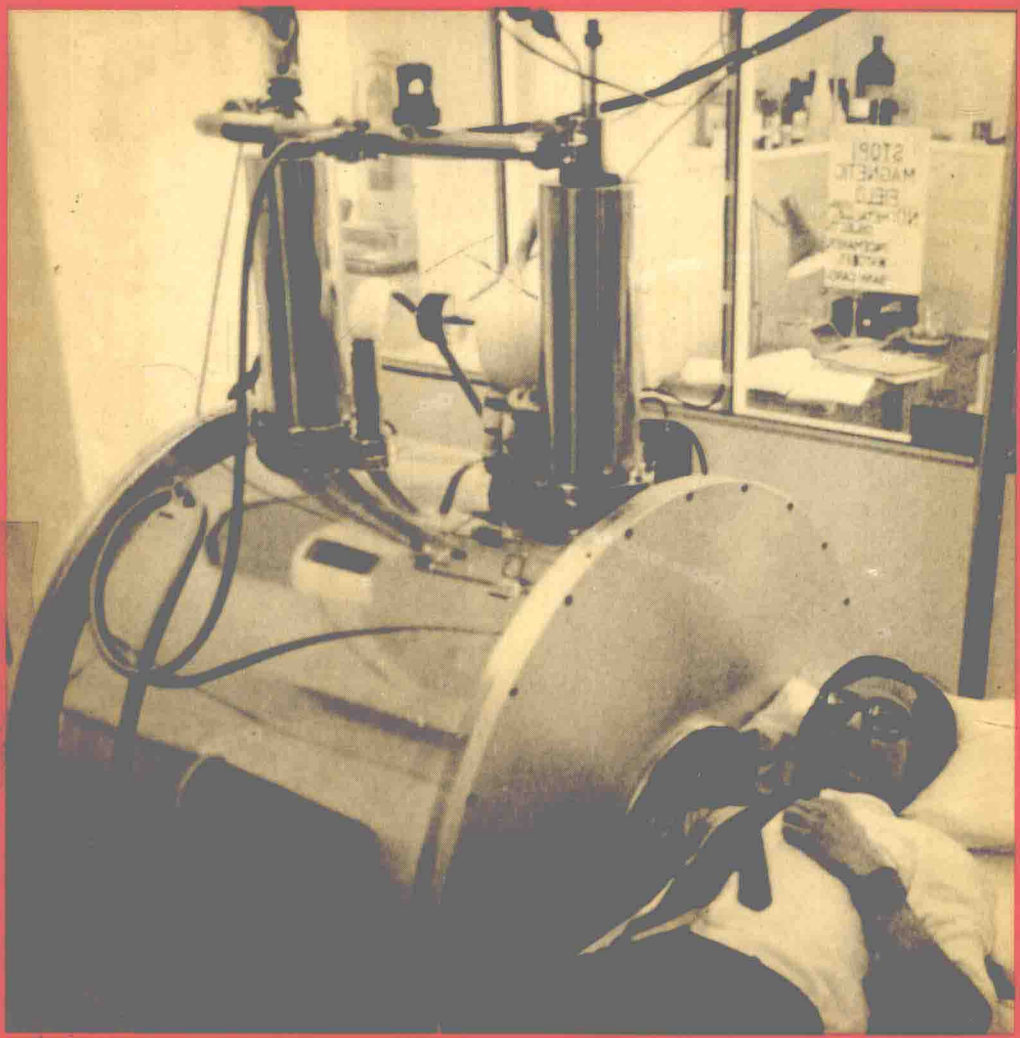
Ciba Foundation Symposium 87

Metabolic acidosis



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Symposium on Metabolic Acidosis, held at the Ciba Foundation, London, 12–14 May 1981.
Editors: Ruth Porter (Organizer) and Geralyn Lawrenson

The hydrogen ion in normal metabolism: a review

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Abstract. The production of hydrogen ions (H^+) by metabolic processes is described, with particular emphasis on glycolysis and ketogenesis. Total metabolic production of H^+ is approximately 150 g day^{-1} but utilization closely balances production, so that intracellular and extracellular H^+ production is maintained within narrow limits. H^+ is generated at several sites in glycolysis but no net H^+ production occurs unless the ATP formed is hydrolysed. The other main source of metabolic H^+ production is ketogenesis. Here H^+ accumulation depends on both the relative dominance of ketone body production over utilization and the loss of base in urine. The H^+ is produced during the synthesis of 3-hydroxy-3-methylglutaryl-CoA and not because of dissociation of acetoacetic acid. Lipolysis and re-esterification of fats are additional major producers of H^+, while net H^+ production also occurs with pathological accumulation and incomplete combustion of other organic acids. Many metabolic systems are sensitive to the changes in pH. These effects have been examined *in vivo* using an ammonium chloride acidaemia model in the rat. Severe insulin resistance and impaired glucose metabolism in liver and muscle were found. One mechanism involved inhibition, by H^+, of the binding of insulin to its receptors. Further mechanisms include inhibition of key glycolytic enzymes including phosphofructokinase. It is concluded that too little attention is paid to metabolic production of hydrogen ions and to their effects, in turn, on metabolism.

Although the total concentration of hydrogen ions (H^+) in the body is low, H^+ turnover, which amounts to more than 150 mol day^{-1}, exceeds that of any other single metabolite. This turnover is associated with remarkably small changes in H^+ concentration in the body, because of exquisitely controlled homeostatic mechanisms. Concentrations of H^+ are generally higher intracellularly than extracellularly and in certain cases extremely high concentrations may be found, e.g. in gastric cells. Within cells, concentrations of H^+ also show considerable variation, as transport is not simply electrochemical or

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by diffusion. Elaborate mechanisms exist to maintain stable concentrations of H^+. Thus, buffer systems are found in all cells and body compartments. Transport mechanisms are particularly important in mitochondria where organic acids, such as pyruvic, citric and glutamic acids, are actively taken up in the non-ionized form but dissociate rapidly within the mitochondrion. The resultant H^+ has to be eliminated. This occurs through a variety of mechanisms such as (indirectly) CO_2 formation, or via the malate shuttle or phosphate extrusion. Similar mechanisms occur in specialized tissues such as lung and kidney where H^+ can be eliminated free, in buffered form, or as CO_2.

The first part of this review will describe the main biochemical pathways that produce H^+, with particular emphasis on those which are important in pathological states. In the second section the metabolic effects of pathological accumulation of H^+ will be described, with particular emphasis on changes in muscle and liver metabolism in experimentally induced acidaemia in the rat.

Metabolic production of hydrogen ions

In the normal adult human, H^+ turnover amounts to 150 mol day^{-1}. Net production amounts to 15 mol in the form of CO_2, which is removed by the lungs, and 50 mmol as non-volatile acids (H_2SO_4, H_3PO_4 and organic acids). H^+ from the latter is excreted by the kidney, buffered by phosphate (P_i) or ammonia. There is also a dietary input of organic and inorganic acid that is removed by similar routes.

Most metabolic production of H^+ occurs through hydrolysis of ATP (see Krebs et al 1975). Thus:

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+ \qquad (1)$$

Approximately 30% of the H^+ produced is buffered by HPO_4^{2-}, to form $H_2PO_4^-$. The respiratory chain is also a major source of H^+, formed when Fe^{3+} reacts with hydrogen donors. Thus:

$$2Fe^{3+} + 2H \longrightarrow 2Fe^{2+} + 2H^+ \qquad (2)$$

H^+ is also formed each time the nicotinamide nucleotides are reduced. Thus:

$$NAD^+(NADP^+) + 2H.R \longrightarrow NADH(NADPH) + H^+ + R \qquad (3)$$

It can be estimated from these reactions that 10 H^+ is formed per molecule of O_2 used. There is, however, no net gain of H^+ in normal circumstances because the H^+ will be removed again when NADH, NADPH and the reduced cytochromes are reoxidized (e.g. $NADH + \frac{1}{2}O_2 + H^+ \longrightarrow NAD^+ + H_2O$) and also when ATP is resynthesized (see below).

The other major metabolic source of H^+ is CO_2 production. Thus:

$$CO_2 + H_2O \longrightarrow H_2CO_3 \longrightarrow H^+ + HCO_3^- \quad (4)$$

This is responsible for 10% of metabolic H^+ production. Local concentrations of CO_2 could become extremely high in poorly perfused cells and lead to extreme changes in pH. In general, however, CO_2 diffuses out of cells extremely rapidly and, apart from in the red cell, kidney and lung, i.e. the sites of carbonic anhydrase (carbonate dehydratase, EC 4.2.1.1) activity, this diffusion will not result in very large shifts in H^+.

The above reactions can become important as net generators of hydrogen ions if they become irreversible. This can occur, for example, in anaerobic states, as discussed below, or in respiratory disease. The other potentially important metabolic source of H^+ is the incomplete oxidation of fuels. An example of this is if ketone bodies accumulate during the combustion of fatty acids. We shall discuss glycolysis and ketoacidosis in detail as examples of these processes but it should be remembered that accumulation of any organic acid, as in the organic acidaemias (see Wadman et al 1982, Leonard 1982), will cause similar problems.

Glycolysis

Several reactions in glycolysis generate H^+. Thus:

$$\text{glucose} + ATP^{4-} \longrightarrow ADP^{3-} + \text{glucose-6-phosphate} + H^+ \quad (5)$$

$$\text{fructose-6-phosphate} + ATP^{4-} \longrightarrow ADP^{3-} + \text{fructose-1,6-diphosphate} + H^+ \quad (6)$$

$$\text{glyceraldehyde 3-phosphate} + NAD^+ + HPO_4^{2-} \longrightarrow \text{1,3-diphosphoglycerate} + NADH + H^+ \quad (7)$$

However, several reactions also utilize H^+. Thus:

$$\text{1,3-diphosphoglycerate} + ADP^{3-} + H^+ \longrightarrow \text{3-phosphoglycerate} + ATP^{4-} + HPO_4^{2-} \quad (8)$$

$$\text{phosphoenolpyruvate} + ADP^{3-} + H^+ \longrightarrow \text{pyruvate} + ATP^{4-} + HPO_4^{2-} \quad (9)$$

$$\text{pyruvate} + NADH + H^+ \longrightarrow \text{lactate} + NAD^+ \quad (10)$$

Contrary to general belief, and as emphasized by Zilva (1978) and Krebs et al (1975), the net result of glycolysis is not H^+ production. The major overall equation is:

$$C_6H_{12}O_6 + 2ADP^{3-} + 2HPO_4^{2-} \longrightarrow 2CH_3.CHOH.COO^- + 2ATP^{4-} + 2H_2O \quad (11)$$

At physiological pH, approximately 30% of the phosphate will be $H_2PO_4^-$ and this will yield H^+. In the main, however, lactate, and not lactic acid, is the end-product. This must be set against the observation that 'lactic acidosis' is generally accompanied by significant H^+ production. However, lactate accumulation usually occurs in anaerobiosis when the lactate dehydrogenase (EC 1.1.1.27) step serves to regenerate NAD^+ so that glycolysis can continue. In this state ATP will be hydrolysed rapidly and this generates H^+ as outlined above. Thus, if one combines reactions (1) and (11):

$$C_6H_{12}O_6 \longrightarrow 2CH_3.CHOH.COO^- + 2H^+ \qquad (12)$$

Therefore glycolysis *per se* is not accompanied by H^+ production, but ATP hydrolysis must always occur. Under normal aerobic conditions there will be both continuous rapid hydrolysis and resynthesis of ATP and further metabolism of lactate, so there will be little net production of H^+.

These equations are an oversimplification of the true *in vivo* state (see Gevers 1977). ATP is primarily present as $MgATP^{2-}$ which has a pK of 5.4 for proton uptake. Similarly, at pH 7.2, ADP will be present as equal amounts of ADP^{3-} and ADP^{2-}. If the ATP is complexed with Mg^{2+} and the ADP is free then:

$$\begin{aligned} \text{glucose} + ADP^{3-} + ADP^{2-} + 2P_i^{2-} + 2Mg^{2+} \longrightarrow \\ 2 \text{ lactate}^- + 2MgATP^{2-} + H^+ \end{aligned} \qquad (13)$$

so, potentially, anaerobic glycolysis *could* generate a proton and, if the pH were to fall, a theoretical yield of 2 H^+ could result. The key to this possibility is the free intracellular Mg^{2+} concentration. It is probably about 1 mmol l^{-1} (Veloso et al 1973), at which concentration most of the ADP will also be complexed. Hence:

$$\text{glucose} + 2MgADP^- + 2P_i^{2-} \longrightarrow 2 \text{ lactate}^- + 2MgATP^{2-} \qquad (14)$$

and, again, no net gain in H^+ will result.

On similar grounds the hydrolysis of ATP is not necessarily accompanied by a stoichiometric release of H^+. Thus, Wilkie (1979) suggests that the true equation at pH 7.2 is:

$$\begin{aligned} 0.36ATP^{3-} + 0.64ATP^{4-} + H_2O \longrightarrow \\ 0.33ADP^{2-} + 0.68ADP^{3-} + 0.72P_i^{2-} + 0.28P_i^- + 0.76H^+ \end{aligned} \qquad (15)$$

This was presented alternatively by Gevers (1979) as:

$$MgATP^{2-} + H_2O \longrightarrow MgADP^- + 0.72P_i^{2-} + 0.28P_i^- + 0.72H^+ \qquad (16)$$

The argument extends beyond glycolysis into oxidative phosphorylation. It is assumed that mitochondrial ATP synthesis consumes protons. There is, however, no hard evidence in favour of this view and Brand & Lehninger

(1977) have concluded that there is no net production or utilization of H^+ during oxidative phosphorylation *in vivo*. However, if ATP is being hydrolysed to yield H^+ then an equivalent amount is probably being formed. In the anaerobic state net hydrolysis will occur in the cytoplasm to yield H^+. This will diffuse rapidly out of the cell and, at least in muscle, probably diffuses out even more rapidly than lactate (Benade & Heisler 1978). The yield of H^+ cannot exceed the contents of total ATP and, similarly, of NADH, which are relatively small (1 mmol kg^{-1}), but this still amounts to considerable H^+ production.

The further metabolism of lactate is also more complex than it first appears. In gluconeogenesis from lactate, for example, there is net production of H^+, and not consumption as generally assumed. Thus:

$$2\ \text{lactate}^- + 4\text{ATP}^{4-} + 2\text{GTP}^{4-} \longrightarrow \text{glucose} + 4\text{ADP}^{3-} + 2\text{GDP}^{3-} + 6\text{HPO}_4^{2-} + 4\text{H}^+ \quad (17)$$

However, regeneration of ATP and GTP will use up approximately $6\,H^+$ so that there is a *net* loss of about $2H^+$, the overall reaction being:

$$2\ \text{lactate}^- + 2\text{H}^+ \longrightarrow \text{glucose} \quad (18)$$

It is important to view these reactions in a morphological context. In exercise lactate will be generated by muscle, and ATP levels within muscle will fall; thus, there will be net production of H^+ by muscle. The lactate will pass into the bloodstream and will eventually be cleared by the liver (and to a lesser extent by the kidney) with net utilization of H^+. Therefore H^+ must pass from muscle to liver to maintain or to restore normal pH in the two tissues. Sestoft et al (1981) have suggested that the normal metabolism of lactate is balanced by hepatic production of other carboxylic acids, e.g. ketone bodies. H^+ from ketogenesis in the liver (see below) will therefore balance H^+ consumption for lactate metabolism, and the converse will occur in peripheral tissues. If lactate is not transported to the liver, as in circulatory collapse, for example, irreversible acidaemia may result. If liver pH falls below 7 the liver starts to produce rather than consume lactate (Lloyd et al 1973), and a vicious circle can ensue.

Substrate cycles

Some substrate cycles are also capable of generating H^+. The best examples are lipolysis and re-esterification in adipose tissue, and glucose storage as glycogen, with later production of glucose again, in liver.

Glycogen does not require ATP for conversion to glucose-6-phosphate.

$$\text{glycogen} + \text{P}_\text{i}^{2-} \longrightarrow \text{glucose-6-phosphate}^{2-} \quad (19)$$

Thus, in the conversion of glucosyl residues to glucose, no protons are involved:

$$\text{glucose-6-phosphate} + H_2O \longrightarrow \text{glucose} + P_i \quad (20)$$

If glycogen synthesis from glucose is now added, reaction 5 will yield one proton, as will the ensuing step involving UTP utilization.

$$\text{glucose-6-phosphate} \longrightarrow \text{glucose-1-phosphate} \quad (21)$$

$$\text{glucose-1-phosphate} + MgUTP^{2-} \longrightarrow \text{glucosyl residue} + MgUDP^{1-} + 2P_i^{-} + H^+ \quad (22)$$

Overall, therefore, cycling of glucose residues to glycogen through glucose-6-phosphate and back (i.e. temporary storage of glucose as glycogen) will yield two protons.

Similar arguments apply to triglyceride synthesis and hydrolysis in adipose tissue. In synthesis the main proton-generating step is production of the CoA derivatives. Thus:

$$\text{Palmitate}^- + MgATP^{2-} + CoA^{4-} \longrightarrow \text{palmitoyl-CoA}^{4-} + AMP^{2-} + 2P_i^{2-} + Mg^{2+} + H^+ \quad (23)$$

In adipose tissue, glycerol phosphate will be produced from glucose:

$$\text{glucose} + 2MgATP^{2-} + 2NADH \longrightarrow 2 \text{ glycerol 3-phosphate}^{2-} + 2MgADP^{-} + 2NAD^+ \quad (24)$$

The glycerol phosphate will combine with three fatty acyl-CoA molecules to form triglyceride. Overall, the reaction will be:

$$\text{glucose} + 6 \text{ palmitate}^- + 8MgATP^{2-} + 2NADH \longrightarrow 2 \text{ triglyceride} + 14P_i^{2-} + 2NAD^+ + 2MgADP^{-} + 6AMP^{2-} + 6H^+ \quad (25)$$

Thus, in adipose tissue H^+ is generated when triglyceride is laid down. In liver this is further exacerbated by the production of an additional H^+ through the glycerokinase (EC 2.7.1.30) reaction. Lipolysis also yields H^+:

$$\text{triglyceride} \longrightarrow 3 \text{ palmitate}^- + 3H^+ + \text{glycerol} \quad (26)$$

so that, overall, adipose tissue has considerable potential for H^+ production. H^+ will be consumed elsewhere when palmitate is metabolized but, inevitably, the adipocyte will tend to lower its own internal pH. Some of the excess H^+ produced represents ATP hydrolysis which will probably be counteracted by regeneration of ATP.