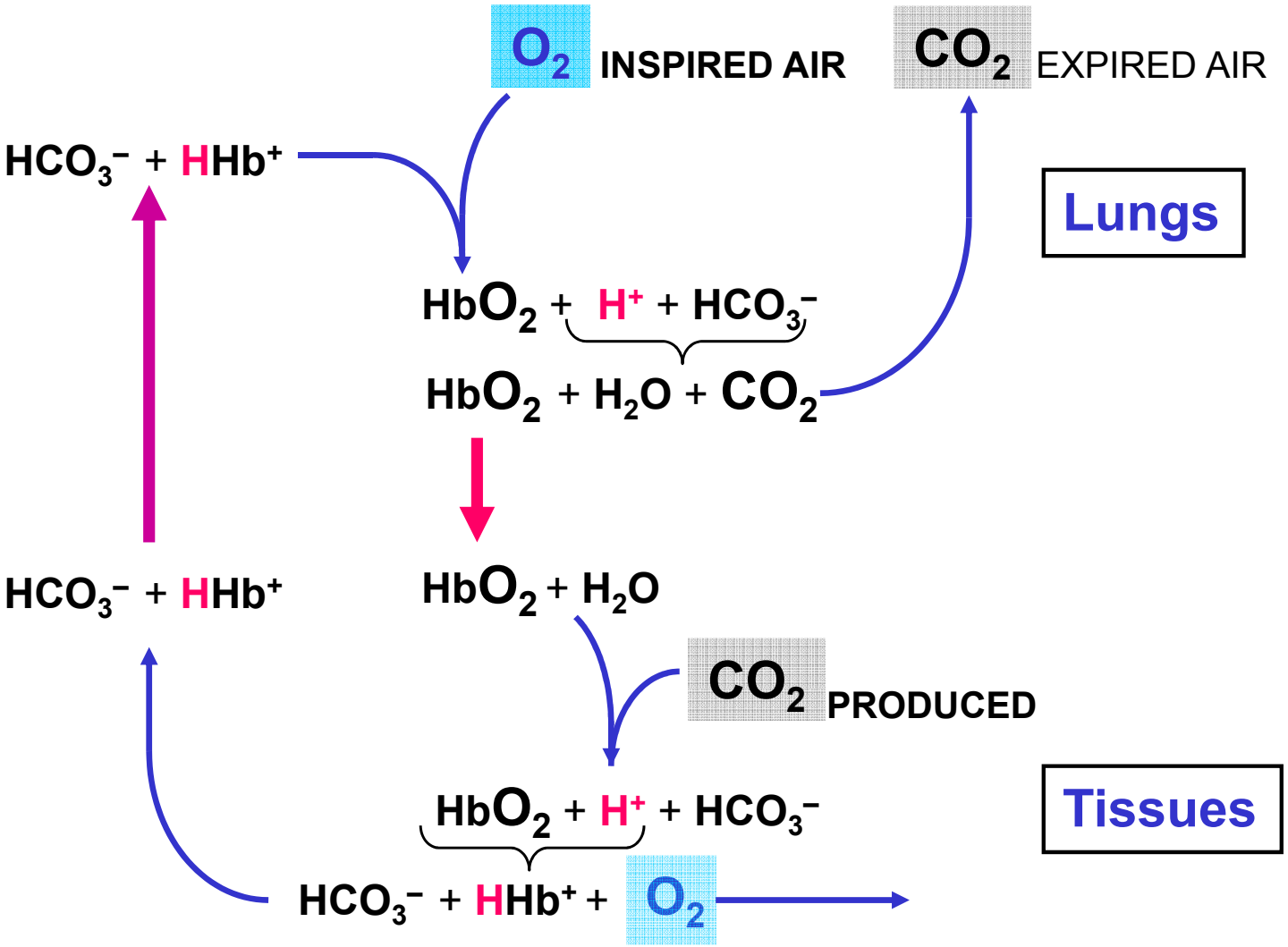
Oxygen and CO_2 transport

Acid- base balance

Biochemistry II
Lecture 9

2008 (J.S.)

Transport of O_2 and CO_2



Inspired and expired air – partial pressures:

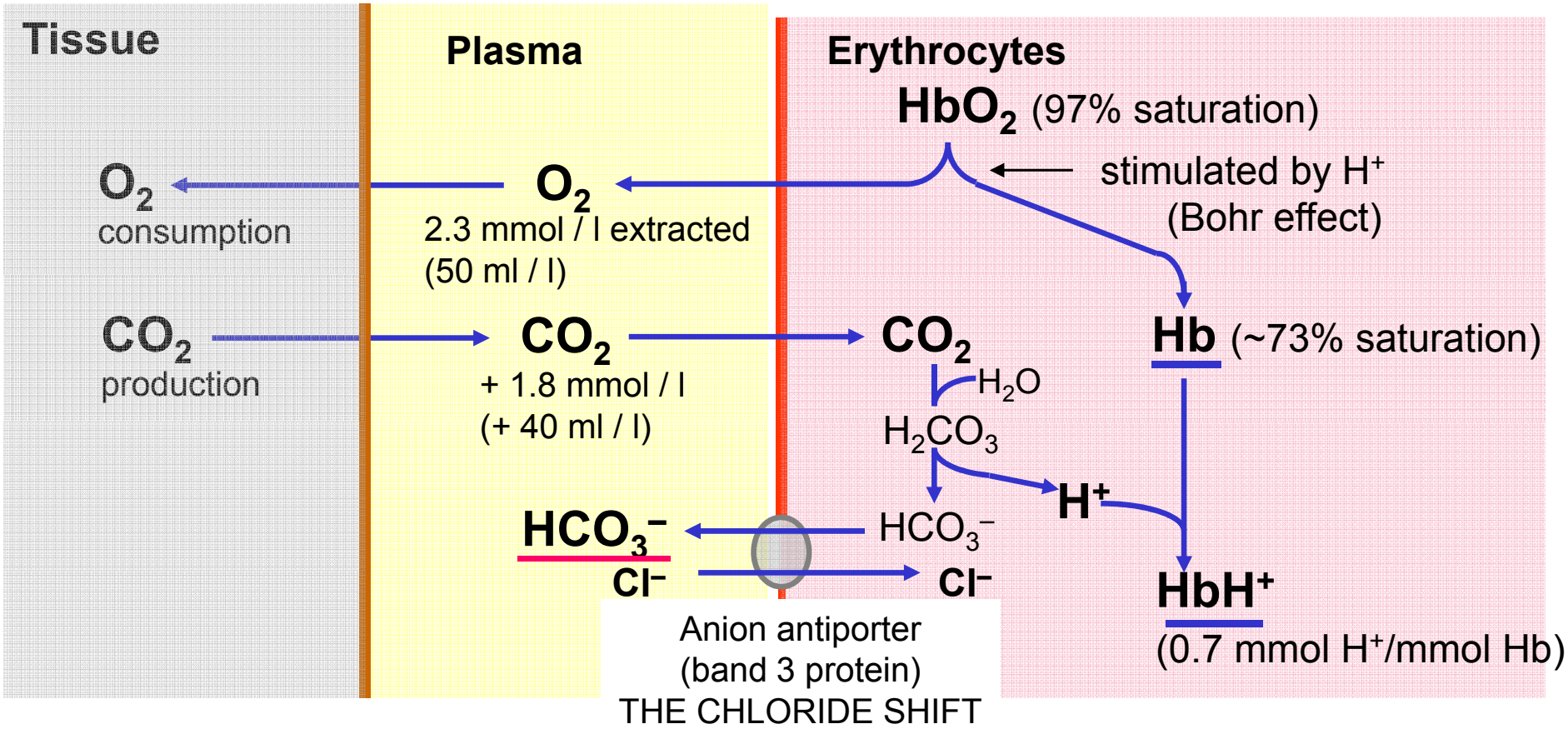
	pO_2	pCO_2	pH_2O
Inspired air	21 kPa	0.03 kPa	0.76 kPa
Expired air	15.3 kPa	4.4 kPa	6.3 kPa

Partial pressures of oxygen and carbon dioxide in blood:

	pO_2	pCO_2
Arterial blood	**12.0** (9.2 – 15.5) kPa	**5.3** (4.6 – 6.0) kPa
Mixed venous blood (central)	**6.7** (5.3 – 8.0) kPa	**6.7** (5.4 – 8.0) kPa

Arterial blood ≈ 9.2 mmol O_2 / l
(200 ml O_2 / l)

Blood in capillaries:



(Central) venous blood ≈ 6.9 mmol O_2 / l
(150 ml O_2 / l)

Transport of oxygen

Molecular oxygen in blood exists in **two forms**:

– O_2 associated with haemoglobin, and

– O_2 dissolved but not associated with any other substance.

The concentration of total O_2 $\boldsymbol{c\ tO_2 = c\ HbO_2 + c\ O_2}$.

Freely dissolved O_2 is given by $pO_2 \times \alpha O_2$ (the concentrational solubility coefficient in blood αO_2 = 0.01 mmol/l kPa (at 37 ºC); the solubility of dioxygen is very low when compared with CO_2 with the αCO_2 = 0.23). Only about **2 %** of the oxygen is transported physically dissolved in the blood.

Haemoglobin (4Fe) can bind four molecules O_2, at complete saturation.

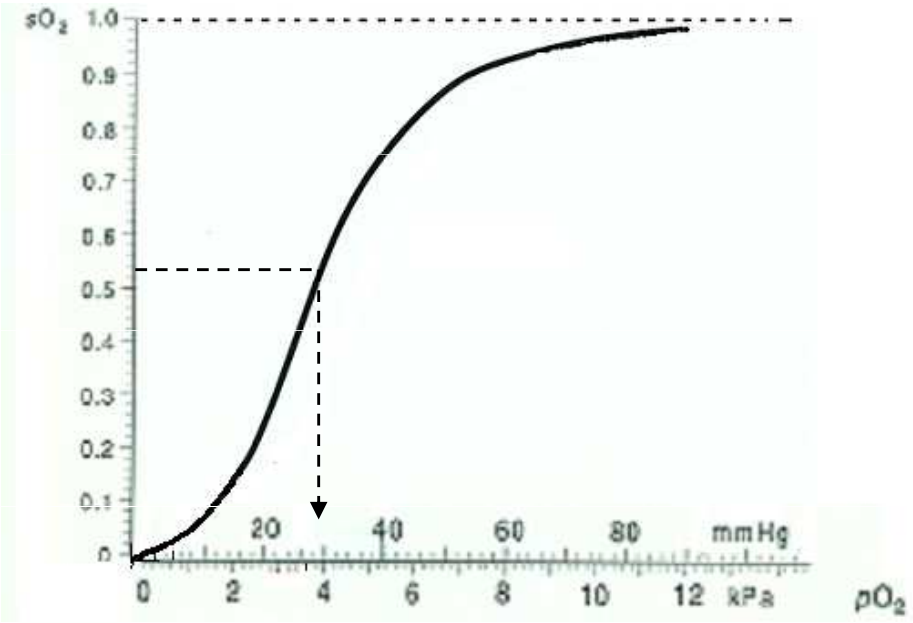
At *c* tHb(1Fe) = 9.2 mmol/l, blood is able to bind 9.2 mmol O_2 /l, i.e. 234 ml O_2 /l (37 ºC), when the blood does not contain any methaemoglobin (MetHb) or carbonylhaemoglobin (HbCO).

The reference method for haemoglobin in human blood measures the total haemoglobin concentration, which includes also MetHb and HbCO which have lost permanently or temporarily the capability of reversible O_2 binding at physiological pO_2.

$$\boldsymbol{c\ tHb} = \boldsymbol{c\ HbO_2} + c\ HbH^+ + c\ HbCO + c\ MetHb$$

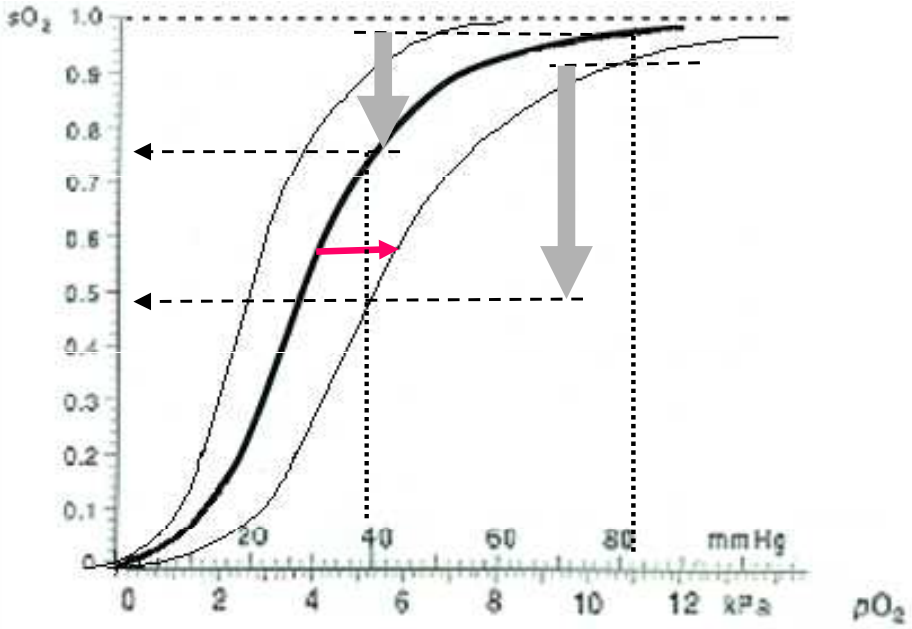
Oxygen associated with haemoglobin

The oxygen saturation curve
(the oxygen dissociation curve, ODC)



Position of the ODC:
*p*50 cca 3.55 kPa,
normal range 3.0 – 4.0 kPa

Shift to the right:
acidification (Bohr effect)
increase in pCO_2
increased temperature
increased 2,3-BPG



Normally, the arterial blood is approximately 97% saturated with oxygen. After release of oxygen to the tissue, mixed venous blood is about 75% saturated.

Factors that decreased the Hb-O_2 affinity (right-shifted curve) facilitate the release of oxygen. In the case of increased Hb-O_2 affinity, release of oxygen is impeded.

Oxygen uptake

The arterial oxygen tension (**a**)$\boldsymbol{pO_2}$ indicates the uptake function of the lungs. Under normal physiological circumstances the available haemoglobin in the arterial blood becomes approximately **97 % saturated** in the pulmonary capillaries.

The remaining 3 % will normally not become oxygenated due to anatomic shunts (or the match between ventilation and perfusion in the lungs that is expressed by the alveolar-arterial pO_2 difference AaΔpO_2).

Oxygen transport

depends on the concentration of O_2 transported by haemoglobin:

$$(a)\boldsymbol{c\ tO_2} = c\ \text{tHb} \times sO_2 \times (1 - \text{COHb} - \text{MetHb}) + pO_2 \times 0.01$$

Normal range: women 7.1 – 8.9 mmol/l
men 8.4 – 9.9 mmol/l

Oxygen release

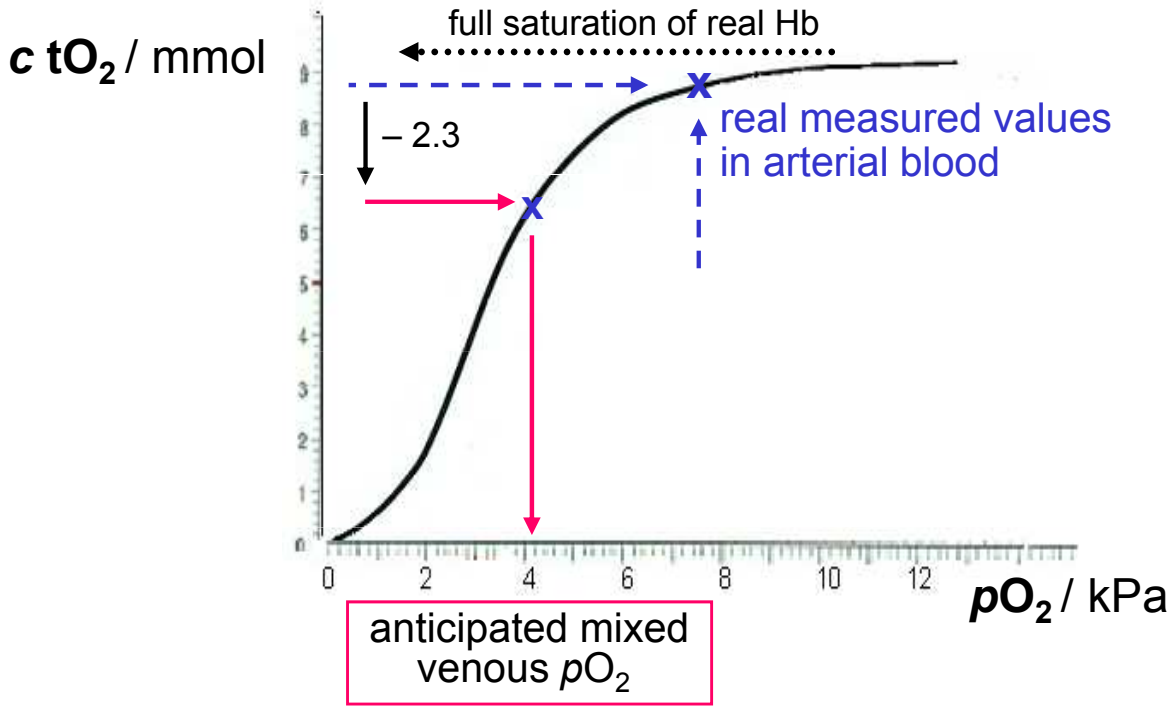
to the tissue is determined by the **oxygen tension gradient between the capillaries and the tissue**, and by the **affinity of haemoglobin to oxygen** (the position of the saturation curve, its normal range *p*50 3.4 – 3.8 kPa).

A rough estimate can be obtained from the **blood oxygen absorption curve**.

The crucial quantity in the O_2 supply to the tissues is the end-capillary pO_2 and the **central mixed-venous blood ($\bar{v}$)pO_2** (the extraction oxygen tension).

Low values (< 4.5 kPa) are indicative of tissue hypoxia. The critical value at 3.5 kPa reflects the minimum O_2 supply to sustain aerobic metabolism.

Under normal physiological circumstances the rate of oxygen consumption is fairly constant,
the **arterio-venous oxygen content difference avΔcO_2** is about **2.3 mmol/l**.



Transport of CO_2

Carbon dioxide is the end product of cellular metabolism. Adult humans produce about 300 – 400 litres CO_2 per day (~ 15 – 20 mol/d) and this amount may be higher, e.g., with the increase in physical activity. Molecules of carbon dioxide are nonpolar. They freely diffuse across plasma membranes. In tissues, capillary blood CO_2 content increases by 40 – 50 ml CO_2 / l.

The total blood CO_2 content is in the range **450 – 550 ml / l**, from which

more than 85 % in the form of **HCO_3^-**,
about 5 % **physically dissolved CO_2 and H_2CO_3**, and
approximately 10 % as **carbamino compounds** R–NH-COO^-.

Carbamino compounds originate by attaching CO_2 to amino groups –NH_2 of various proteins, predominantly of haemoglobin within red blood cells.

Acid-base balance

Metabolism produces large quantities of acids:

– **CO_2** is the product of numerous decarboxylations included in oxidative breakdown of nutrients (tissue respiration, **15 – 20 mol CO_2 daily**).
Carbon dioxide dissolves in water to form **volatile carbonic acid**:

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

The **lungs** control the exchange of carbon dioxide between the blood and the external atmosphere.

– **Non-volatile acids** – products of the metabolism of sulfur-containing amino acids cysteine and methionine (sulfuric acid), phosphorus-containing compounds (phosphoric acid), and some carboxylic acids (e.g. lactate, acetoacetate, and 3-hydroxybutyrate, unless they are completely oxidized to CO_2 and water).
They represent about **30 – 80 mmol H^+ per day** that cannot be removed through the lungs, and must be excreted **by the kidney** into the urine.

Although thre is a large production of acidic metabolites in the body, concentrations of H^+ ions in biological fluids are maintained in the very narrow range:

$[H^+]$ in blood = 45 – 35 nmol / l

pH = 7.35 – 7.45

The human body is more tolerant of acidaemia (acidosis) than of alkalaemia (alkalosis).

Steep decreases in pCO_2 or increases in $[HCO_3^-]$ may be life-threatening. The limit blood pH values compatible with life are

pH 6.80 i.e. $[H^+] \approx 160$ nmol / l , four times higher than the normal $[H^+]$,

pH 7.70 i.e. $[H^+] \approx$ 20 nmol / l , decrease in $[H^+]$ by more than 50 %.

Changes in hydrogen ion concentration are minimized by means of

- **buffer systems**, both intracellular and extracellular,
- **removal of CO_2** through pulmonary ventilation, and
- **reabsorption of HCO_3^- and H^+ ion excretion** in the renal tubules;
- **the liver** also has certain role in maintaining the acid-base status.

Proteins and phosphates are the main **intracellular buffers**.
H^+ ions pass plasma membranes in exchange for K^+ ions – acidaemia may thus lead to an increased plasma K^+ concentration, and alkalaemia to hypokalaemia.

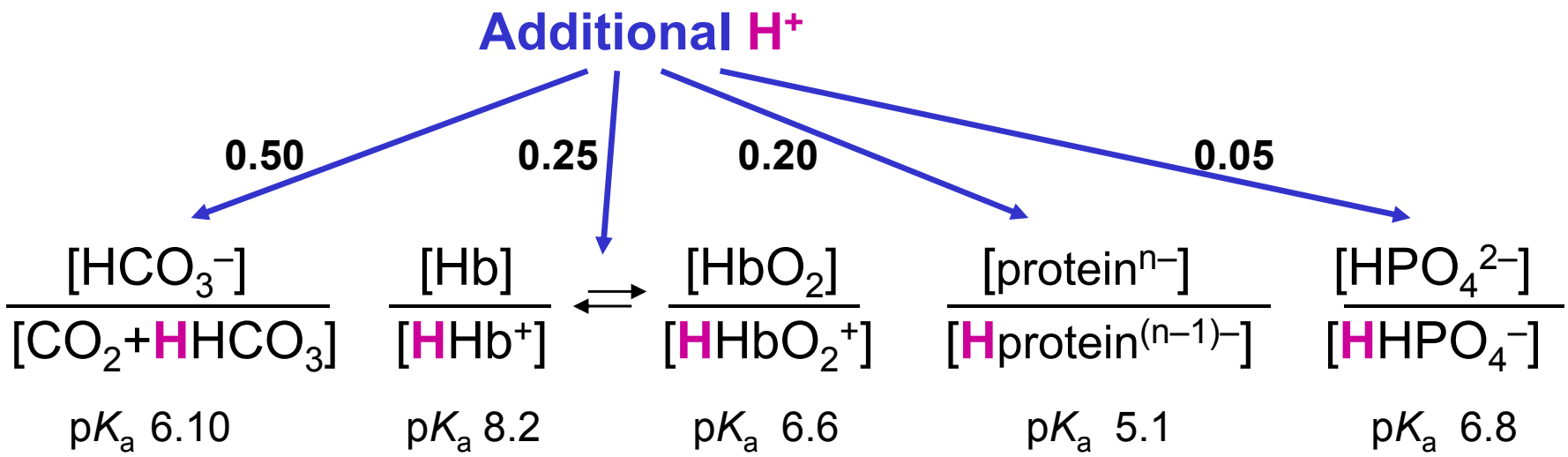
Buffer systems comprising the total blood buffer bases (BB_b)

Buffer system	Blood	Plasma	Erythrocytes
HCO_3^-	0.50	0.27	0.23
Haemoglobin	0.45	–	0.25
Plasma proteins		0.20	–
Phosphate	0.05	0.01	0.04
Σ	**1.00**	0.48	0.52

Concentration of buffer bases **in blood** BB_b 48 ± 3 **mmol / l**
Concentration of buffer bases **in plasma** BB_p 42 ± 3 **mmol / l**

Cooperation of buffer systems

If the concentration of H^+ increases, the H^+ increment is distributed to all buffer systems proportionally to their contribution to the total buffer bases.



Each of these buffers has its own Henderson-Hasselbalch equation, from which it is possible to calculate the resulting change in the ratio $c_{base}/c_{acid.}$

The knowledge of the change in the ratio c_{base}/c_{acid} *of* one of the blood buffers enables to deduce the changes in the other buffer systems.

The hydrogen carbonate (bicarbonate) buffering system

Carbonic acid H_2CO_3 is a weak acid, that originates in the reaction of carbon dioxide with water:

$$\mathbf{CO_2} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons \mathbf{H^+} + \mathbf{HCO_3^-}$$

In blood plasma, CO_2 equilibrates with H_2CO_3 very slowly, but the reaction is catalyzed by extremely efficient **carbonate dehydratase** within erythrocytes.

The "effective" constant of H_2CO_3 dissociation is

$$K_{eff}(H_2CO_3) = \frac{[H^+]\,[HCO_3^-]}{[CO_2 + H_2CO_3]}$$

and for plasma (37 °C) $pK_{eff} = 6.10$

Henderson–Hasselbalch equation for HCO_3^-/H_2CO_3 in blood:

$$\mathbf{pH} = \mathbf{p}K_{\mathbf{eff}}(H_2CO_3) + \mathbf{log}\,\frac{[\mathbf{HCO_3^-}]}{[\mathbf{CO_2 + H_2CO_3}]}$$

← metabolic component

← respiratory component

In these equations, the concentrations $[HCO_3^-]$ and $[CO_2+H_2CO_3]$ are expressed in **mmol/l**, not in the basal SI unit mol/l !

$$\mathbf{pH} = 6.10 + \mathbf{log}\,\frac{[\mathbf{HCO_3^-}]}{0.23 \times \mathbf{p}CO_2}$$

$\mathbf{p}CO_2$ in kilopascals;
(the solubility coefficient of CO_2 is 0.03 if pCO_2 is measured in mmHg)

At pH 7.40, the **ratio $c(HCO_3^-)/c(CO_2+H_2CO_3)$ equals 20**, e.g., 24 mmol/l / 1.2 mmol/l..

The HCO_3^-/H_2CO_3 buffer is the most important of the blood buffering systems
- it depends on the intensive cellular metabolism, and
- changes in this system also reflect changes in other buffers.

Parameters used in the evaluation of acid-base status

The classical concept of Astrup, Siggaard-Andersen, et al.

Modern blood analyzers measure pH and pCO_2,
as well as many supplementary parameters (e.g. **pO_2**, concentrations of **haemoglobin**, **Na^+**, **K^+**, änd **Cl^-**).

Normal values in **arterial blood**:

pH = 7.40 ± 0.05
pCO_2 = 5.33 ± 0.5 kPa

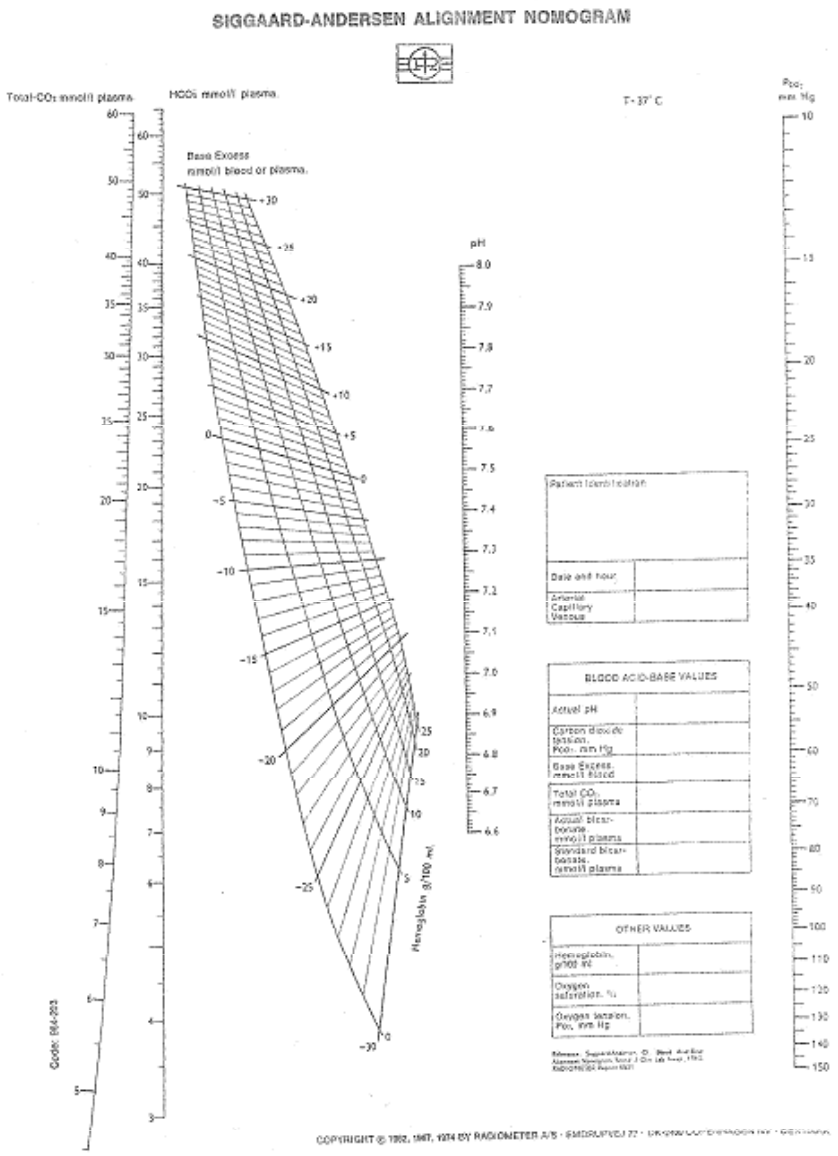
Values of other parameters are estimated by calculations based on known or derived relationships to the measured analytes:

actual [HCO_3^-] = 24.0 ± 3.0 mmol / l by calculation based on Henderson-Hasselbalch equation

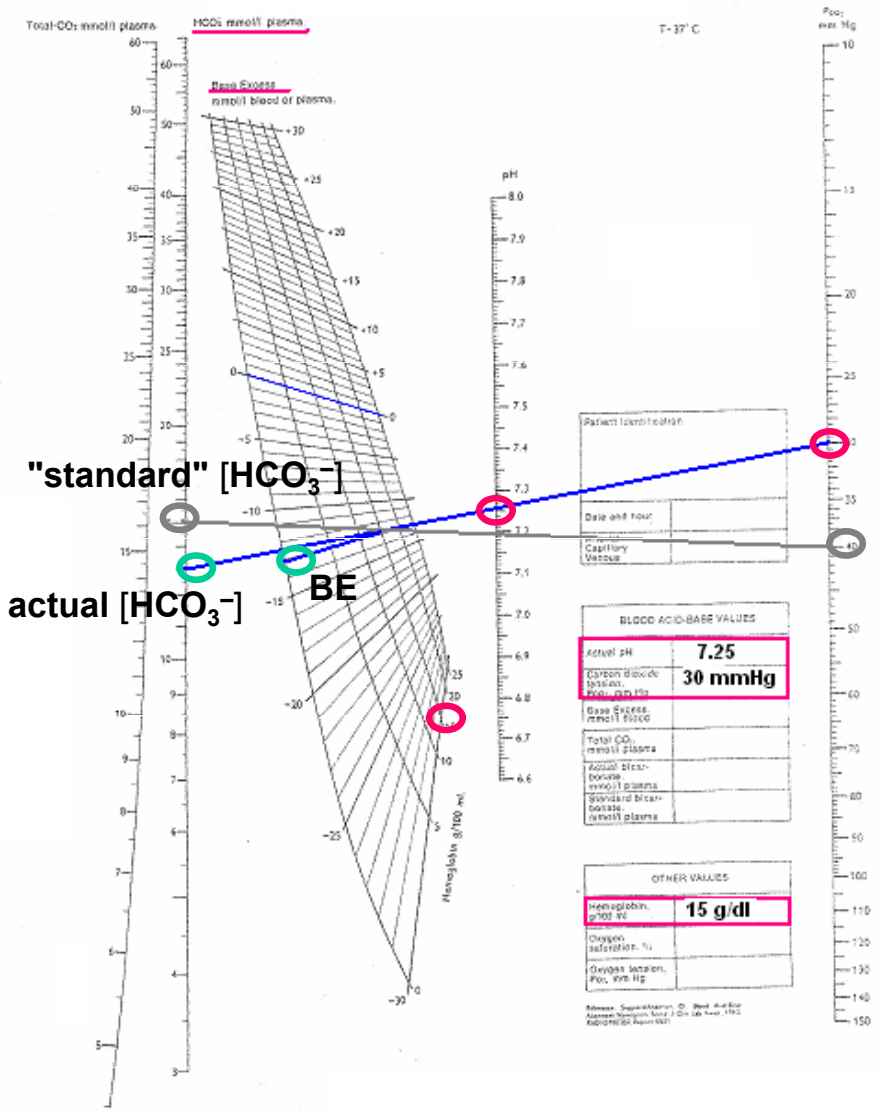
"**standard**" [HCO_3^-] is calculated, when pCO_2 value is outside the normal range; it is the value that would be measured in a given sample, if the sample had normal pCO_2 and was saturated by O_2.

base excess BE (positive or negative), normal range **0 ± 3 mmol / l**;
BE is the excess or the deficit in buffer base concentration, in which the sample would differ (at normal pCO_2 and saturated by O_2) from the normal buffer base concentration in blood 48 ± 3 mmol / l.

Values of actual [HCO_3^-], "standard" [HCO_3^-], and base excess can be also acquired from the Siggaard-Andersen alignment nomogram:



Example:



Buffer base plasma BB_p

- normal value **42** ± **3** **mmol / l**

Components:

hydrogen carbonate	~ **24** **mmol / l**	
plasma proteins	~ **16** **mmol / l**	(albumin ~ 12 mmol / l)
phosphates ($HPO_4^{2-}/H_2PO_4^-$)	~ **2** **mmol / l**	

Buffer base concentration in blood BB_b

- normal value **48** ± **3** **mmol / l**

BB_b is higher than that of plasma (by about 6 mmol / l). Due to haemoglobin and organic phosphates, the concentration of buffer bases in red blood cells is about 56 mmol / l.

As an approximate estimate of BB_{blood} can serve the value of BB_{plasma}, to which $0.67 \times c$(haemoglobin 1Fe) is added.

Basal terms

Blood pH values lower than 7.35 - **acidaemia**,
higher than 7.45 - **alkalaemia**.

Processes evoking these deviations

- **acidosis** – the accumulation of H^+ in the body that results in acidaemia,
- **alkalosis** – a decrease in H^+ concentration in the body

Classification of acid-base disorders

Respiratory disorder

- the **primary change in *p*CO_2** due to low pulmonary ventilation or a disproportion between ventilation and perfusion of the lung.

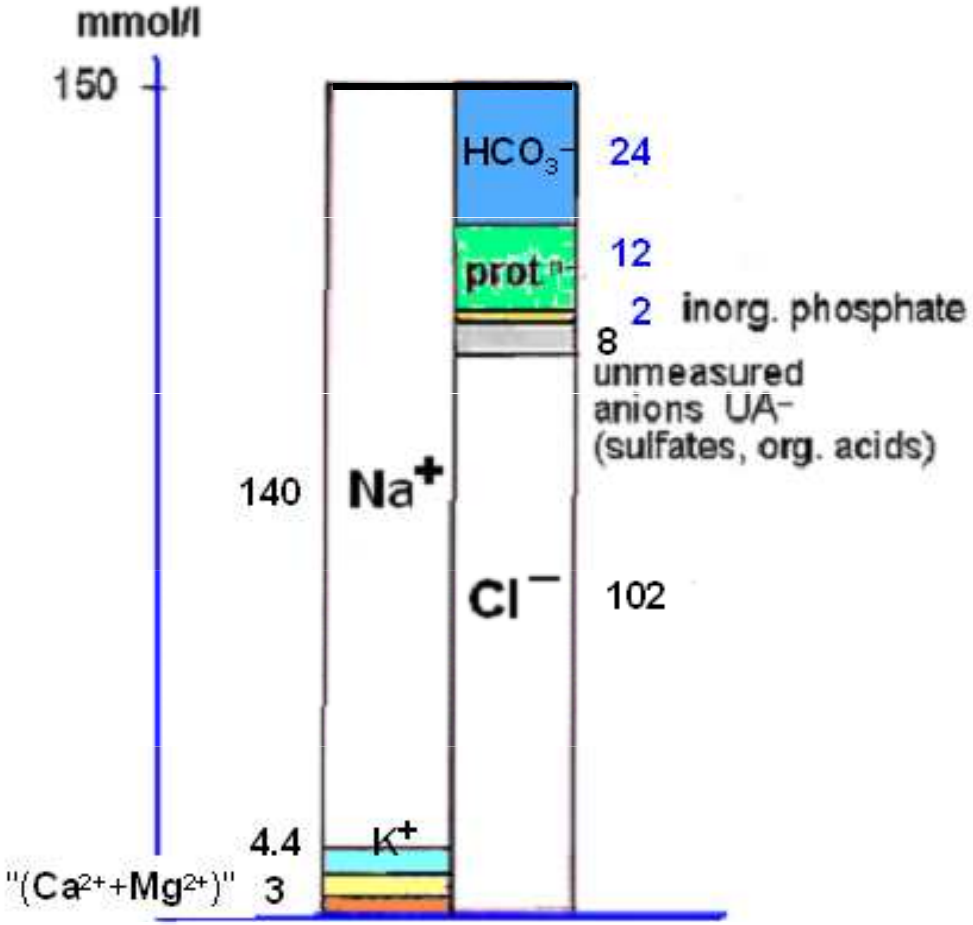
Metabolic disorder

- the **primary change in buffer base concentration** (not only HCO_3^-, but also due to changes in protein, phosphate, and strong ions concentrations).

Quite pure (isolated) forms of respiratory or metabolic disorders don't exist in fact, because of rapid initiation of compensatory mechanisms; however, full stabilization of the disorder may settle in the course of hours or days.

Ions in blood plasma / serum

Changes in main plasma ions concentrations may have considerable effects on acid-base status. It is very useful to know the values even if pH, pCO_2, and base excess appear to be in the normal range.



Deviations of [Cl^-], and namely changes in [Na^+]/[Cl^-] ratio indicate metabolic acid-base disorders.

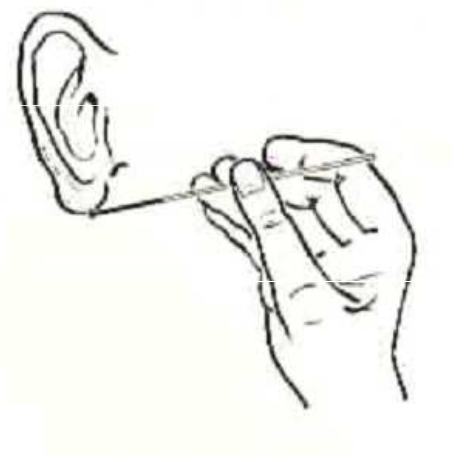
Both acidaemia and alkalaemia may be causes of life-threatening changes in K^+ concentrations.

Anaerobic sampling of blood

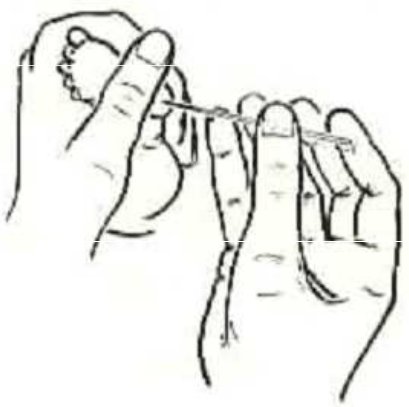
Arterial blood from a. femoralis

"Arterialized" capillary blood

from the ear lobe



from the heel in infants



The blood sample is usable during the limited time period:

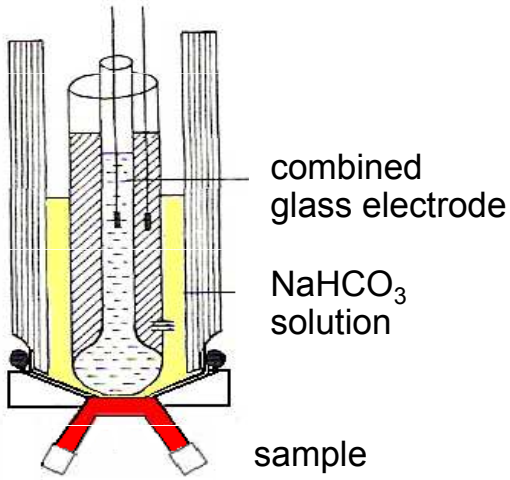
up to **4 hours after sampling, if chilled in ice-cold water**;

at room temperature, measurement of pO_2 within **5 minutes**,

the other acid-base parameters within **30 minutes**.

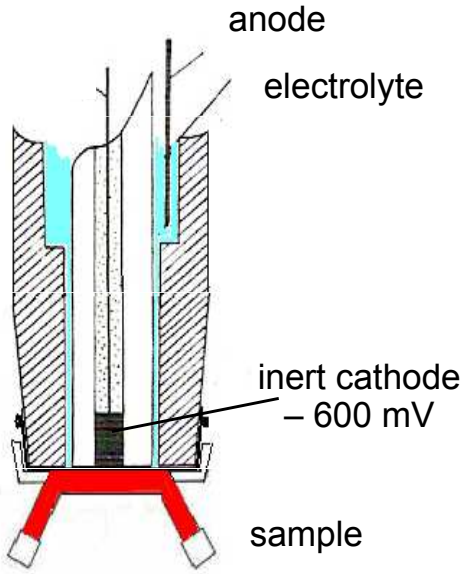
Direct measurement of pCO_2 and pO_2

pCO_2 electrode



CO_2 diffuses from the sample through silicone membrane into the $NaHCO_3$ solution, the change in pH is measured (combined glass electrode).

pO_2 electrode
(Clark's oxygen electrode)



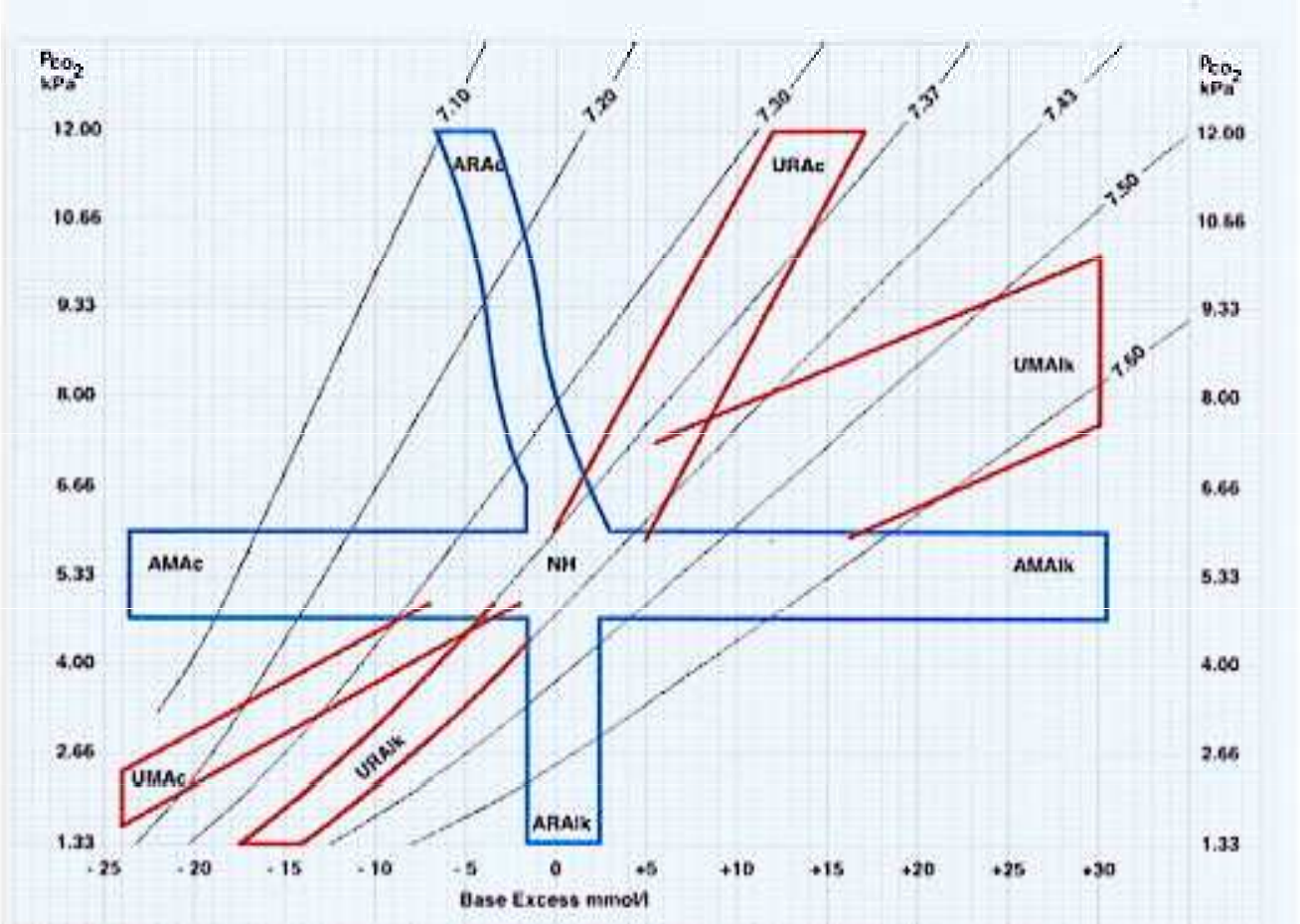
O_2 diffuses from the sample through polypropylene membrane into the electrolyte, where it is reduced to peroxide ion O_2^{2-} (principle of polarography); electric current proportionate to pO_2 is measured.

Evaluation of an acid-base disturbance type and its compensation
(the concept of P. Astrup and O. Siggaard-Andersen)

Entries:

pCO_2 / kPa - measured

BE / mmol/l
calculated or acquired
from the S.-A. nomogram

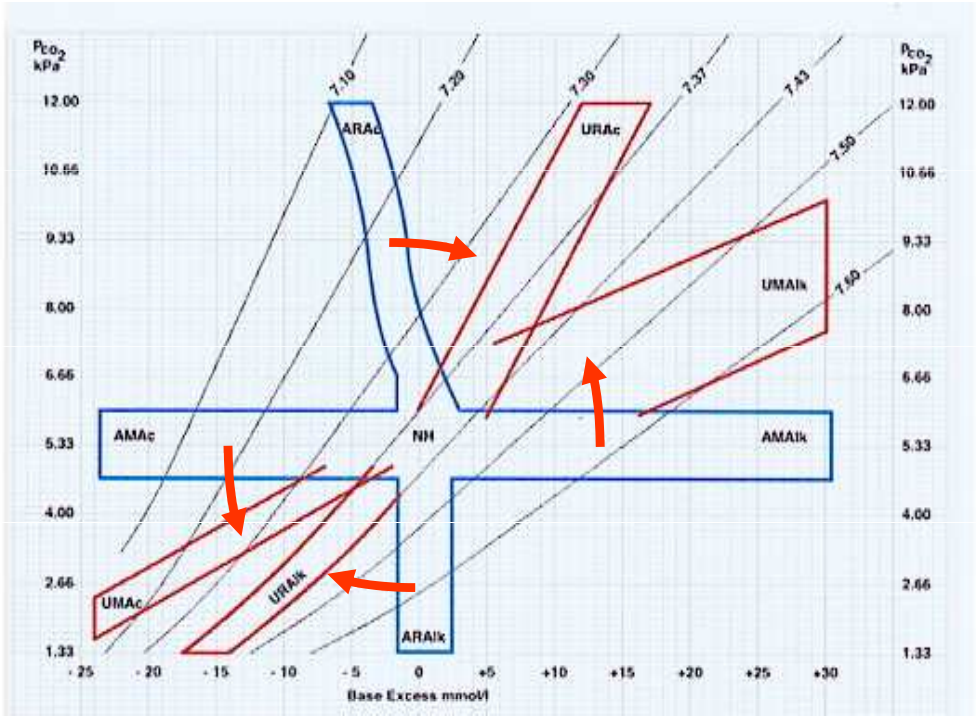


ARAc – acute respiratory acidosis **ARAlk – acute respiratory alkalosis**
AMAc – acute metabolic acidosis **AMAlk – acute metabolic alkalosis**
U – stabilized disturbance
NH – area of physiological values

Engliš, M.: Prakt. Lék. 1972: 52, 558

Acute respiratory or metabolic disorders – **blue** areas

Stabilized respiratory or metabolic disorders
(effect of compensatory activities of the lung or the kidney) – **red** areas



The primary **respiratory disorder** leads to a compensatory change in HCO_3^- reabsorption by the kidney, which reaches its maximal effectivity in **5 – 7 days**.

In the primary **metabolic disorder**, a change in blood pH evokes a rapid change in the pulmonary ventilation rate (during **2 – 12 hours**).

Combined acid-base disorders

Mixed respiratory and metabolic acid-base disorders result in a greater change in blood pH than simple disorders.

Metabolic acid-base disorders may sometimes escape our attention, if they are caused by changes in independent variables of opposite direction. In these **mixed metabolic disturbances**, the blood pH and [HCO_3^-] or base excess can have normal values, in spite of this the composition of body fluids is changed remarkably.

For example, a starving patient (→ ketoacidosis) is losing chlorides due to intensive vomiting (→ hypochloridaemic alkalosis) and the mixed disturbance may pass unnoticed.

New concept of the evaluation of metabolic components in acid-base disorders was proposed by Stewart, Fencl, et al.

It is based on the idea, that acid-base status is determined by **three independent variables**: pCO_2, strong ion difference, and non-volatile weak acids (proteins and phosphates).

Interpretive concept of acid-base disorders (Stewart, Fencl, et al.)

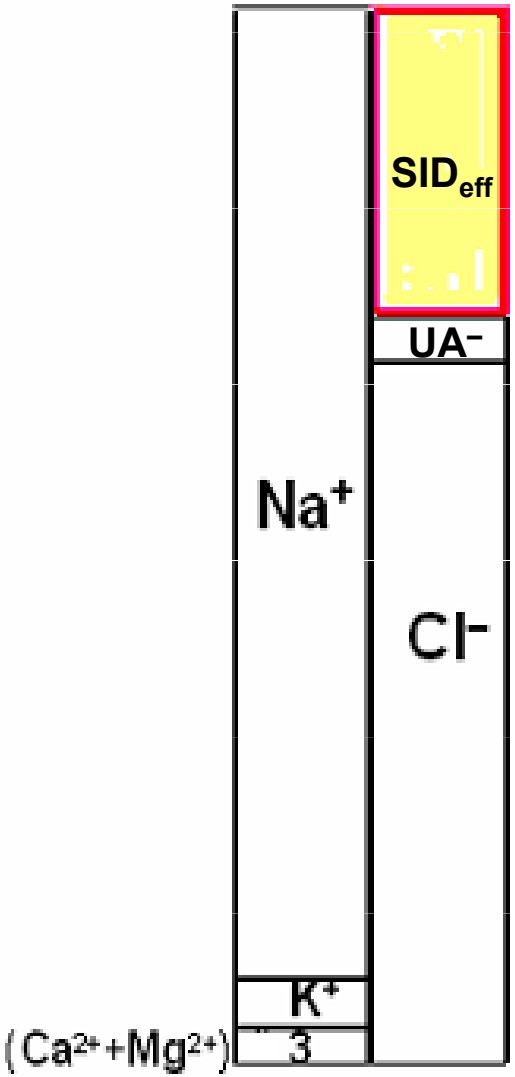
Acid-base status of the body is determined
by **three independent variables:**

- **pCO_2**,
- **balanced concentrations of "strong" ions** expressed by means of the strong ion difference (SID_{eff}), and
- concentration of **non-volatile weak acids** that act as buffer bases (plasma proteins and inorganic phosphates).

Primary acid-base disorders thus can be classified as

- **respiratory**, initiated by an increase or decrease in pCO_2, and
- **metabolic (non-respiratory)** that may have their causes in
  - **abnormal values of SID_{eff}** due to
    - water excess or deficit (changes in both SID and $[Na^+]$, or
    - imbalance of strong ions (excess or deficit of chloride ions, excess of unmeasured anions;
  - **changes in non-volatile buffer bases** caused by
    - decrease in plasma albumin concentration, and
    - decrease or increase in plasma phosphate concentration.

Effective "strong ion difference" (SID_{eff})



$$SID_{eff} = [Na^+] + [K^+] + 3 - [Cl^-] - [UA^-]$$

The value SID_{eff} determinates the concentration of plasma buffer bases BB_p.

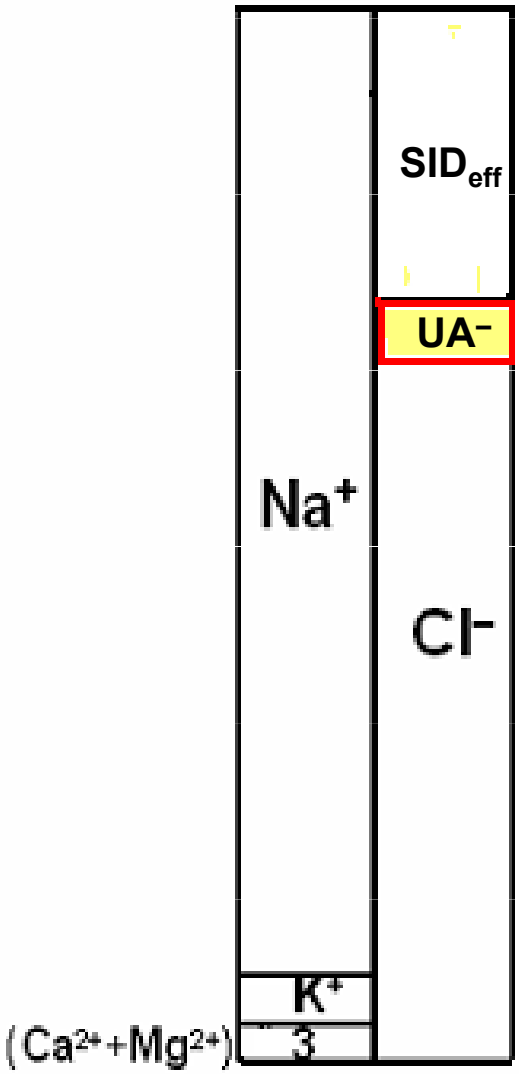
Normal range = 42 ± 3 mmol / l)

SID_{eff} then can be calculated from measurable concentrations of plasma buffer bases::

$$SID_{eff} = [HCO_3^-] + 0.28\ \mathbf{Alb}(g/l) + 1.8\ [P_i]$$

Strong ion **ratio** $[Na^+]+[K^+] / [Cl^-]$ (normal value 1.35 – 1.43) is occasionally used as another sign of strong ion imbalance that is typical for hyperchloridaemic acidosis or hypochloridaemic alkalosis).

Unmeasured anions (UA^-)



$$[UA^-] = [Na^+] + [K^+] + 3 - [Cl^-] - SID_{eff}$$

Normal range **8 ± 2 mmol / l**

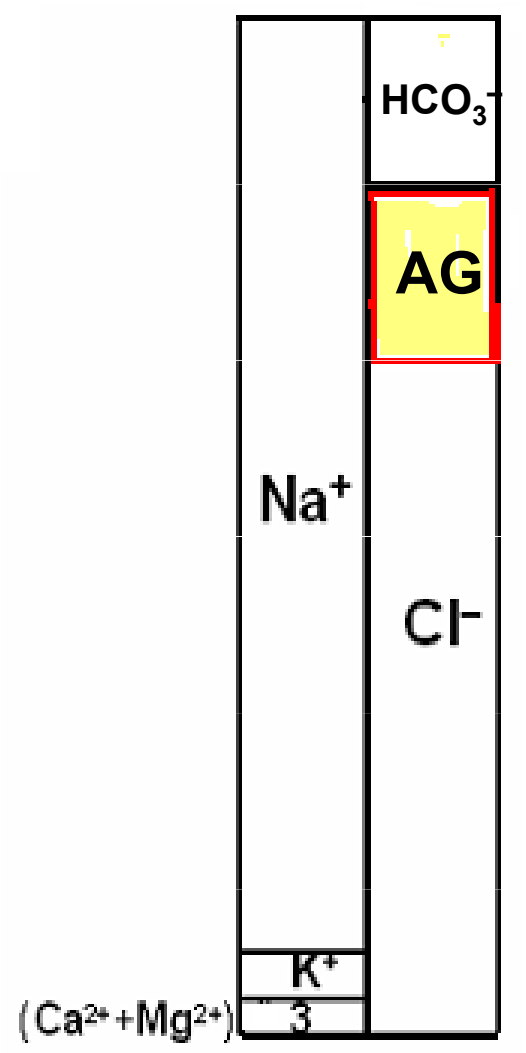
Components: **sulfate**
lactate
acidic ketone bodies
the other carboxylic acids

Corrected value for water content:

$$[UA^-]_{corr} = [UA^-] \times 140 / [Na^+]_{measured}$$

Anion gap (AG) – a simple accessory parameter

that may call an attention to the **possible increase in UA^-:**



$$AG = [Na^+] + [K^+] + 3 - [Cl^-] - [HCO_3^-]$$

Usual values 19 ± 2 mmol/l.

AG represents the "space" filled in by
unmeasured anions,
proteins, and
phosphates.

In hypoproteinaemia, AG value should be corrected:

$$AG_{corr} = AG_{observed} + 0.25 \times (Alb_{ref} - Alb_{measured})$$

(decrease in albumin by 1 g/l enables an increase in HCO_3^- by 1 mmol/l)

Assessment of the metabolic components in acid-base disorders

(Stewart and Fencl)

Entries:	pH	
	Na^+	mmol/l
	K^+	mmol/l
	Cl^-	mmol/l
	HCO_3^-	mmol/l
	phosphate	mmol/l
	albumin	g/l

Modification of laboratory data:

– **correction of** [Cl^-] value for the actual water content:

$$[Cl^-]_{corr} = [Cl^-] \times 140 / [Na^+]_{measured}$$

– calculation of SID_{eff}:

$$SID_{eff} = [HCO_3^-] + 0.28\ Alb(g/l) + 1.8\ [Pi]$$

– calculation of UA^- value and its **correction** for actual water content:

$$[UA^-]_{corr} = ([Na^+] + [K^+] + 3 - [Cl^-] - SID_{eff}) \times 140 / [Na^+]_{measured}$$

– calculation of the **electric charge of albumin** (dependence on pH):

$$[Alb^-] = 0.125 \times Alb(g/l) \times (pH - 5.17)$$ or the value found in the table 1

– calculation of the **electric charge of phosphates** (dependence on pH):

$$[P_i^-] = [P_i] \times (0.309 \times pH - 0.469)$$ or the value found in the table 2

Table 1 **Electric charge of albumin** (mmol/l) – the dependence on pH

g / l	pH			
	7,00	7,20	7,40	7,60
10	2,3	2,5	2,8	3,0
20	4,6	5,1	5,6	6,1
30	6,9	7,6	8,4	9,1
40	9,2	10,2	11,2	12,2
50	11,4	12,7	13,9	15,2

Table 2 **Electric charge of phosphates** (mmol/l) – the dependence on pH

mmol / l	pH			
	7,00	7,20	7,40	7,60
0,5	0,8	0,9	0,9	0,9
1,0	1,7	1,8	1,8	1,9
1,5	2,5	2,6	2,7	2,8
2,0	3,4	3,5	3,6	3,6
2,5	4,2	4,4	4,5	4,7
3,0	5,1	5,3	5,5	5,6
3,5	5,9	6,1	6,4	6,6
4,0	6,8	7,0	7,3	7,5

Quantitative assessment of the ABS metabolic components (Stewart and Fencl)

Analyte		Reference value	Real value	Acidosis		Alkalosis	
Na^+	mmol/l	140		–		+	
$Cl^-_{corr.}$	mmol/l	102		+		–	
$UA^-_{corr.}$	mmol/l	8.0		+		–	
$Phosphate^-$	mmol/l	2.0		+		–	
$Albumin^-$	mmol/l	12.0		+		–	

Engliš, M., Jabor, A., Kubáč, P., Červinka, I.:KBM 2006, 4, 225

Examples:

Analyte		Reference value	Real value	Acidosis		Alkalosis	
Na^+	mmol/l	140		–	excess water	+	dehydration
$Cl^-_{corr.}$	mmol/l	102		+	diarrhoea	–	vomiting, HCl loss
$UA^-_{corr.}$	mmol/l	8.0		+	lactate, ketone bodies,, formate, glycolate	–	-
$Phosphate^-$	mmol/l	2.0		+	renal failure	–	-
$Albumin^-$	mmol/l	12.0		+	-	–	hypoalbuminaemia

Engliš, M., Jabor, A., Kubáč, P., Červinka, I.:KBM 2006, 4, 225