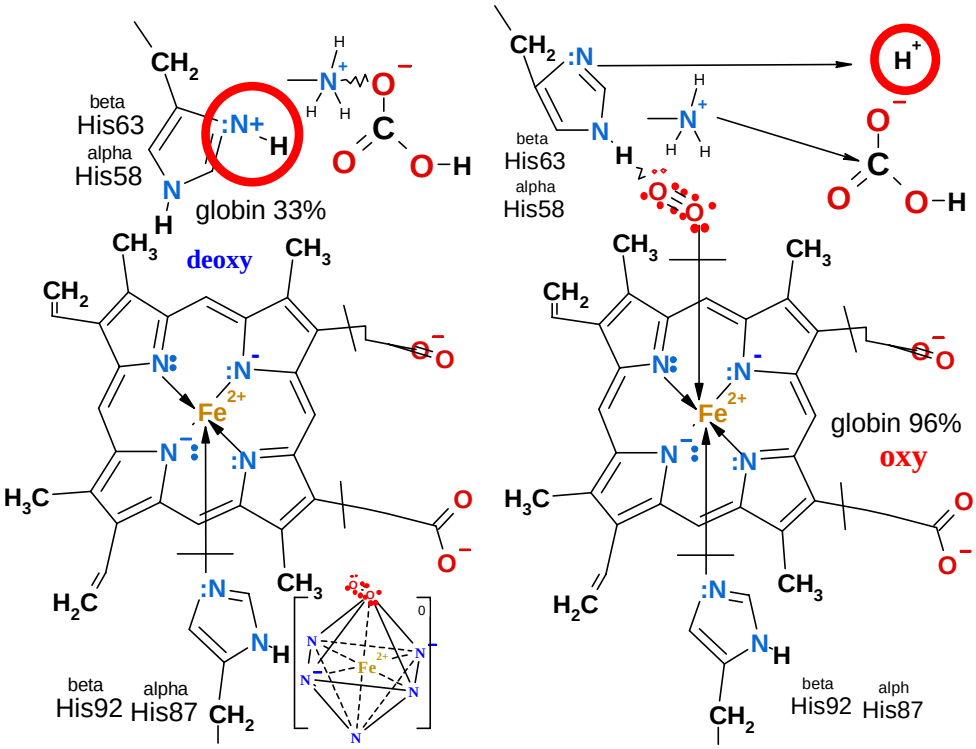
Shuttle deoxy - oxy **hemoglobin** with Carbonic Anhydrase enzyme in **O2** , **CO2** metabolism stabilize physiologic pH=7.36 and oxygen arterial concentration $[O_{2blood}]=6\cdot10^{-5}$ M

I) Oxygen O_2 from **AIR** 20.95% $O_2\uparrow$gas assimilation reaction dissolution in water to form O_{2aqua} exothermic ΔH_r=-55,7 $^{kJ}/_{mol}$ and exoergic ΔG_r= -27,7 $^{kJ}/_{mol}$ as water soluble **1)** $O_{2AIR}+H_2O \Leftrightarrow H_2O+O_{2aqua}$ +Q+ΔG. Penetrate in Human body through aquaporins osmosis by concentration gradient $[O_2]=9{,}768\cdot10^{-5}$ M to venous blood $[O_2]=1{,}85\bullet10^{-5}$ M ΔG_{O2}= RT**ln**($[O_{2Blood}]/[O_{2aqua}]$)= - 4,29 $^{kJ}/_{mol}$ exoergic movement: 2) $O_{2aqua}+H_2O \xrightarrow{Aquaporins} H_2O+O_{2aqua}+\Delta G$ ΔG_{H2O} =RT**ln**($[H_2O]_{right}/[H_2O]_{left}$)= -8,3144*310,15***ln**(0,305/0,2)= -1.088 $^{kJ}/_{mol}$ exoergic ΔG_{O2+}= -5,379$^{kJ}/_{mol}$. $4O_{2aqua}$ from blood plasma adsorbs **deoxy** hemoglobin Hb_T of inspired fresh AIR releases four protons $4H^+$, $4HCO_3^-$: $4O_{2aqua}+(H^+His63,58)_4Hb_T$·salt bridges$(HCO_3^-)_4 \Leftrightarrow Hb_R(O_2)_4+4H^++4HCO_3^-$ stabilizing arterial concentration $[O_2]=6\cdot10^{-5}$ M. $[O_{2Blood}]=6\bullet10^{-5}$ M concentration sensitive equilibrium $(H^+His63,58)_4Hb_T \leftrightarrow Hb_R(O_2)_4$ shift to right regulates erythrocytes glycolysis metabolite BPG^{5-} as two **phosphate 2,3-esters** G^- $H_2COPO_3^{2-}$-$HCOPO_3^{2-}$-COO^- glycerate dihydroxy acid salt with homeostasis concentration $[BPG^{5-}]$=5 mM, so BPG^{5-} pushed out of **cavity** to stabilize and store reserves 459 times higher as arterial blood concentration $[O_{2Blood}]=6\bullet10^{-5}$M amount $[O_{2amount}]=459*6\bullet10^{-5}$ M=0,02754 M.



O2Solutions.pdf. Oxygen adsorbs by donor-acceptor bond on iron(II) Fe^{2+} in coordination center of heme and releases four protons H^+ $Hb_R(O_2)_4$**+4H$^+$**. Proton water sticks $H^++H_2O \rightarrow H_3O^+$ forms hydroxonium ion. ***In tissues*** desorbed oxygen $[O_{2desobed}]$ restore oxygen concentration $[O_2]=6\cdot10^{-5}$ M in blood plasma 459 times and deoxy–hemoglobin capture four protons H^+ $(H^+His63,58)_4Hb_T$ so keeps continuously pH=7,36±0,01.

Oxygen desorbed Krebs cycle converts to mitochondrial oxidative phosphorylation product CO_{2aqua}. **II)** pathway with carbonic anhydrase (**CA**) shift to right concentration gradient CO_2 produces amount 0,0339 M HCO_3^-. **Shuttle deoxy** hemoglobin Hb_T capture $[H^+]$=0,0275 M. So is stabilized constant pH=7,36±0,01 value.

II) $Q_{aqua}+CO_{2aqua}+2H_2O \xleftarrow{CA} H_3O^++HCO_3^- \xleftarrow{Membrane} H_3O^++HCO_3^- \Leftrightarrow H_2O+H_2CO_3+Q_{gas} \leftrightarrow H_2O+CO_2\uparrow_{gas}+H_2O$.
endothermicΔH_r= +9.75 $^{kJ}/_{mol}$; athermic ΔH_r= 0 $^{kJ}/_{mol}$; exothermic ΔHr=-9.76 $^{kJ}/_{mol}$; endothermic ΔH_r= +20.3 $^{kJ}/_{mol}$;
endoergic ΔG_r= +58.4 $^{kJ}/_{mol}$; exoergic ΔG_r=-22.5-1,96 $^{kJ}/_{mol}$;; exoergic ΔGr=-58.2 $^{kJ}/_{mol}$; exoergic ΔG_r= -8,54 $^{kJ}/_{mol}$;
II) $Q_{aqua}+CO_{2aqua}+2H_2O \xleftarrow{CA} H_3O^++HCO_3^- +Q \xleftarrow{Membrane} H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$.
endothermicΔH_r= +9.75 $^{kJ}/_{mol}$; endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; summary endothermic ΔH_r= +64,25 $^{kJ}/_{mol}$;
endoergic ΔG_r= +58.4 $^{kJ}/_{mol}$; exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$; summary exoergic ΔG_r= -23,7 $^{kJ}/_{mol}$;

Shuttle is venous **deoxy** Hb_T , adsorbs four molecules $4O_2$ from fresh AIR, acidify water medium with $4H^+$, promoting CO_2 breathe out: Each H^+ and HCO_3^- ion amount $[H^+]=459*6\bullet10^{-5}$ M =0,0275 M=$[HCO_3^-]$ shifts equilibrium to right $H^+ +HCO_3^-+ Q \leftrightarrow H_2O +CO_2\uparrow_{gas}$ via membrane channels. So pH=7,36 remains constant, as one bicarbonate ion and one hydrogen ion produce one CO_2 right side.

The epithelial cell surface of ***lungs*** has the specific building surface as square area is: S=950 nm x 950 nm= 0.9 μm^2 on super thin 0.6 nm layer within water small volume: $0.5415\bullet10^{-3}$ μm^3 = $0.5415\bullet10^{-18}$ L. Created acidity in thin water layer volume increases up to pH=5.5 if one proton H^+ crosses the membrane channels reaching the surface so hydrogen ion concentration is: $[H_3O^+]=10^{-pH}=10^{-5.5}$ M. Respiration of fresh AIR in lungs Hemoglobin released protons H^+ during oxygen adsorbtion for total amount concentration:

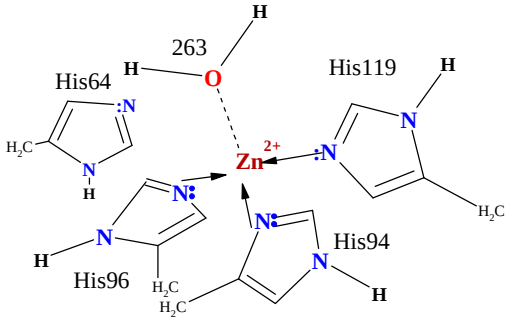
$[O_{2adsorbed}]=[H_3O^+]=459*6\bullet10^{-5}$ M= 0,02754 M forms hydrogen ion $[H_3O^+]_{right}/[H_3O^+]_{left}=10^{-5,5}/0{,}0275$ concentration gradient, which drives exoergic **ΔG** = -22,5 $^{kJ}/_{mol}$ proton movement through epithelial cell membrane proton channels: $H_3O^+_{left} \xleftarrow{proton\_channel} H_3O^+_{right}$ **+ΔG**. General process $H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$ require heat supply endothermic ΔH=54,5 $^{kJ}/_{mol}$ to drive spontaneous ΔG= -82,0679 kJ/mol products evaporation $CO_2\uparrow_{gas}$ and $H_2O\uparrow_{gas}$ keeping moisture H_2O on surface of membrane. Hydrogen ions water acidity shift endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$ and exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$ decomposition $H_3O^++HCO_3^-$ breath out to AIR $CO_2\uparrow_{gas}$ with $H_2O\uparrow_{gas}$:
endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; $H_3O^++HCO_3^- +Q \xleftarrow{Membrane} H_2O+CO_2\uparrow_{gas}+H_2O\uparrow_{gas}$ + ΔG_r= -82,1 $^{kJ}/_{mol}$. exoergic .

Brønsted Acid/Base **CA** and hemoglobin **shuttle** enzymes of O_2 , CO_{2aqua} /HCO_3^- + H^+

Enzyme **Carbonic anhydrase** (**CA**) made acid/base equilibrium H_2O-CA-CO_2/HCO_3^- + H_3O^+

There are **shuttle** buffer systems, that act in the human organism and allow **pH** of the organism to be stabilized constant in narrow interval allowed changes (**pH** = $7.36^{+0,02}_{-0,01}$) despite the fact, that organism produces great amount of metabolic [CO_{2Krebs}]= 0,0275 M. The **CA** made amount of acidic products is [H_3O^+]=[HCO_3^-]=0,01695 M compensated by buffer solution. **CA** buffer of blood are connected to **shuttle** hemoglobin captured proton H^+ by oxygen O_{2aqua} desorbtion due to Krebs product CO_{2aqua} target cells ***in tissues***:

Hydrogen carbonate buffer system carbonic anhydrase equilibrium keeps weak acid CO_{2aqua} and bicarbonate ions at homeostasis normal amounts [HCO_3^-]=0.0154 M, [CO_{2aqua}]=0.0076 M, referring to 56,23 mL (50-60 mL) released volume CO_2 from 100 mL blood as *alkaline reserve* 2,036 / 1 in clinic evaluation.



Carbon dioxide forms by oxidation of carbohydrates, of fats and of proteins. Bicarbonate is created as product in hydration $2H_2O$ of CO_{2aqua} by CA enzyme Zn^{2+} ion active coordination center. It's location in enzyme carbonic anhydrase Zn^{2+} ion coordination pocket:

$$CO_{2aqua} + 2H_2O \rightleftharpoons CA(Zn^{2+}) \rightleftharpoons H_3O^+ + HCO_3^-$$

$$H_2O+CO_{2aqua}+(Zn^{2+}<=OH^-CA+H^+)=>HCO_3^-+(H_2O(263)=>Zn^{2+}\ CA) + H^+$$

$Hb_R(O_2)_4$+4H^+ ⇔ 4O_{2aqua} + $(H^+His63,58)_4Hb_T$ stabilizing arterial concentration [O_2]=6·10^{-5} M in blood. Deoxy hemoglobin $(H^+His63,58)_4Hb_T$ capture four protons 4 H^+ at histidine residues and 4 HCO_3^- in venous hemoglobin form of erythrocytes deoxy $(H^+His63,58)_4Hb_T$ (Tense state). In **lungs shuttle** absorbs oxygen in arterial oxy hemoglobin $(O_2His63,58)_4Hb_R$ (**Relax** state) releasing 4 H^+ and 4 HCO_3^- .

1) First of four human buffer systems is enzyme **CA** made Brønsted Acid/Base endothermic equilibrium: **Q**+CO_{2aqua}+2H_2O $\leftarrow^{CA}\rightarrow$$H_3O^+$+$HCO_3^-$ which consume heat **Q** of Krebs cycle complexes exothermic reactions. Shift to right supported by high water 2H_2O concentration [H_2O]^2 =(993,36/18,0153)^2 = 55,139^2 =3040,4 and by low stabilized pH=7,36±0,01 of hydrogen ions H_3O^+ concentration [H_3O^+] =$10^{-7,36}$ M in products. CO_{2Krebs} as bicarbonate salt bridge linked HCO_3^-…$H_3{}^+N$— and equal produced protons [H^+]=[CO_{2Krebs}]=0,0275=[HCO_3^-] captures deoxy $(H^+His63,58)_4Hb_T$ **shuttle** and brings to ***lungs***. ***Lungs*** evaporates $CO_2\uparrow_{gas}$+ $H_2O\uparrow_{gas}$ endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$, but exoergic ΔG_r= -82,1 $^{kJ}/_{mol}$:

$$H_3O^+ + HCO_3^- + Q \leftarrow^{Membrane}\rightarrow H_2O + CO_2\uparrow_{gas} + H_2O\uparrow_{gas} + \Delta G_r\ .$$

Symbol $(H^+His63,58)_4Hb_T$ to a **Shuttle** molecule of deoxy hemoglobin is inconvenient to write every time the complicated structure of hemoglobin. Deoxy hemoglobin is capturing and oxy hemoglobin completely deprotonated 4 H^+ and 4 HCO_3^-. Equilibrium is oxygen concentration [O_2]=6·10^{-5} M sensitive:

$$4O_{2aqua} + (H^+His63,58)_4Hb_T \leftarrow [O_{2aqua}]=6\cdot10^{-5}\ M \rightarrow (O_2His63,58)_4Hb_R + 4H^+.$$

Lungs venous blood hemoglobin saturation with oxygen 459 times restore circulated arterial blood [O_2]=6·10^{-5} M amount in one liter O2Solutions.doc Adsorbed four 4O_{2aqua} $(O_2His63,58)_4Hb_R$+4H^++4HCO_3^- in products release four protons 4 H^+ and bicarbonate ions 4 HCO_3^- , promoting evaporation $CO_2\uparrow_{gas}$+$H_2O\uparrow_{gas}$ on ***lungs*** epithelia surface, and removing out of organism [H^+]=459*6*10^{-5} =0,0275 M amount H^++H_2O=>H_3O^+ , that is equal to total by respiration evaporated [$CO_2\uparrow_{gas}$] =0,0275 M amount.

Shift to the left $(O_2His63,58)_4Hb_R$+4H^++4HCO_3^- from deoxy captured **shuttle** $(H^+His63,58)_4Hb_T$ oxygen depending concentration [O_2]=6·10^{-5} M adsorbtion-desorbtion equilibrium explain pH stabilization at 7.36.

That explain, why pH is not changed, despite Krebs cycle acid $CO_{2\ aqua}$ product which involved in **CA** equilibrium. Henderson-Haselbalh homeostasis pH value expression leave the ratio[HCO_3^-]/[CO_{2aqua}]=2,0263 practicaly unchanged as intact both concentrations bicarbonate [HCO_3^-] and carbon dioxide [CO_{2aqua}]:

7.36 = pH = pK+log([HCO_3^-]/[CO_{2aqua}])=7.0512+log([HCO_3^-]/[CO_2]) and anti logarithm is being alkaline reserve [HCO_3^-]/[CO_{2aqua}] =$10^{(pH-pK)}$ = $10^{(7.36-7.0512)}$ = $10^{0.3088}$ = 2,0361/1. ***Lungs*** when in venous blood erythrocytes deoxy $(H^+His63,58)_4Hb_T$ (Tense) **Shuttle** hemoglobin by oxygen O_{2aqua} adsorbtion release of protons H^+ and HCO_3^- so in ***Lungs*** evaporates carbon dioxide $CO_2\uparrow_{gas}$ as breathed out in AIR.

In such a way two equilibria stabilize arterial oxygen concentration [O_{2aqua}]=6·10^{-5} M with **shuttle** hemoglobin by oxygen adsorbtion-desorbtion and **CA** buffer system made value pH=7,36 with Krebs cycle drive the exchange metabolism of O_2 and CO_2 respiration to interface human body / environment.

2) Second buffer system, that is present in blood, is the protein buffer system. This one has to be explained a little more, as it differs from the usual buffer systems that are composed from weak acid/salt or weak base/salt. Like hemoglobin **Hb** proteins are long chain polypeptides of amino acids with four type protolytic acid groups:

Amino Acid	pK_{aCOOH}	pK_{aNH3+}	$pK_{aRgroup}$
Isoleucine	2.36	9.68	
Valine	2.32	9.62	
Leucine	2.36	9.60	
Phenylalanine	1.83	9.13	
Cysteine	1.96	10.28	8.18
Methionine	2.28	9.21	
Alanine	2.34	9.69	
Proline	1.99	10.96	
Glycine	2.34	9.60	
Threonine	2.11	9.62	
Serine	2.21	9.15	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07
Histidine	1.82	9.17	6.00
Aspartate	1.88	9.60	3.65
Glutamate	2.19	9.67	4.25
Asparagine	2.02	8.80	
Glutamine	2.17	9.13	
Lysine	2.18	8.95	10.53
Arginine	2.17	9.04	12.48

-COO⁻ deprotonated carboxyl negative anion salt groups, protonated positive charged ammonium groups **-NH$_3^+$**, neutral phenolic acid **–OH** and **-SH** neutral sulfhydryl groups.

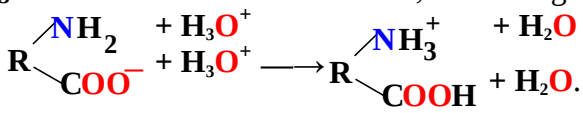
In physiologic medium pH=7,36 ±0.01
Carbonic acid groups deprotonated negative charged **-COO⁻** and amino groups **R-NH$_3^+$** protonated positive charged.
Table given maximal $pK_{a\text{-}COOH^-}$ value smaller about 7,36:
$pK_{a\text{-}COOH^-}$=4.25<7,36 and
given smallest $pK_{a\text{-}NH3+}$ value grater about 7,36 < 9,04 = $pK_{a\text{-}NH3+}$
20 amino acids have four protolytic pK_a equilibria in 47 groups:

1. **R-COOH** ⇔**R-COO⁻** +**H$^+$**, 22 groups of 47
2. **R-NH$_3^+$** ⇔**R-NH$_2$** + **H$^+$** 22+1 group of 47
3. **Tyrosine**-phenol-**OH**⇔**Tyrosine**-phenolate-**O⁻** +**H$^+$** one group,
4. **Cysteine-SH** ⇔**Cysteine-S⁻⁻** + **H$^+$** one group .

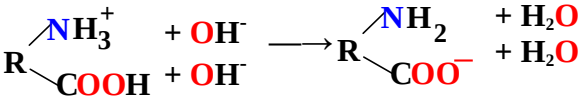
NpK_a number of parallel protolytic equilibria average pK_a value is calculated as $pK_a=(\Sigma\ pK_{a\ R\ group}+ pK_{a\text{-}NH3+}+ pK_{a\text{-}COOH})/NpK_a$

In *Ostwald's dilution law* calculates one the pH of solution at concentration C logarithm: $pH=\frac{pK_a - \log C}{2}$ =.....

14th page: http://aris.gusc.lv/BioThermodynamics/Data_bookSpring2015CT.pdf properties and acidic properties protonated ammonium groups **–NH$_3^+$**. If an acid is added to solution, containing protein like hemoglobin Hb, the **H$_3$O$^+$** ions will react with basic amino group and basic carboxylate group The strong acid **H$_3$O$^+$** will be transformed into water the weak base **H$_2$O** .

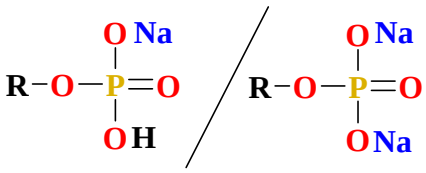


If a strong base is added to protein-containing solution, the **OH$^-$** ions react with the carboxylic groups and the strong base **OH$^-$** will be transformed into water the weak acid **H$_2$O**.

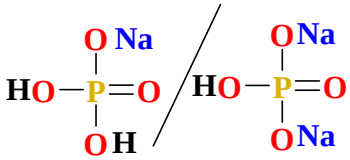


3) Biological important phosphate buffer system **NaH$_2$PO$_4$/Na$_2$HPO$_4$** pK=7,199 we will study as practical work.

4) Biological ubiquities exist besides the inorganic phosphate buffer system, buffer systems of the organic esters of phosphoric acid so as ATP (adenosine tri phosphate), ADP (adenosine di phosphate), CTP, CDP, GTP, GDP, TTP, TDP, UTP, UDP, NADH B_3 vitamin, $FADH_2$ B_2 vitamin, phospho proteins, glucose phosphate, fructose phosphate, etc. :



If there are any difficulties to understand the structure of last two groups of compounds , remember, that phosphoric acid can be shown in structure as in the ester of phosphoric acid one of the hydrogen atoms is replaced by an organic radical. Practically the buffer system consists of a mono substituted and bi substituted salts of the ester. Likely as for phosphates **NaH$_2$PO$_4$/Na$_2$HPO$_4$**.



Not all of these **4** buffer systems act in the same organism body water solutions.

In *erythrocytes* main are bicarbonate buffer with **shuttle** hemoglobin-based proton oxygen **O$_{2aqua}$** sensitive exchange: **(O$_2$**His63,58)$_4$**Hb$_R$** **+4H$^+$**←[**O$_{2aqua}$**]=6·10^{-5} M → **4O$_{2aqua}$** + **(H$^+$**His63,58)$_4$**Hb$_T$** . Krebs cycle product **CO$_{2aqua}$** exchanged to bicarbonate buffer solution: **Q**+**CO$_{2aqua}$**+**2H$_2$O** ←$\overset{CA}{\rightarrow}$**H$_3O^+$**+**HCO$_3^-$**.

In blood *plasma* dominate enzyme **CA** bicarbonate **pH=7.36**±0,01, protein and phosphate buffer solutions.

In sweat, urine and digestive apparatus dominates bicarbonate system and phosphate system is too present.

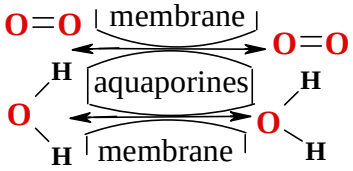
Besides the normal "chemical" mechanisms of buffer action in maintaining constant **pH=7.36**±0,01, with deoxy hemoglobin **(H$^+$**His63,58)$_4$**Hb$_T$** (Tense state), oxy hemoglobin **(O$_2$**His63,58)$_4$**Hb$_R$** (**Relax** state) and with carbonic anhydrase **CA** driven bicarbonate buffer systems are a joint physiological mechanism of action, which carries out the exchange of breathed in **O$_2$** and breathed out **CO$_2$** between AIR in ***lungs*** and tissues on interface human body / environment**.**

Human **shuttle hemoglobin-bicarbonate** buffer system and Krebs cycle driven respiration from AIR O_2 and breathed out CO_2 action **physiologic** mechanism

Before we have to order three molecules involved in the buffer systems. The **shuttle oxy** hemoglobin, second is **carbonic anhydrase CA** with constant value pK=7.0512 and **shuttle deoxy** hemoglobin:

oxy $Hb_R(O_2)_4$ + $4H^+$ <=> **deoxy** $(H^+His63,58)_4Hb_T$ + $4O_{2aqua}$, where completely deprotonated 4 H^+ **oxy** Hb_R but **deoxy** hemoglobin Hb_T capturing four protons 4 H^+ and 4 HCO_3^- as desorbing four oxygen $4O_{2aqua}$ molecules. **Shuttle** and **carbonic anhydrase CA** stabilize exchange process from AIR O_2 to breathed out in to AIR CO_2.

Two **I** and **II pathways** are happen of gradual reactions: **I)** $O_{2AIR} + H_2O \overset{aquaporin}{\rightleftharpoons} H_2O + O_{2aqua}$



Process ***in lungs*** **I) Pathway** first reaction on cell wall membrane aquaporins penetrating water H_2O with oxygen O_{2aqua} by rate 10^9 sec^{-1} reach erythrocyte cells and oxygen concentration in blood plasma significant changes from **venous** blood $[O_2]$=1,85•10^{-5} M to arterial blood plasma in water becomes $[O_2]$=6·10^{-5} M.

Bisphospho glycerate BPG^{5-} drive hemoglobin O_2 adsorbtion⇔desorbtion equilibrium sensitive to concentration. It saturates arterial **shuttle oxy** hemoglobin with oxygen 459 times over $[O_2]$=6·10^{-5} M stored reserve 0,0275 M and pushed out of **shuttle deoxy** hemoglobin bisphospho glycerate BPG^{5-} releases $4H^+$ and 4 HCO_3^-.

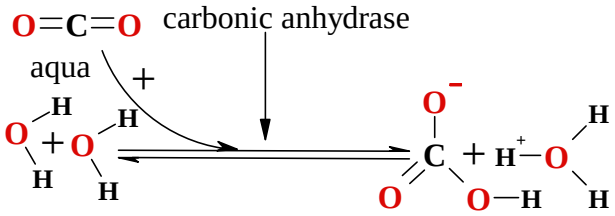
$4O_2$+$(H^+His63,58)_4betaVal1(NH_3^+PO_4^{2-})_2Hb_TG$-↔$(His63,58)_4Arg^+His^+betaVal1(NH_3^+)_2Hb_R(O_2)_4$+$4H^+$+$BPG^{5-}$

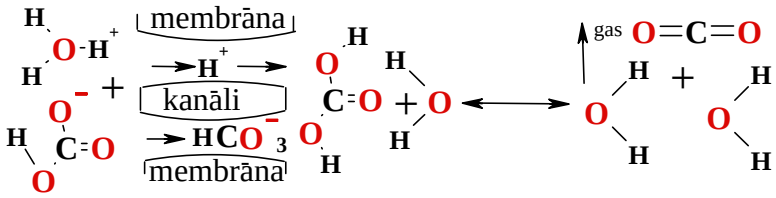
Each adsorbed oxygen molecules O_{2aqua} on hemoglobin releases proton H^+ which increases acidy on epithelial cell surface of ***lungs***. The epithelial cell surface of ***lungs*** has the specific building: super thin 0.6 nm water layer on surface S=950 nm x 950 nm= 0.9 μm^2 as square within small volume $0.5415•10^{-3}$ μm^3=$0.5415•10^{-18}$ L in liters created acidity increases up to pH=5.5 if one proton crosses the membrane channel reaching the surface and that cause fast decomposition of carbonic acid H_2CO_3 to evolving CO_2↑ gas is breathed out to AIR.

II) pathway start from metabolic Krebs cycle oxidation with oxygen O_{2aqua} produces CO_{2aqua} ***in tissues*** cells:

Q_{aqua}+ CO_{2aqua} + $2H_2O$ ←$\overset{CA}{\longrightarrow}$$H_3O^+$+$HCO_3$- $\overset{membrane}{\rightleftharpoons}$ H_2O+H_2CO_3+ $Q_{(gas)}$ ⇌ H_2O + CO_2↑$_{gas}$ + H_2O

Enzyme Carbonic Anhydrase (**CA**) drive to right equilibrium mixture in three gradual reactions first is endothermic: **Q** + $2H_2O$ + CO_{2aqua}←$\overset{CA}{\longrightarrow}$$H_3O^+$ + HCO_3^-.





Second gradual **exothermic** reaction forms Carbonic acid H^++HCO_3^-→$\overset{membrane}{\longrightarrow}$$H_2CO_3$+**Q**. Proton H^+ and bicarbonate HCO_3^- through channels drive concentration gradients for $[H_3O^+]_{right}/[H_3O^+]_{left}=10^{-7,36}{}_{right}/0,0339$ and for bicarbonate ions $[HCO_3^-]_{right}/[HCO_3^-]_{left}$=0,0154 M_{right}/0,0339 M_{left} breathing out of organism to AIR gas CO_2↑$_{gas}$.

Third gradual reaction on ***lung*** epithelial cell surface (outside organism) with absence **CA** decomposes carbonic acid H_2CO_3 to gas CO_2↑$_{gas}$ in endothermic reaction: H_2CO_3 + **Q(gas)**→H_2O + CO_2↑$_{gas}$. Heat supply is important for support the breathing out of organism.

Processes ***in tissues***. As soon as the *arterial* blood reaches ***tissues***, the following reactions occur.

Metabolic CO_{2aqua} product enzyme Carbonic Anhydrase (**CA**) converts to HCO_3^- bicarbonate and hydroxonium H_3O^+ ions according pH=7.36 ***alkaline reserve*** 2.036/1=$[HCO_3^-]/[CO_2]$= 0,0339 M/0,01665 M .

1) Tissues blood oxygen concentration little decreases below $[O_{2aqua}]=6\cdot10^{-5}$ M arterial concentration. Oxygen concentration sensitive **shuttle** equilibrium $(O_2His63,58)_4Hb_R+4H^+ => 4O_{2aqua}+(H^+His63,58)_4Hb_T$ shifts right restoring 459 times arterial concentration $[O_{2aqua}]=6\cdot10^{-5}$ M level amount from reserves of **oxy** hemoglobin $(O_2His63,58)_4Hb_R$. Hemoglobin desorbing oxygen reach decreased venous blood level $[O_2]=1,85\bullet10^{-5}$ M ***in lungs***. Each desorbed oxygen replaces proton H^+ at distal histidine His63,58 in hemoglobin $(H^+His63,58)_4Hb_T$ (Tense state) and bind produced metabolic product HCO_3^- prevent acidity effect stabilizing pH=7.36 constant.

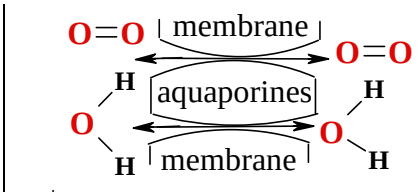
2**)** Krebs cycle metabolite CO_{2aqua} endothermic reaction with water in ***tissues*** drive carbonic anhydrase shift equilibrium to right $Q + CO_{2aqua} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ forming ratio 1/2,0361 = $[CO_{2aqua}]/[HCO_3^-]$. Enzyme Carbonic Anhydrase (**CA**) equilibrium shifts reaction towards bicarbonate anion to prevent of carbonic dioxide accumulation, according Le Chatelier's due to high water $[H_2O]$ concentration 55.3 M, low hydrogen cat ion concentration $[H_3O^+]=10^{-7.36}$ M, enzyme **CA** constant pK=7.0512 value as friendly for physiologic pH=7,36 value. **CA** absence out side human organism as isolated with cell membranes shifts to some fold more acidic as enough at pH=5,5 on the surface for spontaneous carbonic acid bubbling $Q + H_2CO_3 \rightarrow H_2O + CO_2\uparrow_{gas}$.

We follow full cycle of the process, going back the content of venous blood , that to know what mechanism of enzymes: carbonic anhydrase (**CA**) and **shuttle** molecules hemoglobin work in living organisms.

First, hemoglobin are **shuttles** molecules of oxygen $[O_{2aqua}]=6\cdot10^{-5}$ M concentration sensitive equilibrium ***in lungs*** $(O_2His63,58)_4Hb_R + 4H^+ \rightarrow 4O_{2aqua} + (H^+His63,58)_4Hb_T$ stabilize arterial blood concentration to prevent deficiency (hypoxia) and avoid oxidative stress limiting oxygen concentration. **Shuttle** ***in tissues*** desorbs oxygen by proton replaces prevent acidose and stabilize pH=7,36. **Shuttle** in ***lungs*** adsorbs oxygen releasing protons on epithelial cell surface so keeping acidity pH=5,5 promote decomposition of carbonic acid out in AIR.

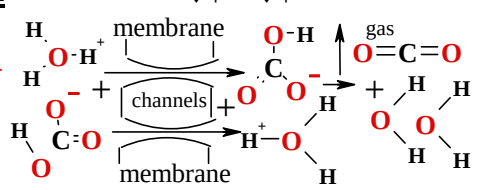
Second, enzyme **CA** equilibrium $H_2O/CA/CO_{2aqua}$ stabilize at pH=7,36 so prevent acidose. Evaporation: endothermic ΔH_r= +54,5 $^{kJ}/_{mol}$; $H_3O^+ + HCO_3^- + Q \xleftarrow{Membrane} H_2O + CO_2\uparrow_{gas} + H_2O\uparrow_{gas}$ + ΔG_r= -82,1 $^{kJ}/_{mol}$. exoergic. Equilibrium keep surface moisture H_2O be side breath out to AIR carbon dioxide $CO_2\uparrow_{gas}$ and water vapor $H_2O\uparrow_{gas}$. For moisture membrane proton channels are permeable H^+, unless **proton** H^+ impermeable for dray **channels**. Therefore membrane is equipped by aquaporins, which are water and solute oxygen O=O permeable in both directions: $O=O + H_2O$ aquaporin channels $\Leftrightarrow H_2O + O=O$. **AQP1** transfer rate is $3\cdot10^9$ per second.

For protons crossing the membrane through proton channels, necessary water molecules locate both side of the membrane and aquaporins are supplier of water H_2O molecules to moisture **alveolar lungs** surface.



$(H^+)_4Hb_T + O_{2aqua} \leftrightarrow (O_2)_4Hb_R + 4H^+$

$\downarrow\uparrow \leftrightarrow \downarrow\uparrow$



Free energy change **ΔG**= -60 $^{kJ}/_{mol}$ for Reaction of H_2CO_3 formation is **exoergic ΔG**<0 negative therefore promotes spontaneous neutralization reaction $H_3O^+ + HCO_3^- <=> H_2CO_3 + H_2O + \Delta G$

Inside the cell–cytosol

CA with water consumes heat **+Q**

$CO_2 + 2H_2O + Q \xleftarrow{CA} H_3O^+ + HCO_3^-$

aqua exothermic

alveolar surface in **lungs** consuming **+Q** heat and evolving water + H_2O supporting surface moisture

$H_2CO_3 + Q \rightleftharpoons CO_2\uparrow_{gas} + H_2O$

endothermic reaction

$H_3O^+ + HCO_3^- <==========> H_2CO_3 + H_2O + \Delta G$

Human **pH**=7,36 of blood **Henderson Haselbalh CA** equation homeostasis

Main buffer system **CA** using hemoglobin **shuttle** stabilizes pH=7,36 and arterial level $[O_{2aqua}] = 6 \cdot 10^{-5}$ M:
deoxy **hemoglobin**$(H^+His63,58)_4Hb_T$(Tense state)<=>**oxy hemoglobin**$(O_2His63,58)_4Hb_R$(**Relax** state)**+4**H^+

Carbonic Anhydrase (**CA**) driven – bicarbonate $2H_2O^{/CA/}CO_{2aqua}$ / $H_3O^+ + HCO_3^-$ buffer system

Organism store H^+ and HCO_3^- as Krebs cycle metabolic product carbonic dioxide, if **CA** produced buffer system acidic form CO_{2aqua} and H_3O^+. For this reason, the acid form have to be transported out of organism in two metabolites through proton channels H^+ across membranes and through bicarbonate channels HCO_3^- with deoxy hemoglobin **shuttle** $4O_{2aqua} + (H^+His63,58)_4Hb_T <=> (O_2His63,58)_4Hb_R + 4H^+$ capturing proton in distal histidine and salt bridge linked $HCO_3^- \ldots H_3^+N$- bicarbonate. Effective of controlled acid form's is breathing out $CO_2\uparrow_{gas}$, that stabilize **pH** of blood **pH=7.36** by metabolites exchange via AIR with oxygen O_2 respiration in and carbon dioxide CO_2 breathing out.

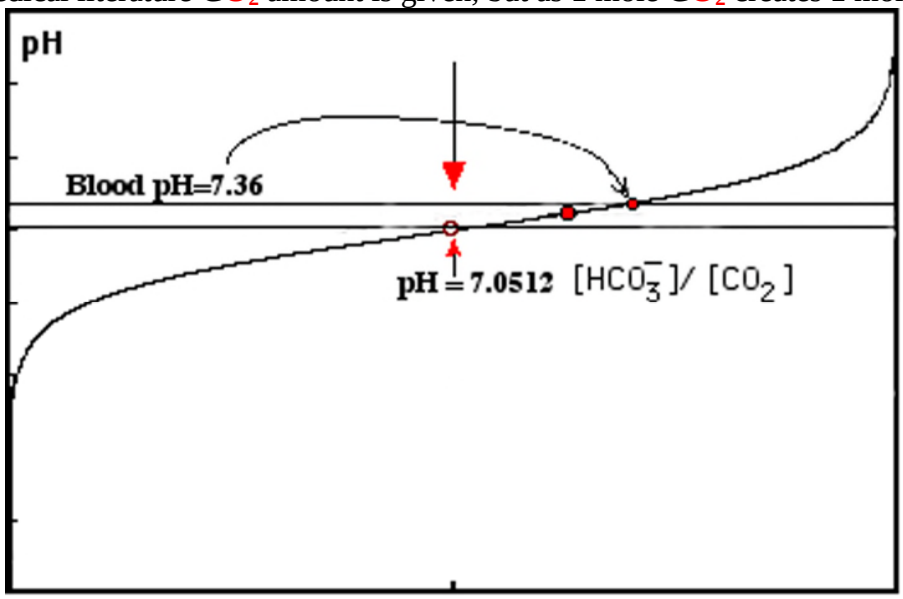
Carbonic anhydrase **CA** make conversion of CO_{2aqua} to bicarbonate anion HCO_3^- in to water medium fast and establish acid-base **Q** $+CO_{2aqua} + 2H_2O \xleftarrow{CA} H_3O^+ + HCO_3^-$ **endothermic** equilibrium at pH=7,36 as producing right side reaction products $H_3O^+ + HCO_3^-$ demanding to heat. So Heating **+Q** shifts equilibrium right side and as soon as H^+ concentration increase as three Krebs cycle product CO_{2aqua} forms two H_3O^+ and HCO_3^-. Instantly carbonic anhydrase **CA** equilibrium is by respiration shifted to left as CO_2 evaporated out consuming H^+ and HCO_3^- in **lungs** and acid concentration $[H^+]$ **remains** stabilized at homeostasis level **pH**=7.36. If concentration H^+ decreases, so increases **pH>7.36**, carbonic anhydrase equilibrium is shifted to the right and the extra amount of HCO_3^- through **kidneys** passes into urine and is transported out and pH stabilizes to homeostasis **pH=7.36** level according Le Chatelier's theorem.

The Brønsted acid is blood-water solution CO_{2aqua}**,** which in. The dissolved into water H_2O (into blood) carbonic dioxide CO_{2aqua} occurring in cell converted with carbonic anhydrase **CA** to $H^+ + HCO_3^-$**.** The water H_2O and carbonic dioxide CO_{2aqua}, finally, is acid in direct equilibrium with HCO_3^- base plus ions H^+**.**

Carbonic anhydrase equilibrium constant pK=7.0512 decreases concentration acid form CO_{2aqua} into water avoid accumulation therefore hydrogen carbonate HCO_3^- and hydrogen ions H^+ are involved into blood **pH** formation according buffer solution

Henderson-Haselbalh equation: $$7.36 = pH = pK + \log\left(\frac{[HCO_3^-]}{[CO_{2aqua}]}\right) = 7.0512 + \log\left(\frac{[HCO_3^-]}{[CO_{2aqua}]}\right);$$

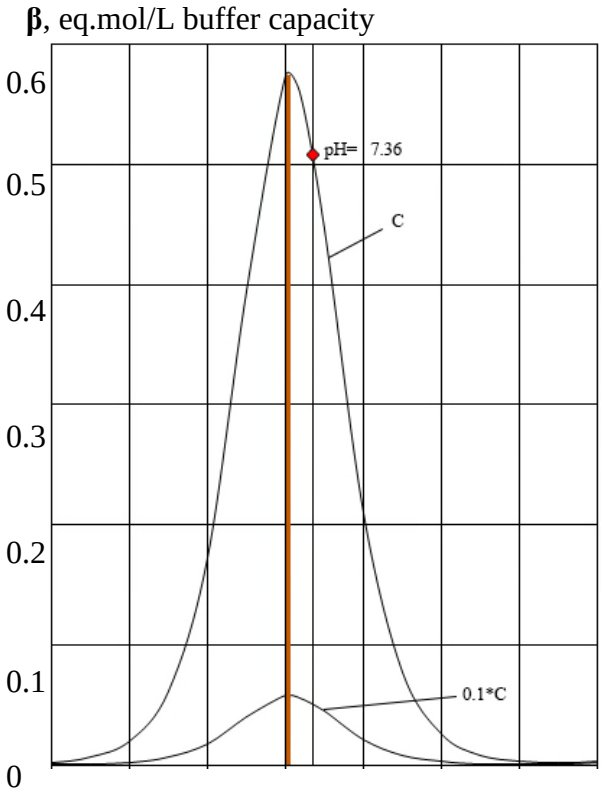
$$\frac{[HCO_3^-]}{[CO_{2aqua}]} = 10^{(pH-pK)} = 10^{(7.36-7.0512)} = 10^{0.3088} = \frac{2.0361}{1}$$ the ratio $[HCO_3^-]/[CO_{2aqua}]$ being approximately **2/1**. In medical literature CO_2 amount is given, but as **1** mole CO_2 creates **1** mole $H_2O^{/CA/}CO_{2aqua}$**,** it is the same.



Buffer region middle point is the over inflection point in graph ○: **pH=pK$_a$ =7.0512**; $[HCO_3^-]/[CO_2]$ =1 is one as well buffer component concentrations are equal $[HCO_3^-]=[CO_2]$ as well as bicarbonate **salt** $[HCO_3^-]$ concentration is equal to Brønsted **weak acid** dissolved in blood CO_2 concentration $[CO_2]$. Alkaline reserve at **7.36** = **pH** is **normal** as $\frac{[HCO_3^-]}{[CO_{2aqua}]} = \frac{2.0361}{1}$.

HCO_3^- **0%**	**50%**	100% **salt –** buffer system **base**
CO_2**+** $2H_2O$ **100%**	**50%**	0% **weak acid** buffer component

As soon as H^+ concentration grows for some reason, Carbonic anhydrase **CA** equilibrium is shifted to left and channeling H^+ and HCO_3^- transported CO_2 out by respiration in **lungs** so acid concentration [H^+] stabilizes. If concentration H^+ decreases, carbonic anhydrase **CA** equilibrium is shifted to the right and the extra amount of HCO_3^- through **kidneys** passes into urine. Bicarbonate channels in **kidney** cells are open at higher values of **pH**>7.36 from side of blood circulation, but **lungs** channel transport are opened for H^+ and HCO_3^- at lower values **pH**<7.36. assuming C=1M= [HCO_3^-]+[CO_{2aqua}]



This value **pK=7.0512** is carbonic anhydrase made equilibrium constant very friendly to blood **pH=7.36**. As most of metabolism products are acidic, the organism has to have a reserve of alkalinity. For this reason the ratio between HCO_3^- and CO_{2aqua} concentrations is **2/1**. The **pH** value of physiological conditions blood homeostasis is **7.36.**

*The **alkaline reserve*** 2.036/1=[HCO_3^-]/[CO_{2aqua}] at blood pH=7.36 can be controlled by adding H_2SO_4 to a sample of **100** mL blood reacts with included in salt HCO_3^- and the CO_{2aqua} is liberated. If **56.23** mL (**50-60** mL) of gaseous CO_2 are liberated from **100** mL of blood, the controlled ***alkaline reserve*** in homeostasis is **normal** and total ***alkaline reserve*** amount concentration **0.023M** = [HCO_3^-]+[CO_{2aqua}] is in homeostasis **normal** as sum of [HCO_3^-] = **0.0154 M** and [CO_{2aqua}]=**0.0076M**.

Two types of **diseases** occur, if the acid-base balance is distorted in the organism ***alkalosis*** and ***acidosis***.

1) *Respiratory alkalosis* occurs, if **lungs** are hyperventilated, for example, during anesthesia. If CO_{2aqua} concentration decreases **pH>7.36 alkalosys** due to hyperventilation, the blood vessels are broadened and their tonus is lowered as a result of it, therefore O_2 supply to brain is shortened.

For this reason it is necessary to use AIR mixtures of O_2 and CO_2 during anesthesia instead of pure oxygen. If respiratory alkalosis occurs for other reasons than hyperventilation of **lungs**, the ratio **2/1** of the buffer components can be re-established in a longer period of breathing normal, CO_2-containing AIR 350 ppm.

2) *Respiratory acidosis* occurs in the cases, when the concentration of CO_2 in the AIR is increased. The result of this is that the action of breathing muscles becomes more difficult. Again, this can be canceled, if the patient starts breathing normal AIR. Hoverer, if increased CO_2 content in the AIR lasts long, metabolic acidosis occurs **pH**<7.36. Metabolic acidosis hemoglobin reserves depleted oxygen concentration below venous [O_2]=1,85•10^{-5} M.

For this reason only the concentrations of carbonic dioxide CO_{2aqua} into water H_2O (avoid carbonic acid H_2CO_3 formation) and bicarbonate HCO_3^- and hydrogen ions H^+ are included into equation for blood **pH**.

There are two sequences, which drive enzymes **CA** and **shuttle** hemoglobin governed gradual reactions

I) $O_{2AIR}+H_2O \overset{aquaporin}{\rightleftarrows} H_2O+O_{2aqua}$; $4O_{2aqua}+$ **deoxy**$(H^+His63,58)_4Hb_T <=[O_2]=6\cdot10^{-5}$ M=>**oxyHb**$_R(O_2)_4+4H^+$,

Glycolysis, Krebs cycle products $C_6H_{12}O_6+6O_{2aqua}+6H_2O \leftarrow \overset{Krebs\ Cycle}{} \rightarrow 6CO_{2aqua}+12H_2O \leftarrow \overset{CA}{} \rightarrow 6H_3O^+ +6HCO_3^-$

II) $Q_{aqua}+CO_{2aqua}+2H_2O <= \underline{CA} => H_3O^+ +HCO_3^- \overset{membrane}{\rightleftarrows} H_2O+H_2CO_3+Q(gas) \rightleftarrows H_2O+CO_2\uparrow_{gas}+H_2O$.

II) process first gradual reaction enzyme Carbonic anhydrase **CA** made equilibrium:

Free energy consumes ΔG_r for reaction endoergic: $CO_{2aqua}+2H_2O+\Delta G+Q <= \overset{CA}{} => H_3O^+ +HCO_3^-$

Enthalpy heat consumed ΔH_r for reaction endothermic: $\Delta H_r=\Delta H°_{H3O}-\Delta H°_{HCO3}-2\Delta H°_{H2O}-\Delta H°_{CO2}$=9,7576 $^{kJ}/_{mol}$

= -285,81-689,93-(2*-285,85-413,7076) = -975,74+985,3276=9,7576 $^{kJ}/_{mol}$ endothermic

Endothermic as needed heat supply to drive reaction forwards.

Entropy decrease ΔS_r **<0** negative as enzyme Carbonic Anhydrase **CA** governed reaction**:**

$\Delta S_r=\Delta S°_{H3O}+\Delta S°_{HCO3}-2\Delta S°_{H2O}-\Delta S°_{CO2}$=-3.854+98.324-(2*69.956+117.57)=94.47-257.482= -163.0134 $^{J}/_{mol/K}$.

$\Delta G_r=\Delta H_r-T*\Delta S_r$=9,7576-(298,15*-0,1630134)=58,36 kJ/mol endoergic **free** energy accumulated in products by **CA** governed reaction. $\Delta S_{dispersed}$= - ΔH_r/ T = -9.5876 /298.15= -32,727 J/K/mol.

$\Delta S_{total}=\Delta S_r+\Delta S_{dispersed}$=-163,0134-32,7271= -195,7405 J/K/mol. $T\bullet\Delta S_{total}$=-0,1957405*298,15= **-58,36** kJ/mol

$\Delta G_r=\Delta G°_{H3O}+\Delta G°_{HCO3}-2\Delta G°_{H2O}-\Delta G°_{CO2}$=-213,275-586,94-(2*-237,19-385,98)= +60,145 $^{kJ}/_{mol}$ endoergic.

Carbonic anhydrase make the equilibrium constant $K_{eq} = 10^{-7.0512}$ or exponent pK_{eq} = 7,0512 is constant and very close to **pH** value 7,36. Water concentration [**H**$_2$**O**]=55.3 M is constant so included in value

$K_{eq}=K\bullet[H_2O]^2=10^{-10.224}\bullet(997,07/18,0153)^2=10^{-7.0512}$ M$=10^{-pKeq}$ exponent value pK_{eq}=7.0512.

II) process first gradual reaction driven by enzyme **CA** $CO_{2aqua}+2H_2O \leftarrow \overset{CA}{} \rightarrow H_3O^+ +HCO_3^-$

$10^{-7.0512}=K_{eq}=[H_2O]^2\cdot K=[H_3O^+][HCO_3^-]/[CO_{2aqua}]$ as $K=[H_3O^+][HCO_3^-]/([CO_{2aqua}]\cdot[H_2O]^2)=10^{-10.224}$

$\Delta G=-RTln(K)=-8.3144\bullet298.15\bullet\ln(1\bullet10^{-10.224})$= 60.145 $^{kJ}/_{mol}$ where R=8,3144 J/mol/K and T=310,15 K (25˚C).

$K_{eq}=EXP(-\Delta G_r/R/T)*[H_2O]^2=(10^{-10.5372})*[H_2O]^2=(10^{-10.5372})*(997,07/18,0153)^2=10^{-7.0512}=10^{-pKeq}$

II) process second gradual reaction concentration gradient and electrochemical membrane potential bicarbonate ion HCO_3^- and proton H^+ 1. $E_H=P\bullet lg([10^{-pH}{}_{extraMit}/10^{-pH}{}_{Mitochon})$=0,06154***lg**(10^2,36)=0,14523 **V**

2. $E_{HCO3-Mitochon}$= **-P•log**([$HCO_3^-{}_{cytosol}$]/[$HCO_3^-{}_{Mitochon}$])= -0,06154***log**(0.0154/0,0338919)= 0,0210821 **V**

E_{sum}=0,14523+0,0210821=0,1663168 V =$E_{membrane}$; $\Delta G_F=nFE$=-1*96485*0,1663168= -16,0471 kJ/mol

3. $\Delta G_{HCO3-}=RTln([HCO_{3cytosol}]/[HCO_{3Mitoch}])$=8,3144*310,15***log**(0,0154/0,0338919)= -2,0341094 kJ/mol

4. $\Delta G_{H+}=-RTln([H_3O^+]_{extraMit}/[H_3O^+]_{Mitochon})=-RTln(10^{-7,36}/10^{-5})$=-8,3144*310,15***ln**(10^**2,36**)= -23,3943 $^{kJ}/_{mol}$

Total $\Delta G_{total}=\Delta G_F+(\Delta G_{HCO3-}+\Delta G_{H+})$=-16,0471 +(-2,0341094)+(-23,3943)= -41,4755 $^{kJ}/_{mol}$ exoergic transfer.

II) process third gradual Carbonic acid formation: $H_3O^+ +HCO_3^- \rightarrow H_2O+H_2CO_3+Q$ exothermic.

$\Delta H_r=\Delta H°_{H2O}+\Delta H°_{H2CO3}-\Delta H°_{H3O}-\Delta H°_{HCO3}$= -285.85-699,65-(-285.81-689.93)= =-985.5+975.74= -9.76 $^{kJ}/_{mol}$

$\Delta S_r=\Delta S°_{H2O}+\Delta S°_{H2CO3}-\Delta S°_{H3O}-\Delta S°_{HCO3}$=69.956+187-(-3.854+98.324)=256.956-94.47= 162,486 $^{J}/_{mol/K}$.....

$\Delta S_{dispersed}$ = - $\Delta H_r/T$ = 9.76 /298.15= +32.735 J/K/mol....

$\Delta G_r=\Delta H_r-T*\Delta S_r$= -9.76 -298.15*0.129751=-38.695 kJ/mol **exoergic** reaction is driven by concentration gradients through **proton** and **bicarbonate channels** of **membrane**.

$\Delta G=\Delta G°_{H2O}+\Delta G°_{H2CO3}-\Delta G°_{H3O}-\Delta G°_{HCO3}$=-237.19-623.17-(-213.275-586.94)=-860.36+800.215= -60.145 $^{kJ}/_{mol}$.

$\Delta S_{total}=\Delta S_r+\Delta S_{dispersed}$=32.735 +162,486 =129.751 J/K/mol....

II) process fourth gradual reaction is non-enzymatic decomposition $H_2CO_3 <=> CO_2\uparrow_{gas}+H_2O$;

$\Delta G=\Delta G°_{H2O}+\Delta G°_{CO2}-\Delta G°_{H2CO3}$= -237.19-385.98-623.17)= -623.17+623.17=0.0 $^{kJ}/_{mol}$ is **anenergic** or neutral.

Enthalpy change decomposition reaction of carbonic acid **Q** + $H_2CO_3 \rightleftarrows CO_2\uparrow_{gas}+H_2O$ endothermic

Substance	$\Delta H°_r$, $^{kJ}/_{mol}$	$\Delta S°_r$, $^{J}/_{mol/K}$	$\Delta G°_r$, $^{kJ}/_{mol}$
H_3O^+	-285.81	-3.854	-213,275
HCO_3^-	-689.93	98.324	-586,94
$H_2O\uparrow_{gas}$	-241,8352	188,7402	
H_2O	-285.85	69.9565	-237,191
$CO_2\uparrow_{gas}$	-393.509	213.74	-394,359
CO_{2aqua}	-413.7976	117.5704	-385,98
H_2CO_3	-699,65	187.00	-623,17

$\Delta H=\Delta H°_{H2O}+\Delta H°_{CO2}-\Delta H°_{H2CO3}$= +20.291 $^{kJ}/_{mol}$

=-286-393.509-(-699.65)= -679.509+699.65= +20.291 $^{kJ}/_{mol}$

is endothermic exactly with the **cooling** effects.....

$\Delta S_{dispersed}$ =-$\Delta H_r/T$ = -20.291/298.15= -68.056 J/K/mol....

$\Delta S_r=\Delta S°_{H2O}+\Delta S°_{CO2}-\Delta S°_{H2CO3}$= +96,696 $^{J}/_{mol/K}$....

= 69.956+213,74-(187.)= 257.482-94.47=+96,696 $^{J}/_{mol/K}$....

$T\bullet\Delta S_{total}$=28,64 $^{J}/_{K/mol}\bullet$298,15 K=**+8.539** kJ/mol

bound TΔSn← lost free energy ΔGreverse$_{reaction}$←....

endothermic $\Delta H°_{reaction}$ =+20,291 kJ/mol; cooling Q= -20,291 kJ/mol spontaneous $\Delta G°_{reaction}$ =-8,539 kJ/mol .