on the fractional blood volume in the respective tissues. In white muscle tissue this influence is negligible, as demonstrated by a comparison of DMO values and pH values found in tissue homogenates (Table 6).

The nonbicarbonate buffer value of blood or tissues represents an important parameter for the evaluation of nonrespiratory proton loads. For tissues this parameter is usually determined by titration with CO_2 or fixed acid or base (Heisler and Piiper 1971). The influence of metabolism on the buffer values or the response of metabolism to the titration procedure is usually neglected. Nevertheless, this buffer value is often treated as the buffer value that is valid for control conditions, whereas it is closer to the value in fatigued muscle, for example. The resulting error may be substantial, especially in anaerobic tissue with high levels of phosphagen. High-energy phosphates may be depleted and the accumulation of P_i results in an increase of up to 100% in buffer value. The response of metabolism to pH changes in the homogenate is eliminated by using the above-mentioned methodological approach for the removal of Ca^{2+} and Mg^{2+} from solution. A change in the contribution of P_i to intracellular buffering is monitored by following the change in free inorganic phosphate concentrations in the homogenate (H.-O. Pörtner, in preparation). Table 7 shows the buffer values (β_{NB}) evaluated in squid and toad muscle homogenates with and without considering phosphate accumulation. β_{NB} values valid for control conditions are low, and this analysis reveals that the values obtained in homogenates should be treated with caution insofar as their reflection of the physiological status of the respective tissue is concerned.

TABLE 6. Comparison of intracellular pH values ($\bar{x} \pm$ SD) in muscle tissues of different animals under resting conditions

	Muscle	Temp. (°C)	pH_i	Technique	Source
Bufo marinus	Sartorius	20	7.28±0.03	Homogenate (5)	H.-O. Pörtner and D. P. Toews, in preparation
			7.27±0.10	DMO (10)	Boutilier *et al.* 1987
	Gastrocnemius	20	7.25±0.02	Homogenate (5)	H.-O. Pörtner and D. P. Toews, in preparation
			7.28±0.09	DMO (10)	Boutilier *et al.* 1987
Sipunculus nudus	Body wall	15	7.29±0.01	Homogenate (4)	H.-O. Pörtner, in preparation
			7.27±0.05	DMO (10)	H.-O. Pörtner 1987*b*
			7.32±0.14	DMO (5)	H.-O. Pörtner *et al.* 1984*b*
Salmo gairdneri	Epaxial	15	7.30±0.01	Homogenate (5)	Y. Tang, H.-O. Pörtner, and R. G. Boutilier, in preparation
		12–14	7.30±0.08	DMO (9)	Hobe *et al.* 1984
		15	7.21±0.12	DMO (9)	Milligan and Wood 1986

NOTE: At the temperature indicated, pH was determined in tissue homogenates under full metabolic control (see text). Values obtained using this technique are in good agreement with mean values found in studies with DMO, but show less variability. Numbers in parentheses show sample size.

TABLE 7. Nonbicarbonate buffer values (β_{NB}, $mmol \cdot pH^{-1} \cdot kg$ wet $weight^{-1}$) of squid (*Illex illecebrosus*) mantle muscle (15°C) and toad (*Bufo marinus*) gastrocnemius muscle (20°C), determined by CO_2 equilibration of muscle homogenates

	Squid	Toad
β_{NB}		
Measured value	33.5±5.4 (5)	25.7±4.9 (6)
Control value	17.7	20.0

NOTE: Metabolism was controlled by a solution containing nitrilotriacetic acid and potassium fluoride. Both tissues are muscle containing high amounts of phosphagen. Despite metabolic control, buffer values in the homogenate had to be corrected for the accumulation of free inorganic phosphate (H.-O. Pörtner, in preparation). Obviously, care has to be taken that metabolic changes in homogenates are considered before buffer values are assigned to any physiological status of the respective tissue. Values are given as mean ± SD. Numbers in parentheses show sample size.

Conclusions

In conclusion, as a consequence of the traditional separation of the fields of biochemistry and physiology, the important metabolic mechanisms in acid–base regulation and acid–base methodology have only received appropriate consideration in recent years. The integration of physiological and biochemical principles in future studies of acid–base regulation should lead to more profound insights into the integration of metabolic and acid–base control. When the techniques available for studying acid–base regulation are used, the extent to which disturbance of metabolic homeostasis may influence the parameters under investigation should always be borne in mind.

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