**Acid-base physiology as CellML modules**

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The following text is designed to go into the tutorial on CellML, OpenCOR and PMR. It may also form the basis of a review paper.

**Building multiscale models**

Much progress has been made over the last 15 years on establishing model encoding standards (CellML, SBML, FieldML) together with model repositories (PMR, Biomodels) and simulation tools (OpenCOR, etc) based on those standards (refs). CellML also allows a modular approach to model building via imports of separately defined component modules, facilitated by annotation of model variables and parameters so that imported components can be inserted automatically into the composite model (ref). In this section we show how composite multiscale models can be constructed from CellML and FieldML models. We first deal with lumped parameter models based on ODEs and algebraic equations that can be encoded in CellML. The CellML models can be extended to include spatially distributed FieldML models where these are needed.

First, some definitions:

***Compartments*** are well mixed regions of cells, tissues or organs that represent either specific locations or more generic locations that can exist in multiple parts of the body. ***Variables*** are quantities that have units and fixed or variable numeric values and can be associated with compartments. ***Parameters*** are variables with fixed values. ***Models*** contain one or more equations in these variables and can stand alone or be associated with one or more compartments. Note that CellML models require compartments, variables and equations. Until filled with variables and equations, compartments are just empty containers.

Note that the term ‘well-mixed’ signifies that to an acceptable approximation in the model, the variables within a compartment are distributed homogeneously throughout that compartment. Of course no biological region is perfectly homogeneous and, if necessary, a well-mixed compartment can be replaced by more than one compartment or by a spatially distributed model where concentration gradients are governed by diffusion equations. However, it is often useful to start with a simple lumped parameter compartment model in order to understand basic mechanisms.

*Modularity* is an important aspect of multiscale modelling and we create composite models by importing other more basic models, as we will demonstrate. *Model reduction* can also be used to simplify a model for computational efficiency. But *model expansion* is equally important – there may be situations where more biophysical detail is called for under certain conditions, or spatial fields need to be considered as part of a model.

*Reactions* are the binding and unbinding of chemical species (almost always catalysed by an enzyme) and are always reversible under the appropriate conditions. When models include reactions, it is usually with equations that express mass conservation of chemical species and use either first order mass action kinetics or a steady state version in the form of an equilibrium equation. Note that it is often useful in diagrams to show the transfer of mass, energy or information across compartment boundaries and we use arrows to indicate the movement of variables across compartment boundaries, sometimes with the *flux* (time-varying flow of a variable) written beside the arrow. The direction of these arrows is just for explanatory reasons to show normal directions of movement – the fluxes could be in the reverse direction if conditions allowed it.

The formulation of a model and its encoding in CellML and/or FieldML is a completely separate exercise from the numerical solution of that model. Once a model is formulated it can be solved by any solver that can handle the syntax of the model encoding languages. The verification of the solver is an important but separate issue.

We demonstrate these concepts and how they are supported by CellML and FieldML with the following example of acid-base physiology and the control of pH. Note that some of these models use molecular components while others are treated in a more empirical ‘black-box’ fashion – possibly to be replaced at a later stage with a more mechanistic model when the physiological data is available to justify this or the questions being asked of the model require this level of detail.

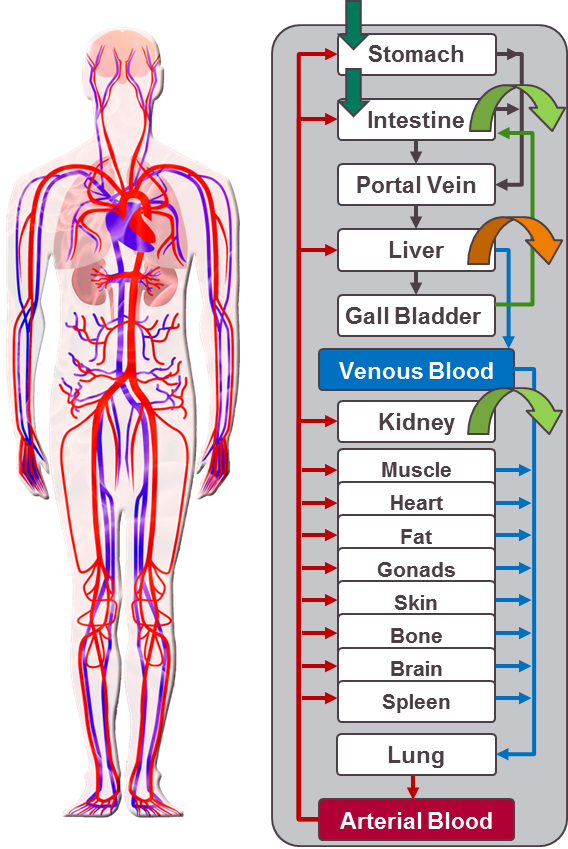
**Acid-base physiology**

Oxidative metabolism in tissue provides the bulk of the body’s energy currency (ATP) by oxidising carbohydrates and fats to carbon dioxide and water. To get rid of this metabolic waste product, is transferred from the tissues to the lungs via the blood, primarily in the form of bicarbonate () and carried primarily by red blood cells (RBCs). The formation of from via the hydration reaction is catalysed by carbonic anhydrase (CA):

.

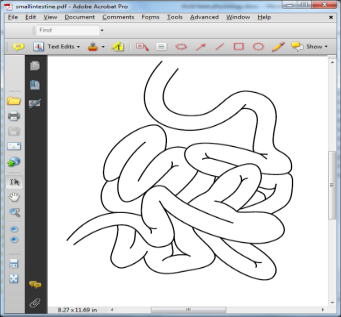
This generates large quantities of in the blood. Buffering this – i.e. controlling pH within the narrow range compatible with life[[1]](#footnote-1) – requires multiple weak acids and bases to be present in blood [1]. Understanding acid-base physiology therefore requires models of several organs: the muscles (as the major source of metabolism and hence metabolic waste products), the lungs (to exhale ) with the respiratory centre in the mid-brain to control respiration rate, the stomach where acid is excreted to maintain a low pH environment hostile to bacteria, the pancreas (where is excreted to neutralise stomach acid passed in the chime to the small intestines), the kidneys (to recover filtered , generate new and excrete filtered buffers) and the arterial, capillary and venous components of the circulation system (each as homogeneous systems containing blood plasma and RBCs). These six organs or organ systems are illustrated, with their inputs and outputs, in Figure 1.

We follow the physiological approach described in the Boron and Boulpaep textbook on *Medical Physiology* [1], the models described in Occhipinti and Boron [2] together with earlier publications from the Boron group in Cleveland [3..8], and models from the Vaughan-Jones and Swietach group in Oxford [9..13].



1

**Kidneys**



**Pancreas**

5

**Blood plasma &  
Red Blood Cells**

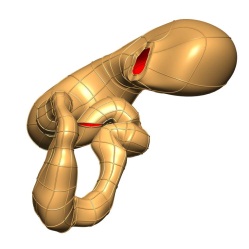


6



2

**Muscles**

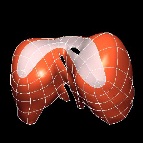


**Stomach**

4

3

**Lungs**



**Diaphragm**

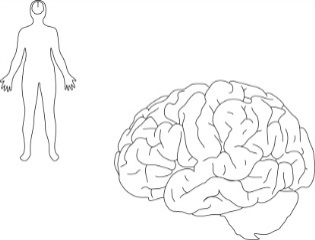
**Vagus (X)**

**Phrenic nerve**

**Glossopharyngeal (IX)**

**Vagus (X)**

,



**Respiratory control**

**Hypoglossal (XII)**

**Figure 1**. A whole body model containing organ level models for ion or gas exchange in the kidneys, muscles, lungs (with respiratory control), stomach, pancreas and systemic circulation.

We first describe some basic equations that will be used in a number of the models below. The first deals with hydration which, when catalysed by carbonic anhydrase, occurs extremely rapidly with an equilibrium constant

.

It is not clear whether the buffering system can be assumed to be in equilibrium, since during rapid changes in *pH* it may not be, but we make this assumption here and examine the consequences of that assumption later. Taking logs and using Henry’s law, , where is the solubility coefficient for in the relevant fluid, yields the Henderson-Hasselbalch equation for the buffer system:

, (1)

where , .

Next, the buffering of by weak acids and weak bases occurs via two types of reaction[[2]](#footnote-3). The first is the dissociation of cationic weak acid () to weak base (), governed by the reaction

, e.g.

Assuming a rapid equilibration, the governing equation for the model is

(2)

Whether these buffers are sufficiently close to equilibrium to make this assumption is debatable [ref].

Similarly, dissociation of uncharged weak acid () to anionic weak base () is governed by the reaction

, e.g.

and at equilibrium

(3)

These reactions hold on both sides of a cell membrane, with the same equilibrium constants on both sides. The neutral species ( and ) move freely down their concentration gradients to equilibrate at equal concentration on either side of the membrane, as indicated in Figure 2. The charged species (, and ), on the other hand, move down their concentration gradients (within a membrane protein channel) until equilibrating with their Nernst potentials,

, and [[3]](#footnote-4).

**Figure 2**. Movement of weak acids and weak bases across the cell membrane. The dotted lines indicate free movement of the uncharged species across the cell membrane. The solid lines indicate the flux of a charged species for which thermally driven diffusion equilibrates the concentration difference with the electrostatic charge difference (the Nernst equation).

Since the reaction is in equilibrium on both sides of the membrane and both and are the same on both sides (free permeation of the uncharged molecule), from (2)

, or

and therefore

. (4)

On the other hand, since the reaction is in equilibrium, and, now we have

, or

and therefore

. (5)

There are many sources of flux across a cell membrane and we list the important ones for the current analysis below. If is the rate of acid extrusion (generally at a metabolic cost) and is the rate of acid loading, the difference between them drives the time rate of change of pH in the cell:

more explanation needed. Is *J* per m2? (6)

(called the fundamental law of pHi regulation) where (m-1) is the surface to volume ratio of the cell, and (mM/pH unit) is the buffering power[[4]](#footnote-5). We consider an open system (the free exchange of energy and mass – in this case - with the atmosphere) only, in which case is . Yet to add an explanation for this. Is this a sufficient approximation? Note that has units of pH\_unit.m-1.mM-1, and have units of mol.s-1.m-2 (or mM.s-1.m) and has units of pH\_unit.s-1.

For a particular cell the acid fluxes and are dependent on a range of protein exchangers, channels and pumps that reside in the cell membrane and are modelled individually. For the cells of interest here these are the acid extruding membrane transporters (NHE, NBC and NDCBE, all driven by the sodium gradient), the acid loading membrane transporters (NBCe1,2 and AE), and various leak channels and ATP-dependent pumps (such as HKA and V-type) shown individually in Figure 3. Note that NHE, NBC and NDCBE depend on the sodium gradient which must be maintained by the ATP-dependent sodium-potassium exchange pump NCX, but since we are not tracking sodium or metabolism, that pump is not included.

The equations governing these exchangers and pumps are provided on the CellML model repository. Most depend on carrier-mediated mechanisms, rather than simple diffusion gradients and therefore obey Michaelis-Menten kinetics where the steady state flux is given by , where and are constants. Here we assume that these models have already been established as CellML models in PMR – see, for example, <https://models.physiomeproject.org/e/236/>.

**Cell compartment**

NBCe1,2

AEs

Leak

Leak

Leak

Mitochondrial metabolism

NHE

NBC

NDCBE

Vtype

ATP

Hv1

HKA

ATP

**Figure 3.** Membrane proteins (channels and transporters) divided into acid extruders (on the left) and acid loaders (on the right), shown here within a composite cell model. Note that the movement of ions across the cell membrane is driven by a sodium gradient (NHE, NBC, NDCBE), a chloride gradient (AE) or a metabolically dependent process (ATP label). Each channel and transporter is modelled with a separate CellML model on the PMR website.

The cells of interest for the tissue level models below are a muscle cell, a renal epithelial cell, a gastric epithelial cell, a pancreatic epithelial cell, the red blood cell or RBC (treated as 3 cases: within the arterial system, within the capillary bed and within the venous system). For the tissue level models we define seven tissue compartments (see Figure 4): alveoli space; renal tubule; gastric interior; pancreas interior; and arterial, capillary and venous blood plasma.

**Cell types:**

**Muscle  
cell**

**Renal  
epithelial  
cell**

**RBC  
arterial**

**RBC  
capillary**

**RBC  
venous**

**Gastric  
epithelial  
cell**

**Pancreas  
epithelial  
cell**

**Pancreas  
interior**

**Gastric  
interior**

**Renal tubule**

**Alveolar  
space**

**Arterial  
blood plasma**

**Venous  
blood plasma**

**Capillary  
blood plasma**

**Tissue**

**components:**

**Figure 4.** Cell and tissue compartments, to which variables and equations are assigned.

Using these cell and tissue compartments, we now define ***composite multicellular tissue models*** for each of the six organ-level models (kidney, muscle, lung, stomach, pancreas and vascular system). Equation (6) is used with appropriate transporters for each parenchymal cell in the organ or organ system tissue model. In each case the parenchymal cells for each organ connect with blood plasma (arterial, capillary or venous). We later accumulate all exchange with blood plasma into one model for the arterial blood plasma, one for capillary blood plasma and one for venous blood plasma. Each model includes the variables shown to the right associated with each compartment. The inputs and outputs for each model are shown above and below the box with an inward or outward arrow, respectively.

**MODEL 1: KIDNEY**

There are several mechanisms to be considered in this model, as shown in Figure 5:

(a) One is the cellular mechanism of *reabsorption* in the kidney. The filtered by the glomerulus is combined with excreted from the epithelial cell (via the ATP-dependent H-pump and HKA pump, and the Na-dependent exchanger NHE) to form and water (via the carbonic anhydrase reaction in the tubule) that diffuses back into the epithelial cell to then regenerate in the blood (via the carbonic anhydrase reaction in the epithelial cell). The kidney reclaims virtually all filtered .

(b) The second is *ammonium excretion*. The kidney synthesizes as a urinary buffer to assist with the excretion of . Filtered in the tubule titrates excreted from the epithelial cell following glutamine metabolism (which produces both and , thereby also generating new into the blood). Conversion of the amino acid glutamine to urea and ammonia, is catalysed by glutaminase in both the liver and the kidney. Under conditions of blood acidosis, excretion in the kidney is enhanced by up-regulating glutaminase production to increase production, and glutaminase production is correspondingly down-regulated in the liver[[5]](#footnote-7).

(c) The third is *titratable acid formation*. When the excreted combines with filtered buffers other than or ammonia, the outcome is the urinary loss of uncharged weak acid and the generation of new into the blood[[6]](#footnote-8).



HA

**Renal tubule**

**Arterial blood plasma**

**Renal epithelial cell**

HA

glutamine

urea

glutaminase

NBCe1,2

AEs

Leak

,

Vtype

ATP

NHE

HKA

ATP

**(a)**

**(b)**

**(c)**

**Figure 5**. Cellular mechanisms in the kidney: (a) reabsorption, (b) ammonium excretion, and (c) titratable acid formation.

In formulating the equations for the kidney model, we assume that every that is transported out of the epithelial cell to the blood plasma is immediately replaced with a from the hydration reaction – i.e. there is no shortage of carbonic anhydrase and there is no shortage of available to feed the reaction. This is also assumed to diffuse with no delay from the renal tubule. Note that we are assuming the cell to be a well-stirred system. In reality there may be significant gradients of and particularly adjacent to transport proteins that influence the trans-membrane fluxes, but here we neglect the possible influence of unstirred layers.

Using sub- or superscripts *rt* for renal tubule, *rec* for renal epithelial cell and *abp* for arterial blood plasma to indicate which compartment a variable belongs to, the equations for this model are:

*(i) Renal epithelial cell*

; (7)

(8)

. (9)

Note that the flux terms in eqn 7 are positive in the direction of the arrows in Fig.5 – i.e. a positive flux of from the epithelial cell to the renal tubule and a positive flux of from the epithelial cell to the capillary. The factor of 3 for the NBC transporter reflects the transfer of 3 ions for every one . The term includes all sources of production within the cell. Note that is constant across all three renal compartments.

*(ii) Renal tubule*

. (9)

. (8)

Note that we are assuming that every transported to the renal tubule from the epithelial cell immediately combines with a filtered to produce water and , which diffuses back to the epithelial cell to continue the cycle. In reality there may be *pH* gradients along the tubule created by diffusion or advection by urinary flow.

With now calculated on both sides of the apical (tubule-epithelial) membrane, the Nernst potential can be calculated and therefore the ratio of and concentrations on either side of the membrane:

*(iii) Arterial blood plasma*

. (10)

The bicarbonate sink term in this equation is considered later when we model and exchange with the RBCs.

**MODEL 2: MUSCLE**

Metabolic activity in muscle produces which diffuses across the cell membranes to the capillary blood, or the is converted to bicarbonate and actively transported, as illustrated in Figure 6.

**Capillary blood plasma**



**Myocyte**

NBCe1,2

AEs

Leak

**Figure 6**. The release of from metabolic activity in muscle into the blood stream.

*(i) Myocyte*

; (11)

*(ii) Capillary blood plasma*

. (12)

The bicarbonate sink term in this equation is considered later when we model and exchange with the RBCs.

Question: Are these 3 bicarbonate transporters all operating in the myocyte? Should transporters in endothelial cells be considered as well? Should other extracellular spaces be modelled as well?

**MODEL 3: LUNGS & RESPIRATORY CONTROL**

There are several ways to develop a model for gas exchange between the pulmonary capillaries and alveolar air. One approach is to develop a detailed biophysical model that takes into account the anatomy and material properties of all the cells and tissues that influence the diffusion of and (see [1] for a detailed discussion of this). Another, more empirical, approach is to represent the volume rate of transport of across the lung endothelium from capillary to alveolar space***[[7]](#footnote-9)*** with Fick’s law using a proportionality constant that takes into account the cross-sectional area and length of the diffusion path and the molecular weight of the gas:

(mol.m-2.s-1)

where (m2) is the surface area for gas exchange, (m) is the distance between the alveolar space and the blood plasma, (0.03mM/mmHg or 0.03mol.m-3.mmHg-1) is the solubility of in the tissue, *MW* is molecular weight (44 for ) and is a proportionality constant [1]. and are the alveolar and capillary partial pressures of (mmHg).   
Are the units for the eqn above correct (is *k* dimensionless?).

The nerves involved in respiratory control are shown in Figure 1 by green dashed lines. Blood gases (, and *pH*) are sensed peripherally by glomus cells in the carotid and aortic bodies at the bifurcation of each of the common carotid arteries and in the aortic arch, respectively, and send feedback signals to the medulla via afferents of the glossopharyngeal nerve (CN IX) and the vagus nerve (CN X), respectively. They respond to hypoxia, hypercapnia and acidosis. Other so-called central chemoreceptors are located within the brain parenchyma in brain extracellular fluid behind the blood-brain barrier. These appear to be responsible for 65-80% of the integrated response but with a slower time constant than the faster acting peripheral receptors. The end result of this feedback is modulation of a respiratory rhythm generator in the medulla that controls the inspiratory phase of breathing via the phrenic nerve output to the respiratory muscles. Lung movement is mainly controlled by the diaphragm, which is excited during inspiration by a signal from the phrenic nerve. It recoils during expiration which is usually passive in mammals, although forced expiration can be induced by the abdominal muscles. The resistance to flow in the upper airway and movements of the tongue are affected by signals from the hypoglossal nerve. The vagus nerve is an important bundle of nerves that carries signals to and from the brainstem. Signals from chemoreceptors and baroreceptors in the aorta as well as signals from stretch receptors in the lungs are transferred via the vagus nerve to the brainstem, and signals that act to slow the heart rate are transferred by the vagus nerve to the heart. Chemoreceptors and baroreceptors are also located in the carotid sinus and their signals are transferred to the brainstem via the glossopharyngeal nerve. The lung itself is a complex organ that comprises of airways and blood vessels that are ‘suspended’ within a delicate network of gas exchange tissue4. The airways, pulmonary arteries, and pulmonary veins converge at the alveoli (microscopic airfilled sacs, with walls comprised of capillaries) where respiratory gas exchange occurs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Afferent nerves** | **from receptors ..** | **located in ..** | **project to ..** |
| glossopharyngeal (IX) | chemoreceptors | carotid sinus | pre-Bötzinger complex |
|  | baroreceptors | brainstem |
| vagal (X) | chemoreceptors | aortic arch |  |
|  | baroreceptors |  |
|  | stretch receptors | upper airway |  |
|  | stretch receptors | atria of heart |  |
| hypoglossal (XII) |  |  |  |
| **Efferent nerves** | **innervating ..** |  |  |
| phrenic | diaphragm |  |  |
| abdominal muscles |  |  |

Table 2.

For the purposes of the present whole body acid-base model we will ignore the details of both gas exchange on the lungs and the feedback control of respiration and just define an empirical relationship between ventilation and blood gas concentrations. This is discussed in [1] and shown in Figure 7 as plots of total ventilation (in L.min-1) as a function of (a) and (b) .

mmHg

0

10

20

30

40

50

60

20

30

40

50

**47**

**110**

= **37** mmHg

Total ventilation (L/min)

0

10

20

30

40

50

60

20

60

100

140

= **49** mmHg

**44**

**36**

mmHg

Total ventilation (L/min)

(a)

(b)

**Figure 7**. Integrated ventilatory responses to changes in (a) and (b) (adapted from [1]).

**MODEL 4: STOMACH**

The acidic environment in the stomach (*pH*≈2), required to kill bacteria and to aid food breakdown, is maintained by the -ATPase pump in parietal cells lining the stomach, as shown in Figure 8.

**Stomach**

**Capillary blood plasma**



**Gastric epithelial cell**

NBCe1,2

AEs

Leak

HKA

ATP

**Figure 8**. Release of via the -ATPase in parietal cells of the stomach.

The acid produced by parietal cells in the stomach has to be neutralised for chyme arriving in the duodenum. This is achieved via the release of cholecysto-kinase (CCK) from … which stimulates the release of from S-cells in the pancreas.

**MODEL 5: PANCREAS**

**Capillary blood plasma**



**Pancreas tubule**

**Pancreatic epithelial cell**

NBCe1,2

AEs

Leak

AEs

**Figure 9**. Release of via the exchanger in S-cells of the pancreas.

**MODEL 6: BLOOD CIRCULATION**

We treat the arterial circulation as one homogenous compartment and the venous circulation as another separate homogenous compartment, in order to allow for the differences in arterial and venous concentrations of , and .

***Exchange of and*** ***between plasma and******red blood cells***

The vascular transport of muscle-generated or recycled from the kidney depends heavily on the gas-channel mediated transport of from plasma to red blood cells where it is hydrolysed to , dissolved or stored as carbamino hemoglobin () via the reaction

within the RBC. Assuming equilibrium,

. (20)

Similarly in the RBC,

at equilibrium gives (21)

And in the plasma

(22)

As indicated in Figure 9, nearly 70% of the newly generated is carried away in the blood stream as bicarbonate (mostly in the RBC) about 20% as carbamino haemoglobin and only 10% in solution.

What bicarbonate transporters are operating on the RBC membrane?

Note that the significance of membrane transporters such as the Rh complex and AQP1 for is controversial (RVJ ref) since diffusion delays in the unstirred layers adjacent to the membrane may be as important as the kinetics of the membrane transporters. The RBC membrane is unusual in being crammed with proteins that limit the area of bilipid membrane available for diffusion, so it is possible that membrane protein-facilitated transport may be more significant here than in other cell types (ref). We ignore these subtleties here and just assume an unimpeded rapid transfer of across the RBC membrane.

Note also that here we are not dealing with the Bohr effect (the enhanced release of from haemoglobin through binding) or the movement of water to supply for bicarbonate production in the RBCs (and their consequent swelling) in the systemic capillaries, but both effects may be readily added to the model.

6%

**Dissolved**

5%

**Capillary blood plasma**

NBCe1,2

AEs

Leak

Pancreatic

S-cells

NBCe1,2

AEs

Leak

Gastric

parietal

cells

Muscle

cells

**Capillary RBC**

**AQP1**

**Rh complex**

AEs

**diffusion**

**Dissolved**

4%

64%

21%

**Figure 10**. Exchange of , and between parenchymal cells and plasma and between plasma and red blood cells. is transported to the RBC by diffusion (dotted line) and via membrane protein channels (Rh complex and AQP1). is stored as , or in solution.

**COMPOSITE MODEL**

Capillary blood plasma model

Muscle model

Gastric model

Pancreas model

RBC

Venous blood plasma model

Lung model

RBC

Kidney model

Arterial blood plasma model

RBC

Bicarbonate  
reabsorption model

Ammonium   
excretion model

Titratable acid   
formation model

Components

**Figure 11**. Block diagram showing how models relate to each other.

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1. Binding proteins are heavily influenced by the dissociation of acidic or basic amino acids, especially histodine, and are therefore dependent on ambient pH. Intracellular metabolism is also influenced by pH - for example, glycolysis and lactic acid formation are inhibited by acidosis (and, conversely, stimulated by alkalosis). K+ channels in a wide variety of cells are inactivated by acidosis. H+ affects smooth muscle in blood vessels (vasoconstriction in pulmonary arterioles and vasodilation in the systemic circulation) and reduces Ca2+ binding by plasma proteins. [↑](#footnote-ref-1)
2. Note that an acid or base (alkali) is any chemical substance that can donate or accept an , respectively [1]. [↑](#footnote-ref-3)
3. *R*≈8.4 J.mol-1.K-1 and *F*≈0.96x105 C.mol-1, therefore at *T*=298K (25°C), *RT*≈2.5kJ.mol-1 and *RT/F*≈25mV. [↑](#footnote-ref-4)
4. A buffer is any substance that tends to minimize changes in pH by reversely producing or consuming H+. The buffering power of a solution is measured by the amount of added acid or base (per unit volume of solution) needed to decrease or increase the solution pH by one pH unit. i.e. a buffer with a high value of requires a lot of additional acid or base to produce a unit change in pH. Without , the buffering power of whole blood is about 25 mM/pH unit (called the non- buffering power). 80% of this non- buffering power is associated with the cellular components of blood. [1] [↑](#footnote-ref-5)
5. The liver also consumes glutamine for urea synthesis, and this is enhanced by an alkalosis-induced increase in glutaminase production in the liver. [↑](#footnote-ref-7)
6. Note that about 70mmole/day of new is needed to neutralise the ~70mmole/day of non-volatile acid - comprised of ~40mmol/day of non-volatile acid produced (in addition to ) by metabolism, ~20mmol/day of dietary strong acid, and the intestinal loss of ~10mmol/day of . There must also be a corresponding excretion of ~70mmol/day of into the urine [2]. [↑](#footnote-ref-8)
7. A 70kg person on a typical western diet produces ~15mol/day or 0.66kg/day of from metabolism. [↑](#footnote-ref-9)