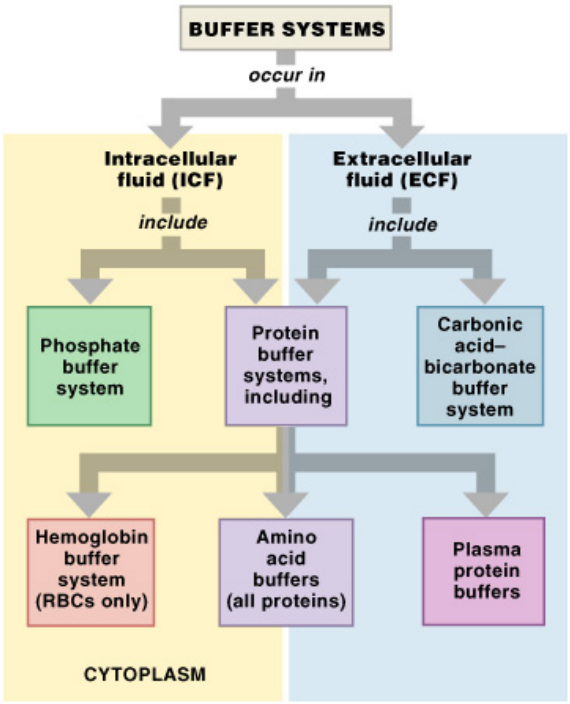
Physiological Buffers

- All about maintaining equilibrium



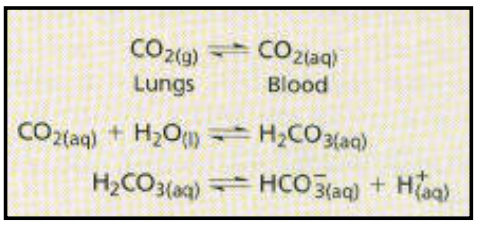
1. Protein buffer systems:
   - help regulate pH in ECF and ICF
   - interact extensively with other buffer systems
2. Carbonic acid–bicarbonate buffer system:
   - most important in ECF
3. Phosphate buffer system:
   - buffers pH of ICF and urine

Carbonic acid Bicarbonate

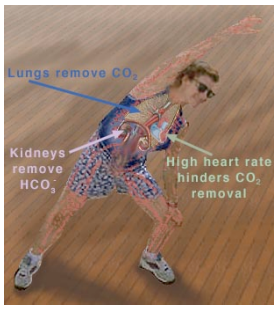
$H_2CO_3 \Leftrightarrow HCO_3^- + H^+$

pKa = 6.4

Why this pair?



Carbonic Anhydrase (CA) catalyzes dissociation of carbonic acid into H^+ and HCO_3^-



Removing CO_2 from the blood helps increase the pH.

Removing HCO_3^- from the blood helps lower the pH.

OVERALL REACTION

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

- Major buffer in blood (pH 7.4) and other extracellular fluids is the **carbonic acid/bicarbonate pair (See Clinical Notes, p. 43)**

$$H_2CO_3 \underset{\text{Carbonic acid}}{} \xrightleftharpoons{pK_a = 6.4} HCO_3^- + H^+ \underset{\text{Bicarbonate}}{}$$

A base added to blood would be neutralized by the following reaction:

$$H_2CO_3 + OH^- \rightleftharpoons HCO_3^- + H_2O$$

The addition of an acidic substance to blood also results in neutralization:

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

- The pH of blood (7.4) is at the upper limit of the buffering capability of this system. Seems ill-suited and inefficient as many metabolic processes generate acids.
- But, we can regulate both CO_2 and HCO_3^-
- Depends on three equilibria:

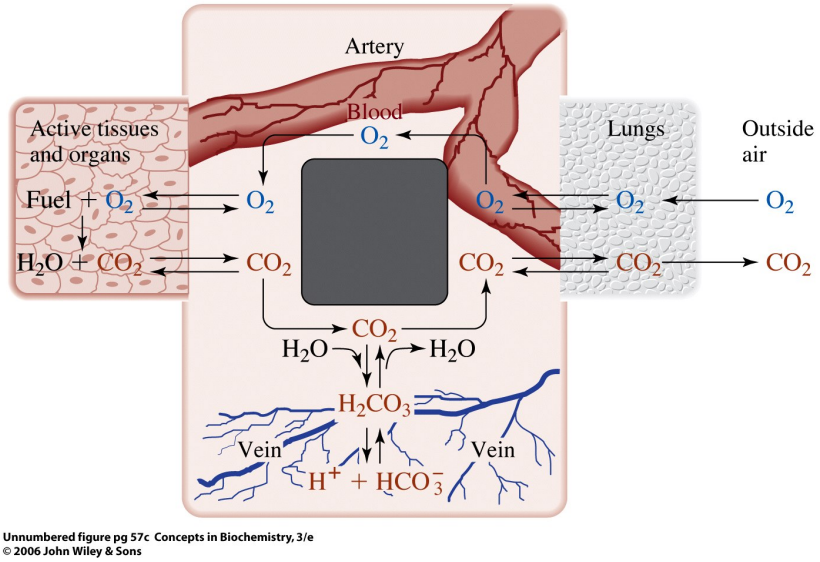
$$CO_{2(g)} \rightleftharpoons CO_{2(aq)} \quad \text{(Lungs / Blood)}$$

$$CO_{2(aq)} + H_2O_{(l)} \rightleftharpoons H_2CO_{3(aq)}$$

$$H_2CO_{3(aq)} \rightleftharpoons HCO_{3(aq)}^- + H_{(aq)}^+$$

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad \text{Net rxn.}$$

- Gaseous CO_2 dissolves in blood to form carbonic acid
- Carbonic acid rapidly dissociates to H^+ and bicarbonate; sped up by presence of enzyme carbonic anhydrase in blood.
  - Concentration of carbonic acid in blood is very low



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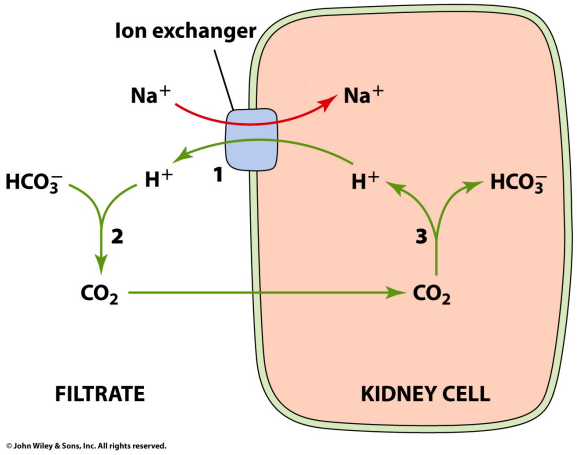
- **Works in reverse as well, H^+ is removed from cells by blood plasma**
  - Neutralized by the reaction with HCO_3^- and leads to **release of CO_2** as gas from lungs; basically eliminates H^+
  - Can also adjust breathing if you need to keep more H^+ in the blood.
- Great system because it can be readily REGULATED!
  - CO_2 regulated by breathing
  - HCO_3^- regulated by kidneys

KIDNEYS:

- Short term adjustments made by changing breathing; longer term adjustments made by the kidneys
- Remove excess acid (hydrogen ion) or bases (bicarbonate) in the urine and keep the bicarbonate concentration in the blood normal
- **HCO_3^- in kidney filtrate is actively reclaimed before being lost in urine**.



1: H^+ leaves kidney in exchange for Na^+ via a Na^+/H^+ exchanger protein

2: Expelled H^+ recombines with HCO_3^- in the filtrate, forming CO_2.

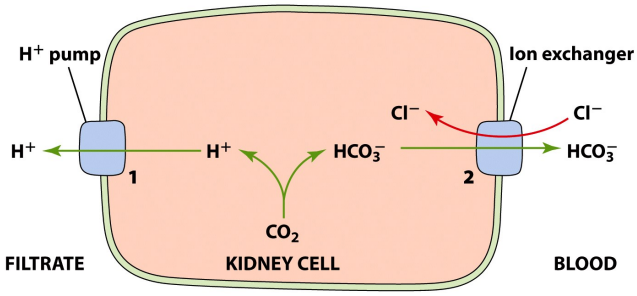
3: CO_2 is non-polar and can diffuse back into kidney cells, where it is converted to H^+ and HCO_3^-

- **Kidneys also generate HCO_3- to offset losses (CO_2 loss, buffering metabolic acids)**

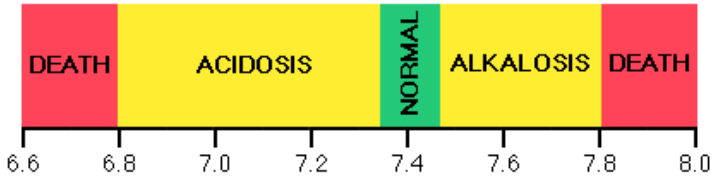
Metabolic activity in kidney generates CO_2, which is converted to H+ and HCO_3^-

1: Cells excrete the H^+ via a H^+ pump, which goes to urine (why urine is acidic)

2: Bicarbonate left in cell returned to blood in exchange for Cl^-



MEDICAL CONDITIONS ASSOCIATED WITH BLOOD pH:



1. **Acidosis**:
   - Blood pH goes DOWN (Becomes ACIDIC)
   - [H^+] goes UP
2. **Alkalosis**:
   - Blood pH goes UP (Becomes BASIC)
   - [H^+] goes DOWN

Normal conditions

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

Excess acid

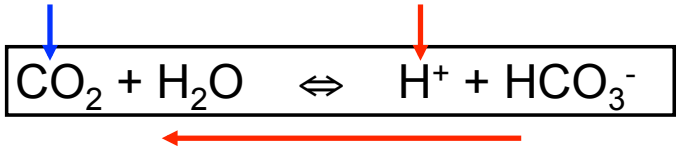
$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightleftharpoons H_2O + CO_2$$
$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightarrow H_2O + CO_2$$
$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Insufficient acid

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

ALKALOSIS:

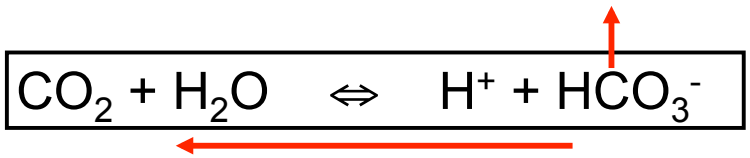
- Characterized by increase in blood pH (pH 7.74)
- Becomes more basic/alkaline
- **RESPIRATORY ALKALOSIS**
    - **Hyperventilation: Breathing rate more rapid than necessary for normal CO_2 elimination**
        - Central nervous system disorders such as meningitis, encephalitis, cerebral hemorrhage
        - Drug or hormone induced physiological changes
        - Anxiety
    - Excessive intake of O_2; Abnormally **low** CO_2 due to excessive exhalation
    - pH goes so **high** that weakness and fainting result
- Hyperventilation causes $[CO_2]$ ↓
- Equilibrium shifts to the LEFT
- $[H^+]$ goes down and pH goes UP



Treatment:

- Correct underlying physiological problem
- In short term, respiratory alkalosis can be helped by breathing a CO_2 rich atmosphere (breathing into a paper bag)
- NH_4Cl infusion (for alkalosis). NH_4Cl dissociates into NH_4^+ and Cl^-. The NH_4^+ (ammonium) is in equilibrium with NH_3 (ammonia) and H^+. Because ammonia is volatile, it is respired through the lungs, leaving behind H^+ and Cl^1 or hydrochloric acid, which lowers the pH.

- **METABOLIC ALKALOSIS:**
- Caused by elevated HCO_3^- concentrations
    - Gain of HCO_3^- or loss of H^+
- Bicarbonate ions interact with H^+ in solution
- Reduced H^+ causes alkalosis
- Causes:
    - Addition of bicarbonate
    - Loss of fluids that have low conc. of bicarbonate (vomiting, diuretics)
    - Loss of hydrogen: For every hydrogen ion lost, one bicarbonate is added to the plasma. Vomiting; low potassium shifts K+ into plasma and H+ into cells
- Compensation:
    - Respiratory: hypoventilation (more CO_2)
    - Increased HCO_3^- loss at kidney

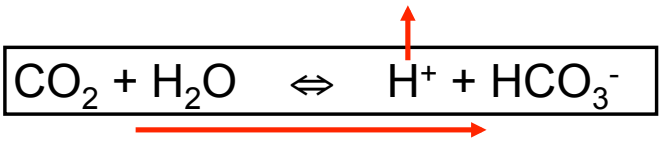


ACIDOSIS:

- Blood pH goes **DOWN**
- $[H^+]$ goes **UP**

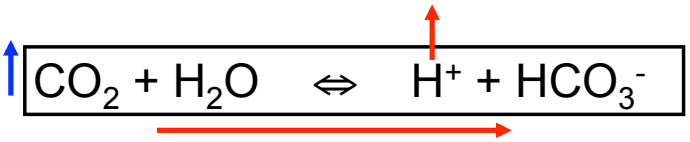
Although a slight drop in pH from 7.4 to 7.2 does not sound significant, this involves an increase in H^+ concentration from 3.9×10^{-8} M to 6.3×10^{-8} M, an increase of > 60% !

- **TWO TYPES:**
  - **Metabolic Acidosis**
    - Causes:
      Uncontrolled diabetes (ketoacidosis)
      Starvation diets
      High-protein/low fat diets
      Overproduce acidic compounds called ketone bodies that lower blood pH
      Sudden surges in LACTIC ACID during exercise



  - **Respiratory Acidosis**
    - Causes
    - Obstructive lung disease
    - Hypoventilation (too much CO_2)
    - Disease states that prevent efficient expiration of CO_2

- $[CO_2] \uparrow$ (can't be expelled)
- Equilibrium shifts to the RIGHT
- $[H^+] \uparrow$; pH $\downarrow$



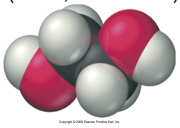
Treatments:

- **Treat the cause** - Stop alimentary loss of base; correct hypoxia; reduce renal acid load by diet; give insulin; treat shock with intra-venous fluids and stop hemorrhage etc
- In short term, can **ventilate**
- Commonly, **bicarbonate** is infused intravenously ($NaHCO_3^-$)

The Danger of Antifreeze

- Each year, thousands of pets die from consuming antifreeze
- Most brands of antifreeze contain ethylene glycol; taste sweet taste & give initial effect of drunkenness

ethylene glycol
(aka 1,2–ethandiol)



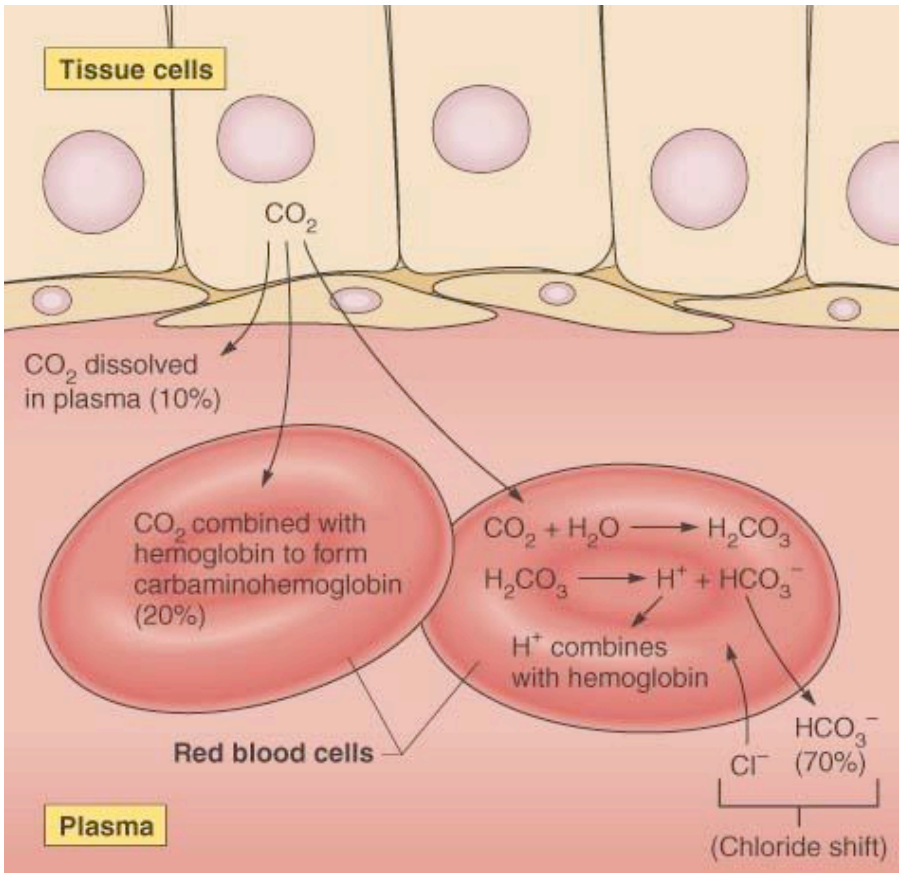
- Metabolized in the liver to glycolic acid
  - $HOCH_2COOH$ (weak acid) (pKa 3.83)
- Can overwhelm the buffering ability of HCO_3^-, causing the blood pH to drop: ACIDOSIS
- the treatment is to give the patient ethanol, which has a higher affinity for the enzyme that catalyzes the metabolism of ethylene glycol

Other Physiological Buffering Systems:

A. HEMOGLOBIN BUFFERING SYSTEM

- Transports oxygen from lungs to peripheral tissues
- Transports carbon dioxide from tissues to lungs for exhalation

- Hemoglobin is a buffer for both CO_2 and H^+.
- CO_2 diffuses across RBC membrane from tissues.
- The CO_2 can bind directly with hemoglobin and be released in the lungs. (20%)
- The CO_2 that reacts with water forms carbonic acid that then dissociates into bicarbonate (70%) in RBC
  - Bicarbonate ions diffuse into plasma in exchange for chloride ions
  - H^+ binds to hemoglobin and released in RBCs in lungs to combine with bicarbonate & reform CO_2 for exhalation



B. PHOSPHORIC ACID SPECIES

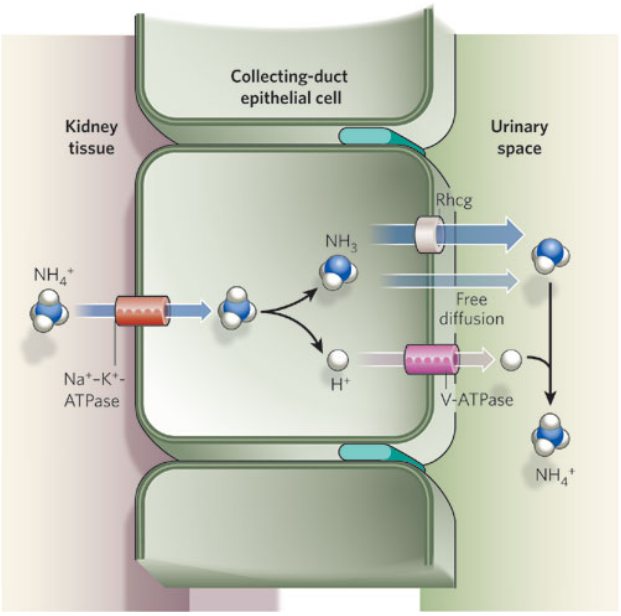
- **$H_2PO_4^-$ and HPO_4^{-2}** conjugate pair (pKa = 7.2) (dihydrogen phosphate/monohydrogen phosphate)
- Used a lot in labs to mimic cellular conditions
- Use the H-H equation to prepare these buffers

C. IONIZABLE GROUPS ON AMINO ACIDS IN PROTEINS ASSIST IN BUFFERING

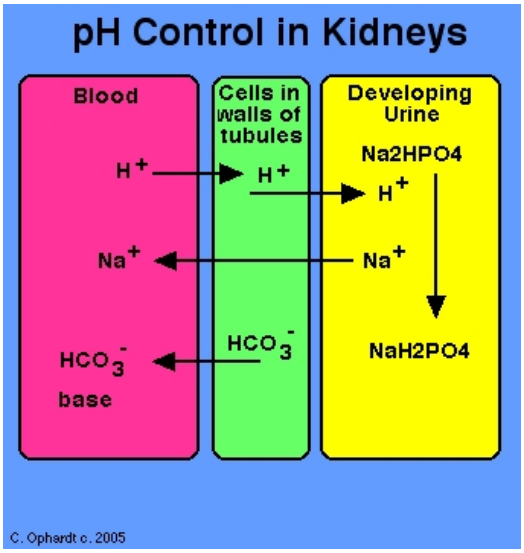
D. BUFFERS IN URINE:

- The ability to eliminate large amounts of H^+ in a normal volume of urine depends on the presence of buffers
  - without buffers, would need to dilute the H^+ in urine with 1000x more water!

1. **Carbonic acid–bicarbonate buffer system**
2. **Phosphate buffer system:** $H_2PO_4^{-1}$ is the weak acid, and HPO_4^{-2} is the conjugate base. (these two provided by filtration)
3. **Ammonia buffer system:**
   - Tubular deamination of glutamine generates NH_3, that is transported into the tubule and buffers H^+ by becoming NH_4^+, that is transported into urinary space
   - Bicarbonate is reabsorbed along with Na^+



Control of bicarbonate reabsorption and H+ excretion



AMINO ACIDS I

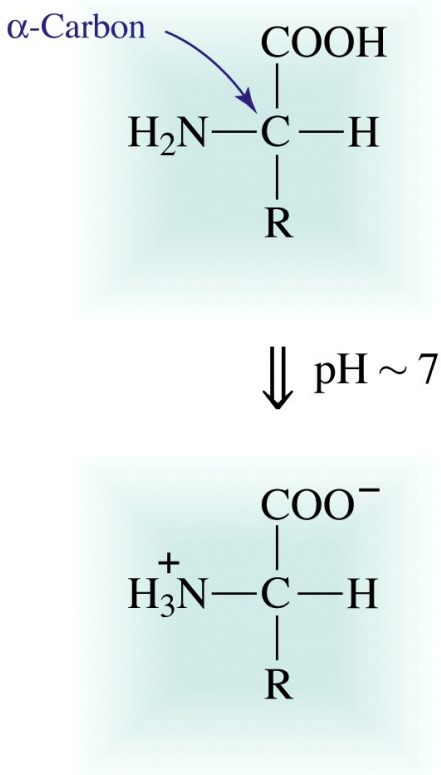
- All 20 amino acids in pure form are white, crystalline, high-melting solids

- Amino acids act as: enzymes (catalysts), metabolic intermediates, carriers of energy and waste products and hormones.
- Amino acids are the building blocks of proteins
- Proteins are the most abundant macromolecules in living cells. May be 0.1 million different proteins in humans. Play pivotal role in almost every biological process.
- Generally, proteins composed of the 20 naturally occurring amino acids
- Only one way to link amino acids together – **peptide bond**
- Protein structure and function defined by sequence and type of the amino acids
- Proteins display great diversity in function and structure

- **DEFINITION:**
  Any organic molecule with at least one CARBOXYL group (organic acid) and at least one AMINO group (organic base)

GENERAL STRUCTURE OF THE 20 AMINO ACIDS:

At physiological pH (~7.4), amino acids exist as **zwitterions** – positive and negative charge on the same molecule. Dependent upon the pKa of the group. For example:



Carboxylic acid functional group

Amine functional group

R = biochemical shorthand for "side chain"
Protonation of COOH and H_2N depends on pH of the solution

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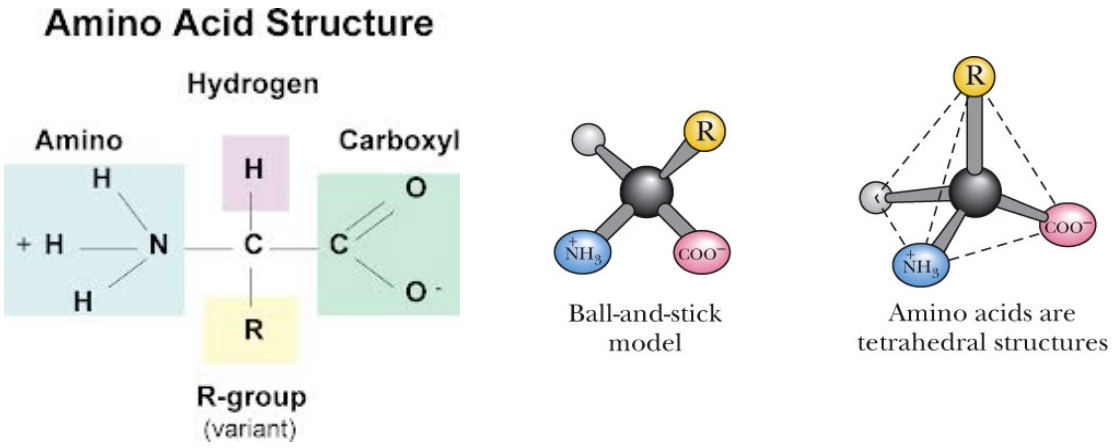
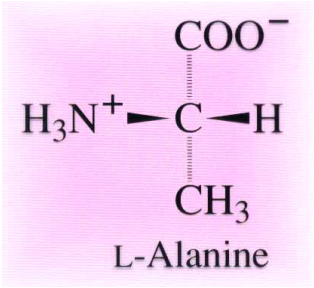


General anatomy of an amino acid.
Except for proline and its derivatives, all of the amino acids commonly found in proteins possess this type of structure.

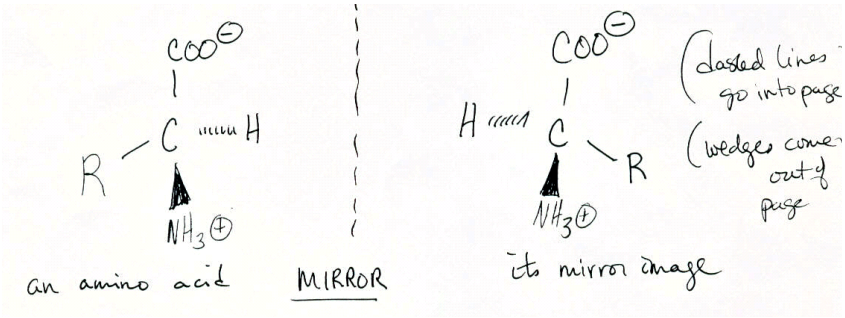
Have both ACID and BASE groups in same molecule

STEREOCHEMISTRY OF AMINO ACIDS:



- Stereochemistry imparts certain characteristics to a compound.
  - Every compound has a mirror image
  - Sometimes mirror images of a molecule are superimposable in space with the original object and some are not
  - NON-superimposable mirror images = **CHIRAL**
    - Have no plane of symmetry
    - **General rule: Carbon atom with 4 DIFFERENT atoms or groups bonded to it is chiral**.
    - Other Chiral examples:
      - Hand or foot
      - Right hand is a mirror image of your left hand – note how they cannot be superimposed
  - Superimposable mirror images = **ACHIRAL**
    - Achiral examples:
      - Two plain coffee mugs
      - You can turn the mirror image of the plain mug in space to make it superimposable.

- Each amino acid (like your hand) also has a mirror image.



These images are NOT superimposable; No matter how you turn the mirror image in space you won't regain the original. Note no plane of symmetry.

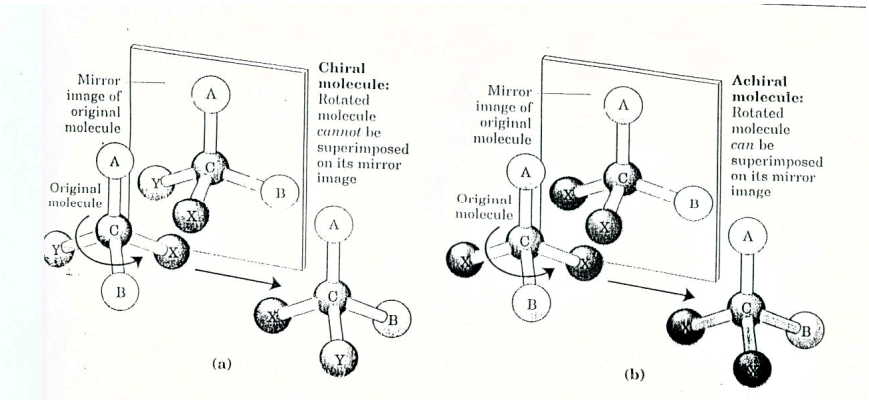
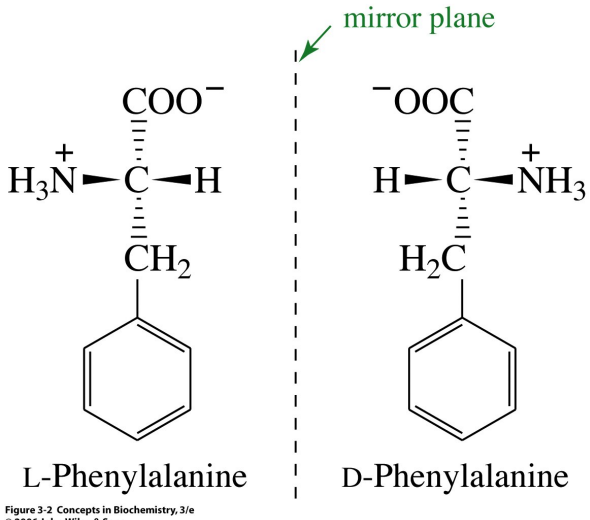


figure 3–9
Molecular asymmetry: chiral and achiral molecules. **(a)** When a carbon atom has four different substituent groups (A, B, X, Y), they can be arranged in two ways that represent nonsuperimposable mirror images of each other (enantiomers). Such a carbon atom is asymmetric and is called a chiral atom or chiral center. **(b)** When a tetrahedral carbon has only three dissimilar groups (i.e., the same group occurs twice), only one configuration is possible and the molecule is symmetric, or achiral. In this case the molecule is superimposable on its mirror image: the molecule on the left can be rotated counterclockwise (when looking down the vertical bond from A to C) to create the molecule in the mirror.

Chirality movie:
http://cwx.prenhall.com/petrucci/medialib/media_portfolio/text_images/083_Chirality.MOV

These are called ENANTIOMERS = non-superimposable mirror images

- Most amino acids (except glycine) have **four** different groups attached to the a-carbon and therefore are **chiral or have a chiral center.**
- For glycine, R is hydrogen. Therefore, the mirror images ARE superimposable and NOT chiral. A plane of symmetry exists.
- **Each amino acid except glycine has 2 ENANTIOMERS**
- The enantiomers are classified based on the ability to rotate polarized light – optically active.
  - Rotate light in either (+) or (-) direction
  - Called **D or L enantiomers** (again non-superimposable mirror images)
  - Both D and L amino acids exist in nature but **only L amino acids are used as building blocks for proteins.**
  - D-amino acids are found in a few rare bacteria in the cell walls.



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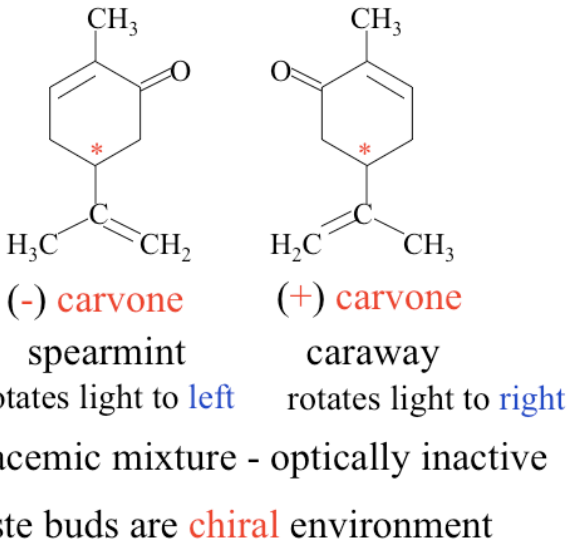
EXAMPLE 1:

Different flavors tasted depending on chirality of one carbon in carvone.

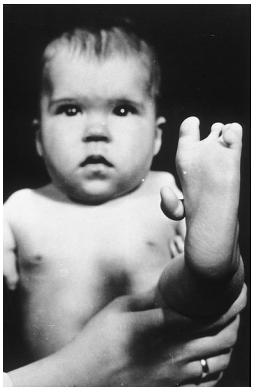
(-) = spearmint
(+) = caraway

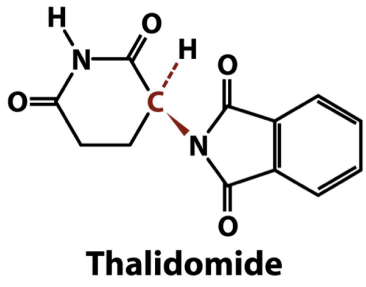
Taste receptors can tell the difference in 3-dimensional structure of the small molecule.



EXAMPLE 2: Thalidomide

- A sedative given to women in late 1950s and early 1960s to help morning sickness.
- Mixture of R- and S- forms.
- R- form is effective
- S- form caused severe birth defects, including appendage.
- Can racemize *in vivo* - Both forms can interconvert.





EXAMPLE 3: Conversion of L → D amino acids in proteins with age

- Part of aging process may include the isomerization of the amino acid **aspartic acid (aspartate)** from L → D in proteins in teeth and eyes.
- Isomerized proteins are less biologically active
- Scientists are identifying the enzymes that change D back to L to try and slow the aging process.

EXAMPLE 4: Isomer Isolation Leads Researchers To More Effective Psychiatric Drugs

In an effort to increase effectiveness and reduce dosing, side effects, and potential drug interactions, pharmacologists are isolating and purifying specific forms of psychiatric drugs.

Mirror image (or racemic) isomers are labeled as "right handed" (termed "R-" or dex-) or "left handed" (termed "S-" or "levo-"). Because the different isomers are difficult to separate, the FDA allows drug companies to include both in medicines, as long as the less-functional isomer is not harmful.

Successes:

- **Dexmethylphenidate** (Focalin) (Treatment of ADHD)
- **Citalopram** (Celexa) (SSRI; Treatment of depression)
    - S-citalopram under the generic name of escitalopram
    - Escitalopram (Lexapro) is nearly four times more potent as an SSRI than the mixture present in the parent compound citalopram
    - Escitalopram (Lexapro) has better side-effect and drug-interaction profiles.

Failure:

- **Prozac** = R is the more effective enantiomer. S is thought to be potentially dangerous.

  Isolating R-fluoxetine could yield a more effective drug with fewer side effects.

  However, late in 2000, Eli Lilly and Company halted development of the R- isomer of fluoxetine (Prozac) after Stage III clinical trials revealed unexpected cardiac side effects (*Psychiatric News*, December 1, 2000). The company had hoped that R-fluoxetine would replace fluoxetine, which was facing imminent expiration of its patent protection.