Upsala J Med Sci 101: 35–68, 1996

REVIEW

Carbon Dioxide Formation and Elimination in Man

Recent theories and possible consequences

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The normal pH in blood and extracellular fluid at 37°C is 7.40. The pH inside ordinary muscle cells is 7.00 at the same temperature (1, 2, 3). Proteins are ionized in just this pH region to such a degree that the activity of most enzymes is optimal (4, 5, 6, 7). However, the pH value varies with the temperature and is higher at lower temperatures and vice versa (1, 2, 8). Different tissues even have different optimal pHs as an effect of different temperatures. It is now thought that one of the primary goals of acid base regulation in the body is to maintain a constant level of protein ionization (4, 7). Thus, acid-base regulation is not directed at maintaining the same pH (pH-stat-regulation) at all occurring temperatures (2, 9, 10), but at preserving an unchanged ionization of histidine (α-state-regulation), which is the only amino acid which takes a more active part in proton exchange within the physiological part of the pH scale where α is defined as the quotient between the ionized and unionized α-amino group of the histidine content in proteins (11).

pH regulation

Many mechanisms work together in the body to keep hydrogen ion activity (the pH value) normal. Perhaps the simplest and most traditional approach is to think of pH regulation as a united effect of metabolic processes and a number of buffers. The carbonic acid and bicarbonate buffers are quantitatively the body´s largest buffer which according to earlier approaches constitutes a unified buffer system and which handles no less than about 20,000 mmol per day. This buffer is very fast and occurs both intra- and extra-cellularly. This buffer is present intracellularly as are, among others, the physico-chemical buffers in the Table 1.

Table 1

	Relative buffer value	Rate of action	Extracellular distribution
Carbonic acid-bicarbonate	12	++++	+++
Phosphate	7	+++	+
Protein	15 approx	++	+
Production or consumption of acid or base			
Phosphocreatine-creatine	26	+++	-
Glucose-glucose-6(P)	-2,6	+	-
Glutamate-glutamine	8	+	-

(Modified from Sahlin, ref 3)

It is important to remember that another type of buffer is also present where acids or bases are produced and consumed. Based on this view the phosphocreatinine-creatine buffer can be considered the body's strongest of the "classical" buffer systems (3).

The pH is more stable intracellularly than extracellularly due to the fact that many buffer systems only work intracellularly. Even more important, however, are the port systems which regulate passage of ions in the cell membranes. A large number of such processes or port systems have been described (for review see Tønnesen, ref 12).

Electron neutral processes

1. Simple diffusion of uncharged acids or bases.
2. Na^+/H^+ antiport
3. Na^+ -coupled Cl^-/HCO_3^- antiport.
4. Na^+ -independent Cl^-/HCO_3^- antiport.
5. H^+/K^+ ATPase
6. H^+ -lactate-cotransport
7. Cl^--/formate$^-$- antiport
8. Na^+ -lactate$^-$ (acetate)-cotransport

Electrogenic processes

1. Na^+ -nHCO_3^-- cotransport(symport)
2. Proton-translocating ATPase
3. Proton channels
4. HCO_3^-- channels

Traditional view of metabolism

According to the traditional view, carbohydrates and fats are metabolized in mammals to carbon dioxide and water. According to the same view proteins produce carbon dioxide, water and ammonia, the latter of which is converted to urea via the urea cycle.

Alternative view of metabolism

If we consider metabolism in mammals of compounds containing a **carboxylate ion**, this can be described schematically, according to Atkinson &Camien (13), as

$$CH_3\,(CH_2)_{n-2} - COO^- \Rightarrow (n-1)CO_2 + HCO_3^- \qquad (1)$$

Traditionally the carboxyl group is not considered to be charged, and then this reaction leads directly to the formation of carbon dioxide and water. The fact is, however, that the carboxyl group is in most cases a carboxylate (an)ion, since the pK_a for the carboxylate group is approximately 3. Oxidation of each carboxylate ion at a physiological pH results in production of an HCO_3^- ion (bicarbonate ion = "bicarbonate").

The same is true especially for ***proteins***: These are absorbed and metabolized containing ionized amino acids. At hydrolysis of proteins out in various organs and tissues at a physiological pH, a dipole is formed where generally both the amino group and the carboxylate group are charged. The following metabolism (5, 13) which occurs in mammals can, at a physiological pH, be simplified to:

$$\begin{array}{l} \qquad\qquad NH_3^+ \\ \qquad\qquad | \\ CH_3 - (CH_2)_{n-3} - CH - COO^- \Rightarrow (n-1)CO_2 + HCO_3^- + NH_4^+ \end{array} \qquad (2)$$

Notice that protein metabolism results in molar equivalent amounts of bicarbonate ions and ammonium ions in normal man approximately 1 mol per day. According to Atkinson & Camien (13), bicarbonate production is just as great as for gluconeogenesis. Furthermore, additional bicarbonate ions are produced for each carboxylate or carboxamide group in the side chains of aspartate, glutamate, asparagine and glutamine. This latter bicarbonate production is balanced in part by production of sulphuric acid from cysteine and methionine. In summary: According to Atkinson & Camien (13) the total metabolism in a normal individual forms approximately 1 mol HCO_3^-, 1 mol NH_4^+ and 10-15 mol CO_2 daily. The metabolic production of acid from protein is minute.

In this connection it must be pointed out that the above calculation of endogenous bicarbonate production has been criticized (14), primarily because it does not take into account the use of bicarbonate in the synthesis of urea from ammonia. As a result of this, Atkinson and his co-workers´ reasoning is not shared by the majority of researchers in the area (13, 14, 15, 16, 17). Instead the classical view that metabolism of glucose and triglycerides produce CO_2 and water still pertains. Also, the metabolism of most amino acids, i.e. 13 out of the 20, are generally metabolized to glucose and triglycerides after deamination without any net generation of acid or base (14). However, as pointed out by Atkinson & Camien (13) nonvolatile acids are produced when a neutral amino acid is converted to a number of protons and its conjugate base, as for exemple when sulphur containing amino acids are metabolized (Table 2):

methionine or cysteine $\Rightarrow$ glucose + urea + $2SO_4^{2-}$ + $4H^+$

But even if a heavy diet (3300 kcal daily with 45% as carbohydrates, 40% as fat, and 15% as protein) is considered, if results in a combustion of approximately 1000 mmol amino acid recidues of which 12 mmol are cysteine and 24 mmol methionine producing 72 mmol H^+ as sulphuric acid. In addition conversion of cationic amino acid (lysine, arginine, and some histidine) residues into neutral products and H^+ results in a generation of another 138 mmol H^+ daily. In contrast metabolism of anionic amino acids (glutamate$^-$ and aspartate$^-$ but not their amide derivatives glutamine and asparagine) to neutral end products will remove H^+ by generation of 100 mmol bicarbonate from the same diet. To this proton removal we must add metabolic contributions from other dietary organic anions such as acetate$^-$, lactate$^-$, gluconate$^-$, malate^{2-}, and citrate^{3-} (corresponding to approximately 60 mmol of bicarbonate) that are oxidised to neutral end products, mainly carbon dioxide, and glucose and triglycerides. The metabolism of these organic anions, whether it occurs in the liver or by the intestinal flora will consume H^+. Totally it follows that the above diet would result in a net production of approximately 50 mmol H^+ (14, 15).

This production of acid is balanced by a greater generation of bicarbonate from other amino acids (13, 14, 16, 17). Out of quantitative reasons the metabolism of glutamine seems the most interesting. The metabolism of glutamine is exclusively catalysed by glutaminase and can in a simplified form be written (14).

$^+$glutamine$^-$ $\Rightarrow$ $2NH_4^+$ + carboxylate^{2-}

carboxylate^{2-} $\Rightarrow$ glucose^0 or CO_2 + $2HCO_3^-$

Thus, gluconeogenesis from the carbon skeleton of glutamine results in the same bicarbonate production as its total combustion to CO_2 and water. The critical part to understand is that H^+ ions are removed or bicarbonate generated by these metabolic

processes quite independent of what happens to the NH_4^+ produced. If, however, the NH_4^+ is eliminated in the urine without previous metabolism to urea this means that bicarbonate is not consumed in the urea cycle which is equivalent to production of the same amount of protons from the point of acid-base balance (vide infra!). From the quantitative point of view, however, it must be emphasised that in normal acid-base balance the renal ammonium ion excretion is much smaller than the hepatic ammonium ion metabolism leading to urea production. The total bicarbonate production from amino acid metabolism amounts to more than one mol per day (14) but these metabolic processes take place within the different metabolic compartments of the body where acid-base balance is maintained (14). However, from the point of whole-body acid-base balance there is no question that unless the bicarbonate produced is protonised it could not possibly be eliminated as CO_2 through the lungs. Thus, a substantial part of the systemic arterio-venous difference of carbon dioxide over the lungs is created by the liver.

Table 2. Daily production of HCO_3^-, NH_4^+, and SO_4^{2-} in the catabolism of a daily normal intake of 100 g protein according to Atkinson & Camien (13).

Product	Source	mmol per 100 g protein	Product	Source	mmol per 100 g protein
HCO_3^-			**NH_4^+**		
	Peptide bonds glu, asp, glu-NH_2 aspNH_2	909 183 1092		Peptide bonds glu-NH_2 + asp-NH_2 lysine hist x 2 arg	909 80 61 42 39 1131
SO_4^{2-}					
	Cysteine	19			
	Methionine	15			
		34			

Irrespective of whether the reader of this paper agrees with Atkinson´s theory, this means in short that catabolism of carbohydrates, fats and proteins results in all the carbon atoms being converted to either so-called "conjugate base" or "conjugate acid" in the HCO_3^-/CO_2-system, which constitutes the main extracellular buffer system. This system can be characterized in the following way (according to Henderson-Hasselbalch):

$CO_2 + H_2O \Rightarrow H_2CO_3$ $pK_1 = 2.5$ (a)

$H_2CO_3 \Rightarrow HCO_3^- + H^+$ $pK_2 = 3.6$ (b)

$pH = 3.6 + \log([HCO_3^-]/[H_2CO_3])$ (c)

After addition of (a) and (b) H_2CO_3 is eliminated:

$CO_2 + H_2O \Rightarrow HCO_3^- + H^+$ $pK_3 = pK_1 + pK_2 = 6.1$ (d)

$pH = 6.1 + \log([HCO_3^-]/[CO_2])$ (e)

If the pH of the blood and in the rest of the body is to remain constant, however, HCO_3^- and CO_2 must be eliminated at the same rate as they are produced in the body.

As stated above the metabolism of proteins leads, in addition, to production of NH_4^+, which must also be eliminated. Hydrolysis of a peptide formation results in a carboxylate anion which forms HCO_3^- as the $-NH_3^+$ group in the amino acid forms NH_4^+. Hydrolysis of the side chains´ amide formations in glutamine and asparagine results in additional carboxylate ions which form HCO_3^-. Furthermore, since the ammonium ion (not ammonium) constitutes the chemical compound which is formed from amino acids, such as glutamine, before appearing in the urine, it has not undergone any net exchange of protons with buffers during its passage out via the kidneys, and have consequently not caused any shift in the pH of the blood. Accordingly, the contention that is sometimes seen that NH_3 serves as a proton carrier or proton eliminator is not considered to be correct by Atkinson and coworkers (13) as well as by others (14, 15).

The carbon dioxide content in plasma (reviewed by Nunn, ref 18) is of great interest to us in discussions of acid-base balance and is commonly expressed as the partial pressure of carbon dioxide CO_2 (arterial $PaCO_2$ or venous PvO_2). In alveolar air there is normally a partial pressure of carbon dioxide ($PACO_2$) of about 5.3 kPa = 40 mm Hg) and the corresponding partial pressure of the gas is present in arterial blood ($PaCO_2$). Practically speaking there is a diffusion equilibrium for CO_2 between alveolar air and pulmonary capillary blood. The speed of exhalation of CO_2 thus rises and falls with alveolar ventilation, the size of which is dependent on the breathing center in the medulla oblongata and its reactions to changes in the pH and PCO_2 in the blood. It has long been a matter of discussion as to whether the breathing center is directly affected by changes in PCO_2 and/or indirectly affected by simultaneous shifting of the pH. Effects of changes in PCO_2 on ventilation are illustrated in Fig. 1 .

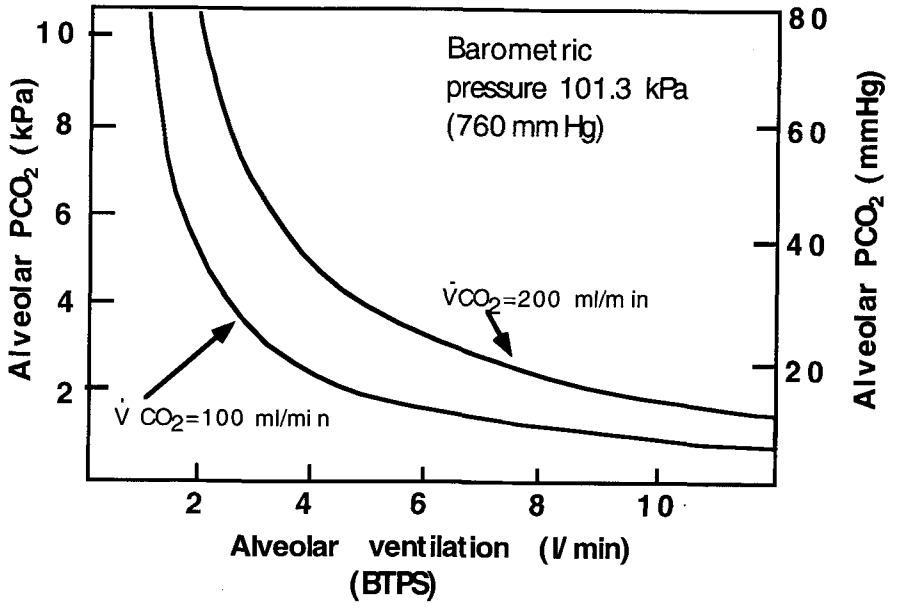

Fig. 1

The effect of CO_2 output and alveolar ventilation on alveolar PCO_2 according to Nunn (18). The lowest curve shows the relationship between ventilation and alveolar PCO_2 for a carbon dioxide output of 100 ml/min while th upper curve shows the same relationship when the carbon dioxide output is 200 ml/min (STPD). By courtesy of the author and Butterworth-Heinemann Ltd.

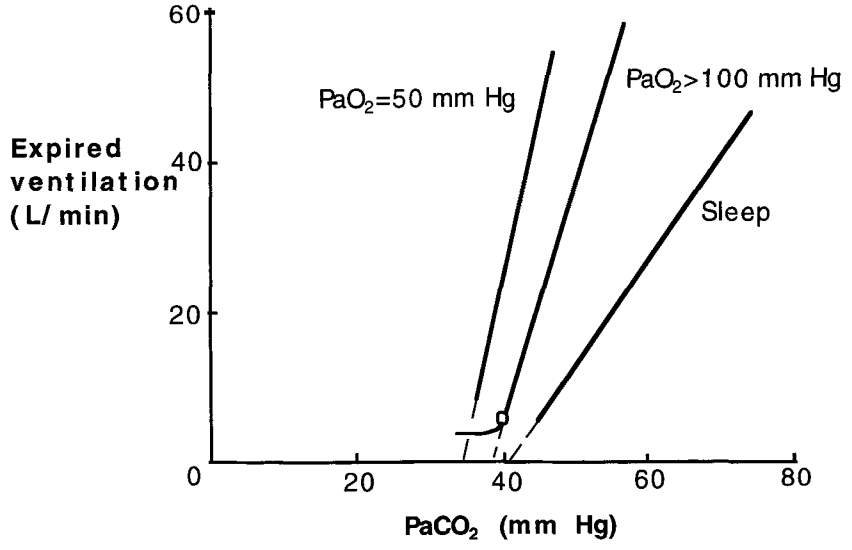

Fig. 2

The CO_2-ventilation dose response curve according to Staub (19). Middle line represents normal conditions with an arterial PO_2 > 100 mm Hg. Normal operating point is shown by the open circle where P_ACO_2 = 40 mm Hg and V_E = 6 L/min the curve being defined by the slope of the straight portion and the

extrapolated x-axis intercept. Increasing inspired CO_2 causes a steep rise in ventilation to to about 60 L/min at PCO_2 = 55 mm Hg, resulting in a sensitivity of 3.6 L/ (min · mm Hg P_ACO_2). $PACO_2$ below normal decreases ventilation, but it does not stop. For low PaO_2 the set point (X-intercept) is shifted to the left and there is an increase in the slope (sensitivity). Sleep is an example of a depressed dose-response curve in which the set point is shifted to the right and the slope is reduced, indicating decreased sensitivity. By courtesy of the author and Churchill Livingstone Inc. New York.

The sensitivity of the breathing center to rises in $PaCO_2$ is, however, also dependent on PaO_2, so that a low oxygen saturation decreases the activity in the breathing center. A combination of a high CO_2 and low oxygen supply is more unfavorable to respiratory activity than the same CO_2 concentration with a high administration of oxygen. An hypoxia thus depresses the breathing center simultaneously in the same way as it depresses function in other parts of the central nervous system. An oxygen deficit with a low PaO_2 instead exerts its breathing stimulation effect via the peripheral chemoreceptors in the aortic arch and the glomus carotium, and this effect can most likely be modified by the hydrogen ion (H^+) concentration in the blood. An excess of CO_2 and a shortage of oxygen will thus stimulate respiration but these do not work independently of each another (Fig 2). For additional studies of breathing regulation, other overviews can be consulted (18).
The air contains 0.03% carbon dioxide (CO_2). When a mixture of gases containing 2% CO_2 is inhaled, the respiratory minute volume rises by approximately 30%, primarily due to an increase in the depth of respiration. At higher concentrations of CO_2 an increase in depth and frequency of respiration occurs with a maximal stimulation (minute volume approximately 10 times the resting value) at 9-10% CO_2 in the inspired gas. At higher concentrations ventilation declines again and the narcotic and toxic effects of carbon dioxide on the central nervous system take over. At a concentration of 30-50% in inspired air, narcosis occurs and ventilation is smaller. Thus, these high concentrations result in a pronounced depression of respiration. If the breathing center is simultaneously affected by hypoxia, opiates, hypnotics or sedatives, there is an inhibition even at lower concentrations of CO_2. In pronounced cases of influence on the breathing center, an inhibition occurs at CO_2 concentrations as low as 5% in inspired air.

The neutralization of hydrogen ions (H^+) which is caused by other buffer systems in the serum is not revealed in the determination of standard bicarbonate. Of the anions in the plasma, in practice only HCO_3^- and protein$^-$, form a weak acid with H^+ and consequently are able to enter into the buffer system within the pH range in question. This constitutes the "buffer base" (BB) in serum and is normally equivalent to 41.7 mmol/L (17 mmol/L of which is protein). When H^+ is neutralized, BB decreases. Thus BB provides a principle of the non-respiratory component in the acid-base balance (18, 20, 21). However, an appreciable part of the metabolic as well as the respiratory

supply of hydrogen ions is buffered by Hb in the red blood cells. The value of BB in whole blood (NBB) thus varies with the haemoglobin (Hb) value, so that for each g/L of Hb the value increases with 0.042 mmol/L. If NBB for plasma is 41.7, the corresponding value for blood with a Hb concentration of 120 g/L = 41.7 + 120 · 0.042 = 46.7 mmol/L.

The difference between the current BB and NBB is an expression of the surplus or deficit of hydrogen ions in mmol/L irrespective of how this value is affected by the Hb value. This difference (20) is called "base excess" (BE): The expression is perhaps a bit awkward, since with acidosis with an excess of H^+ and a deficit of base (BE negative) the value must be expressed as a negative excess, while with alkalosis and an excess of base the value of BE is positive. The value of BE, however, is an adequate expression of the metabolically determined shift in the acid-base balance and indicates by definition (NBB-BB=BE) how many mmols of acid or base must be supplied at a normal PCO_2 in order to reset the pH to the normal value of 7.38. Consequently, BE is zero if the pH is 7.38 at a PCO_2 of 40 mm Hg. The Astrup nomogram has been combined with curves for BB and BE which were presented by Siggaard-Andersen (22). It was later found that the BE-value was calculated for the entire extracellular volume, and thus, a correction was introduced so that the Hb content was calculated as distributed throughout the entire extracelluar volume. This corrected BE value is called "standard BE" and is the same as calculating BE at a Hb concentration of 5 g/L (23).

Landbound mammals cannot eliminate HCO_3^- and NH_4^+ via the lungs as they can with CO_2. The alternative, namely complete excretion of HCO_3^- and NH_4^+ via the kidneys would cause extremely great problems, since an adult person is considered to produce (13) up to 1 mol HCO_3^- and 1 mol NH_4^+ per day. If the daily production of the latter metabolite were eliminated via the kidneys, the quotient between NH_4^+ concentration in the urine and blood would be so high that this excretion would require large quantities of energy for an active pumping of ammonium ions via the kidneys, and in addition, because the ammonium ion is so small and very diffusible, an extensive backflow through the membranes of the kidneys would be expected. The same considerations can be applied to HCO_3^-, but here there would also be a risk of precipitation of carbonates in the kidney tubules. If it were possible to eliminate all the HCO_3^- produced daily by metabolism via a urine flow of one liter, the pH in the urine would need to increase to approximately 9, if one assumes that the CO_2 tension in the urine remained in equilibrium with the PCO_2 in the blood and in the interstitial fluid. At this pH and this carbonate concentration, the solubility products for calcium carbonate would be exceeded at a calcium ion concentration of 0.2 µmol/L or about 0.1% of the normal calcium concentration in plasma. When mammals are considered as a whole, it is clear that their metabolism sometimes produces much more HCO_3^-

than man's, and, in addition, mammals must be able to survive a temporary dehydration with a secondarily low urine production, which should further increase the urine concentration of bicarbonate.

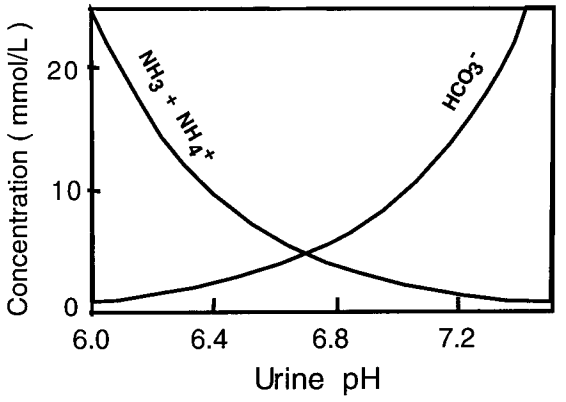

Fig. 3

The calculated concentrations of bicarbonate (HCO_3^-), and ammonia/ammonium ions (NH_3/NH_4^+) in urine as a function of urine pH according to Atkinson & Camien (13) assuming that both NH_3 and CO_2 are in equilibrium across the tubular membrane, and blood and urine concentrations are 1.2 mmol/L for CO_2 and 14 mmol/L for NH_3/NH_4^+. By courtesy of Academic Press.

The curves in Fig. 3 (13) show the concentration of HCO_3^- (that would be in equilibrium with plasma and the peritubular fluid which contains 1.2 mmol/L CO_2 and 14 mmol/L NH_3) as a function of the urine's pH. This concentration of NH_3 corresponds to 1 mmol/L NH_4^+ at pH 7.40, which is much higher than that which can usually be measured in plasma, and has therefore been used to illustrate that the possibility of excretion has not been underestimated. Fig 3 shows that at pH of approx. 6.7, where the concentrations of the two ions are equal, the urine will contain approx. 5 mmol/L of NH_4HCO_3. If urine production were 1 liter per day, NH_4^+ and HCO_3^- would be eliminated at a rate which corresponds only to 0.5% of that by which they are produced (1 mol is produced per 24 h, while about 0.005 mol is eliminated via the urine). From this reasoning it is apparent that elimination via the urine is both physically and chemically impossible for the entire daily production of HCO_3^-. Instead, a large part of the 1 mol HCO_3^- would in this case accumulate daily, which during the first 24 h would increase extracellular pH to approximately 8.0.

CO_2 transport and elimination

According to the traditional view of mamalian metabolism, carbon dioxide and water are formed in the metabolism of carbohydrates and fat and protein. According to the same view the proteins also produce NH_3 which is transformed via the urea cycle to a free water-soluble and nontoxic substance, namely urea. Carbon dioxide is

transported as 1) physically soluble CO_2, 2) carbonic acid, 3) bicarbonate ion, 4) carbamino-CO_2.

Table 3 Normal values for carbon dioxide carriage in blood

	Arterial blood (Hb 95% sat.)	Mixed venous blood Hb 70% sat.	Arterio-venous difference
Whole blood			
pH	7.40	7.367	-0.033
PCO_2 (kPa)	5.3	6.1	+0.8
(mmHg)	40.0	46.0	+6.0
Total CO_2 (mmol/L)	21.5	23.3	+1.8
(ml/L)	48.0	52.0	+4.0
Plasma (mmol/L)			
Dissolved CO_2	1.2	1.4	+0.2
Carbonic acid	0.0017	0.0020	+0.0003
Bicarbonate ion	24.4	26.2	+1.8
Carbamino CO_2	Negligible	Negligible	Negligible
Total	25.6	27.6	+2.0
Erythrocyte fraction of 1 litre of blood			
Dissolved CO_2	0.44	0.51	+0.07
Bicarbonate ion	5.88	5.92	+0.04
Carbamino CO_2	1.10	1.70	+0.60
Plasma fraction of 1 litre of blood			
Dissolved CO_2	0.66	0.76	+0.10
Bicarbonate ion	13.42	14.41	+0.99
Total in 1 litre of blood (mmol/L)	21.50	23.30	+1.80

These values have not been derived from a single publication but represent the mean of values reported in a large number of studies (18).

In the equilibrium

(1) (2)

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

its first step is far too slow and needs to be catalyzed by carbonic anhydrase, which is a zinc containing enzyme of relatively low molecular weight which was discovered as early as 1933 by Meldrum and Roughton (reviewed by Nunn, ref 18). It is actively inhibited by a variety of non-substituted sulphonamides while the substituted sulphonamides are not active as carbonic anhydrase inhibitors. Acetazolamide is the

best known of the active inhibitors and gives complete carbonic anhydrase inhibition at a concentration of 5-20 mg/kg in all organs examined, but otherwise has no pharmacological effects. In experiments with large doses of acetazolamide it has been shown that the effect of carbonic anhydrase is not essential to the continuance of life (24). However, total inhibition increases the PCO_2 gradient between the tissues and alveolar gas and ventilation of the lungs are moreover increased and alveolar PCO_2 decreases (24).

Carbamino binding

In a protein the amino groups comprising a peptide binding between amino acids cannot bind CO_2. Carbamino binding of CO_2 therefore only occurs at the terminal amino group in each protein and at the free amino groups of the basic amino acids lysine and arginine. One single binding position per protein monomer is more than sufficient to explain the amount of carbon dioxide estimated to be transported as carbamino-CO_2. Furthermore, it can be pointed out that in principle no CO_2 molecules are bound as carbamino compounds to plasma proteins, but instead to hemoglobin. Desoxyhemoglobin, however, is about 3.5 times as effective a carrier of CO_2 as is oxyhemoglobin, while PCO_2 has very little influence on the amount of CO_2 bound as carbamino-CO_2 as long as PCO_2 is within the physiological range. While the amount of CO_2 transported in the blood in carbamino binding is small, it can be shown that the difference between the amount carried in venous and arterial blood is around one third of the total arteriovenous difference. Thus, this represents the major part of the "Haldane effect", which constitutes the difference in CO_2 content between oxygenated and reduced blood at a constant PCO_2. In comparison the "Bohr effect" is the amount of oxygen released when the concentrations of carbon dioxide and hydrogen ions are increased, both these "effects" taking place in the peripheral capillary network presumably in order to facilitate release of oxygen from arterial blood.

Bicarbonate elimination

As indicated above, there is no renal excretion mechanism for large amounts of bicarbonate. When this has been pointed out, however, it must be conceded that smaller amounts of bicarbonate are lost via the feces. Bicarbonate must, thus, be turned into carbon dioxide before it can be excreted from the body. This is accomplished by binding a proton and forming carbonic acid, which then dissociates into water and carbon dioxide. The carbon dioxide can then be eliminated from the blood when it passes through the pulmonary vascular bed. Atkinson and Camien (13) have proposed that the way the body eliminates bicarbonate is by the urea synthesis of the liver, where-upon the formation of 1 mol of urea, 2 mols of bicarbonate are used and 1 mol of carbon dioxide is formed. The prerequisite for elimination of bicarbonate

is thus its protonization. Since the human body is considered to produce (13) about 1 mol of bicarbonate per day, which at steady state must be eliminated, 1 mol of protons must be added in order for bicarbonate elimination to take place. As has already been pointed out, the body also produces about 1 mol of NH_4^+ every day, and at first thought protons from NH_4^+ should be donated to bicarbonate. As already stated, however, pKa for the CO_2/HCO_3^- buffer is 6.1, while the same value for the NH_3/NH_4^+ buffer is 9.25. This means that ammonium ions are 1000 times weaker an acid than carbon dioxide/bicarbonate. A direct donation of protons is thus impossible. And even if this proton donation to HCO_3^- was possible, the body would never be able to handle the problem of getting rid of so large an amount of NH_3/NH_4^+ in any way other than via the urea cycle. Nature has solved this problem by transferring, by means of an addition of ATP-bound energy, a very weak acid (the ammonium ion) to such a strong acid (carbamoyl phosphate) that it can release protons.

The total formula for the urea cycle is usually written:

$$3ATP^{4-} + NH_3 + CO_2 + 2H_2O + \text{aspartate}^- \Rightarrow$$
$$\text{fumarate}^{2-} + 2ADP^{3-} + 4P_i^{2-} + AMP^{2-} + \text{urea} + 5H^+$$

Ammonia is the substrate for the urea cycle (25) as a result of the high intra-mitochondrial pH. However, carbon dioxide as such can not be taken up in the formula, but instead is included as bicarbonate (26). Since, however,

$$H_2CO_3 + H_2O \leftrightarrow HCO_3^- + H^+ + H_2O$$

the urea cycle´s true formula can be rewritten:

$$3ATP^{4-} + NH_3 + HCO_3^- + H_2O + \text{aspartate}^- \Rightarrow$$
$$\text{fumarate}^{2-} + 2ADP^{3-} + 4P_i^{2-} + AMP^{2-} + \text{urea} + 4H^+$$

Since a part of the initially consumed ATP can be recovered via the citric acid cycle, the final energy cost is 1 mol ATP per mol of urea formed (27). By means of the urea cycle two protons from NH_4^+ are released for each urea molecule produced. If instead the final products of the urea cycle are considered the formula according to Christensen (5) becomes:

$$2HCO_3^- + 2NH_4^+ \Rightarrow H_2NCONH_2 + CO_2 + 3H_2O$$

It has long been known that urea synthesis constitutes the base for elimination of nitrogen and particularly ammonia, which would otherwise accumulate to toxic concentrations in the bodies of mammals. The other function of urea synthesis, namely to transfer bicarbonate to CO_2 via an energy requiring proton pump (protonisation), however, must be considered to be at least as important biologically. There are at least two main reasons why this function has not been recognized earlier. In the first place, it is not obvious from the equation for urea synthesis that this process involves

the energy requiring transfer of protons from a weak acid to the conjugate base of a stronger acid against an energy gradient, and in the second place the metabolic generation of bicarbonate and the subsequent necessity that it must be eliminated has not been generally appreciated (13).

There has been intense criticism of the above theory proposed by Atkinson and co-workers (13), and a number of objections have arisen. The best articulated ones have been from Walser (16) and Jungas et al (14). Their calculations of the bicarbonate balance have confirmed that oxidation of many amino acids should in fact generate bicarbonate, but in addition Jungas et al (14) have found that the large production of acid (protons) in the urea cycle is balanced within the liver by a local amino acid oxidation generating large amounts of bicarbonate. Furthermore, it now seems to be proven that the same ammonium ion is recirculated and reused for the donation of protons to bicarbonate via an enterohepatic recirculation (16, 28, 29, 30, 31, 32). Endogenously produced urea is thus transported to the intestinal tract from the systemic circulation which together with the nitrous non-protein content of the oral intake is metabolized by the enteric bacteria to ammonia or ammonium ions which are readily absorbed especially in the colon and transported by the portal circulation to the liver where new synthesis of urea, or amino acids, takes place. Fasting adults seem to transport some 4-8 g (220-440 mmol) ammonia per 24h via the portal vein to the liver (28, 29). Our group has documented enteric release of ammonium ions in piglets (33) of the same magnitude.Out of the endogenous urea production 15-30% is hydrolyzed in the gut implying that this amount of ammonium ions (or urea) is recirculating as the faecal content is only 50 mg/24h (29). The clinical habit of treating hepatic encephalopathy with lactulose seems to result in at least a doubling of the faecal bacterial output along with "soluble nitrogen" resulting in a reduced urea production (34). The bacterial destruction of the urea content of the intestinal tract is then also inhibited by the oral simultaneous administration of neomycin (34).

The regulation of hepatic urea and glutamine synthesis has recently been reviewed in detail by Häussinger et al (26). Suffice here to make a short survey of certain facts. The mentioned two processes are regulated in parallel and partly by similar mechanisms especially as regards acid-base balance which influences both of them. However, while ureagenesis is accomplished by low affinity systems including the gluconeogenesis from the amino acids, the hepatic glutaminase and the intramitochondrial carbonic anhydrase located in the periportal hepatocytes, the synthesis of glutamine takes place in the cytosol by a high affinity system including other isoenzymes of the carbonic anhydrase family as well as glutamate uptake into the perivenous hepatocytes. *In vitro* the regulation of the urea cycle seems to be

exerted by the activity of the carbamoyl-phosphate synthetase and the availability of ornithine which, in addition to ammonium ions, determines the concentration of each of the ornithine cycle intermediates and thereby the flux through the cycle. In addition the activity of intramitochondrial carbonic anhydrase, which produces HCO_3^- (a substrate of the carbamoyl-phosphate synthetase) from CO_2 inside the mitochondrion is a third factor of importance. *In vivo* there is a large body of evidence to support that rapid increases in intramitochondrial N-acetylglutamate accompanies an increase in the rate of urea synthesis under various nutritional conditions. In the isolated perfused liver urea production is proportional to effluent pH while glutamine production instead seems to be inversely proportional to the same pH value, the respective processes being approximately equal at normal pH (7.40). This is dependent upon the fact that the glutamine synthetase flux is negatively correlated to, and the glutaminase flux positively correlated to effluent pH. Thus, the increased net glutamine production by the liver in acidosis is due to both a decreased glutamine degradation and to an increased perivenous glutamine synthesis. The ammonium ion concentration leaving the periportal compartment is, thus, critical for glutamine synthetase flux.

The body´s content of carbon dioxide, bicarbonate and carbonate

The total amount of carbon dioxide and bicarbonate in the body is very large, around 120 L, which is about 100 times greater than the total amount of gaseous oxygen (35). Consequently, when ventilation is altered so that it no longer eliminates as much carbon dioxide and bicarbonate as metabolism creates, then the amounts of carbon dioxide and bicarbonate in the body will slowly change (36). The opposite is true when the CO_2 elimination exceeds its production. The content in the body of carbon dioxide-bicarbonate is often described in the form of a 3-compartment hydraulic model (Fig.4) which originated as an analog hydrodynamic model at the Department of Physiology in Uppsala (37), where Henrik Enghoff earlier had realized the importance of the dead space in pulmonary ventilation which he called the "volumen inefficas" (38, 39, 40) and later Torsten Teorell laid the foundation of what later became farmacokinetics (37).

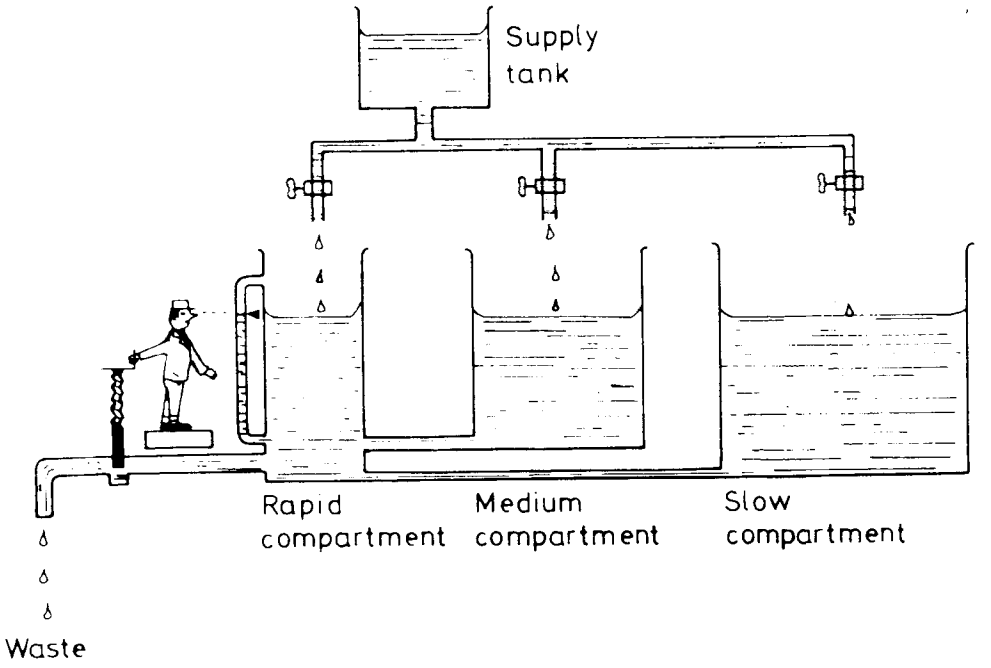

Fig. 4

A hydrostatic analog model of carbon dioxide kinetics according to Nunn (18). By courtesy of the author and Butterworth-Heinemann Ltd.

In the modell the depth in the three different pools corresponds to the carbon dioxide storage room in a rapid compartment (pool), an intermediate compartment and a slow compartment. The rapid pool represents blood, brain, kidneys and other well-perfused tissues, while the intermediate pool represents, in particular, resting muscles and other tissues with a moderate blood flow. The slow and also the largest pool farthest to the right includes, in particular, all fat and bone tissue where carbon dioxide is stored in the form of carbonate. The metabolic production of carbon dioxide is represented by a flow from a tank located up above where the flows can be varied individually. The outflow represents alveolar ventilation where the little man makes sure that the level is just high enough in the rapid pool. This corresponds to the triggering that carbon dioxide performs via the chemoreceptors. When there is hyperventilation the outflow valve opens completely, whereby an exponential decrease in the levels of all three pools occurs and the content of the rapid (and smallest) pool falls most quickly. In contrast to this, in hypoventilation an increase in the levels occurs which is dependent on the balance between inflow and outflow in each of the three pools. With complete apnea the levels in the three pools become dependent on the inflow of carbon dioxide from the metabolism and capacity (distribution volume) in the three pools comprising the body store. It can be seen from

the model that the consequence of a decrease in ventilation is an increase in the CO_2 content of the storage space, which does not constitute a reflection of the amount of time following an increase in ventilation (41). The speed of the increase of the carbon dioxide in the storage space during hypoventilation is in fact much slower than the speed of the decrease during hyperventilation, which is of great benefit to patients with respiratory arrest. With a total respiratory arrest, apnea, the speed of increase of arterial PCO_2 is in the order of 0.4-0.8 kPa per minute (3-6 mm Hg per minute), which constitutes the total result of carbon dioxide-bicarbonate production and the capacity of the body to store these compounds (36). Another example which can be worth considering is what happens when 5% carbon dioxide is instilled in the inspired gas of a patient who is being mechanically ventilated: in this case the arterial PCO_2 will increase by about 2.3 kPa (17.3 mm Hg) in 2 minutes, of which the greater part occurs during the first minute.

It is also important to remember that after a patient´s body temperature has changed more than 1°C or he has fallen asleep or awakened, or after his ventilator settings have been changed, his HCO_3^-/CO_2 store is not in a steady state before at least 60 min have passed (41). In addition, when the patient´s body mass (body weight) is large, and blood flow or ventilator settings are changed, then it takes at least two hours before a new steady state is attained. This can readily be checked by observing the metabolic quotient (carbon dioxide production per min/oxygen uptake per min) which should remain stable during a respiratory and metabolic steady state (41). Thus, it is not until this has been attained that measurements of the patient´s metabolic rate is meaningful.

Renal control

By regulating water and electrolyte losses and excretory waste products in the urine, the kidneys contribute to maintaining the interior environment constant in regard to volume and osmotic pressure - around 285 mosm/L plasma. The kidneys also assist in the control of the hydrogen ion activity by secreting urine of varying acidity, that is to say the concentration of bicarbonate in the plasma is regulated by: a) resorbtion of filtered bicarbonate (Fig.5a), and b) excretion of 50-80 mmol H^+ per day as titratable acid (Fig. 5b). This excretion can be increased in metabolic acidosis and the urine can reach a pH of 5.5 to 4.5. Of the approximately 60 mmol hydrogen ions which are excreted per day, most are bound to phosphate. The hydrogen ion secretion into the tubule urine gives rise to: The salts in the tubular urine - sodium hydrogen phosphate, some sodium sulphate as well as salts of organic acids - which are changed to their acidic form. The result of this proton secretion is called the urine´s titratable acidity. For a review see Alpern et al. (42).

The glomerulus ultrafiltrate has a pH of 7.25, a PCO_2 of 8 kPa (60 mm Hg) and a bicarbonate concentration of 24 mmol/L. In the proximal convoluted tubule (PCT) the

tubular fluid is acidified to approximately pH 6.7 and the bicarbonate concentration lowered to 8 mmol/L, a decrease of the bicarbonate content of the native ultrafiltrate corresponding to 75%. A further HCO_3^- resorbtion takes place in the proximal staight tubule where the proton secretory capacity, however, is far less than in the PCT and in the loop of Henle (a decrease of another 50% of the bicarbonate dilivered from the PCT) after which a final reduction of the bicarbonate concentration to virtually zero takes place in the distal nephrone.

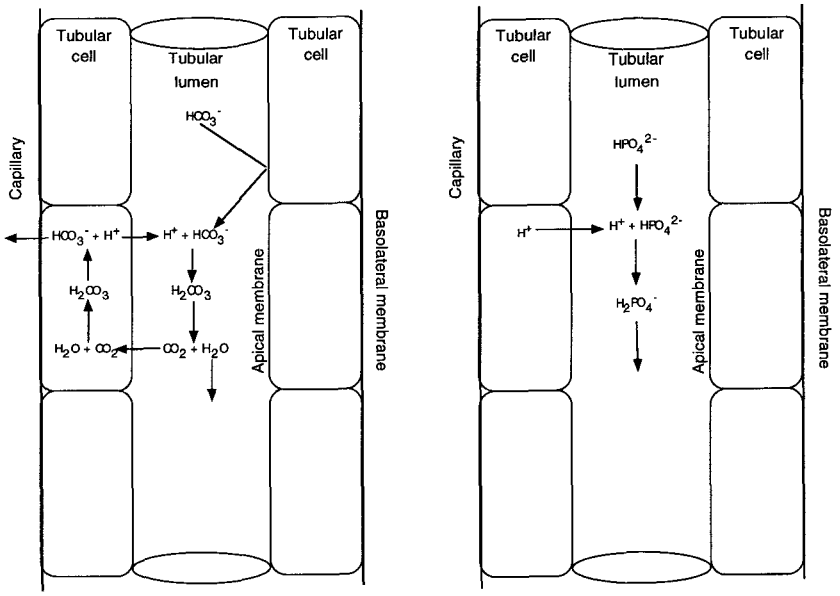

Fig. 5

Main renal mechanisms of urinary acidification

a. By bicarbonate absorbtion b. By phosphate excretion

While hydrogen ions passively enter (or HCO_3^- ions leave) the tubular cell across the basolateral membrane the extrusion of the hydrogen ions across the apical membrane occurs against the electrochemical gradient requiring an active transport (Fig. 5a). The net acidification of the tubular fluid occurs by secretion of protons or, in the case of previous administration of carbonic anhydrase inhibitors, by direct bicarbonate absorbtion across the apical membrane. Contributing to the acidification of the tubular fluid is normally the electrochemically neutral Na^+/H^+ antiporter, the electrogenic sodium-independent proton secretion driven by an H^+-ATPase and ATP, the sodium-dependent and independent electroneutral chloride-bicarbonate exchanger as well as the paracellular HCO_3^- diffusion. The apical membrane of the proximal tubule thus possesses both an H^+-ATPase as well as a Na^+/H^+ antiporter, the latter of which appearing to be the major apical membrane mechanism mediating HCO_3^-

absorbtion. The H^+-ATPase is a form of primary active hydrogen ion transport in that hydrogen ion transport is directly coupled to the metabolism of ATP while the Na^+/H^+ antiporter is secondarily active but not directly coupled to metabolism. Rather, the basolateral membrane Na^+-K^+-ATPase consumes ATP to lower the cell sodium concentration. The Na^+/H^+ antiporter then uses the low cell sodium concentration to drive extrusion of hydrogen ions from the cell to the tubular lumen against an electrochemical gradient for the hydrogen ions. The major transport mechanism for H^+/HCO_3^- on the basolateral membrane of the proximal tubule is the Na^+-$3HCO_3^-$ cotransporter and much evidence suggests that this mechanism mediates most of basolateral membrane HCO_3^- transport secondarily effecting transepithelial hydrogen ion secretion to the tubular lumen.

Regulation of renal urinary acidification

The acidifying process is of interest, also from a clinical point of view. It is mainly achieved by the influence of an increasing luminal pH which leads to stimulation of tubular acidification and, at higher luminal HCO_3^- concentrations also to an increasing paracellular leak of HCO_3^-. A maximal bicarbonate ion tubular absorbtion is, however, soon reached, the active proton secretion appearing to be independent of the luminal HCO_3^- concentration. Regulatory sites seem to be located intracellularly implying that increases in luminal HCO_3^- concentration and pH cause increases in cytoplasmic pH by increasing apical membrane hydrogen ion secretion and the increased intracellular pH then drives basolateral membrane base efflux at a faster rate.

Changes in extracellular volume also influence the rate of proximal tubule sodium as well as bicarbonate ion resorbtion. Thus, volume expansion seems to decrease both bicarbonate and sodium ion resorbtion partly due to the effect of extracellular volume on the Na^+/H^+ antiporter which seems to be influenced by low extracellular volume in much the same way as a diet low in sodium chloride. Especially chronic changes in dietary sodium chloride may regulate proximal tubule HCO_3^- absorbtion through adaptive changes in the proton and HCO_3^- transport.

Chronic potassium ion depletion leeds to a stimulation of proximal HCO_3^- resorbtion while acute changes of the potassium ion concentration in the peritubular capillaries have no effect. The mechanism for this alternation seems to be that potassium ion depletion elicits a tubule intracellular acidification and increased activities of the Na^+/H^+ antiporter and the Na-$3HCO_3^-$ cotransporter and thereby bicarbonate ion reabsorbtion.

Catecholamines and angiotensin II stimulate while parathyroid hormone inhibits proximal tubule sodium bicarbonate absorbtion. $Alpha_2$-agonists increase the

Na^+/H^+ antiporter activity and the corresponding antagonists block HCO_3^- absorbtion. The mechanism seems to be that parathyroid hormone induced activation of adenylyl cyclase seems to inhibit the Na^+/H^+ antiporter activity, in which this activation of adenylyl cyclase and inhibition of the antiporter activity are modulated by angiotensin II and the noradrenaline α_2-receptor.

Ammonium ion excretion

Many textbooks still state that part of the kidneys´ acid-base regulation occur via ammonium ion secretion. However, our understanding of the mechanism of excretion of ammonium ion has changed markedly during the last few years. The previous idea that first ammonia is formed and then it is protonized in the tubular lumen is now considered (13, 15, 30, 42, 43) to be incorrect. Ammonia is in fact never shaped at physiologic pH values, but the ammonium ion is formed in the kidneys or intestine (27, 44, 45) and excreted renally either after conversion to urea or in an unchanged form. Ammonium ions are thus first formed in the proximal convoluted tubule cells from glutamine under the influence of the enzyme glutaminase according to, among others, the two following formulas:

glutamine $\rightarrow NH_4^+$ + glutamate

glutamate $\rightarrow NH_4^+$ + α-ketoglutarate (2-oxoglutarate)

The latter enzymatic reaction is stimulated by glutamate dehydrogenase. The whole sequence of reactions that takes place is enhanced during acidosis, possibly by the decrease in intracellular pH which stimulates the α-ketoglutarate dehydrogenase. The end product of the glutamine/glutamate metabolism is CO_2 and HCO_3^- and water. The formation of the bicarbonate ion from glutamine is in fact regared to be the target of this metabolism during metabolic acidosis.

It now appears that the transport of ammonia/ammonium ions to the lumen of the proximal convoluted tubule occurs via two mechanisms (15). First, NH_3 can diffuse out of the cell across either the basolateral and apical membrane, but tends to exit across the apical membrane because of the lower luminal pH (non-ionic diffusion and trapping). This transport mechanism now seems to play only a minor role. Instead NH_4^+ is predominantly transported across the apical membrane via the Na^+/H^+ antiporter, most likely as a Na^+/NH_4^+ counter-transport where NH_4^+ is replacing H^+. Much of the ammonia that enters the loop of Henle is absorbed, largely as a result of active transport in the thick ascending limb by replacing potassium on the $Na^+/K^+/2Cl^-$ -cotransporter. In this way an accumulation of ammonia/ammonium ion in the renal medulla occurs by a countercurrent multiplication process. From the collecting ductal system an addition of NH_4^+ then occurs by active proton secretion and passive diffusion of NH_3 to the tubular lumen. The clinically important fact is

that the urinary concentration of ammonium ion has no single relation to the urinary pH.

A metabolic acidosis can be produced by administration of ammonium chloride to a person or animal. A negative glutamine balance arises in the entire body which depends first on the fact that there is a very large uptake of glutamine in the kidneys (see review by Welbourne, ref 45). This uptake is in fact 2-3 times greater than that of the intestine, which otherwise has the greatest glutamine uptake. The musculature loses glutamine at the same time and the liver produces some, but the total glutamine release is less than the amount consumed by the kidneys. The muscle simultaneously increases its uptake of glutamate and ammonium ion while the intestine releases large amounts of ammonium ion and lesser amounts of glutamate to the liver. The sum of this process is that the kidneys generate bicarbonate from glutamine at the same time as the intestine consumes less glutamine than normal and at the same time as urinary excretion of ammonium ion increases greatly. Ammonium ion secretion in acidosis and normal kidney function can increase to 250 (maximum 400) mmol per day. The ammonium ion is water-soluble and therefore is transported slowly through the membranes of the tubular cells, and is therefore reabsorbed only to a limited degree. In addition, since pH in the urine is lower than in the peritubular blood, transport from the tubular cell out into the tubular lumen is favoured. When the pH in the urine falls, the amount of ammonium ions in the urine increases. It has previously been thought that this secretion of ammonium ions was a way of discharging protons from the body. To all appearances this is wrong, since ammonium ions in the experiment described are conveyed in the primary urine, and since normally this ion and not ammonia is formed in the metabolism of mammals, the latter compound can thus not have been protonized in the kidneys. The amount of free hydrogen ions in urine with pH 4.5 - 5 is in fact low, even in a metabolic acidosis, and secretion of protons with primary phosphate is limited (usually to around 1/30 mol = 30-35 mmol per day) by the supply of phosphate (around 1 g per day). In such a situation the organism seems to strive to maintain equilibrium in phosphate turnover. One can say that $H_2PO_4^-$ is voided in the urine as a sodium- or ammonium complex.

Significant secretion of base only occurs with the bicarbonate ion. Thus, in the event of an systemic HCO_3^- excess HCO_3^- secretion takes place over the apical membrane the energy of which is provided by an H^+ ATPase in the basolateral membrane which actively extrudes the hydrogen ion after which the bicarbonate ion exits across the apical membrane via the electroneutral Cl^-/HCO_3^- exchanger. This secretion of HCO_3^- occurs only together with Na^+ and K^+, not with NH_4^+ and H^+. Secretion of bicarbonate ion can therefore be insufficient when there is a lack of sodium and

potassium ions in the organism, and then a so-called paradoxical aciduria arises, that is the urine has a low pH despite alkalosis in the blood.

Dysfunction in the system - does it exist?

One piece of evidence traditionally required before accepting a new physiological or biochemical principle whose function is claimed necessary for life is that there should be a dysfunction in the proposed system and this should in case of malfunction lead to serious illness or death. Up till now dysfunction has not been recognized in the acid-base system proposed by Atkinson (13). I should like, however, to offer the hypothesis that dysfunction in this system could cause the part of the Sudden Infant Death Syndrome (SIDS) that can be explained by a respiratory insufficiency (46).

To begin with I think it can be stated that the syndrome strikes apparently previously healthy infants at around 3-5 months of age, and these are most often found dead in their sleep by their parents (47). By definition there are hardly any cases of SIDS (sometimes also called cot death) reported where the death occurred suddenly in an infant who was believed to be awake; on the contrary the pattern seems to be that the infant goes to sleep and seems to sleep peacefully after, according to the parents, having been normally awake. As a rule there have not been any signs of hypoxia (cyanosis) recorded before death. In some cases, however, it has been claimed that signs of chronic hypoxia have been found upon autopsy (46, 47, 48). Furthermore in a recent report, Meny et al (49) have published monitoring records of 6 infants dying suddenly and unexpectedly at home which seem to show that immediately before the occurrence of death bradycardia and possibly obstructive apnoea were more prominent features than central apnoea. It has also lately been recommended that parents of infants under 6 months of age should allow the infant to sleep on its back, since it has been shown that this recommendation has led to a lower incidence of SIDS. Ponsonby et al (50) have published results indicating that lying on the back in bed seems to be the position in which the infant tolerates a high environmental temperature best.

The most important argument for my hypothesis, however, is that, according to Atkinson & Camien (13), deficient protonisation of endogenously produced HCO_3^- could lead to a progressive alkalinisation, which according to what I fear results in a compensatory hypoventilation (Part 1 of the hypothesis). A rise of pH through an increasing HCO_3^- concentration is thus counteracted according to Henderson-Hasselbalch´s equation by an increase in PCO_2, which is an effect of hypoventilation, and likewise a hypoxia-produced lactacidosis. The process finally leads to a more and more pronounced metabolic alkalosis since it can be compensated only in part by a hypercapnia. The tissue hypoxia already discussed arises despite a relatively high

arterial oxygen saturation because tissue oxygen uptake becomes more and more problematic. This is because the already left-shifted oxygen dissociation curve of the possible remaining fetal hemoglobin is further displaced to the left by the progressive alkalosis. Added to this effect is the influence of a possibly elevated body temperature on the PCO_2, which also increases and contributes to the progressive respiratory depression. Thus, hypothetically the end result is an increasing and finally massive metabolic alkalosis which leads to a respiratory depression including systemic hypercapnea and tissue hypoxia despite a relatively good arterial oxygen saturation and thereby the absence of cyanosis. Due to the progressive acid-base disturbance and hypoxia, the infant´s breathing center cannot function normally, and during sleep (51, 52), not seldom after a period of wakefulness with relatively vigorous sensory stimulation possibly leading to a relative hyperventilation, an additional compensatory hypoventilation arises acutely, resulting in an even more serious hypoxia, as a result of the fact that the hypoxic respiratory drive does not function in so small an infant, contributing to the "failure to arouse" which has been described in experimental animals (53). The final result is a bradycardia with secondary respiratory arrest (48, 49, 51) which leads to asystole and death which at the postmortem is given the diagnosis SIDS.

Hypothetically an insufficient protonisation ($HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$) of the striken infant´s endogenously (or bacteriologically) formed bicarbonate ion could be an effect of the infant´s uncompensated shortage of ammonium ions in the liver, which in turn could be due to the fact that the large supply of urea (54, 55) in the breast milk (often twice as much as in the mother´s plasma) is not metabolized, or that oral ammonium ion was not supplied (Part 2 of the hypothesis). The breakdown of urea normally takes place in the intestine (28, 29, 56, 57, 58, 59, 60) by means of urease-producing bacteria (61, 62) which colonize the newborn infant´s intestine, which initially after birth is sterile. This colonization is considered to take place during the first days of life, but according to my hypothesis it is delayed, inhibited or in some other way insufficient in those infants who develop respiratory insufficiency leading to sudden infant death. It has been demonstrated that not all strains of the bifidus bacteria have the ability to metabolise urea (57, 62). Of particular interest is also the fact that as much as 80% of the oral intake of urea is metabolised in the infant's intestine while the corresponding figure is approximately 20% for the adult human (28, 31). On the other hand the bioavailability of orally administered urea seems to be only approximately 20-30% (63, 64). It increases, however, when the infant is receiving a diet deficient in proteins, during recovery from illness, during malnutrition, uraemia or after birth with a low birthweight (65).

It is well known that normal human breast milk contains large amounts of urea (54, 55) comprising some 25% of the total nitrogen content of human breast milk (66, 67).

It is also known that the content of non-protein bound nitrogen in breast milk is larger than can be explained by its content of urea (54). It has also been demonstrated that the urea in breast milk can in part (approximately 20%) be utilized as a substrate (63, 64, 65) for the synthesis of alpha-amino nitrogen compounds (26), i.e. amino acids and proteins. Häussinger and co-workers (26) have demonstrated experimentally that ammonium ions from the portal area, can be used for glutamine synthesis in the liver, particularly during metabolic acidosis. It is well known to intensive care physicians that the intravenous administration of ammonium chloride lowers the base excess value and it is, hence, consequently used in the treatment of metabolic acidosis. When the patient being treated is spontaneously breathing, the administration of ammonium chloride causes a so-called compensatory hyperventilation. I have administered 80 mmol ammonium chloride orally to myself and noted that this substance is obviously rapidly absorbed from the gastrointestinal tract and causes a slight hyperventilation which in turn causes the respiratory quotient=RQ to increase from a resting value of 0.82 to 0.87 and carbon dioxide elimination to increase by about 30 ml/min, and that this appears to continue for nearly an hour (= 1.8L CO_2= 80 mmol). I have been unable to find any information in the literature about the ammonium ion concentration in infants immediately after a SIDS death. It must also be stated that if ammonia/ammonium ion were to be measured, high values are recorded, most likely as a result of postmortal changes (unpublished observations). There is, on the other hand, information about urea concentration in the vitreous body in this patient group which is compared with such values from autopsies on infants of the same age. It is seen that infants who die from SIDS have lower urea concentrations than infants who have died due to other causes (61). Since a normal enterohepatic circulation of urea and ammonium ion (31, 32, 56, 57, 63, 64, 65) hypothetically does not function in SIDS cases, the consequence would be that urea production in this group of small children is less than normal, and since the distribution volume is considered as large as in the control group, this means that urea concentration in the body fluids decreases. Consequently, the low urea concentrations found in infants considered to be victims of SIDS are not incompatible with my hypothesis.

Signs of chronic hypoxia have been found in the autopsies of some SIDS victims (46, 47, 48). However, there is only a single report of low oxygen saturation in infants (45) who later died of SIDS and none has reported signs of hypoxia in the form of cyanosis prior to death. This finding is consistent with the circumstance that a left-shifted oxygen dissociation curve, which is an effect of an alkalosis, more easily results in a fully saturated hemoglobin, but that this change also makes the release of oxygen from oxyhemoglobin in the periphery more difficult, and in extreme circumstances even could lead to tissue hypoxia. The accelerating metabolic alkalosis is probably initially counteracted by a lactic acidosis, but it can be expected that this compensation

mechanism will prove to be insufficient. It must be observed that the endogenous bicarbonate production of the body could be so large that only one or more days´ suspension of protonisation could result in a life-threatening alkalosis (13).

At this stage it is difficult to prove the theory. The above-cited finding that infants who died from sudden infant death syndrome have low concentrations of urea in the vitreous body of the eye indicates, however, that this group of infants form smaller amounts of urea in the liver compared with other infants. This in turn could indicate that either the intestine´s bacterial formation of ammonium ions from urea is defective and/or that the SIDS victims´ metabolism (or enteric bacteriological metabolism) produces more (or eliminates less) bicarbonate ion than that of other infants. The fact that colostrum contains significantly less urea than breast milk some weeks later (54) indicates, however, that there is an adaptation between the newborn infant´s metabolism and the composition of breast milk. Since the amount of breast milk consumed increases gradually, the daily intake of urea also increases. Nothing thus far seems to be published either about ammonium ion intake or the amount of ammonium ion produced in the intestine and how this varies with the infant´s age. It is known, however, that the daily urine excretion of ammonium ion markedly decreases during the course of the first 6 weeks of life, at the same time as the urine excretion of urea increases (69, 70, 71). At the age of 6 weeks the normal infant excretes 0.5-1 mmol ammonia/ammonium ions daily in the urine (69), an amount of ammonium ion which can be regarded as a safety margin which, if needed, at least partly can be used for neutralising excess bicarbonate ions. A somewhat increasing pH and base excess in the normal infant´s capillary blood have been noted during the first 6 weeks of life (70). Thus, it seems probable that breast milk in the newly delivered mother also contains ammonia/ammonium ion (66). In that case, this constitutes a certain protection against a progressive alkalosis. A pilot study of mine comprising three subjects (unpublished observations) showed that early (2-4 weeks) breast milk contains 400-500 µmol/L ammonium ions and 5-6 mmol/L urea, and that the ammonium ion concentration gradually decreased during the first 6 months of the infant's life. An expanded study of the composition of breast milk with special attention to the physical effects of breast milk´s content of non-protein bound nitrogen compounds ought to be undertaken immediately.

Purely quantitative considerations, however, can already be made. First of all, it is clear that breast milk´s content of ammonia/ammonium ion is not as great as to protect the infant from lethal acid-base effects. If, on the other hand, it is assumed that the urea content in breast milk is not split by bacterial urease, and in addition the kidneys´ elimination of bicarbonate ions is relatively small, the result is that a continuous alkalisation will take place and that base excess in this case can increase by slightly more than 4 mmol/L per day. The kidneys are probably able to handle this

demand for bicarbonate ion elimination during a relatively long period of time. The renal bicarbonate ion excretion, however, is highly dependent on a normal volume of urine, and it can probably be assumed to decrease since bicarbonate and urea secretion initially result in an exaggerated diuresis leading to dehydration and/or a shortage of sodium and, as a result of this, a so-called paradoxical aciduria. In this case the result is an accelerating alkalinisation, and within some days it could constitute a serious risk of a metabolic alkalosis which has respiratory effects. Results published by Walser & Bodenlos (28), Summerskill & Wolpert (29) in adults, and Wheeler et al (31, 32) in infants describe a significant enterohepatic circulation of urea-ammonium ions and provide reason to suppose that the absence of bacteria in the intestine, which degrade urea, results in an enhanced alkalinisation exceeding that which was calculated above as the enterohepatic circulation of ammonium ions. In this context urea seems to work as a biological amplifier as regards the digestive system's ability of conveying the indirect proton donator to the urea cycle.

Since it can be shown that colostrum contains ammonium ions, and if and when, in addition, it can be shown that in SIDS the intestine of dead infants does not contain ammonia/ammonium ion producing bacteria, the consequence will be that studies must immediately be undertaken to add physiological amounts of ammonium ion to infant formula during their first 6 months of life. Normal infant pabulum should also contain physiological amounts of urea and an appropriate dose of a ammonium compound. Furthermore, the proposed hypothesis should, if proved to be true, make it possible to routinely check whether the fecal contents of infants exert urease activity, and for those who lack this metabolic function, ammonium compounds could be administered orally, or these infants could be inoculated with an appropriate bacterial strain producing ammonium ions from urea. Another possibility might be to administer appropriatly modified, non-pathogenic ammonium-producing bacteria to all newborn infants in order to bring about the prevention of SIDS.

SUMMARY

Systemic metabolism results in a production of not only carbon dioxide, water and urea but also bicarbonate ions.

Most of these bicarbonate ions are generated during the catabolism of glutamine.

In order to be eliminated as carbon dioxide in the lungs bicarbonate ions must be protonised.

This protonisation of the bicarbonate ion seems to take place in a number of tissue compartments in which acid-base balance is maintained.

One of the most important processes for protonisation of the bicarbonate ion is the hepatic ureagenesis from ammonia/ammonium ions.

A substantial part of the ammonia/ammonium ions are generated during the catabolism of amino acids. Terminal oxidation of glutamine in the gut seems to be of great significance for this process.
In certain conditions the enteric generation of ammonium ions seems so important that an ATP-driven enterohepatic recirculation of ammonium ions/urea constituting an amplifying mechanism for the protonisation of the bicarbonate ion is motivated.

ACKNOWLEDGEMENTS
The author is indebted to Professors M. H:son Holmdahl, L. Paalzow, H. Ulfendahl, and K.J. Öbrink and Dr Sören Englesson in Uppsala and Dr Aage Laerdal in Stavanger for help to locate some of the references of this review.

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