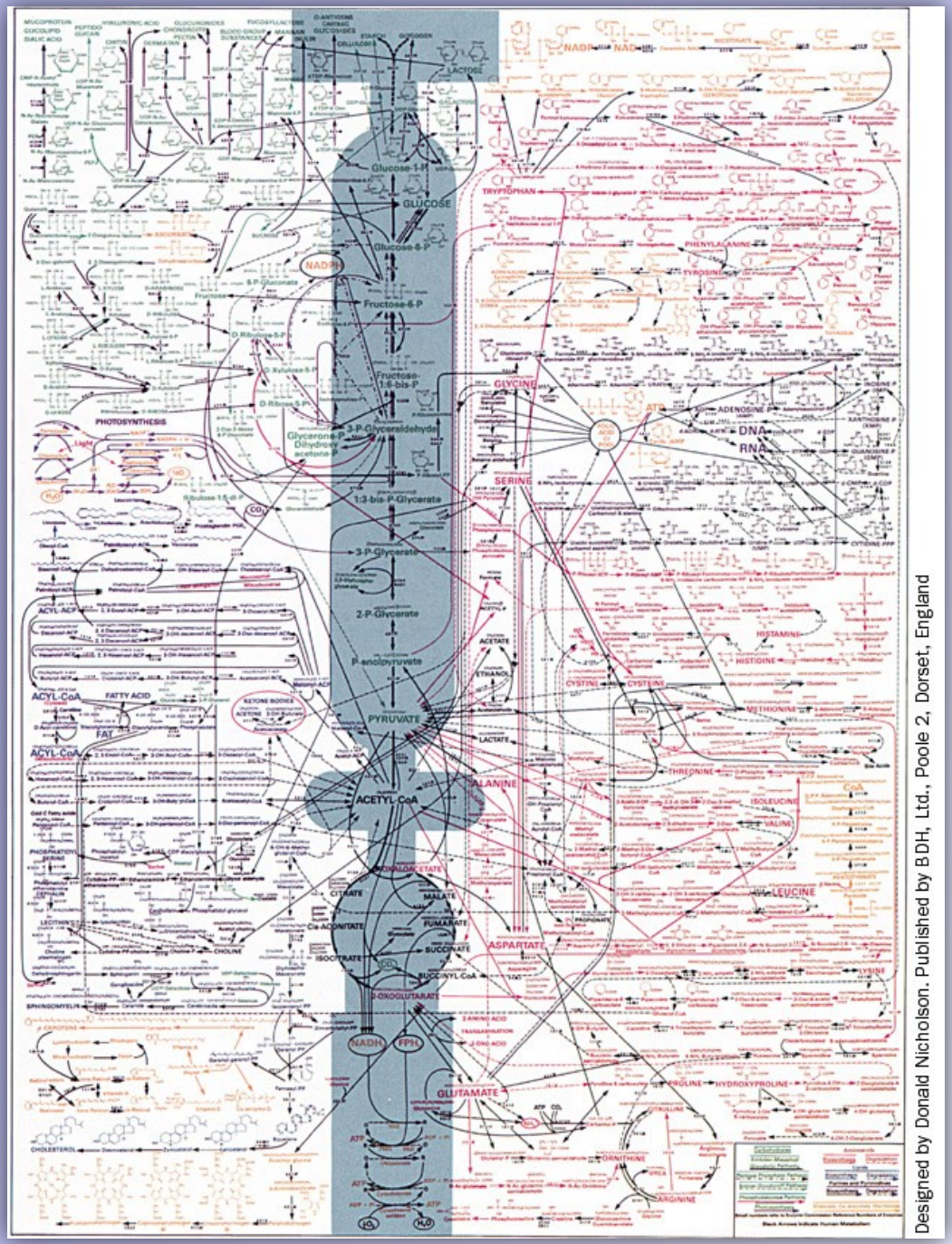
CHEM 539

Molecular Metabolism: Pathways and Regulation

Spring 2015

PPT Set 1: Introduction



Map of the major metabolic pathways in a typical cell

Types of pathways:
catabolic
anabolic
amphibolic
cataplerotic
anaplerotic

Catabolic pathways are convergent.

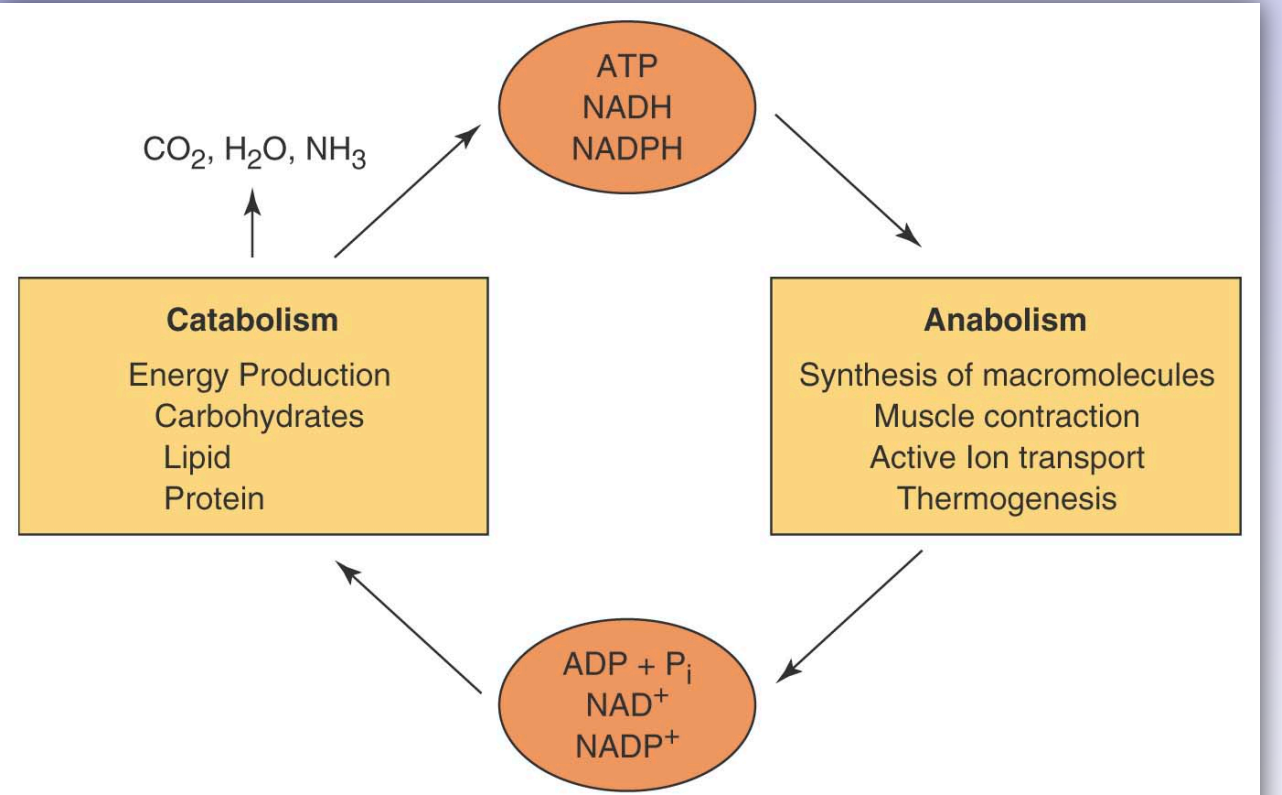
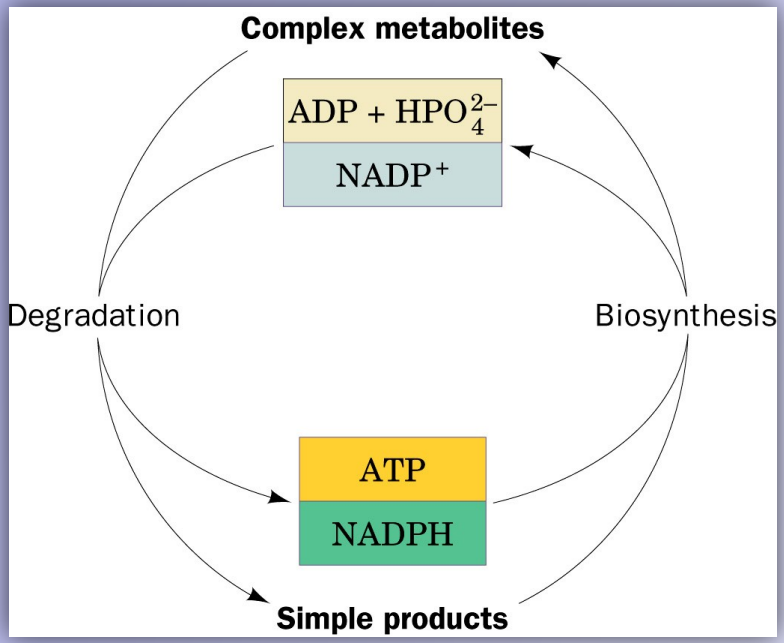


Figure 14.1. Energy relationships between energy production (catabolism) and energy utilization (anabolism).

Anabolic pathways are divergent.

ATP and NADPH are sources of free energy for biosynthetic reactions.



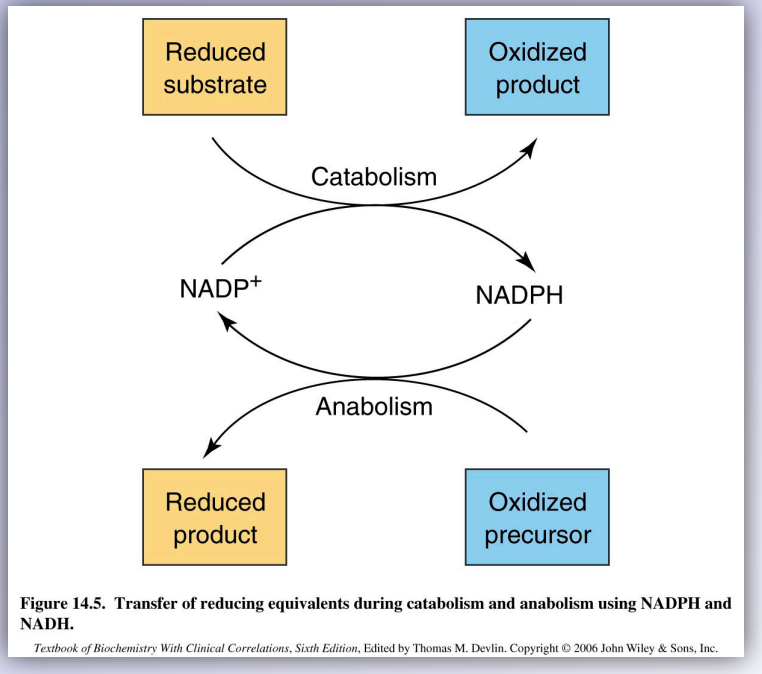
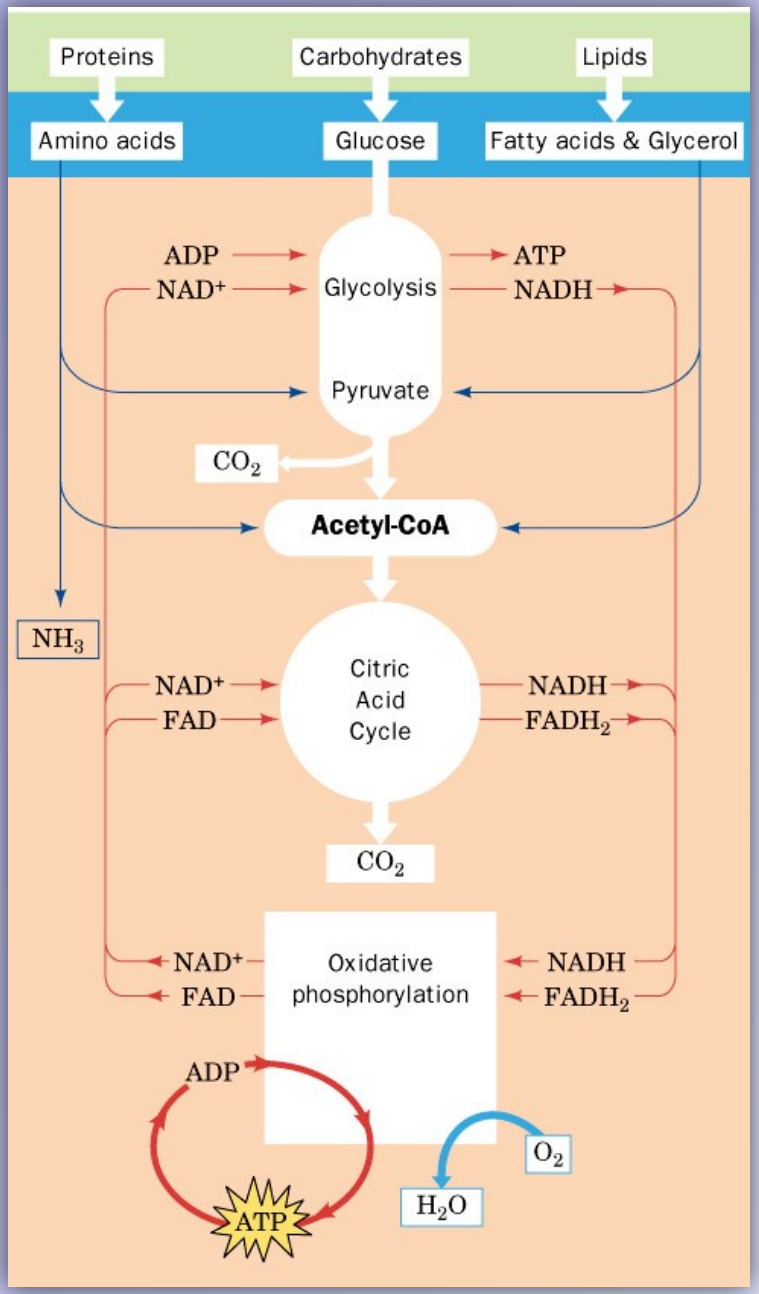


Figure 14.5. Transfer of reducing equivalents during catabolism and anabolism using NADPH and NADH.

Redox reactions (FAD, NAD$^+$) are a major source of free energy in living systems (Nernst equation).



An overview of human catabolism (aerobic)

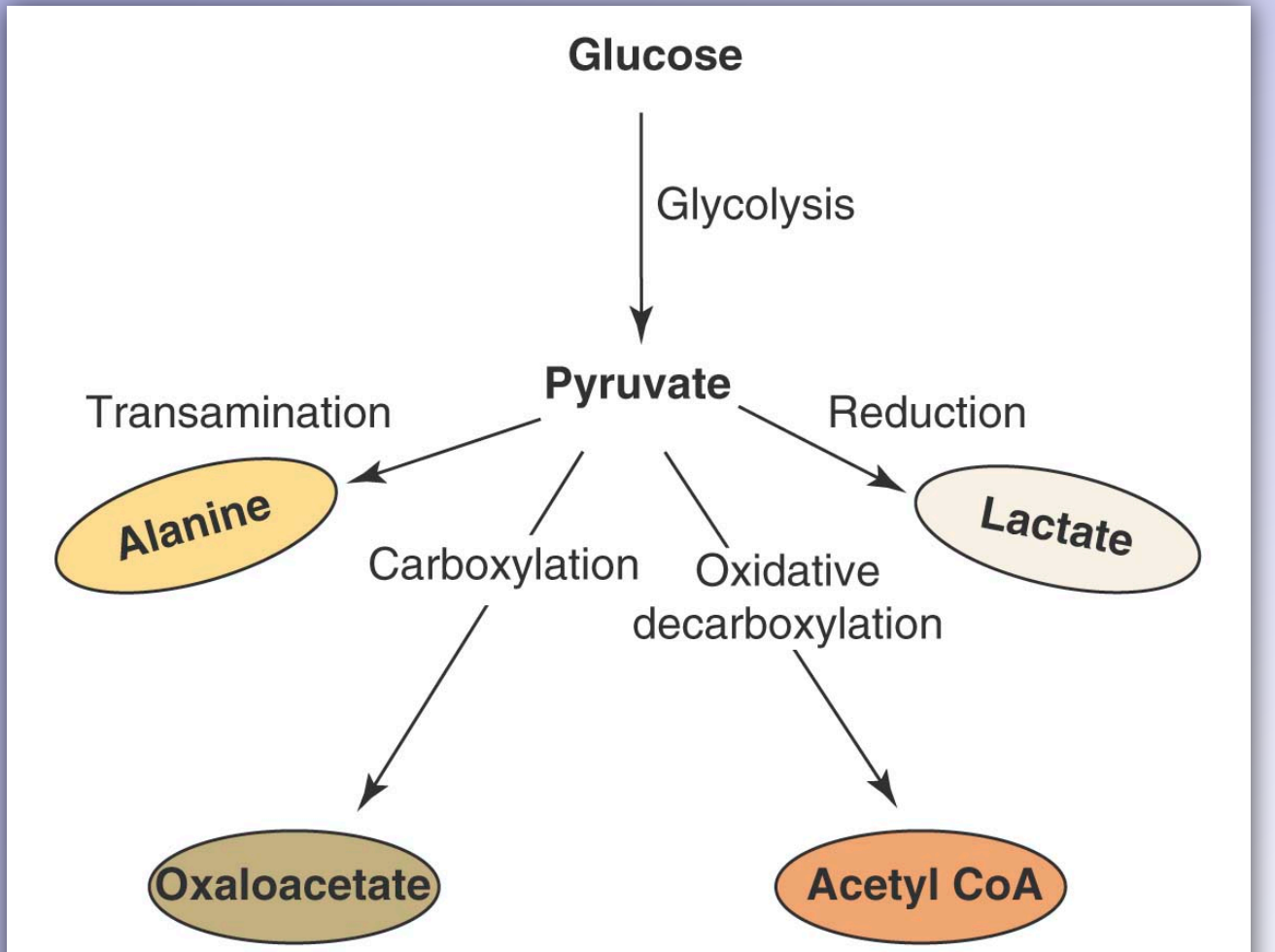


Figure 14.13. Metabolic fates of pyruvate.

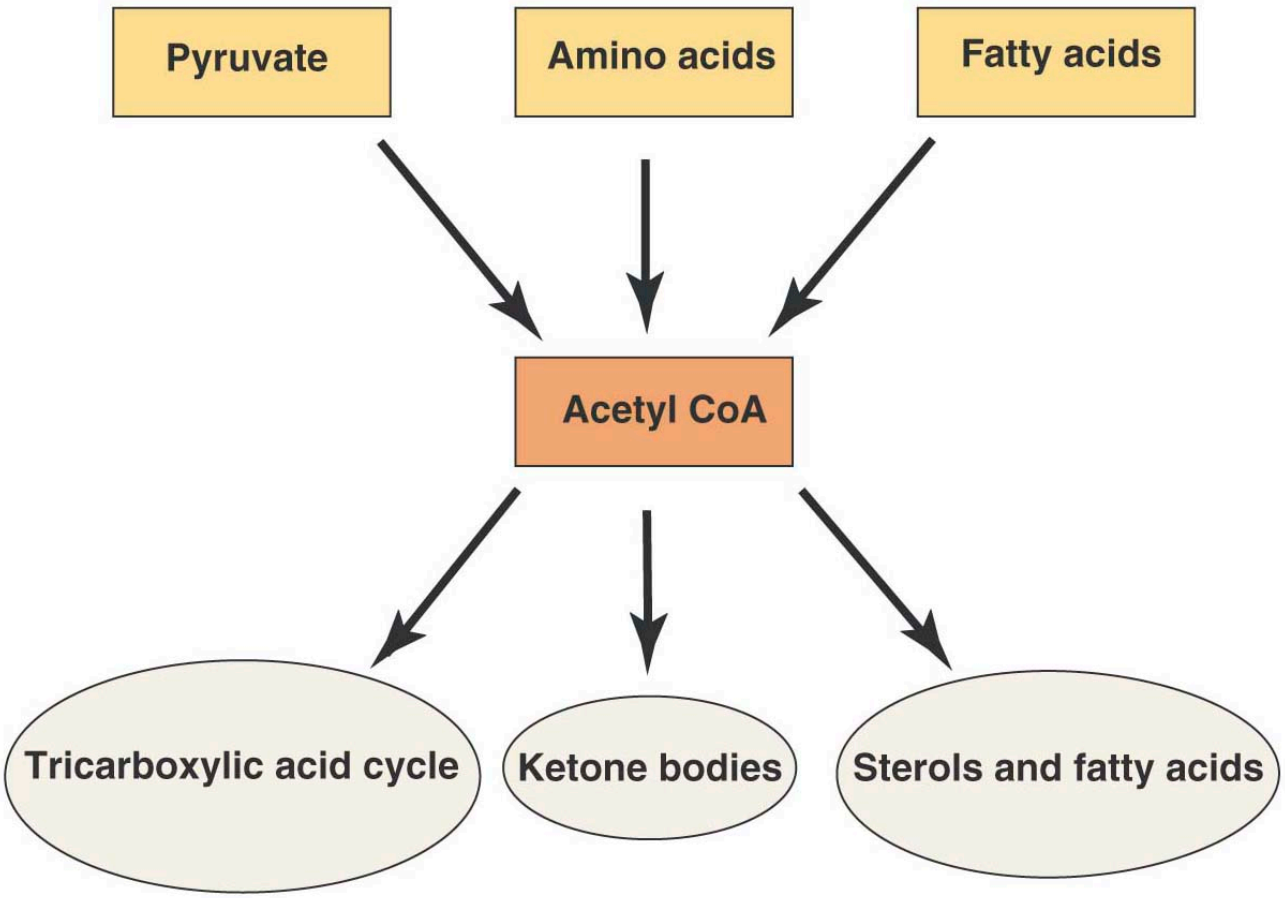


Figure 14.18. Sources and fates of acetylCoA.

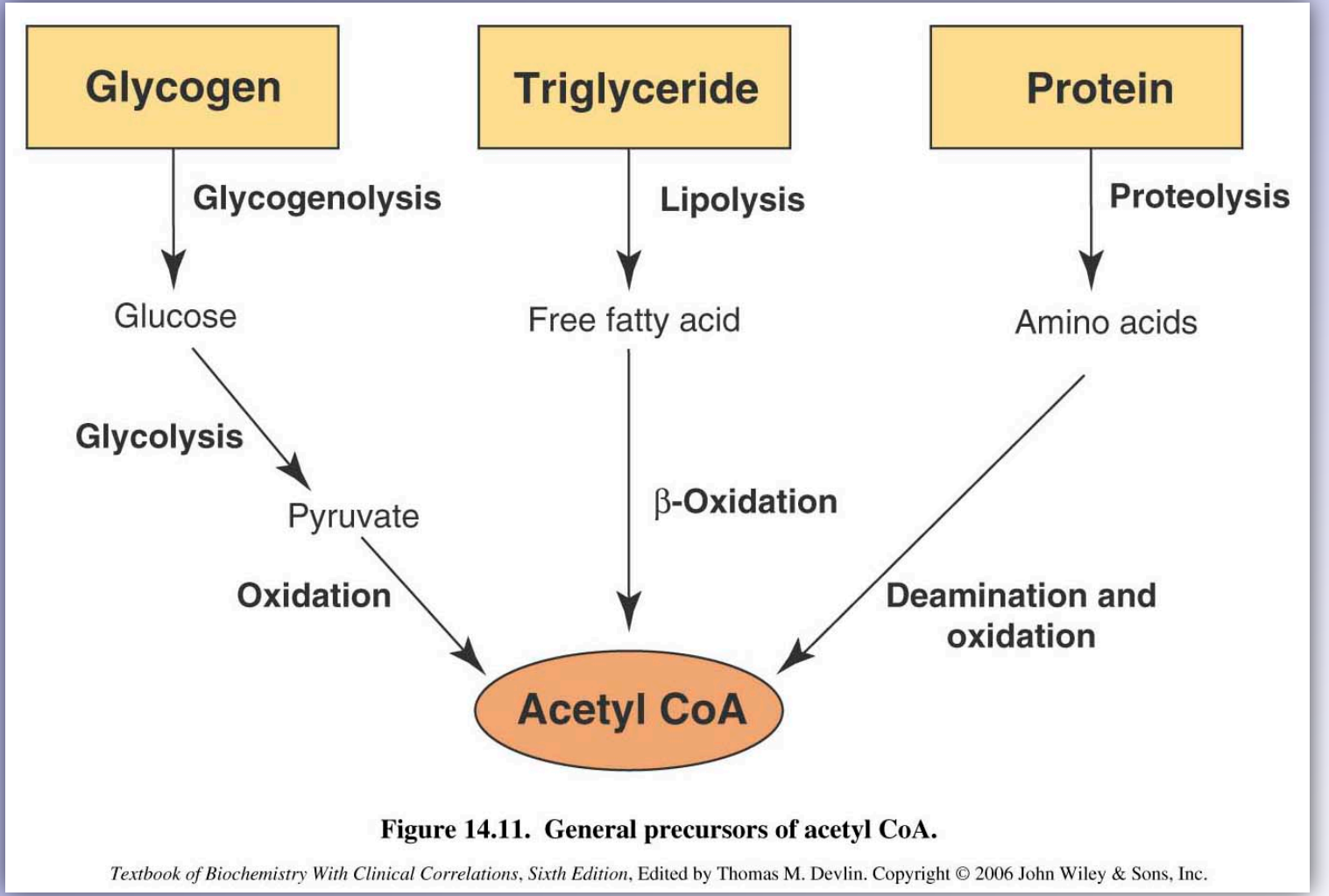
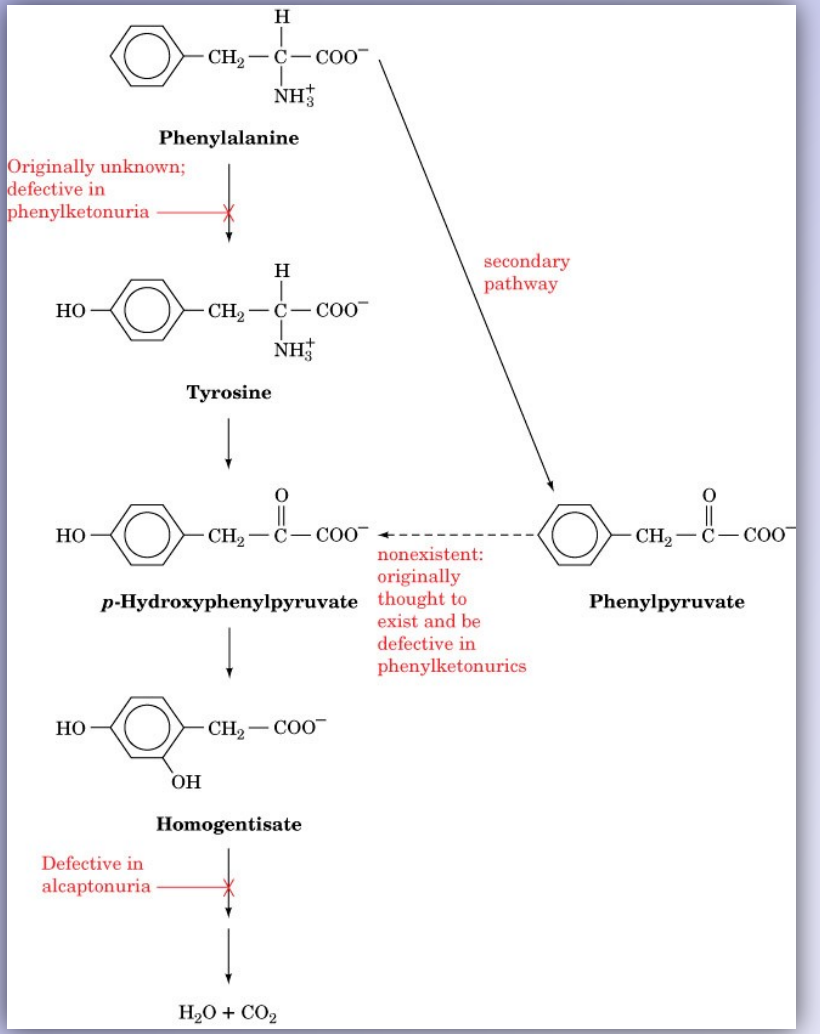


Figure 14.11. General precursors of acetyl CoA.

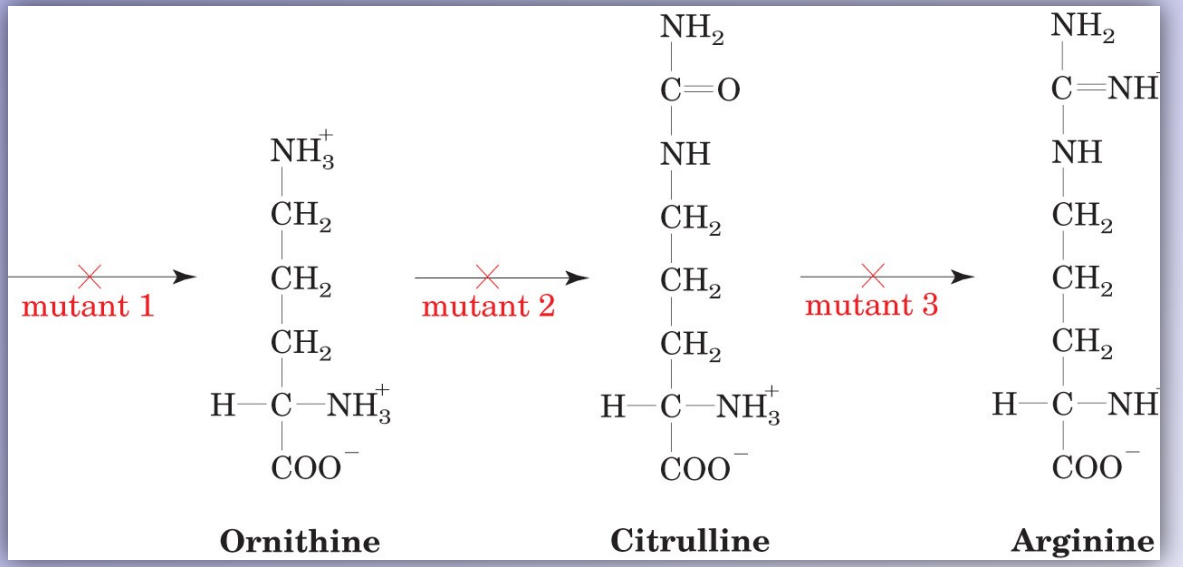
Methodology to elucidate metabolic pathways

Genetic defects cause metabolic intermediates to accumulate (metabolic bottlenecks).



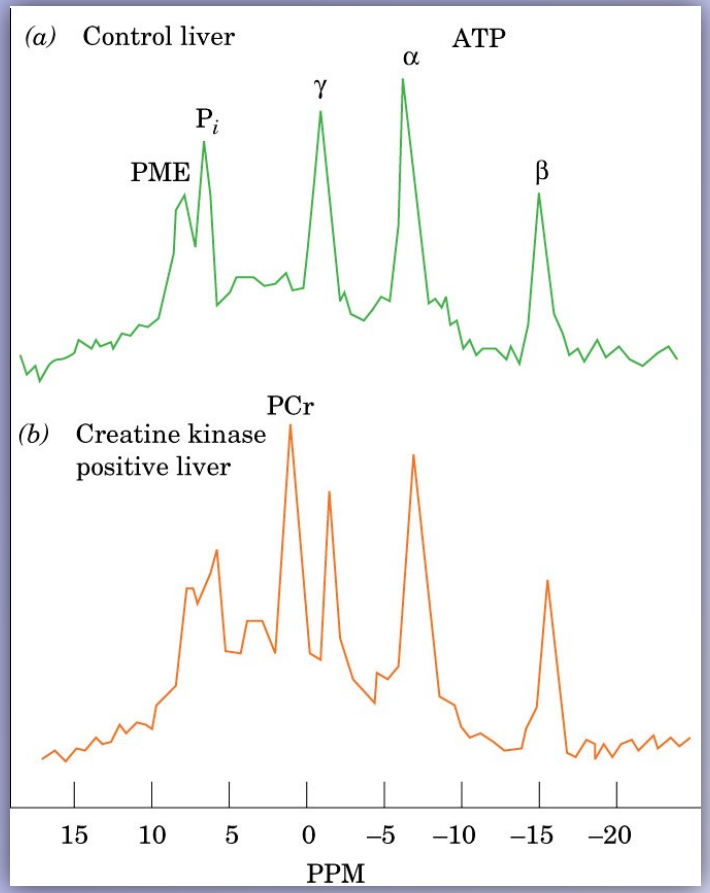
Pathway for phenylalanine degradation in humans

Generating metabolic blocks by genetic manipulation



Pathway of arginine biosynthesis deduced from studies of three arginine-requiring *auxotrophic mutants* (mutants requiring a specific nutrient for growth). In this case, the mutants were isolated after X-ray irradiation.

All mutants grow in the presence of arginine. Mutant 1 also grows in the presence of ornithine or citrulline, and mutant 2 grows in the presence of citrulline. Mutant 3 does not grow on ornithine or citrulline.



Use of transgenic organisms

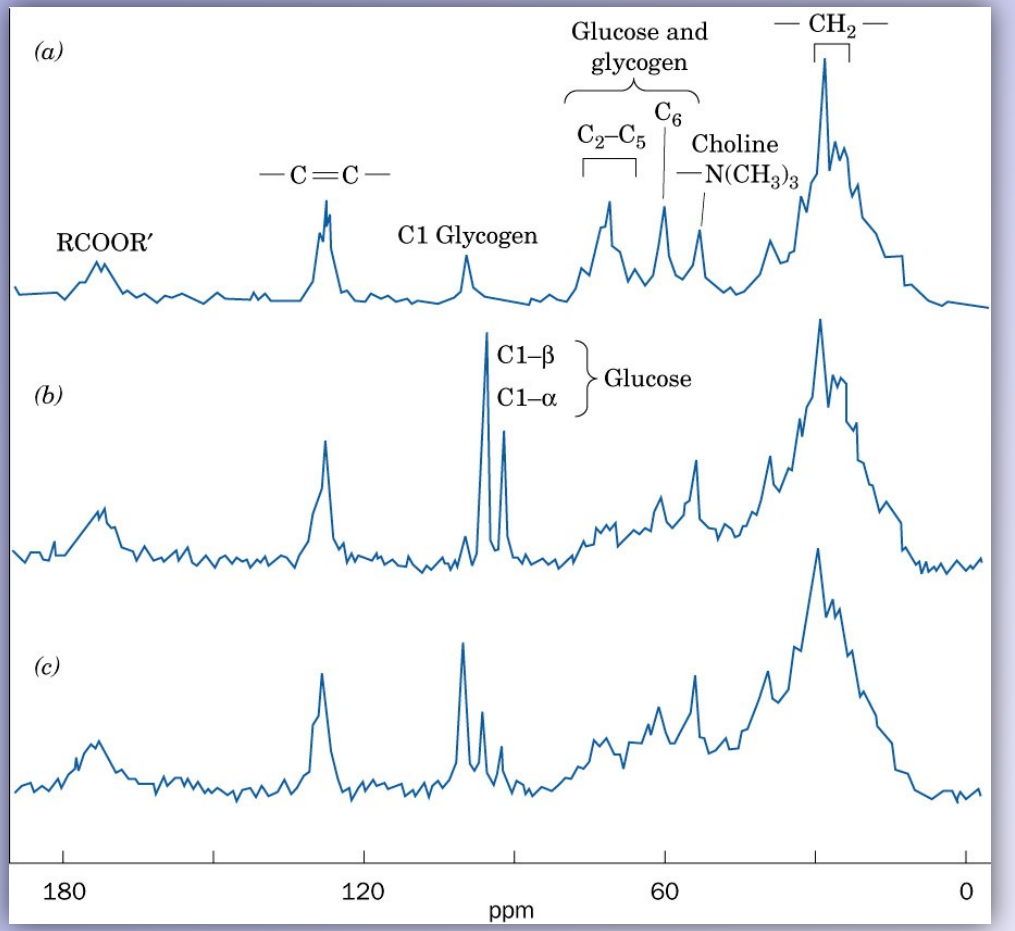
The expression of creatine kinase* in transgenic mouse liver as demonstrated by localized *in vivo* ^{31}P NMR.

A. Normal mouse liver after fed a diet supplemented with 2% creatine

B. Mouse liver transgenic for creatine kinase after feeding the same diet.

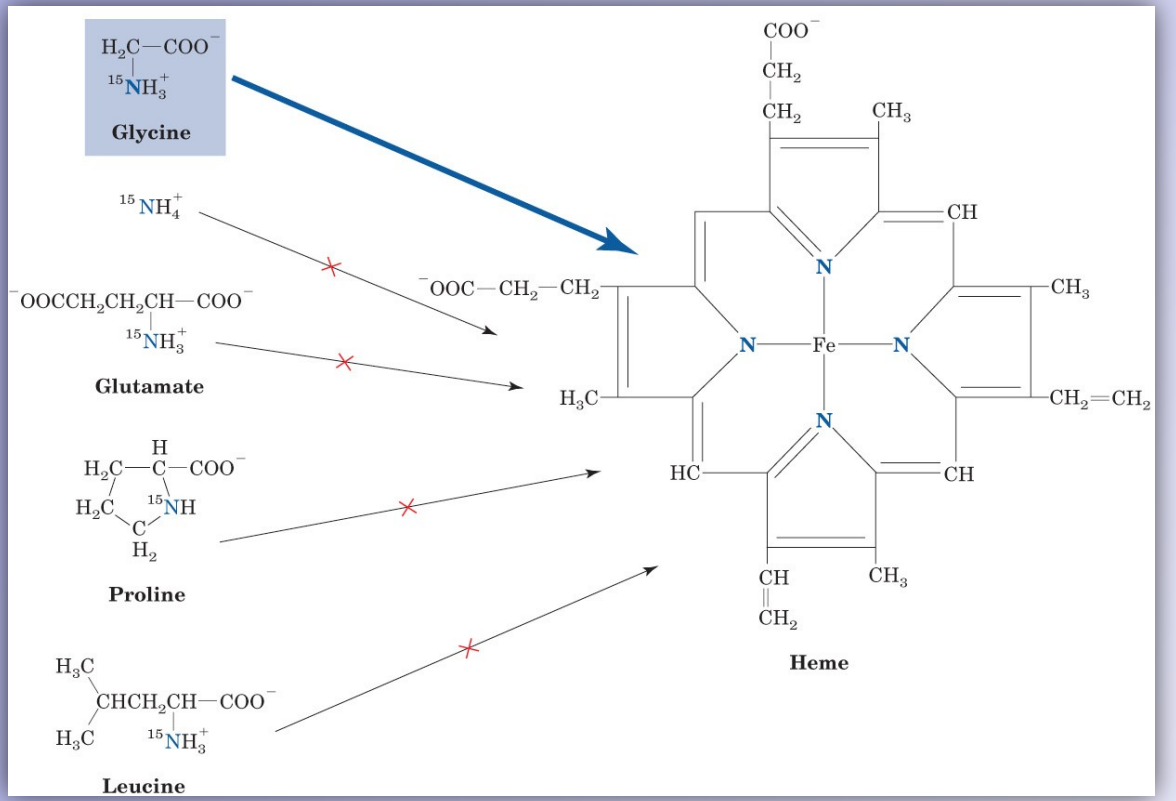
*CK converts creatine into creatine phosphate.

Use of stable isotopes and NMR spectroscopy



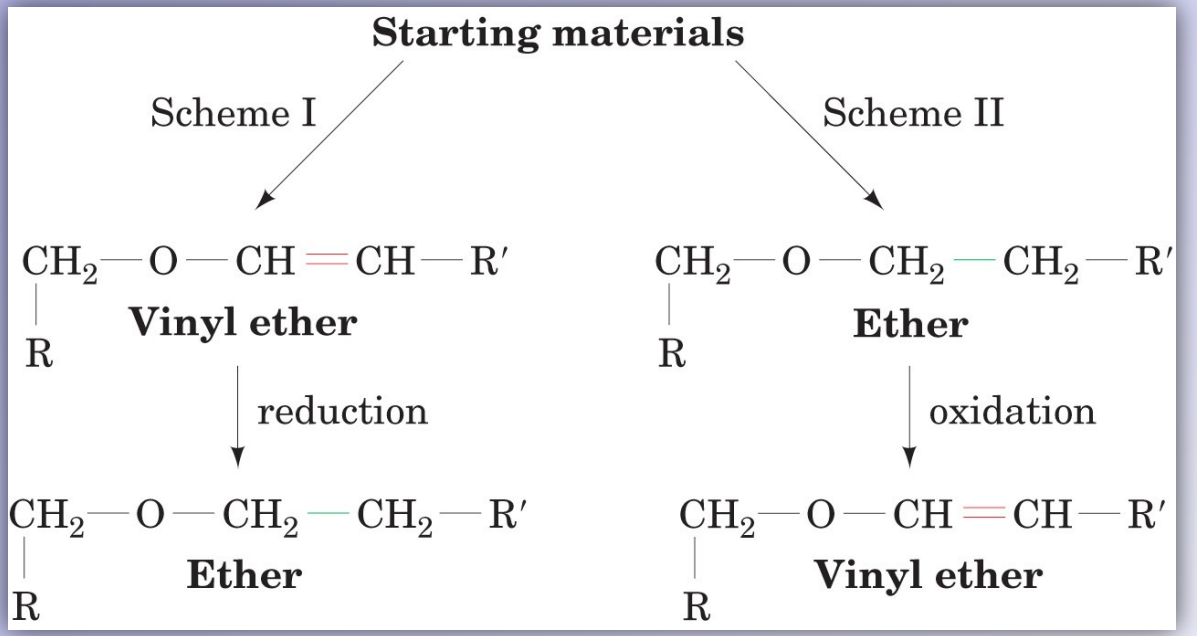
The conversion of D-[1-^{13}C]glucose to glycogen as observed by localized *in vivo* ^{13}C NMR.

Use of stable isotopes to establish metabolic origins of complex metabolites and precursor-product relationships



The metabolic origin of the nitrogen atoms in heme. Only [^{15}N] glycine among the various [^{15}N]labeled precursors tested, serves as a source of nitrogen in the biosynthesis of the porphyrin ring of heme.

Establishing precursor-product relationships (order of appearance of metabolic intermediates)

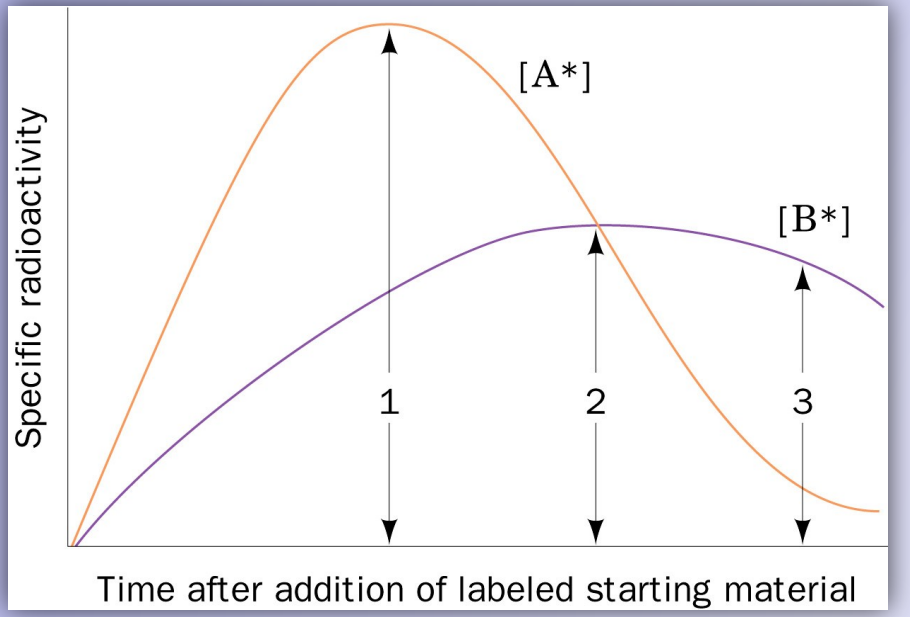


Two possible pathways for the biosynthesis of ether– and vinyl ether–containing phospholipids. The pathway shown in Scheme II is the correct pathway. How is this information obtained?

Radioactive tracers: pulse-chase experiments

A pulse of radiolabeled starting material is administered to an organism and the specific radioactivities of the resulting metabolic products are followed over time.

The flow of a pulse of radioactivity from precursor to product.



Criteria that must be met to establish that A* is the precursor of B*:

1. While the radioactivity of a product is rising, it should be <u>less than that</u> of its precursor.
2. When the radioactivity of a product is at its peak, it should be equal to that of its precursor. The radioactivity of a product peaks <u>after</u> that of its precursor.
3. After the radioactivity of a product has peaked, it should remain <u>greater than that</u> of its precursor.

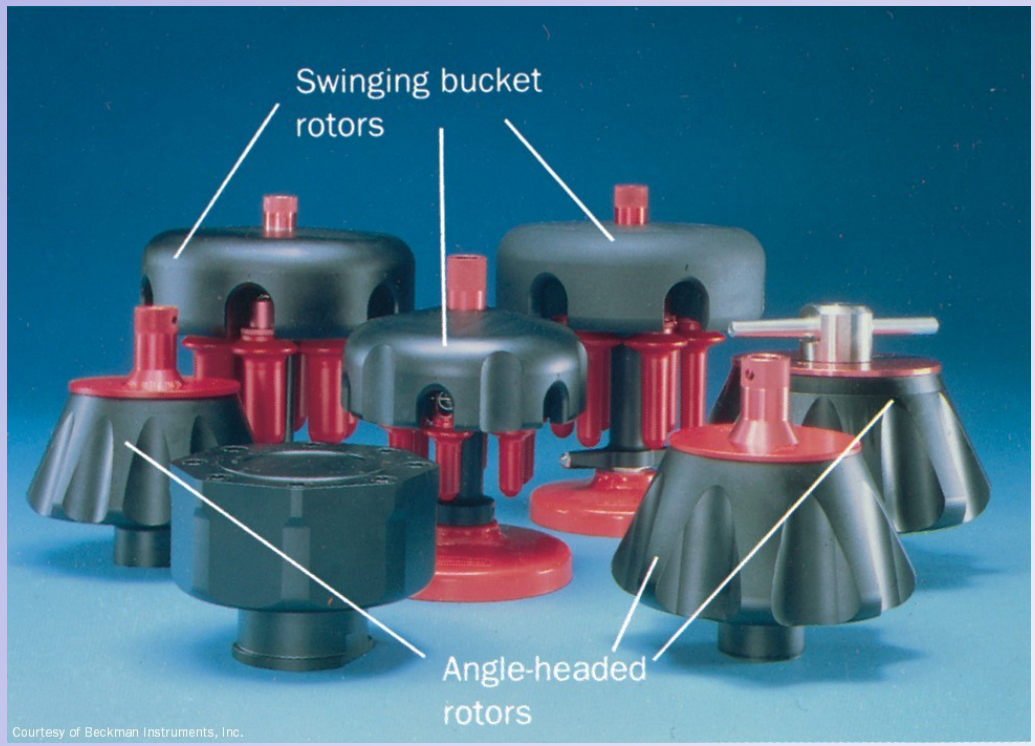
Compartmentation: Eukaryotic cells

Metabolic functions of eukaryotic subcellular organelles

Organelle	Function
Mitochondrion	Citric acid cycle, electron transport and oxidative phosphorylation, fatty acid oxidation, amino acid breakdown
Cytosol	Glycolysis, pentose phosphate pathway, fatty acid biosynthesis, many reactions of gluconeogenesis
Lysosomes	Enzymatic digestion of cell components and ingested matter
Nucleus	DNA replication and transcription, RNA processing
Golgi apparatus	Posttranslational processing of membrane and secretory proteins; formation of plasma membrane and secretory vesicles
Rough endoplasmic reticulum	Synthesis of membrane-bound and secretory proteins
Smooth endoplasmic reticulum	Lipid and steroid biosynthesis
Peroxisomes (glyoxisomes in plants)	Oxidative reactions catalyzed by amino acid oxidases and catalase; glyoxylate cycle reactions in plants

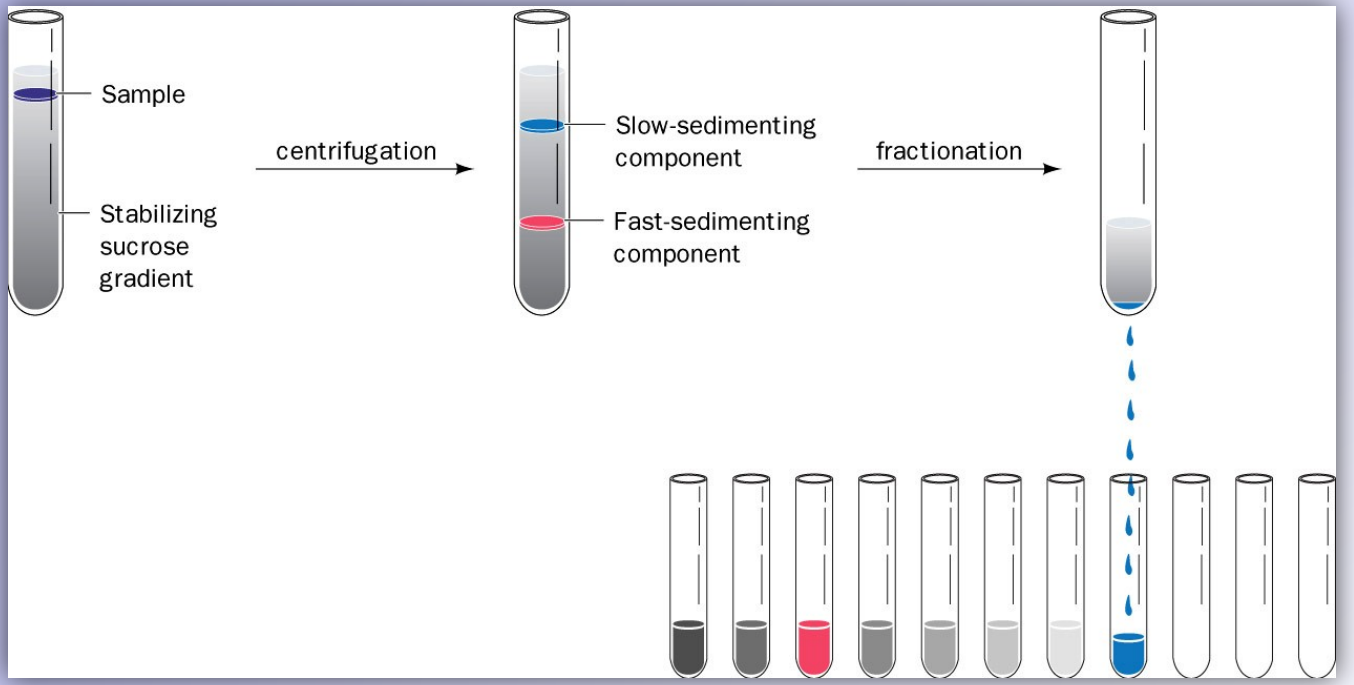
Subcellular organelle isolation in the laboratory

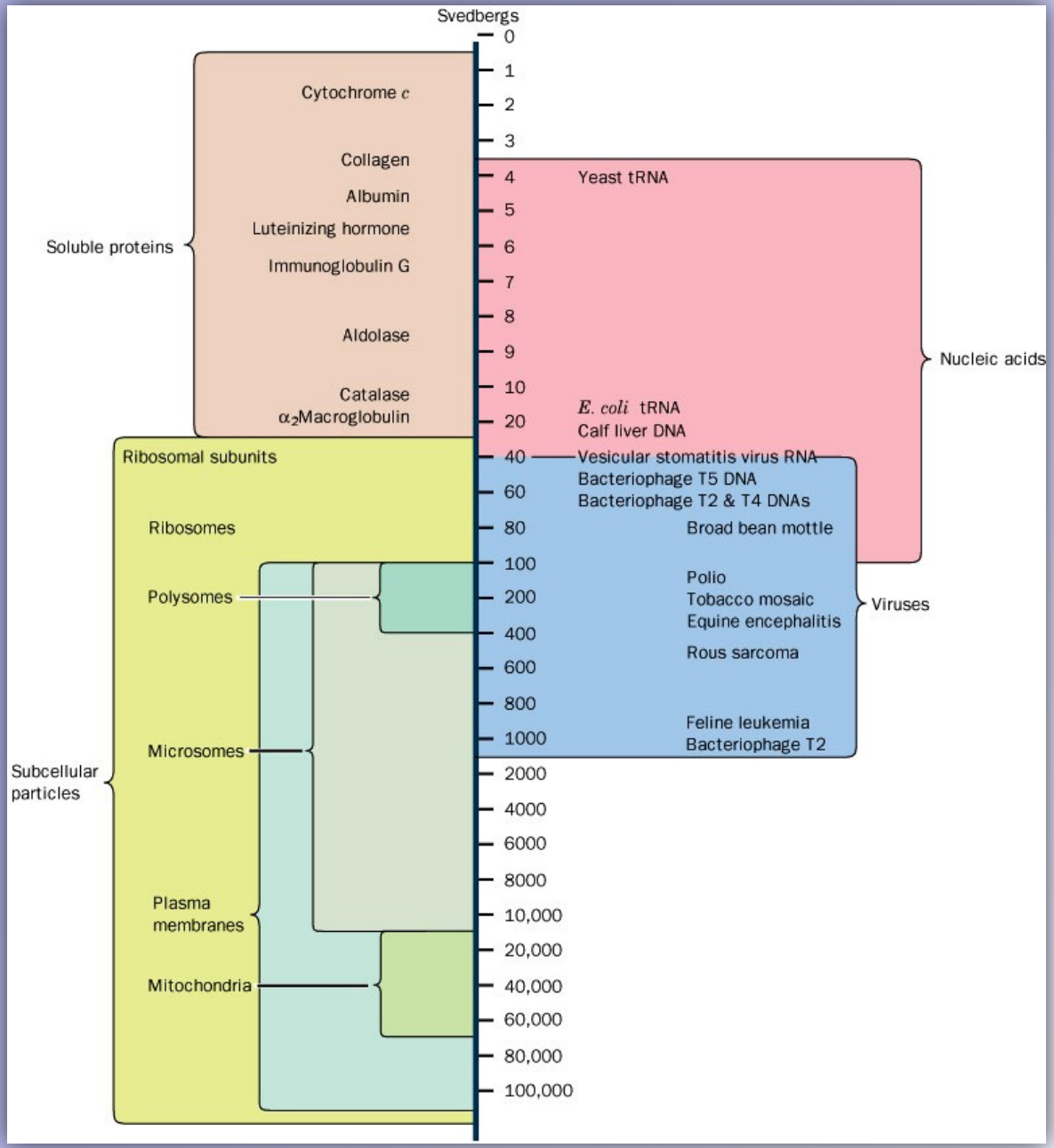
Ultracentrifugation



A selection of preparative ultracentrifuge rotors

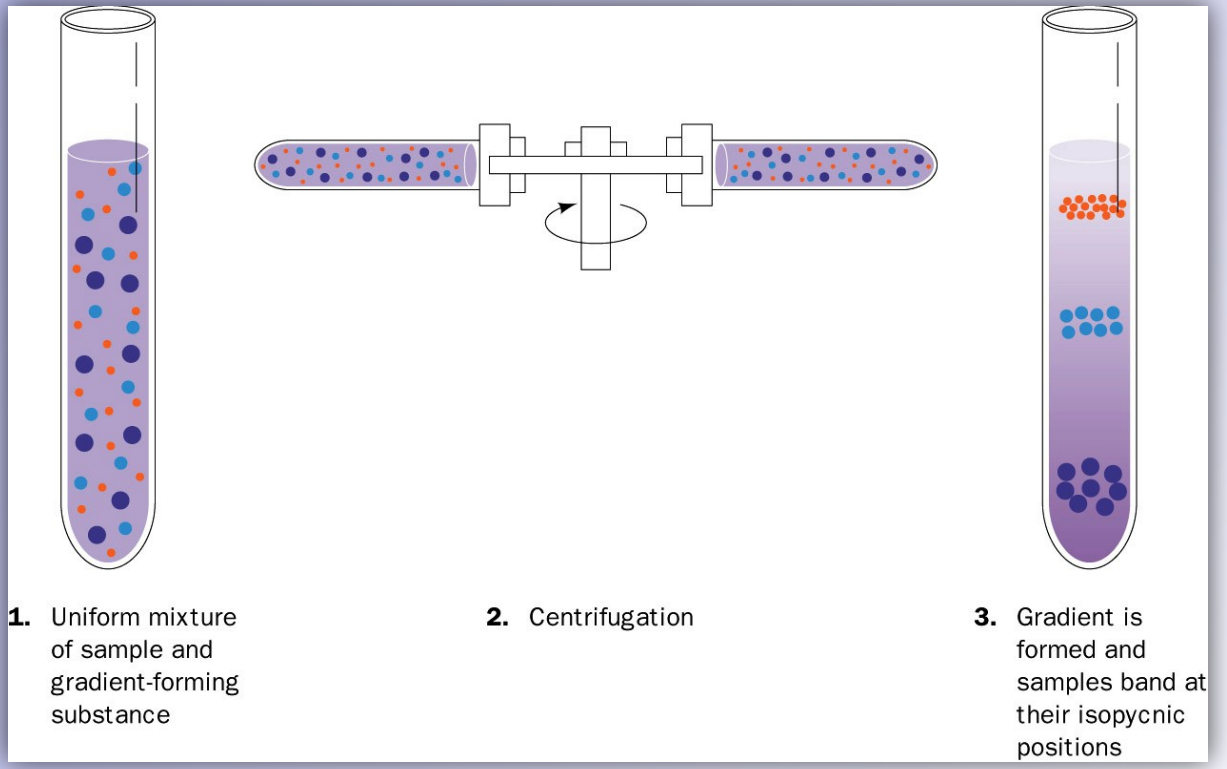
Zonal ultracentrifugation: uses a preformed sucrose density gradient. This method separates similarly shaped macromolecules largely on the basis of their molecular masses (differing sedimentation coefficients).





Sedimentation coefficients in Svedbergs (S) for some biological materials

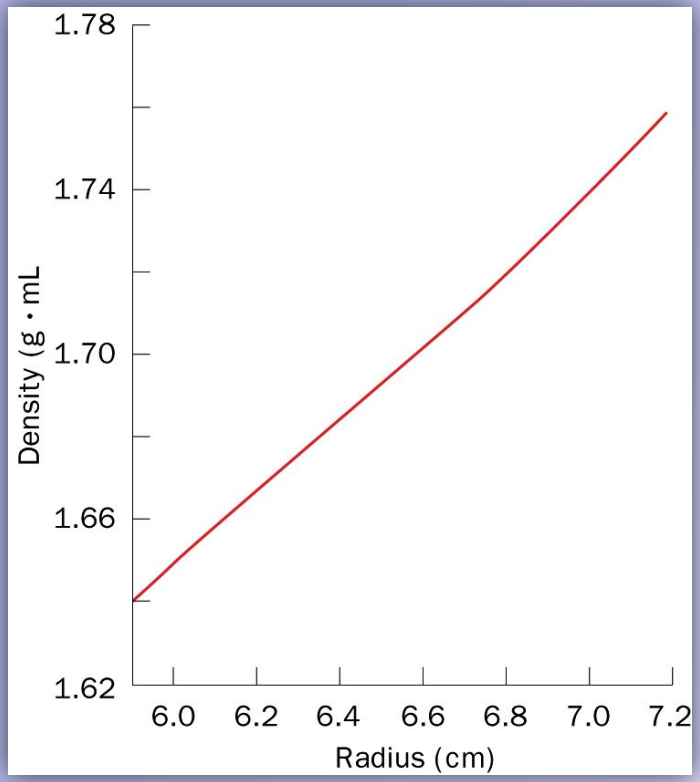
Isopycnic ultracentrifugation (also called equilibrium density gradient ultracentrifugation): Separates particles according to their densities. CsCl or Cs_2SO_4 solutions are spun at high speed to create a density gradient. Sample components band at positions where their densities equal that of the solution. Used for fractionation of **subcellular organelles**, not for fractionation of protein mixtures (proteins have similar densities).



1. Uniform mixture of sample and gradient-forming substance

2. Centrifugation

3. Gradient is formed and samples band at their isopycnic positions



Equilibrium density distribution of a CsCl solution in an ultracentrifuge spinning at 39,460 rpm

Densities of biological material

Material	Density (g/cm^3)
Microbial cells	1.05 - 1.15
Mammalian cells	1.04 - 1.10
Organelles	1.10 - 1.60
Proteins	1.30
DNA	1.70
RNA	2.00

Omics hierarchies in the study of biological metabolism

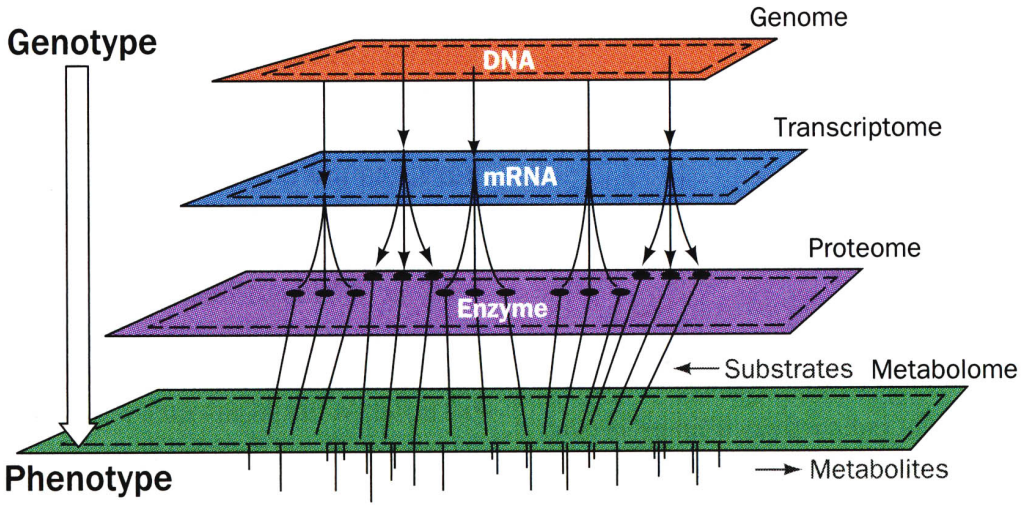


Figure 16-20 **The relationship between genotype and phenotype.** The path from genetic information (genotype) to metabolic function (phenotype) has several steps. Portions of the genome are transcribed to produce the transcriptome, which directs the synthesis of the proteome, whose various activities are responsible for synthesizing and degrading the components of the metabolome.

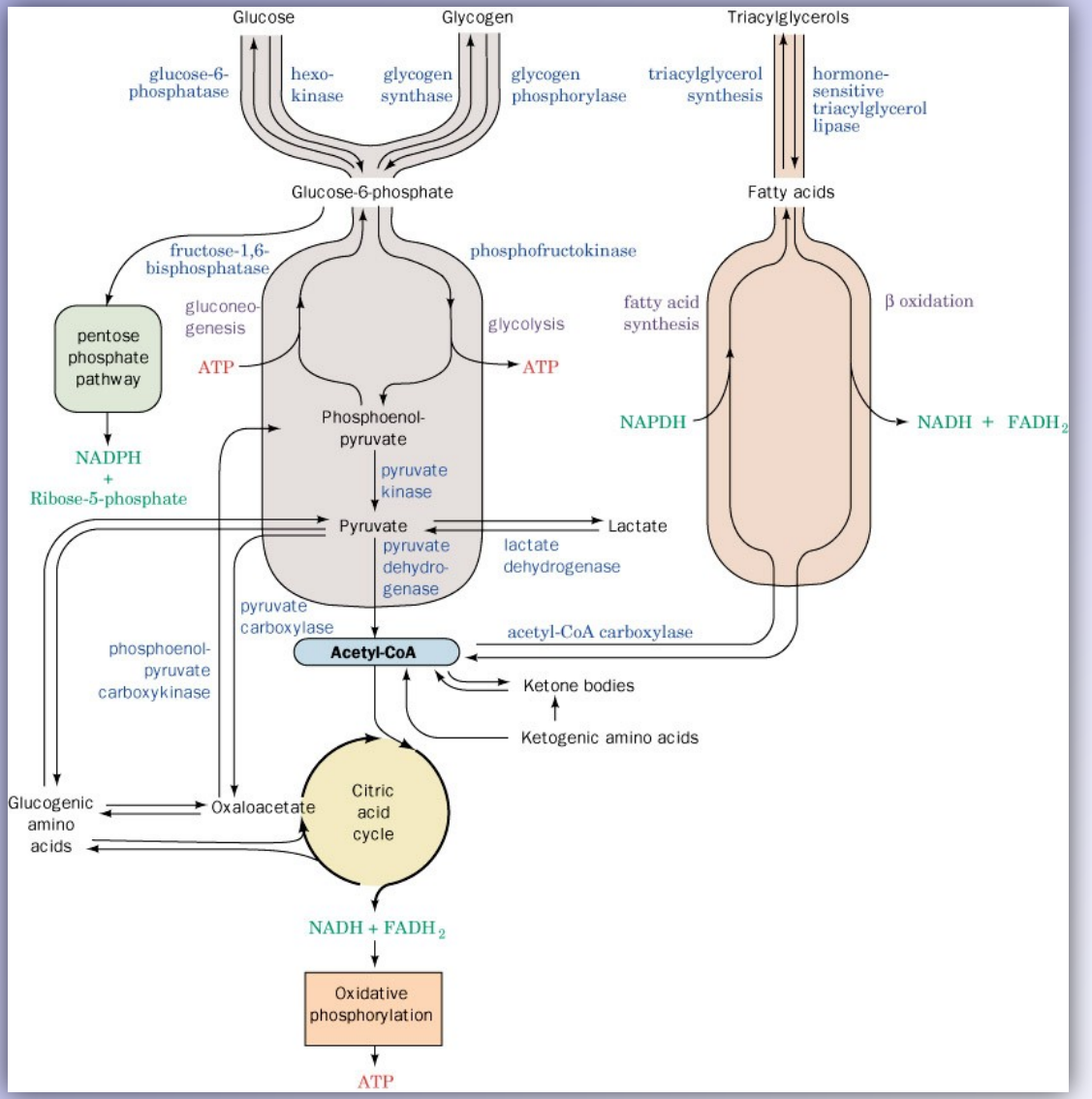
Fuel reserves in a normal 70-kg man

Fuel	Mass (kg)	Calories[a]
Tissues		
Fat (adipose triacyglycerols)	15	141,000
Protein (mainly muscle)	6	24,000
Glycogen (muscle)	0.150	600
Glycogen (liver)	0.075	300
Circulating fuels		
Glucose (extracellular fluid)	0.020	80
Free fatty acids (plasma)	0.0003	3
Triacylglycerols (plasma)	0.003	30
Total		166,000

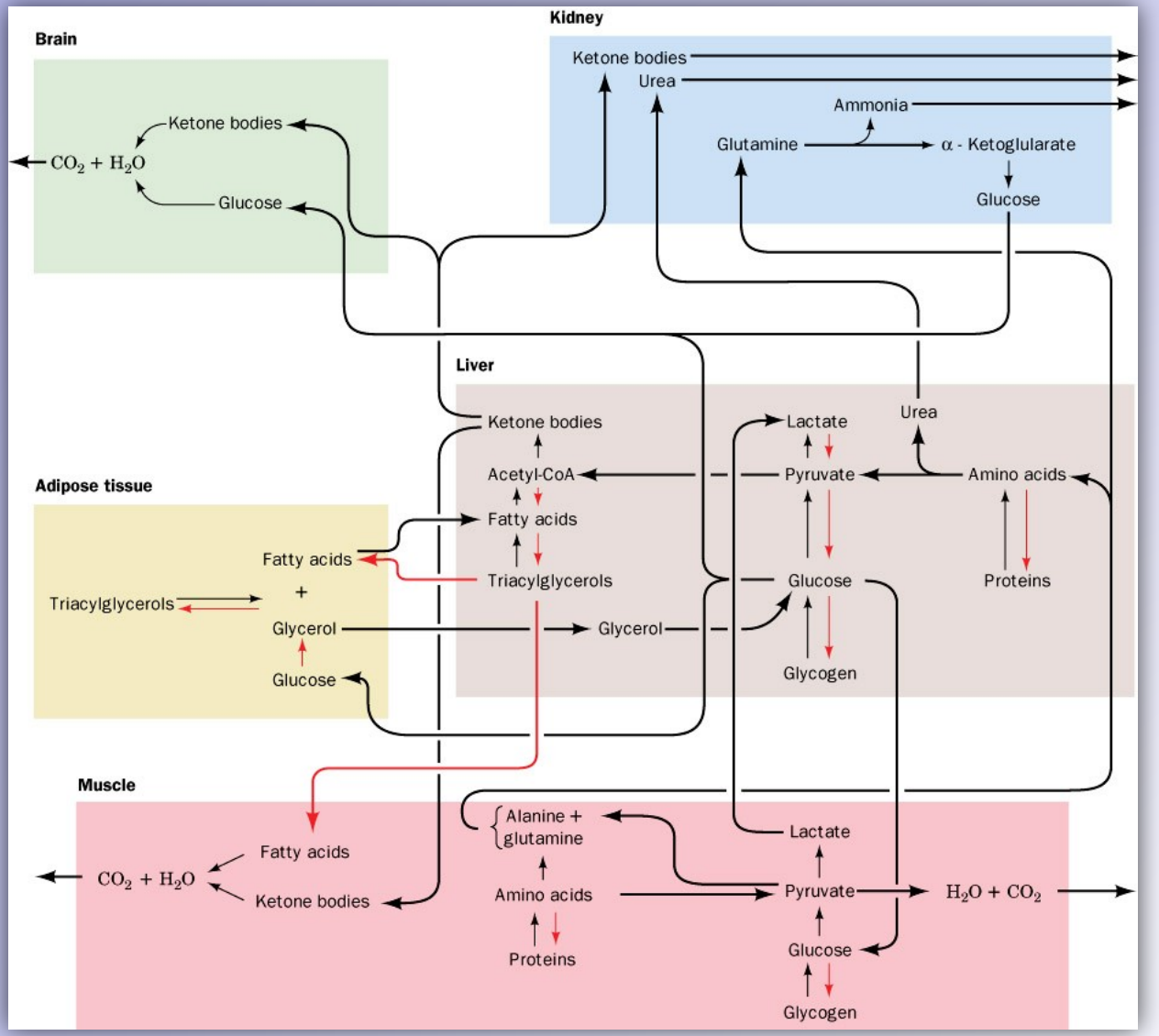
[a]One (dieter's) Calorie = 1 kcal = 4.184 kJ.

Source: Cahill, G.F., Jr., *New Engl. J. Med.* **282,** 669 (1970).

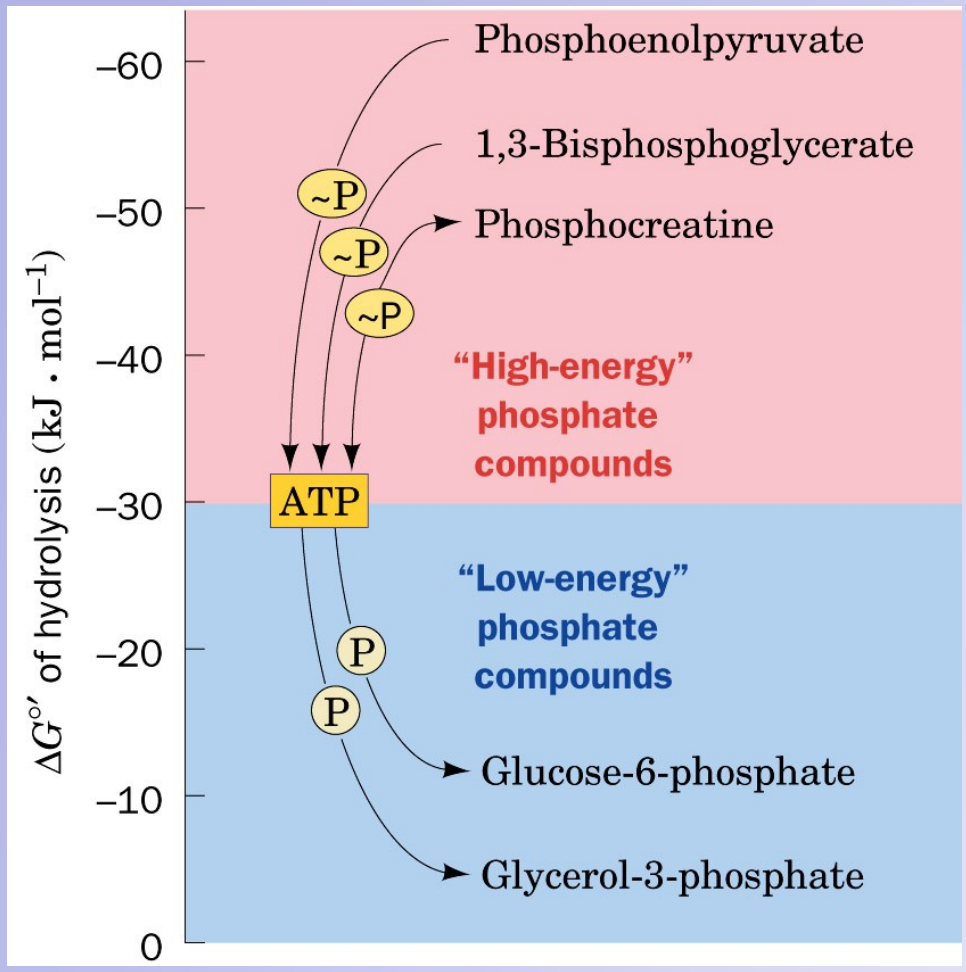
The major energy metabolism pathways in humans



Metabolic interrelationships between brain, adipose tissue, muscle, liver and kidney in humans

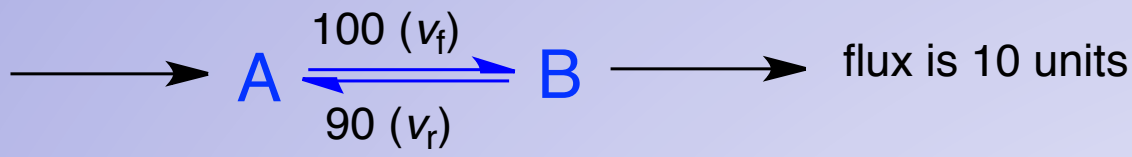


The flow of phosphoryl groups from "high-energy" phosphate donors, via the ATP-ADP system, to "low-energy" phosphate acceptors.

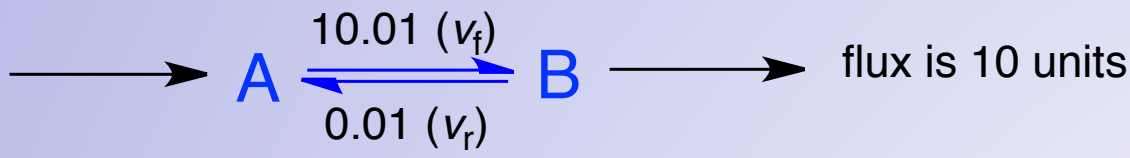


The coupling of catabolism with the generation of high-energy metabolites such as ATP and NADH.

Equilibrium (reversible) and non-equilibrium (irreversible) reactions in metabolic pathways



near-equilibrium reaction *in vivo*



non-equilibrium reaction *in vivo*

The net flux through the pathway is given by (v_f-v_r). In the non-equilibrium reaction, the rate of the forward reaction dominates, so that the net flux is almost identical to this rate. In the near-equilibrium reaction, both forward and reverse rates are almost identical but considerably in excess of the flux.