Advanced Fish Feeding:
8- Absorption & Metabolism of food

Part 1: Nutrient Absorption

Part 2: Nutrient Metabolism

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Part 1: Nutrient Absorption

Most nutrient absorption occurs in the intestine

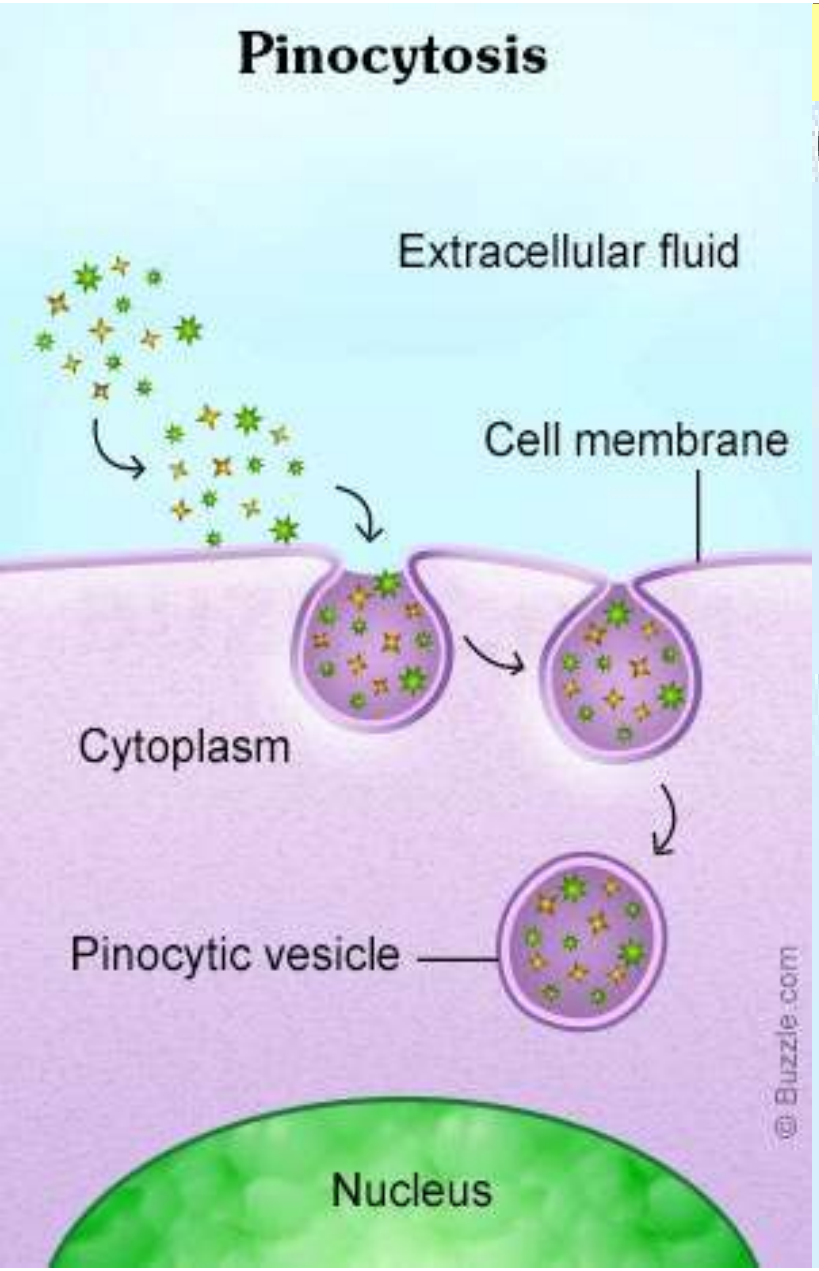
a cross-section of the intestinal luma shows that it is highly convoluted, increasing surface area

absorption through membrane is either by **passive diffusion** (concentration gradient)

or by **active transport** (requires ATP)

or via **pinocytosis** (particle engulfed)

nutrients absorbed by passive diffusion include: electrolytes, monosaccharides, some vitamins, smaller amino acids


Pinocytosis
Extracellular fluid
Cell membrane
Cytoplasm
Pinocytic vesicle
Nucleus
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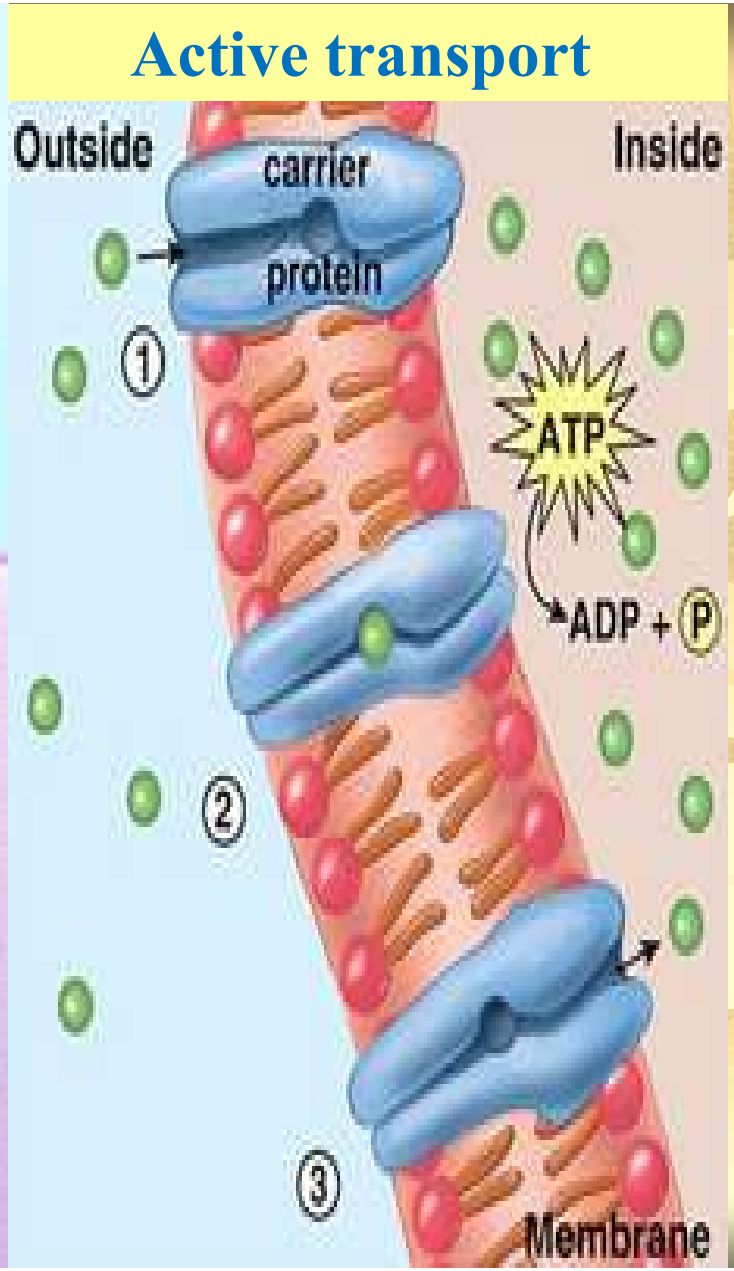
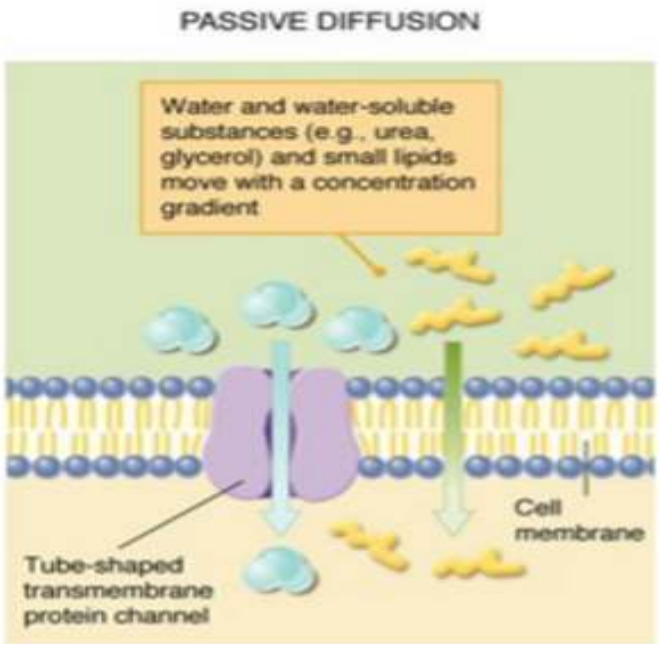
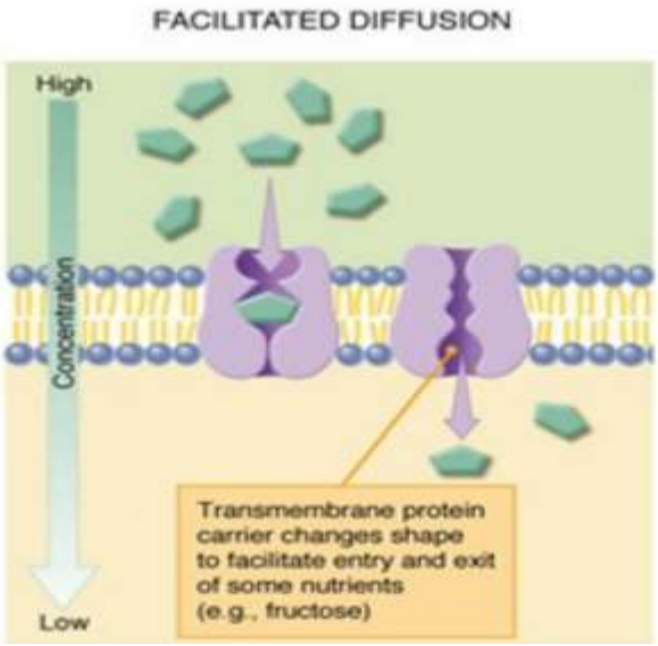
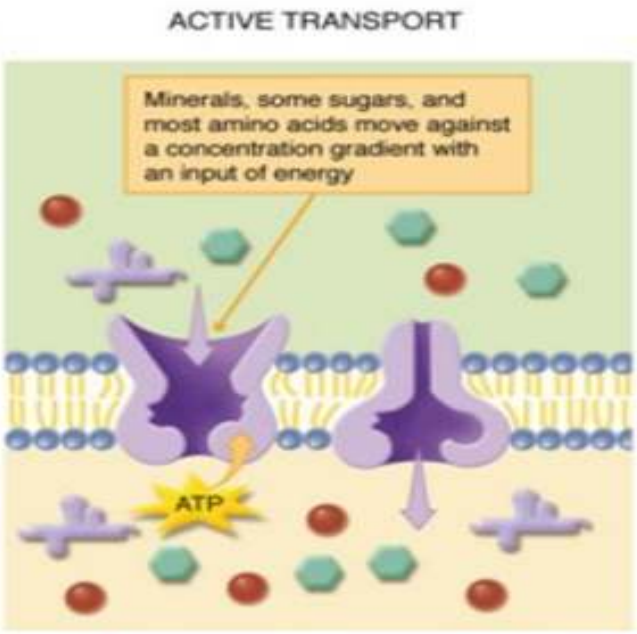

Active transport
Outside
carrier
protein
Inside
1
ATP
ADP + P
2
3
Membrane

Overview of Absorption

- The road to nutrition absorption
  - Passive diffusion
  - Facilitated diffusion
  - Active transport



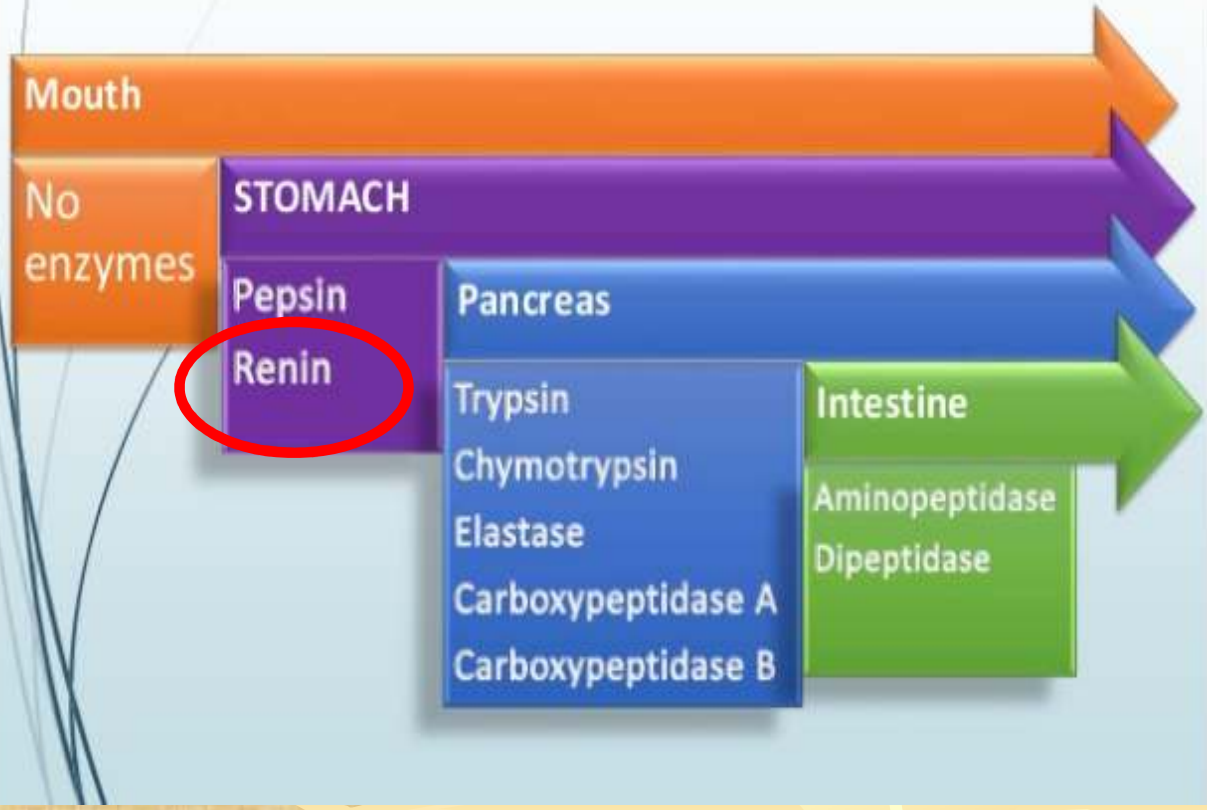




Protein Digestion

- Proteins are broken down to
  - Tripeptides
  - Dipeptides
  - Free amino acids

Digestion of Proteins (Overview)

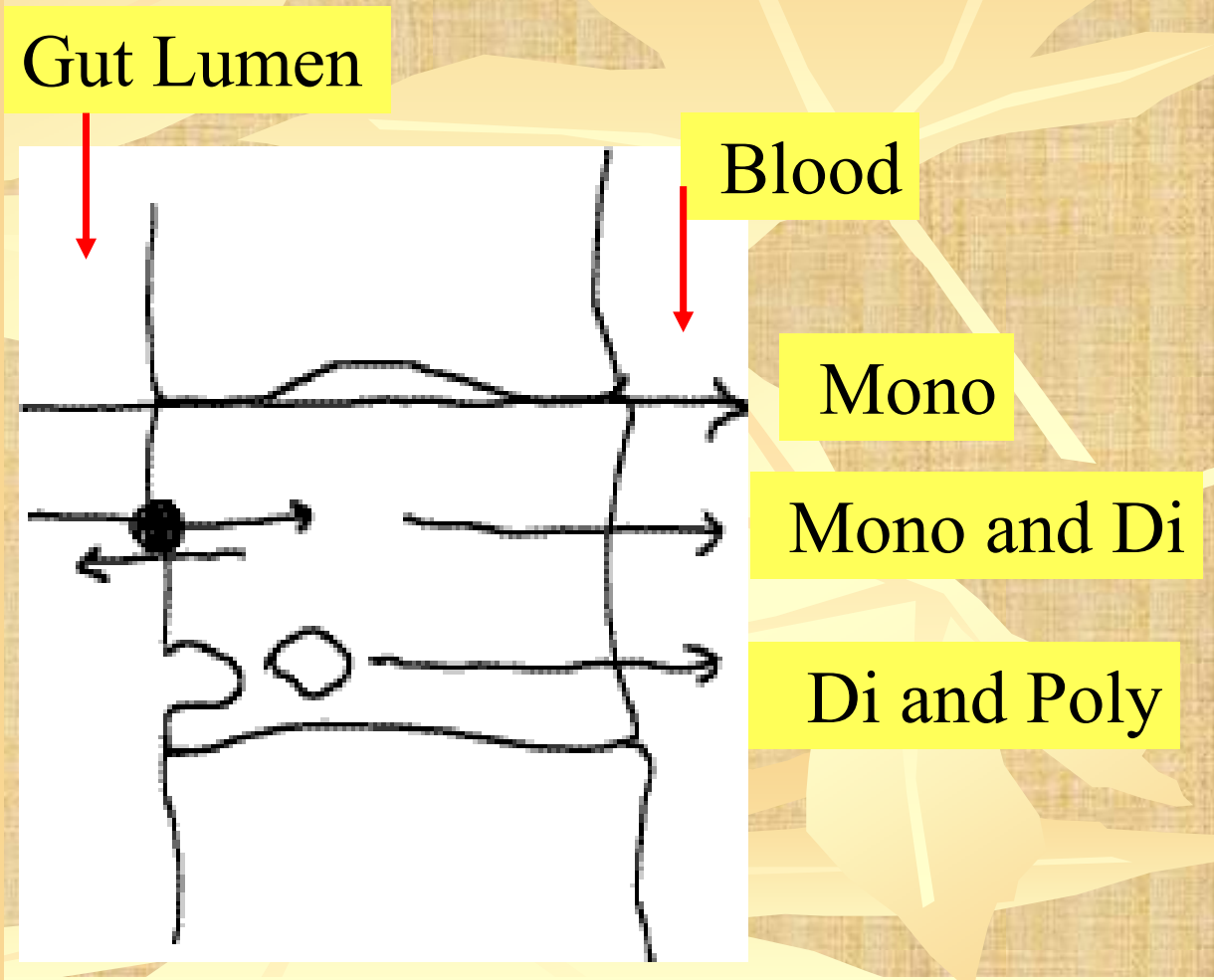


Absorption of amino acids

- Absorption of most of the AAs by active transport mechanism.
- By Na+ dependent active transport system (Na^+ amino acid cotransport)
- An energy requiring process

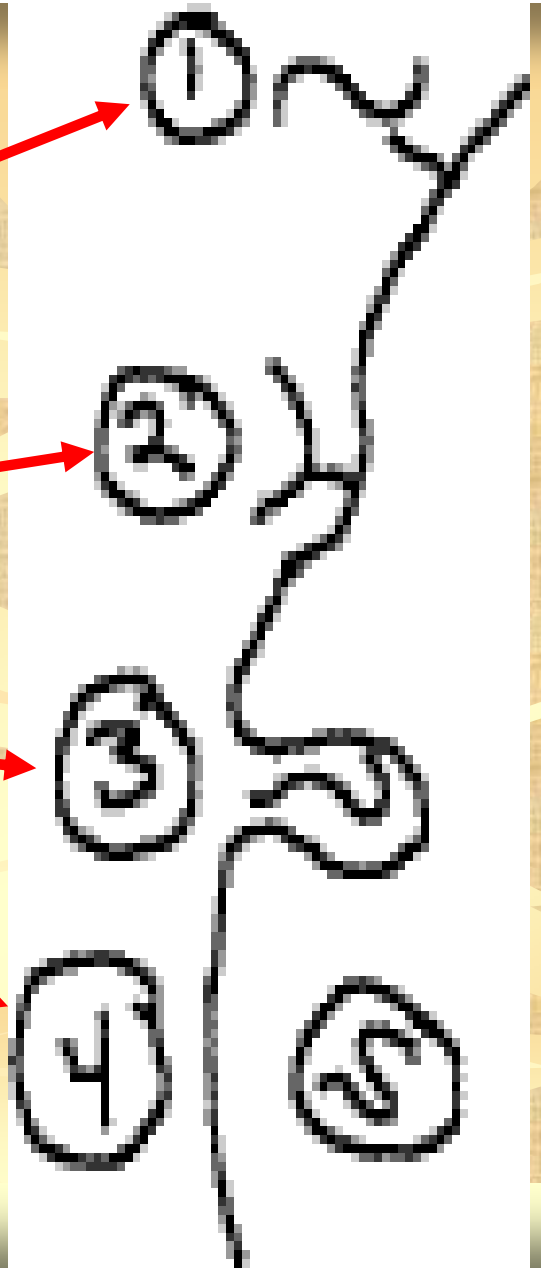
Proteins Absorption

- Mono-peptides (Amino Acids)
  - CoTransport (Antiport) via Na Linked System
  - Movement Between Cells
- Di-peptides
  - CoTransport (Antiport)
  - Pinocytosis
- Poly-peptides
  - Pinocytosis



Trans-Membrane Transport of Macromolecules

- 1 Attachment of molecule to receptor or surface
- 2 Involution of surface
- 3 Engulfment of molecule
- 4 Pinching off and import of macromolecule into the cell



Defects in protein digestion and absorption

- For a **number of reasons**, protein absorption might be incomplete
- **Some of the proteins**, because of their **physical or chemical structure**, are **resistant to proteolytic** attack and therefore **pass** through the small intestine relatively **unmodified**
- Furthermore, the **absorption** of free amino acids and peptides may be **less than 100%,** particularly **if gut function is impaired**

- This **occurs** in a number of clinical conditions, such as **intestinal infection** or **injury**, and when certain **'antinutritional' factors** such as **lectins** or **trypsin inhibitor proteins** are **present in the diet**
- This **unabsorbed protein or amino acid** then **passes** through **into the rectum.**
- **Metabolism by the rectum microflora then occurs, but the amino acids are no longer available to the body, and are excreted in the faeces, mainly in the form of bacterial protein.**

Lipid Digestion/Absorption

- Fats serve a structural function in cells, as sources of energy, and insulation
- the poor water solubility of lipids presents a problem for digestion: substrates are not easily accessible to digestive enzymes
- even if hydrolyzed, the products tend to aggregate to larger complexes that make poor contact with the cell surface and aren't easily absorbed
- to overcome these problems, changes in the physical state of lipids are connected to chemical changes during digestion and absorption

Lipid Digestion/Absorption

Five different phases:

- hydrolysis of triglycerides (TG) to free fatty acids (FFA) and monoacylglycerols
- solubilization of FFA and monoacylglycerols by detergents (bile acids) and transportation from the intestinal lumen toward the cell surface
- uptake of FFA and monoacylglycerols into the cell and resynthesis to triglyceride
- packaging of TG's into chylomicrons
- exocytosis of chylomicrons into lymph

Enzymes Involved in Digestion of Lipids

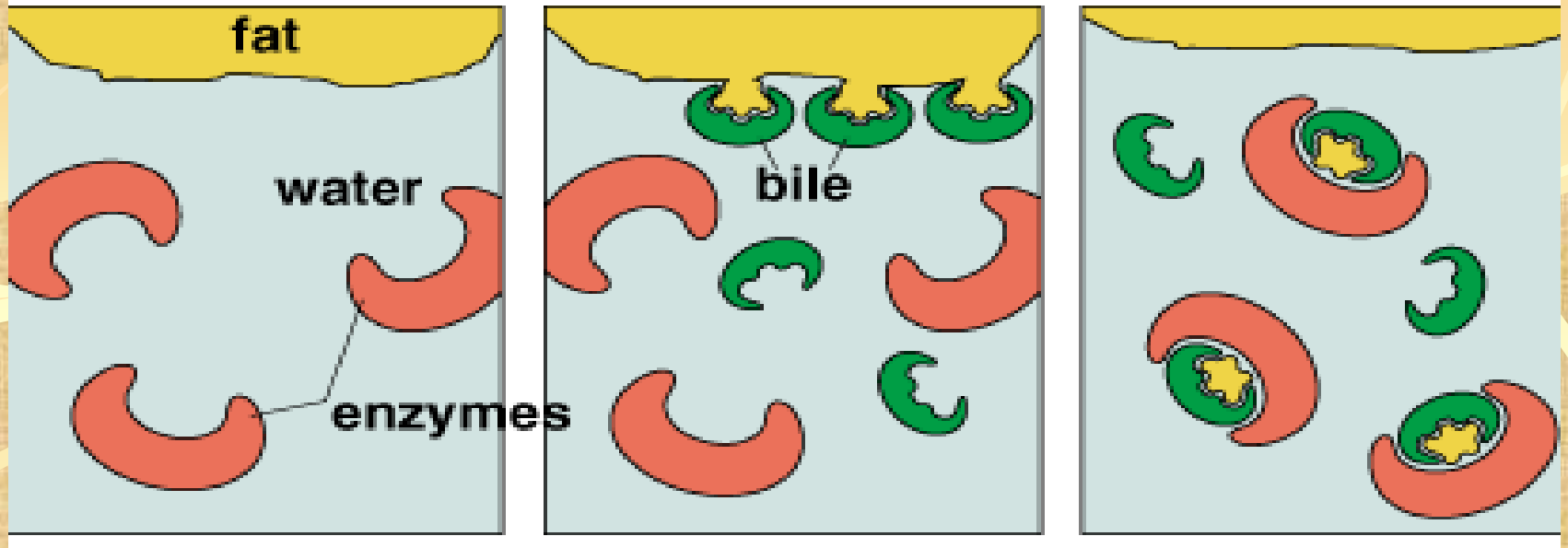
- lingual lipase: provides a stable interface with aqueous environment of stomach
- pancreatic lipase: major enzyme affecting triglyceride hydrolysis
- colipase: protein anchoring lipase to the lipid
- lipid esterase: secreted by pancreas, acts on cholestrol esters, activated by bile
- phospholipases: cleave phospholipids, activated by trypsin

What about Bile???

- These are biological detergents synthesized by the liver and secreted into the intestine
- they form the spherical structures (micelles) assisting in absorption
- hydrophobic portion (tails of FA) are located to the inside of the micelle, with heads (hydrophillic portion) to the outside
- they move lipids from the intestinal lumen to the cell surface
- absorption is by diffusion (complete for FA and monoglycerides, less for others)

Lipids Absorption

- Lipids
  - Bile Emulsification
  - Absorption
  - Conversion to Lipoproteins (Complex Aggregates of Macromolecules)
  - Volatile Fatty Acids Directly Absorbed (Small Sized Molecules with polar/nonpolar groups



Fat and water tend to separate. Enzymes are in the water and can't get at the fat.

Bile (an emulsifier) arrives. Bile has an affinity for both fat and water and can therefore bring the fat into the water.

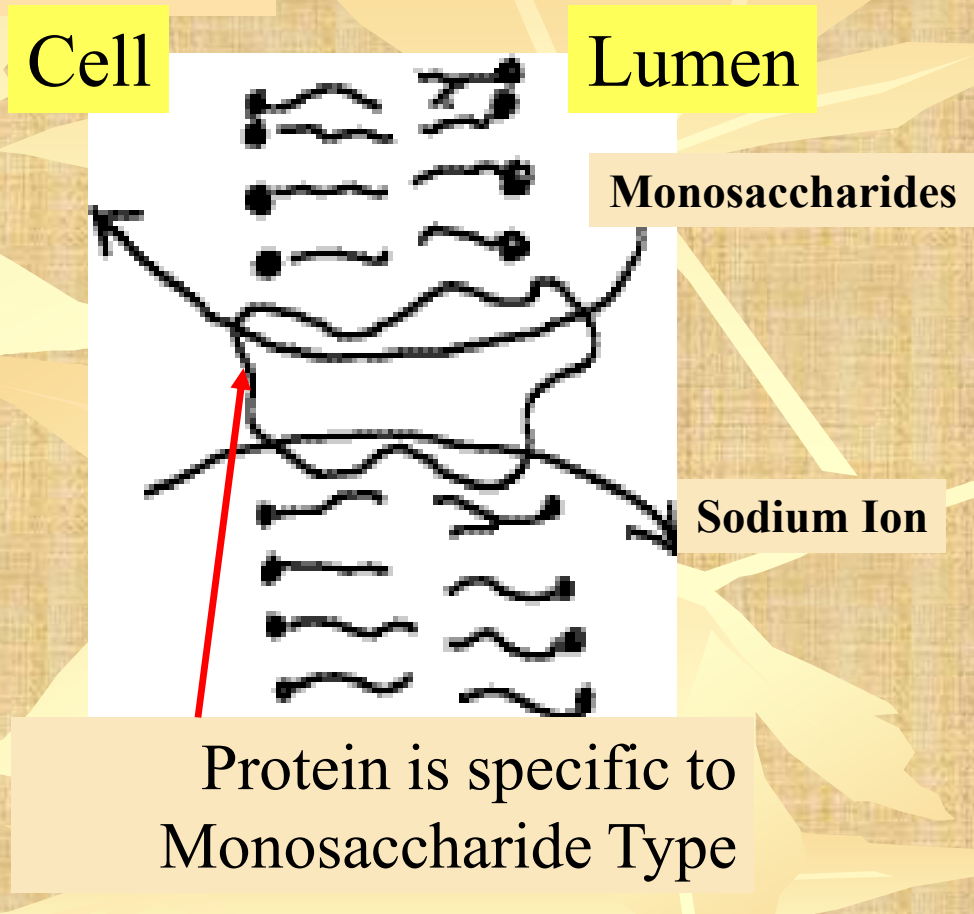
After emulsification, the fat is mixed in the water solution, so fat-digesting enzymes have access to it.

Factors Affecting Absorption of Lipids

- amount of fat consumed (↑fat=↓digestion=↓absorption)
- age (↑ age = ↑ digestion)
- emulsifying agents (↑ digestion = ↑ absorption)
- chain length of FA's (> 18C = ↓ digestibility)
- degree of saturation of FA (↑ sat = ↓ digestibility)
- overheating and auto-oxidation (rancidification (rot) at double bond)
- optimal dietary calcium=optimal FA absorption(↑ Ca=↓ absorption)

Carbohydrates Absorption

- Active Co-transport (Anti-port) of Simple Sugars
- Sodium Ion – Moves out Passively in Response to Solute Gradient
- If Protein Gates Saturated no Further Absorption
- Cellulose, Though Complex Carbohydrate is Fermented into Volatile Fatty Acids



transport Na out of the cell to maintain proper electrochemical gradient (sodium potassium pump)

Digesting and Absorbing Vitamins

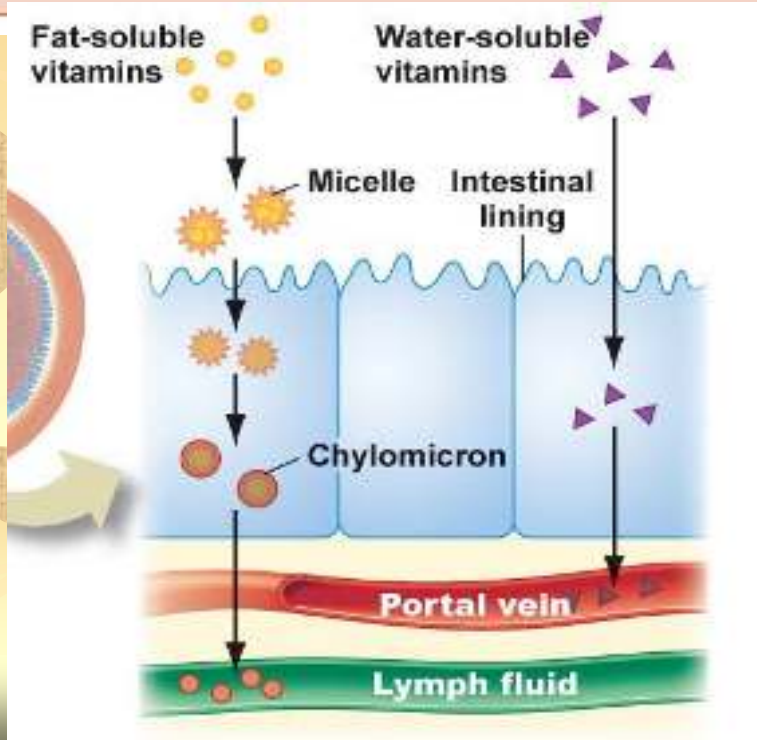
a Vitamins are hydrolyzed in the stomach from the protein complexes found in food.

b Most of the water-soluble vitamins are absorbed in the upper small intestine with the exception of vitamin B_{12}, which is absorbed in the posterior intestine

c The water-soluble vitamins are absorbed directly into the portal vein and transported to the liver, where they are either stored (B_{12}) or sent out into circulation.

d Excess water-soluble vitamins are excreted through the kidneys in the urine.

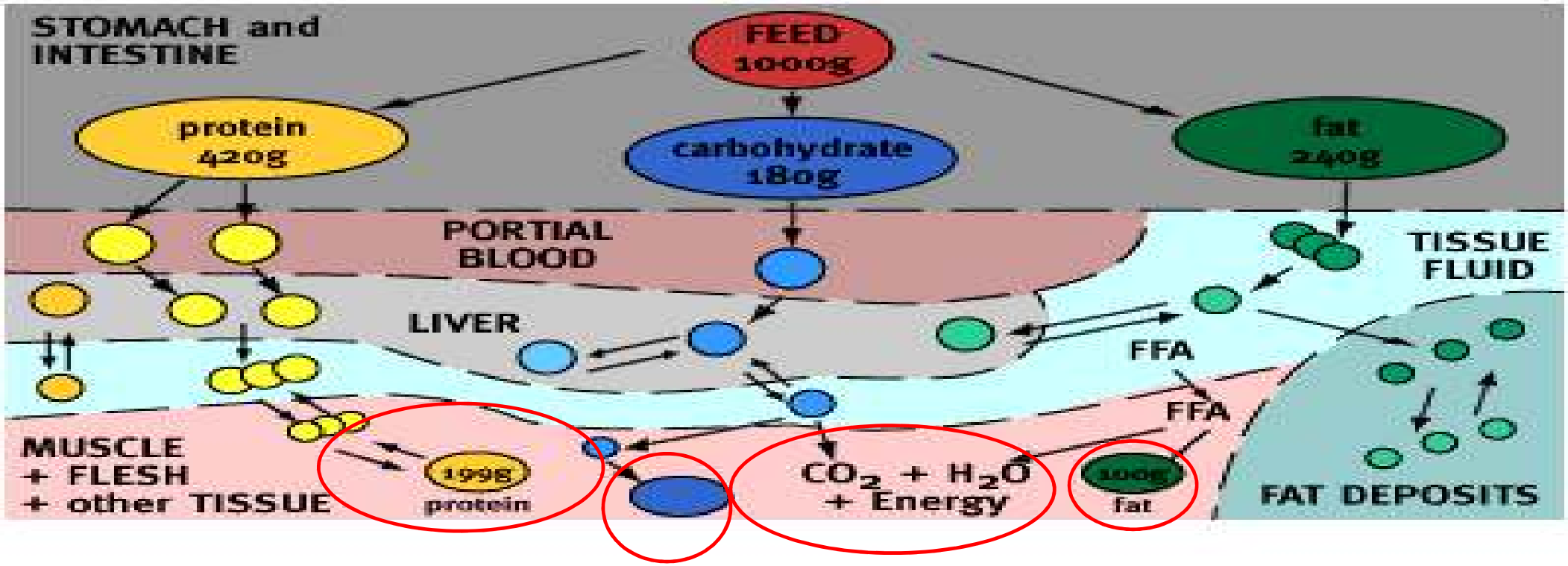
d In the small intestine, the fat-soluble vitamins are transported into the intestinal cells as part of micelles. Once inside the intestinal cells, fat-soluble vitamins are packaged with fat and other lipids into a chylomicron. The chylomicrons travel through the lymph system to the main circulation.



TRANSPORT OF IRON BETWEEN STORAGE AND FUNCTIONAL POOLS

- Iron in the body is recycled extensively between the functional and storage pools.
- It is transported in plasma by an iron-binding glycoprotein called transferrin, which is synthesized in the liver.
- Transferrin is about one third saturated w ing serum /dL in
- The major function of plasma transferrin is to deliver iron to cells, including erythroid precursors, which require iron to synthesize hemoglobin.

or
r-



Amino Acids

Protein

Glucose

Glycogen

Carbohydrate

Triglycerides

Fatty Acids

Free Fatty Acids

Part 2: Nutrient Metabolism

Metabolism: carbohydrates

Metabolism: the biological utilization of absorbed nutrients for synthesis (e.g., growth) and energy expenditure, for most aquatic species, the protein sparing effect of COH is good, COH metabolism has a long time associated with it.

Once COH is ingested/digested, blood levels quickly rise, but require extended periods to decline. This delay response is considered similar in effect to that of diabetes thus, turnover of COH by aquatics is much slower than that of land animals.

Metabolism: carbohydrates

explanation: aquatics often prefer to oxidize amino acids for energy

COH metabolic role:

1) immediate source of energy.
2) energy reserve (glycogen).
3) converted to triglyceride.
4) synthesis of non-essential amino acids.

Metabolism: COH/energy

Normal pathway of converting COH to energy is known as **glycolysis**

1 mole of glucose converted to

2 moles of pyruvate = 6 ATP's

each mole of ATP represents 7.3 kcal energy

overall energy efficiency is 41% (fairly efficient transformation)

Metabolism: COH/energy

The entire oxidation of glucose utilizes two mechanisms: **Glycolysis** and **TCA** cycle

glycolysis takes place in cytosol, TCA in the mitochondria

TCA cycle utilizes a variety of substrates (e.g., amino acids, fatty acids, keto acids) for energy gain each turn on the TCA cycle = 15 ATP (w/2 molecules of pyruvate entering, this equals a total of 30 ATP

All the enzymes for glycolysis/TCA have been identified in fish tissues

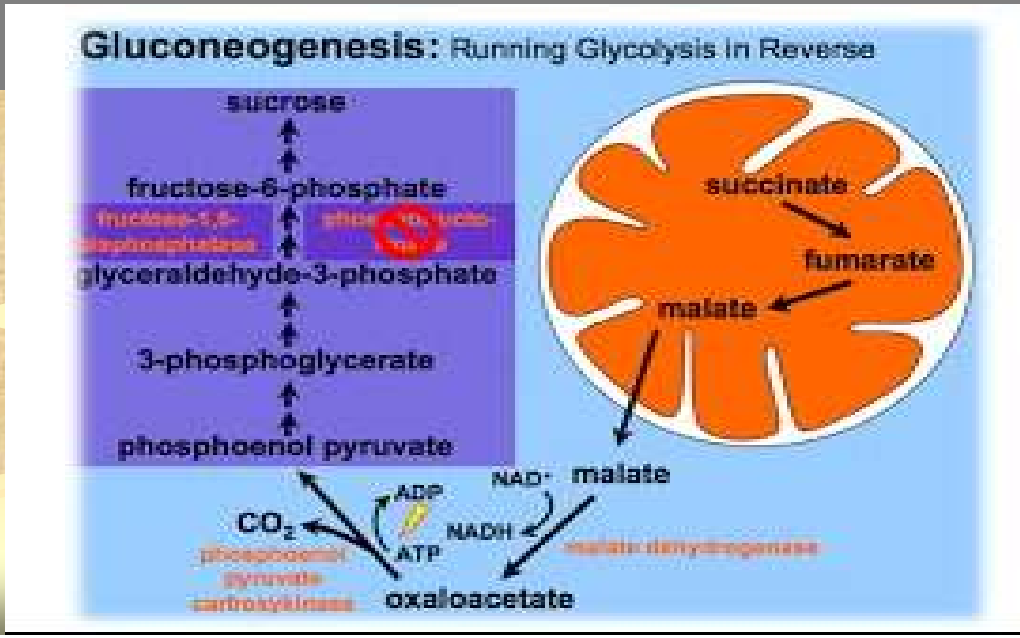
those tissues showing highest enzyme activity are the heart and muscle tissue others include brain, kidney, gills, liver

Metabolism: COH/energy

Glucogenesis : The formation of glucose through the breakdown of glycogen.

Gluconeogenesis: synthesis of glucose as a result of starvation,

is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids.



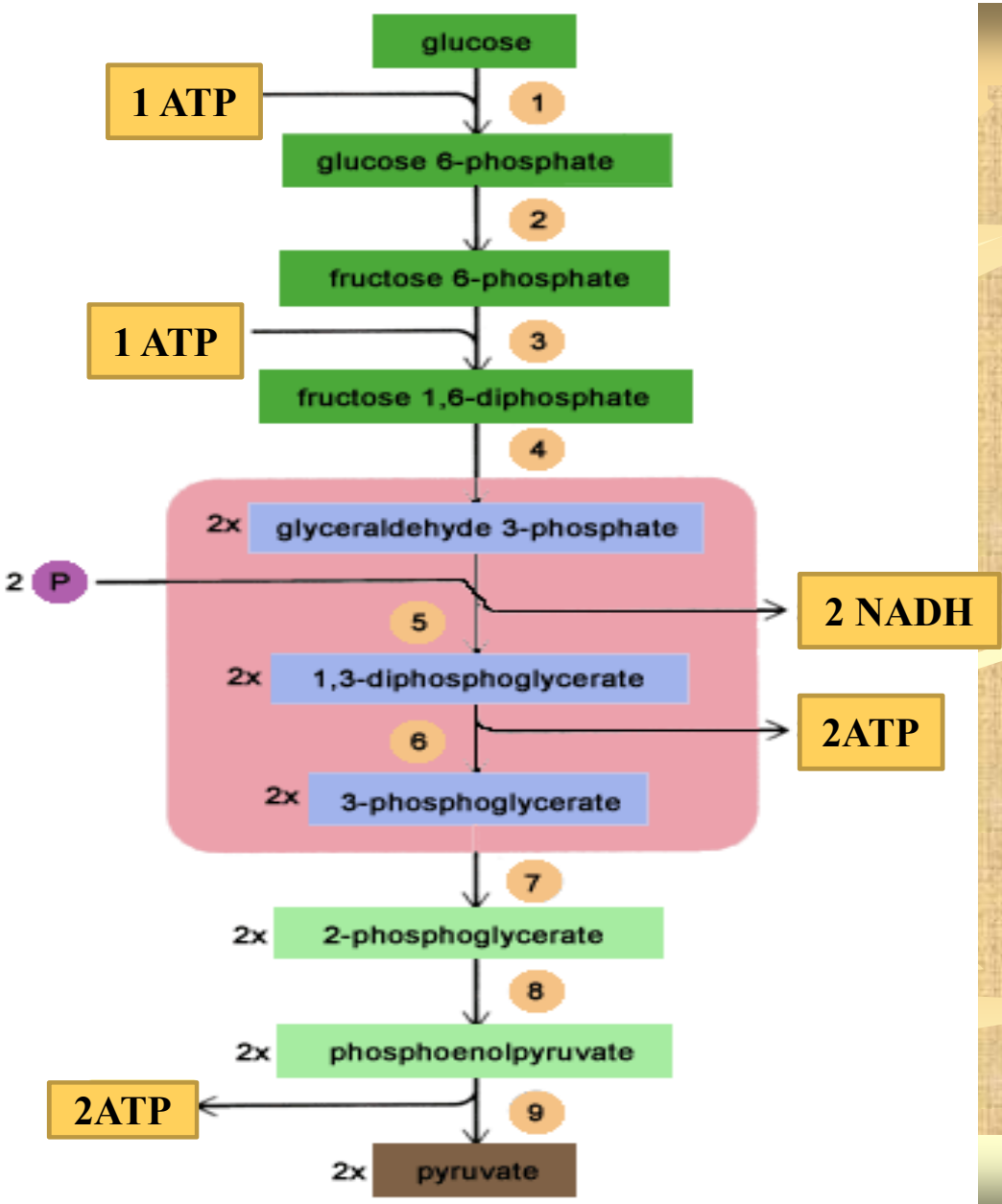
ATP Summary for Glycolysis:

Starting with glucose (six carbons) how many ATP are made using **aerobic glycolysis**? E.T.C = electron transport chain

Step	ATP (used -) (produced +)
1	-1
3	-1
5 - NADH to E.T.C to FAD = 2 step 5 used twice	2 x 2 = +4
6 used twice	1 x 2 = + 2
9 used twice	1 x 2 = + 2
NET	**6 ATP**

Starting with glucose (six carbons) how many ATP are made using **anaerobic glycolysis**? E.T.C = electron transport chain

Step	ATP (used -) (produced +)
1	-1
3	-1
5 - NADH to pyruvic acid to lactic acid. E.T.C. not used	0
6 used twice	1 x 2 = + 2
9 used twice	1 x 2 = + 2
NET	**2 ATP**



Total energetic Balance from Glucose to (2) Pyruvate (Aerobic Glycolysis):

- - 2 ATP + 2 NADH.H + 4 ATP = 2 ATP + 2NADH.H

An excellent animation of glycolysis can be found here.

Total ATP Production

2 ATP – Glycolysis

2 ATP – Citrus Acid Cycle

34 ATP – Electron Transport Chain

38 ATP – Total energy released from one molecule of glucose.

TOTAL ATP Production from one Glucose molecule

Glycolysis: (Net yields)

Stage I.

ATP	**2 ATP**
2 NADH+H$^+$ → 2 FADH$_2$ (to ETC)	**3 ATP**

Stage II. **Conversion of pyruvate to ACoA**

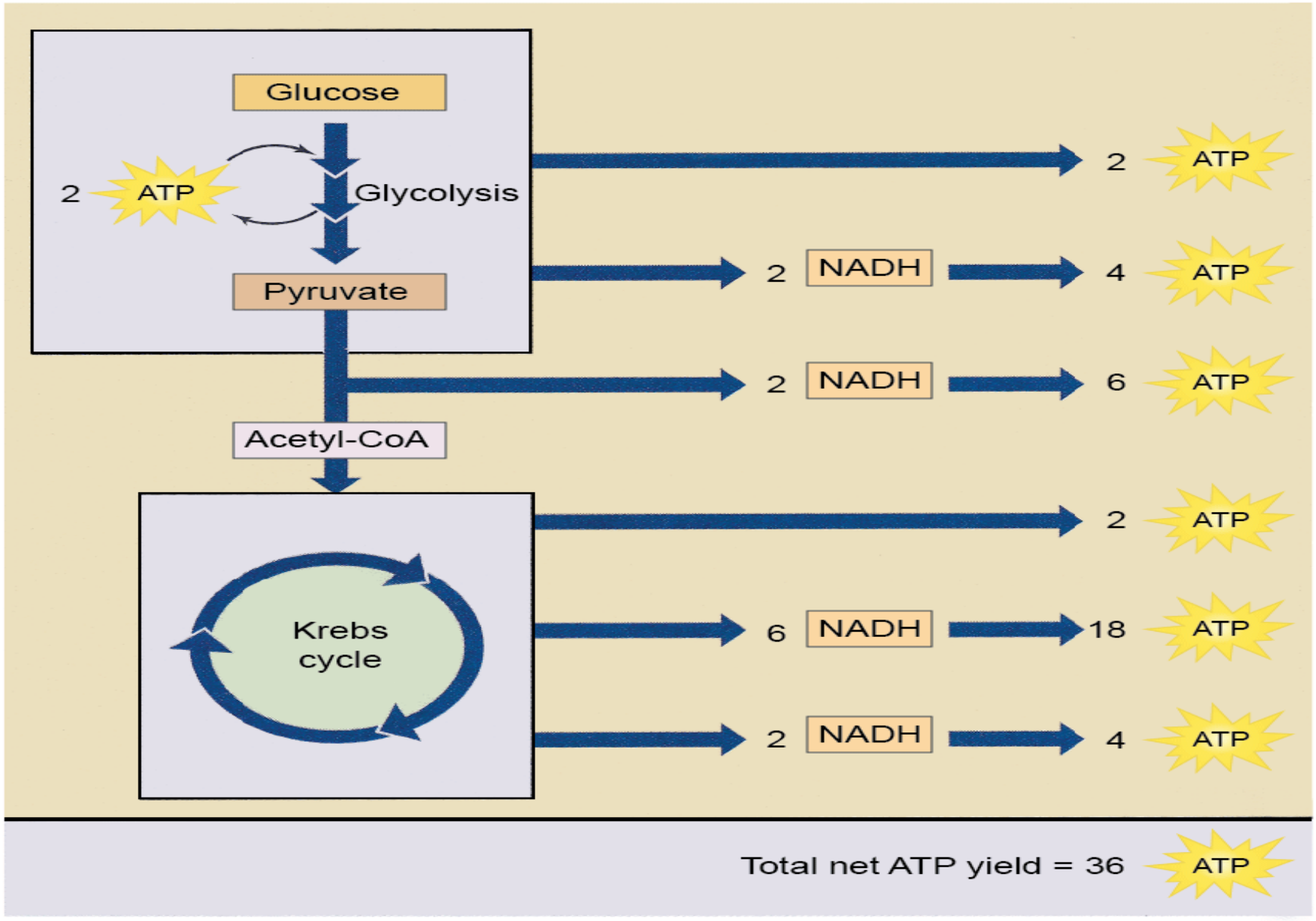
2 NADH + H$^+$ (to ETC)	**5 ATP**

Stage III. **TCA cycle**

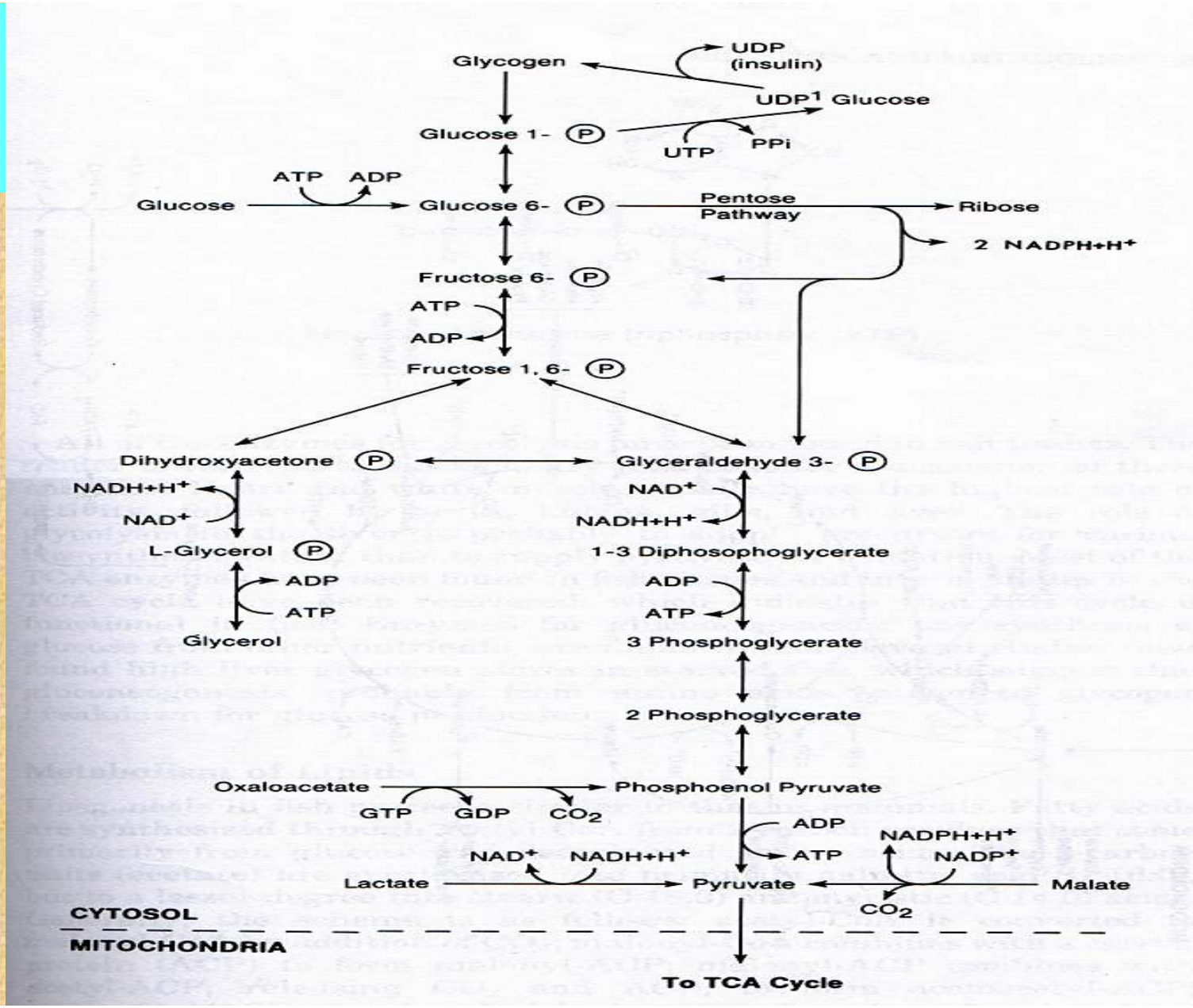
ATP (at one site)	**2 ATP**
NADH+H$^+$ at three steps (to ETC)	**15 ATP**
FADH$_2$ at one step (to ETC)	**3 ATP**

Total ATP from one molecule of glucose = 30 ATP

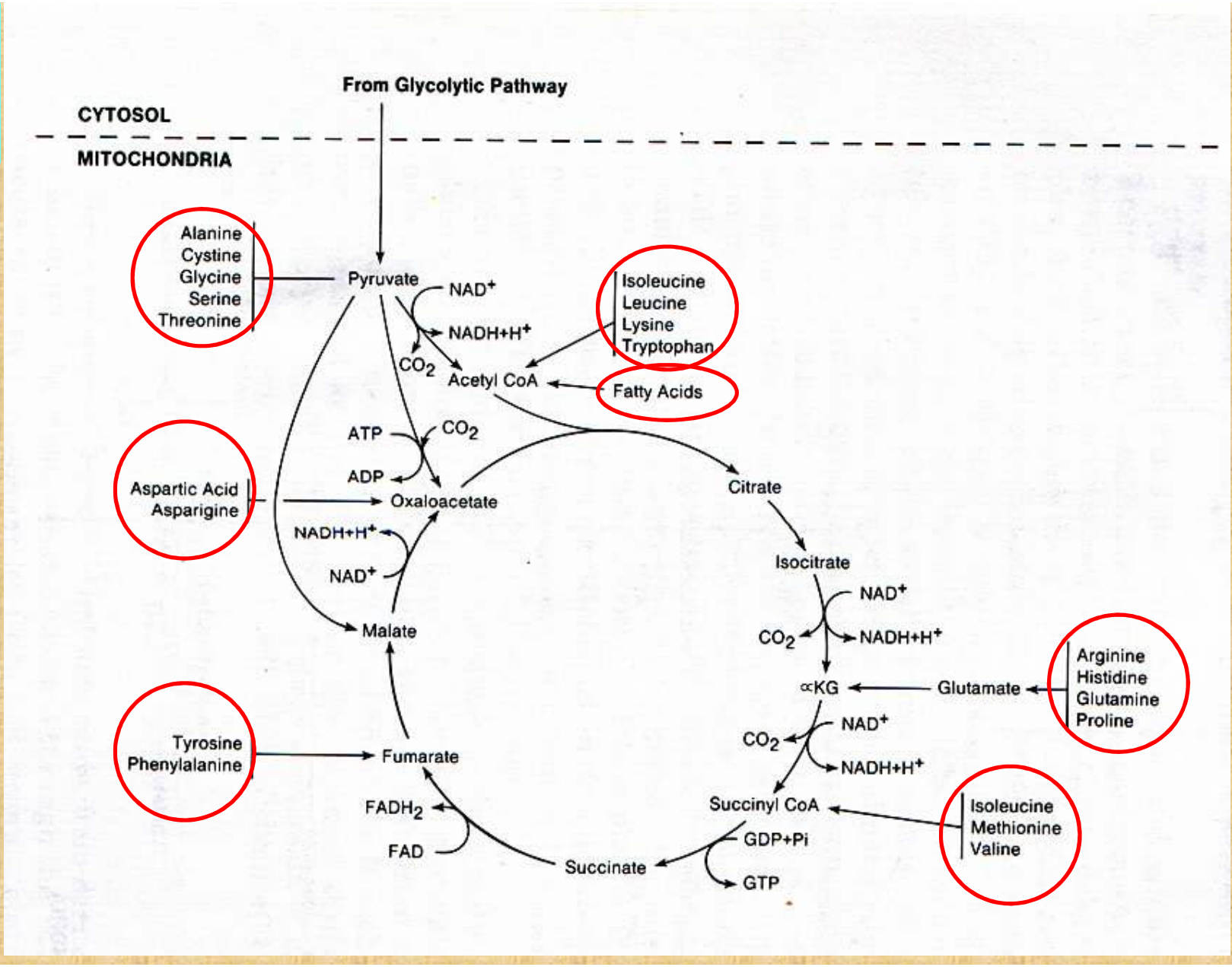
IMPORTANT THOUGHTS: EACH NADH+H YIELDS 2.5ATP AND EACH FADH2 YIELDS 1.5ATP


Glucose
2
ATP
Glycolysis
Pyruvate
Acetyl-CoA
Krebs cycle
2
ATP
2
NADH
4
ATP
2
NADH
6
ATP
2
ATP
6
NADH
18
ATP
2
NADH
4
ATP
Total net ATP yield = 36
ATP

Glycolytic Pathway



Tricarboxylic Acid Cycle



Metabolism: lipids

Formation of lipids is known as **lipogenesis** formation is through compound known as acetyl CoA (entering into TCA cycle)

fats are derived from the carbon skeleton found in all COH and non-essential amino acids

Step 1: COH, NEAA broken down into 2-carbon units known as acetate

Step 2: acetate converted to stearic acid or palmitic acid

responsible enzyme: fatty acid synthetase

Metabolism: fatty acids

Once palmitate (16 C) has been formed, it can be elongated by enzymes in the mitochondria

the ability to chain elongate seldom exceeds 18 carbons in length

FA's (fatty acids) are added to glycerol phosphate (from glycolysis) to form a lipid

primary site for FA synthesis is in liver and adipose

Metabolism: fatty acids

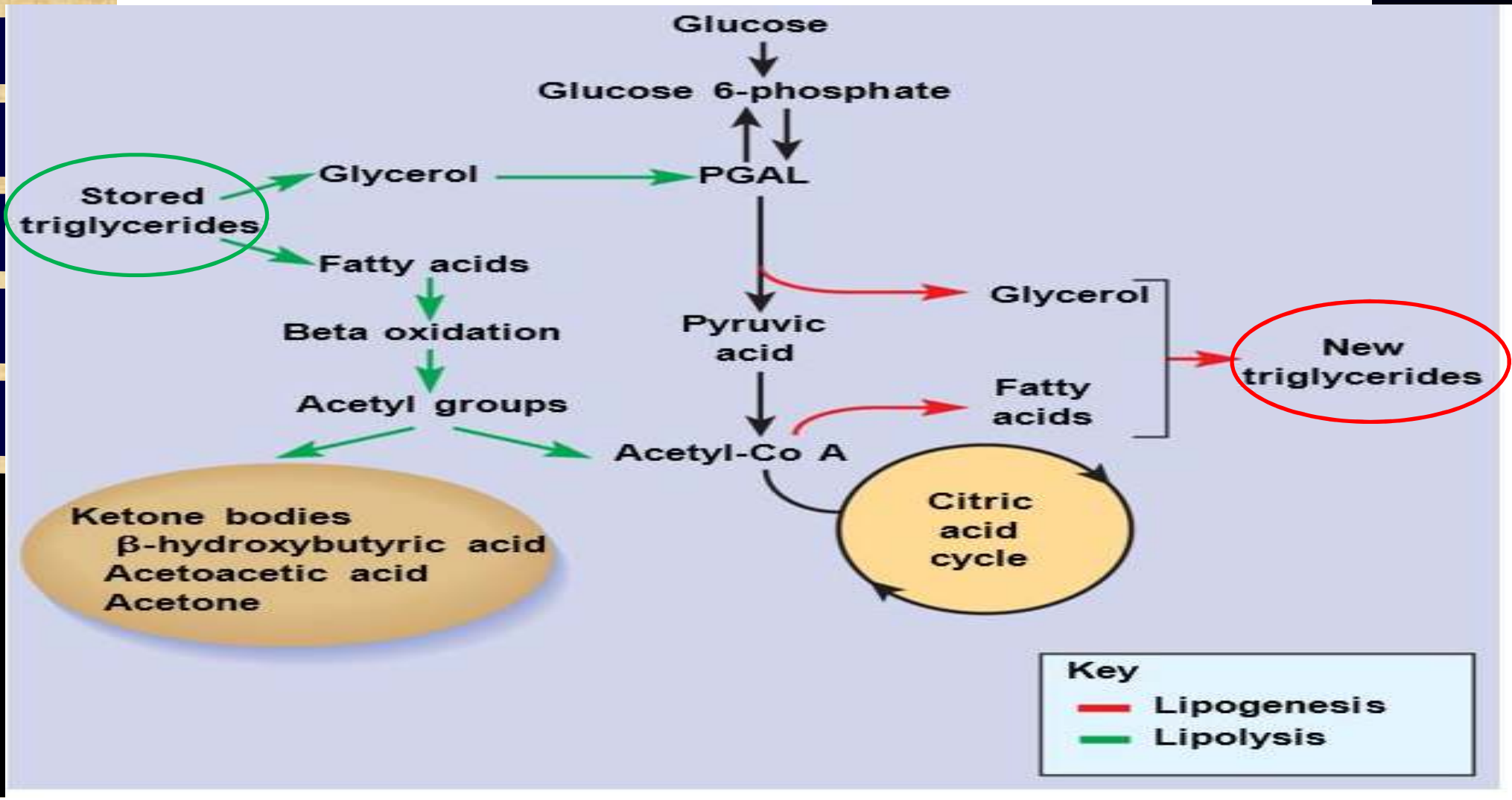
Lipolysis: catabolism or oxidation of fatty acids in fish is similar to that of mammals

once you hydrolyze the fat (remove FA's) the glycerol goes back into glycolytic pathway for energy production.

Release of triglycerides from adipose is under hormonal control

obesity: disease in which individual lacks ability to mobilize triglycerides

Lipogenesis and Lipolysis Pathways



Metabolism: amino acids

Amino acids are "kept" in the body's amino acid pool

release is controlled by liver

sources: dietary and catabolism of proteins

protein metabolism: oxidation followed by energy release, carbon skeleton use for FA synthesis

amino acids, unlike lipids and COH, are not stored in the body

Metabolism: amino acids

Excesses of AA's (amino acids) in pool are deaminated and C-skel burnt for energy or converted to COH/lipid

where do the amino (NH_3) groups go?

They are transaminated (passed to a different C-skel) and eventually either excreted or used for subsequent AA synthesis

Terrestrials excrete urine, birds excrete uric acid, inverts/fish largely ammonia

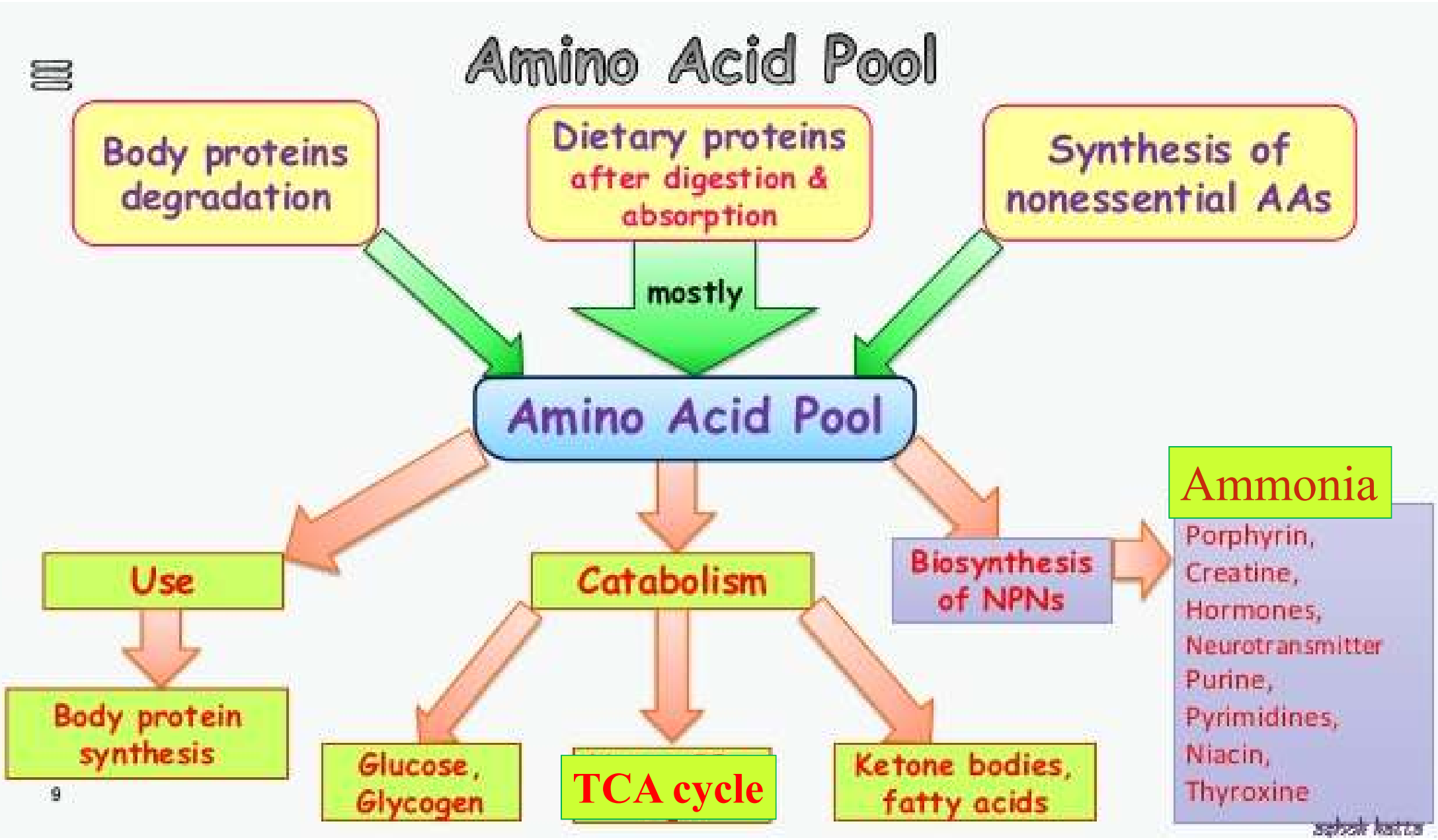
Metabolism: amino acids

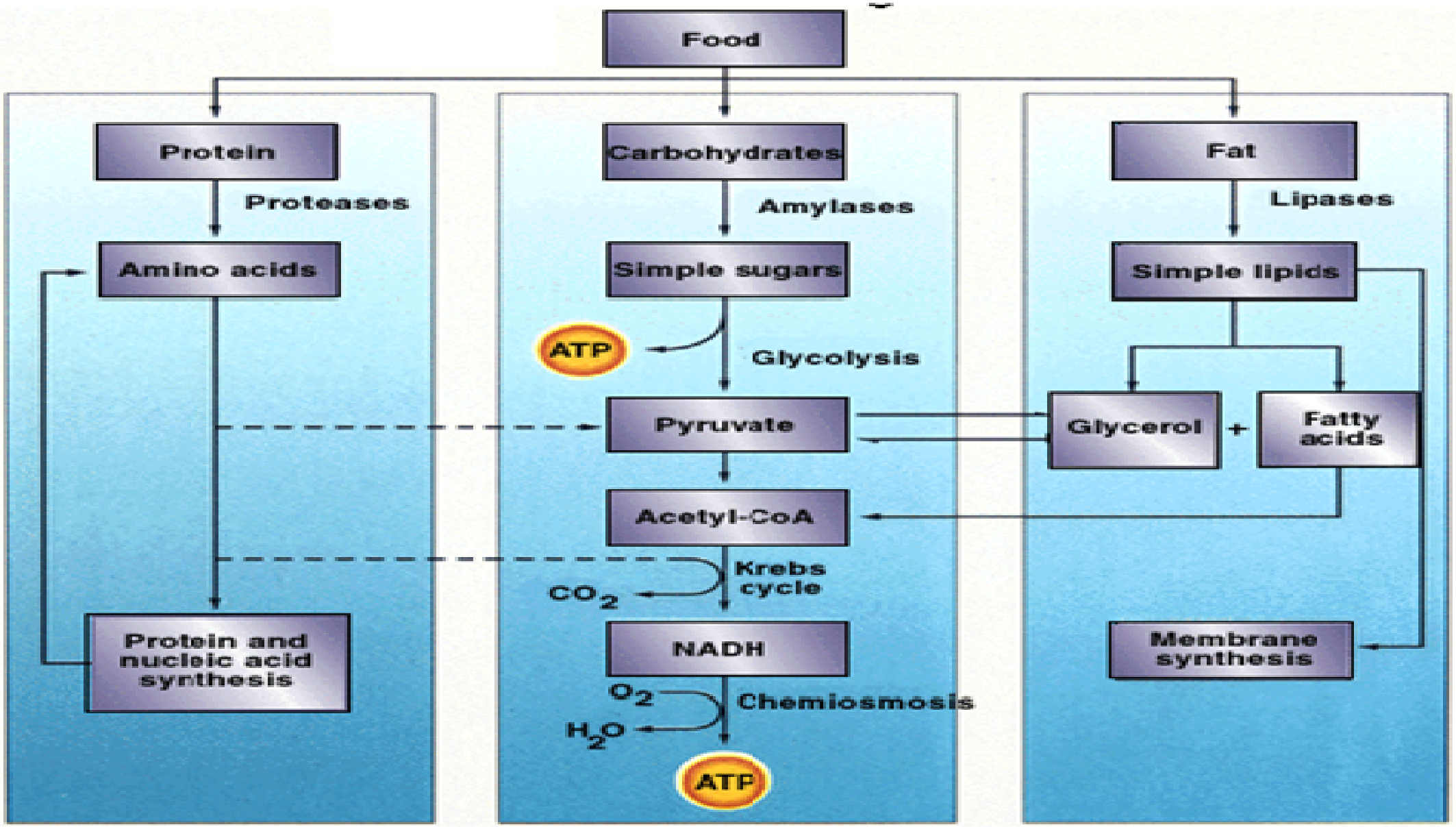
Teleosts excrete a mixture of nitrogenous compounds

most nitrogenous waste excreted thru gills

Excretion of ammonia requires less energy than urea because urea is synthesized

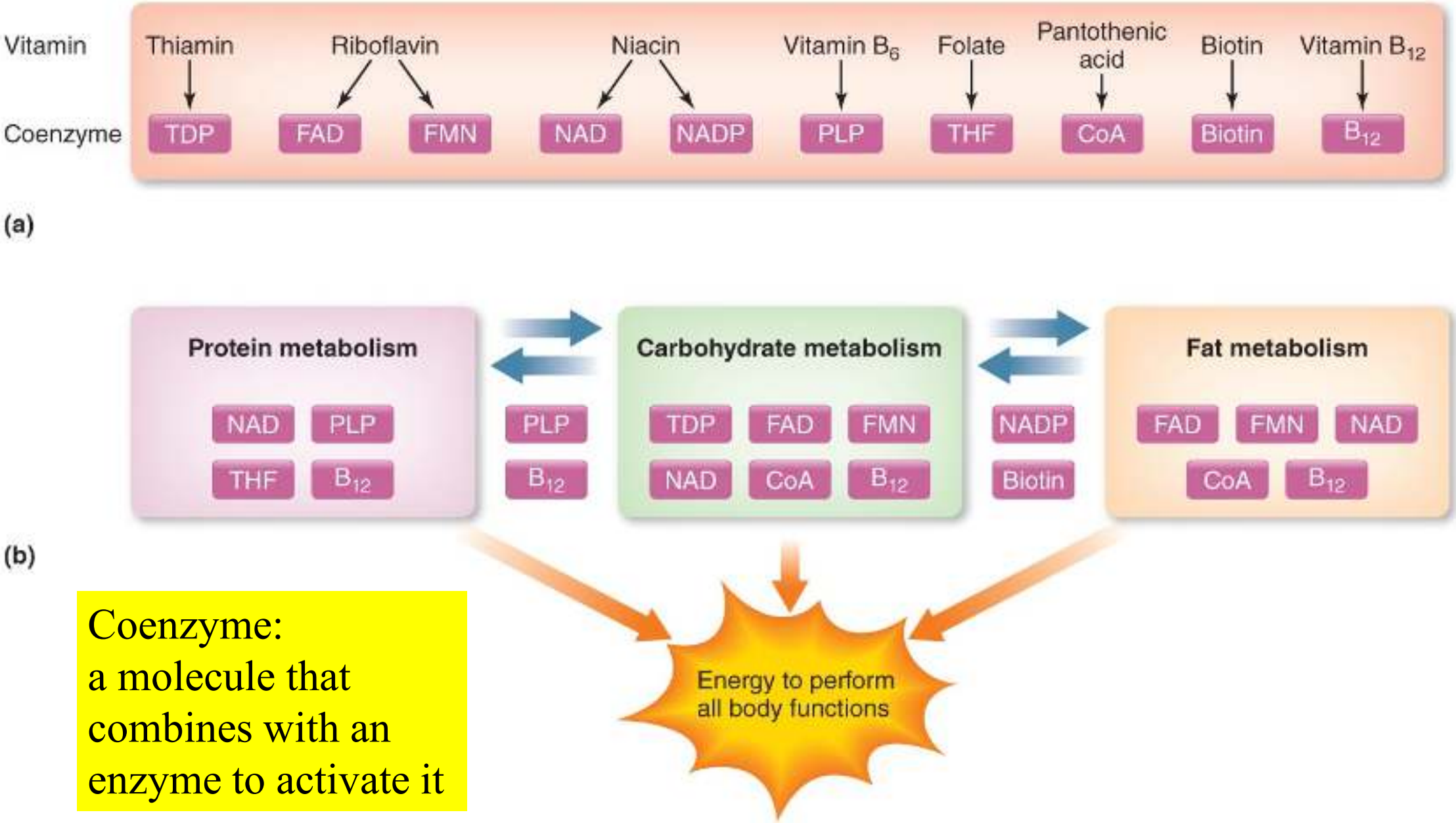
further, excretion of ammonia does not require movement of water across membrane (ie., easy passage)


Amino Acid Pool
Body proteins degradation
Dietary proteins after digestion & absorption
Synthesis of nonessential AAs
mostly
Amino Acid Pool
Use
Catabolism
Biosynthesis of NPNs
Ammonia
Porphyrin,
Creatine,
Hormones,
Neurotransmitter
Purine,
Pyrimidines,
Niacin,
Thyroxine
Body protein synthesis
Glucose, Glycogen
TCA cycle
Ketone bodies, fatty acids
9

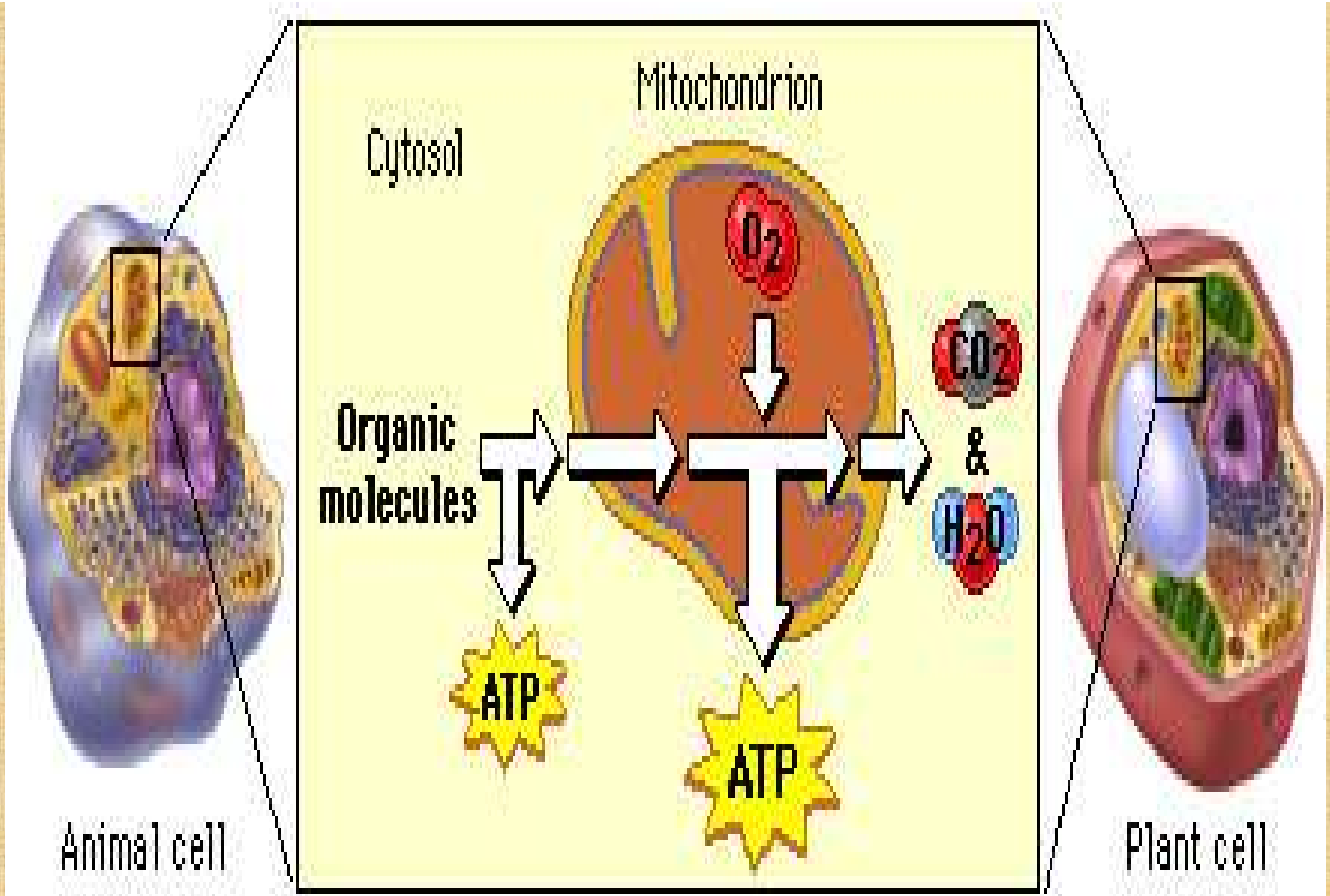

Food
Protein
Proteases
Amino acids
Protein and nucleic acid synthesis
Carbohydrates
Amylases
Simple sugars
ATP
Glycolysis
Pyruvate
Acetyl-CoA
Krebs cycle
CO_2
NADH
O_2
H_2O
Chemiosmosis
ATP
Fat
Lipases
Simple lipids
Glycerol
+
Fatty acids
Membrane synthesis

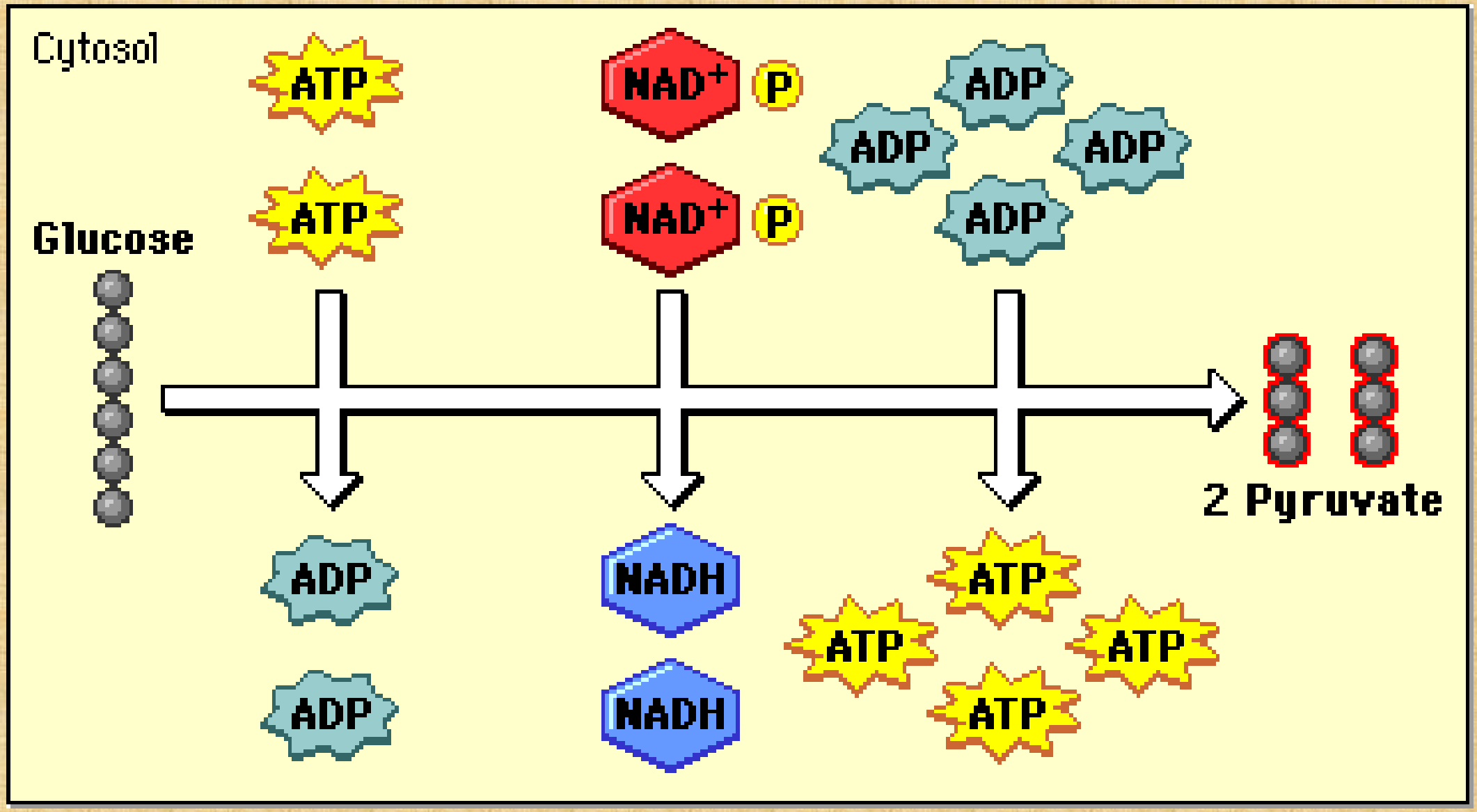
- Vitamins are absorbed unchanged
  - A, D, E & K with other lipids -- B complex & C by simple diffusion and B_{12} if bound to intrinsic factor
- Minerals are absorbed all along small intestine
  - Na+ cotransported with sugars & amino acids
  - Cl- exchanged for bicarbonate reversing stomach
  - Iron & calcium absorbed as needed

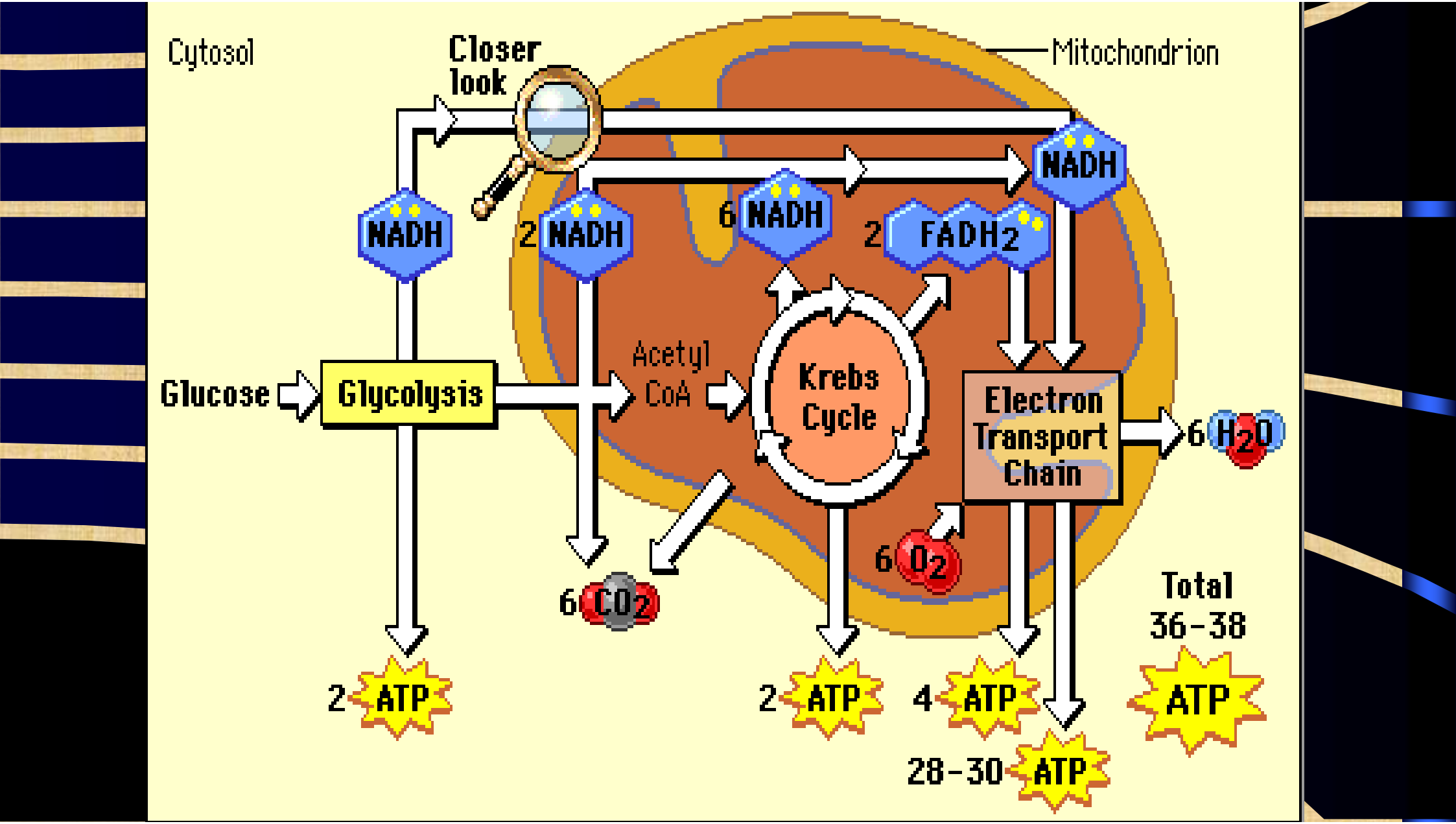
Vitamins and minerals
Do not directly provide energy
Are needed for generating energy from macronutrients
Often function as coenzymes
B-vitamins are particularly important in assisting energy metabolism



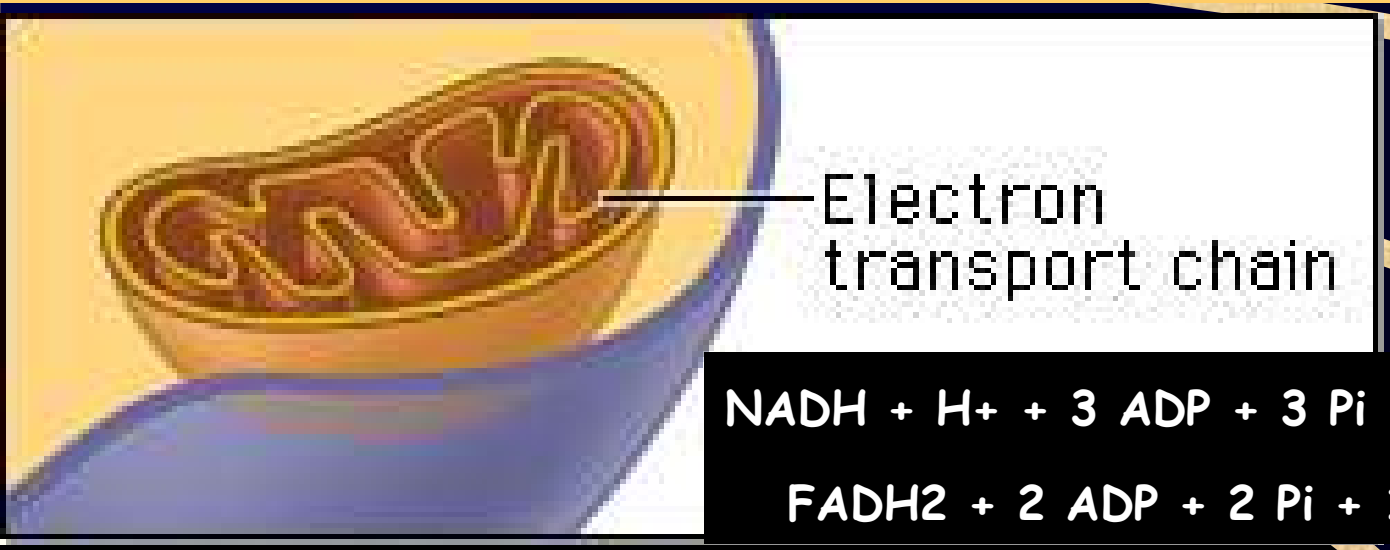
Coenzyme:
a molecule that combines with an enzyme to activate it


Mitochondrion
Cytosol
O2
CO2
&
H2O
Organic molecules
ATP
ATP
Animal cell
Plant cell


Cytosol
ATP
ATP
NAD+
P
NAD+
P
ADP
ADP
ADP
ADP
Glucose
2 Pyruvate
ADP
ADP
NADH
NADH
ATP
ATP
ATP
ATP
ATP

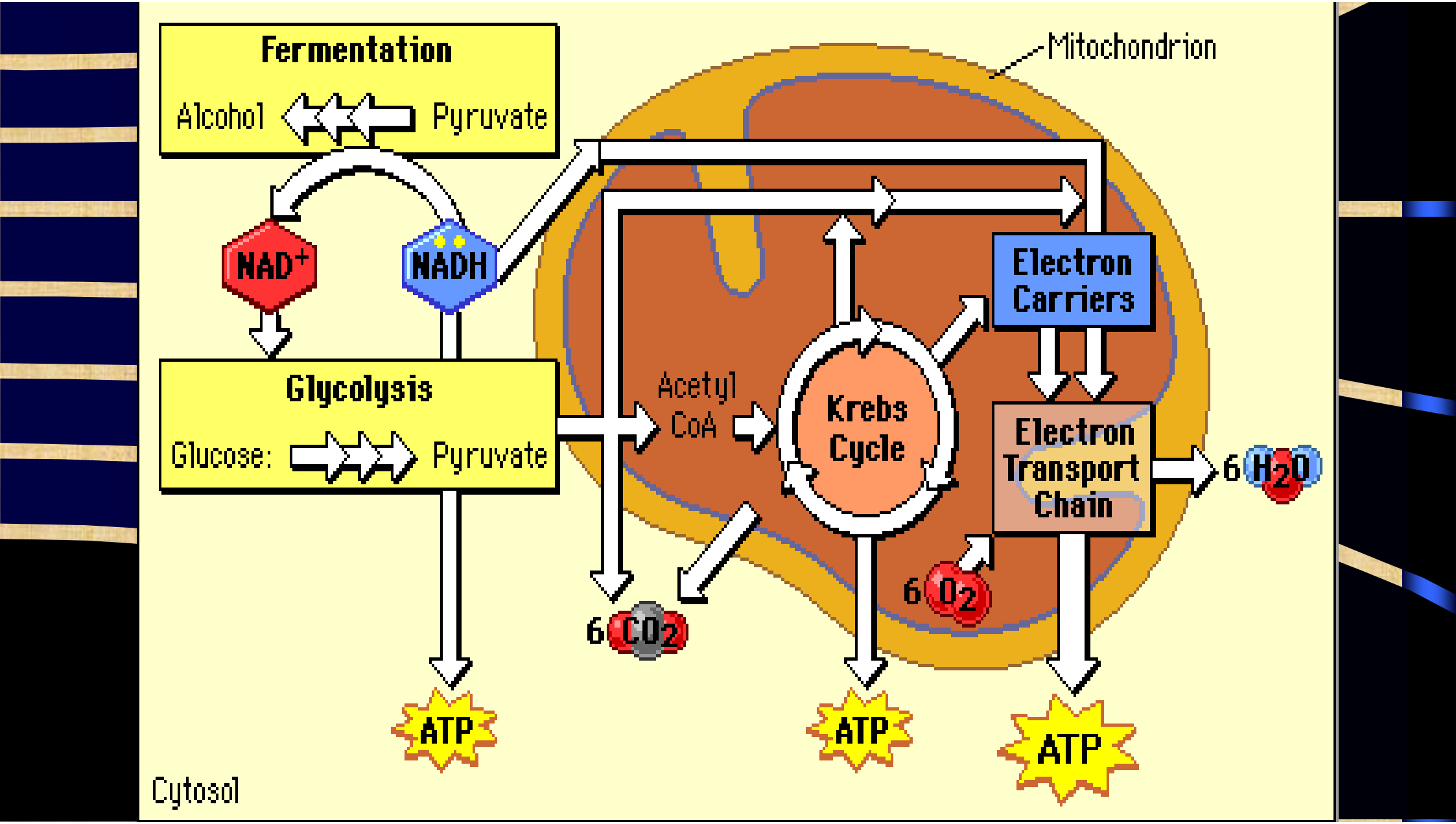

Cytosol
Closer look
Mitochondrion
NADH
2 NADH
6 NADH
2 FADH2
NADH
Glucose
Glycolysis
Acetyl CoA
Krebs Cycle
Electron Transport Chain
6 H2O
6 O2
6 CO2
2 ATP
2 ATP
4 ATP
28-30 ATP
Total 36-38 ATP

Oxidative Phosphorylation via the Electron Transport Chain . The electron transport chain allows the release of the large amount of chemical energy stored in reduced NAD^+ (NADH) and reduced FAD ($FADH_2$). The energy released is captured in the form of ATP (3 ATP per NADH and 2 ATP per $FADH_2$). The electron transport chain (ETC) consists of a series of molecules, mostly proteins, embedded in the inner mitochondrial membrane.



$NADH + H^+ + 3\ ADP + 3\ P_i + 1/2\ O_2 \rightarrow NAD^+ + H_2O + 3\ ATP$

$FADH_2 + 2\ ADP + 2\ P_i + 1/2\ O_2 \rightarrow FAD^+ + H_2O + 2\ ATP$

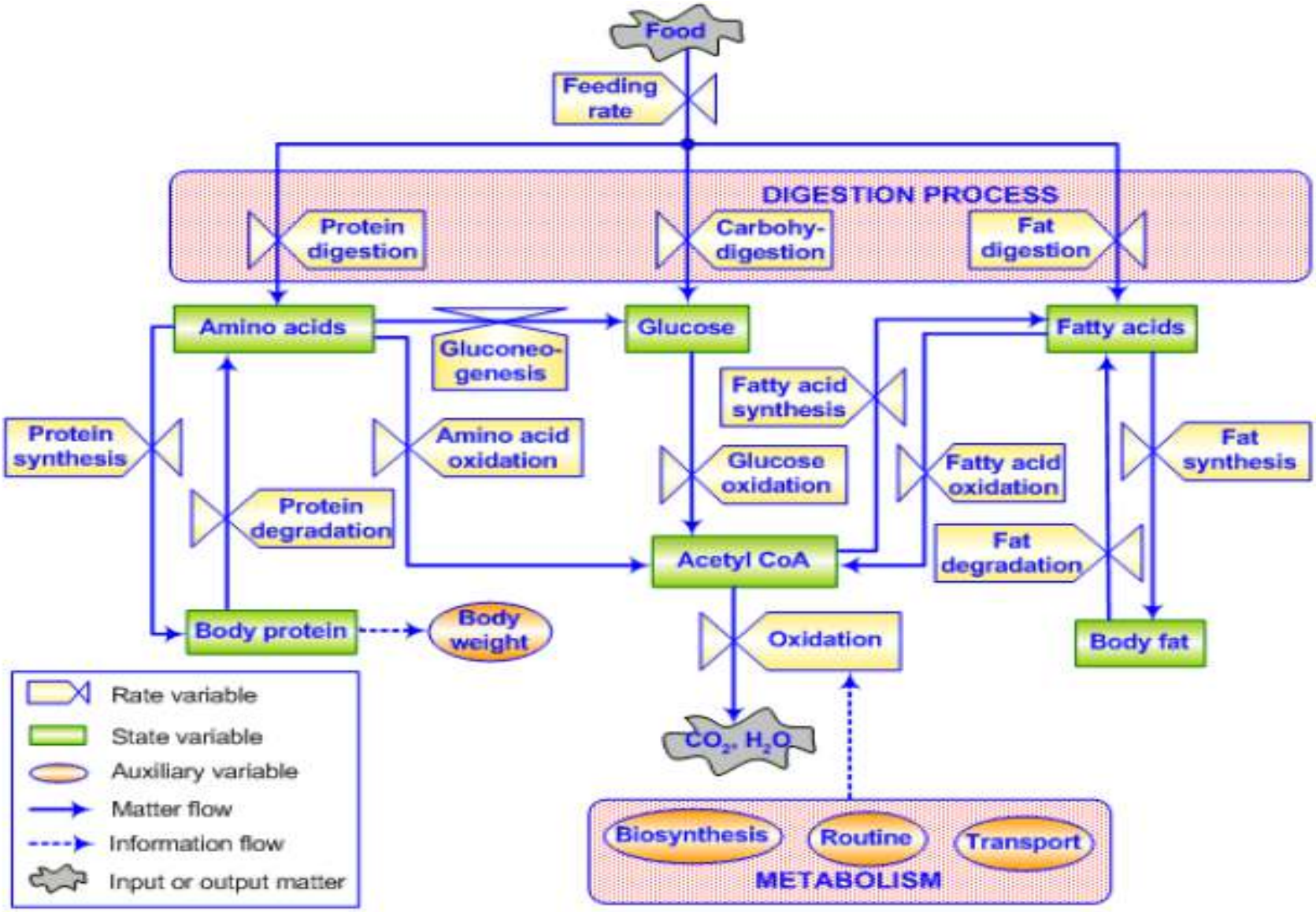

Fermentation
Alcohol
Pyruvate
Mitochondrion
NAD+
NADH
Electron Carriers
Glycolysis
Glucose:
Pyruvate
Acetyl CoA
Krebs Cycle
Electron Transport Chain
6 H2O
6 O2
6 CO2
ATP
ATP
ATP
Cytosol

Fermentation

All cells are able to synthesize ATP via the process of glycolysis. In many cells, if oxygen is not present, pyruvate is metabolized in a process called fermentation.
Fermentation complements glycolysis and makes it possible for ATP to be continually produced in the absence of oxygen. By oxidizing the NADH produced in glycolysis, fermentation regenerates NAD+, which can take part in glycolysis once again to produce more ATP.

There are two types of fermentation. Both types of fermentation regenerate oxidized NAD+, which is necessary for glycolysis and consequently the continued production of ATP by that pathway.
The net energy gain in fermentation is 2 ATP molecules/glucose molecule. In both lactic acid and alcoholic fermentation, all the NADH produced in glycolysis is consumed in fermentation, so there is no net NADH production, and no NADH to enter the ETC and form more ATP.

The majority of tissue in a fish, approximately 60% is the swimming musculature, of which fish have two primary types .Red (slow-twitch, oxidative) fibers are typically located in a superficial lateral wedge between the epaxial and hypaxial regions of white (fast-twitch, glycolytic) fibers. The red muscle is specialized for sustained, aerobic swimming contractions, while the white muscle has a high anaerobic capacity for powerful, short-duration bursts of activity. In tunas, the red muscle position is more internalized compared to ectothermic teleosts, extending from the superficial lateral region in toward the backbone. The lateral wedge in fish may contain red, white, or pink (intermediate, or fast, oxidative-glycolytic) fibers, depending on the species .The internalized position of the red muscle in fish is associated with vascular countercurrent heat exchangers which trap metabolic heat produced during muscle contractions, allowing fish to elevate red muscle temperature above ambient


Food
Feeding rate
DIGESTION PROCESS
Protein digestion
Carbohy-digestion
Fat digestion
Amino acids
Gluconeo-genesis
Glucose
Fatty acids
Fatty acid synthesis
Protein synthesis
Amino acid oxidation
Glucose oxidation
Fatty acid oxidation
Fat synthesis
Protein degradation
Acetyl CoA
Fat degradation
Body protein
Body weight
Oxidation
Body fat
CO_2, H_2O
Biosynthesis
Routine
Transport
METABOLISM
Rate variable
State variable
Auxiliary variable
Matter flow
Information flow
Input or output matter

A. Peptide bond

- In proteins aa's are joined covalently by peptide bonds, i.e., amide linkages b/w α-carboxyl of one aa and α-amino group of another. e.g., valylalanine.

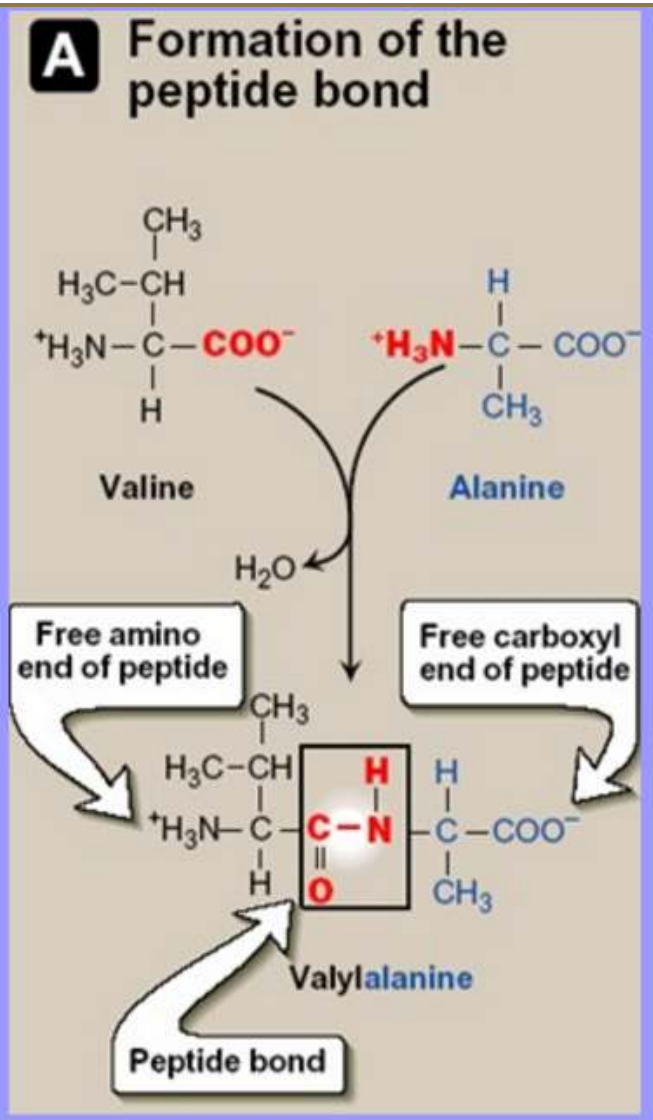
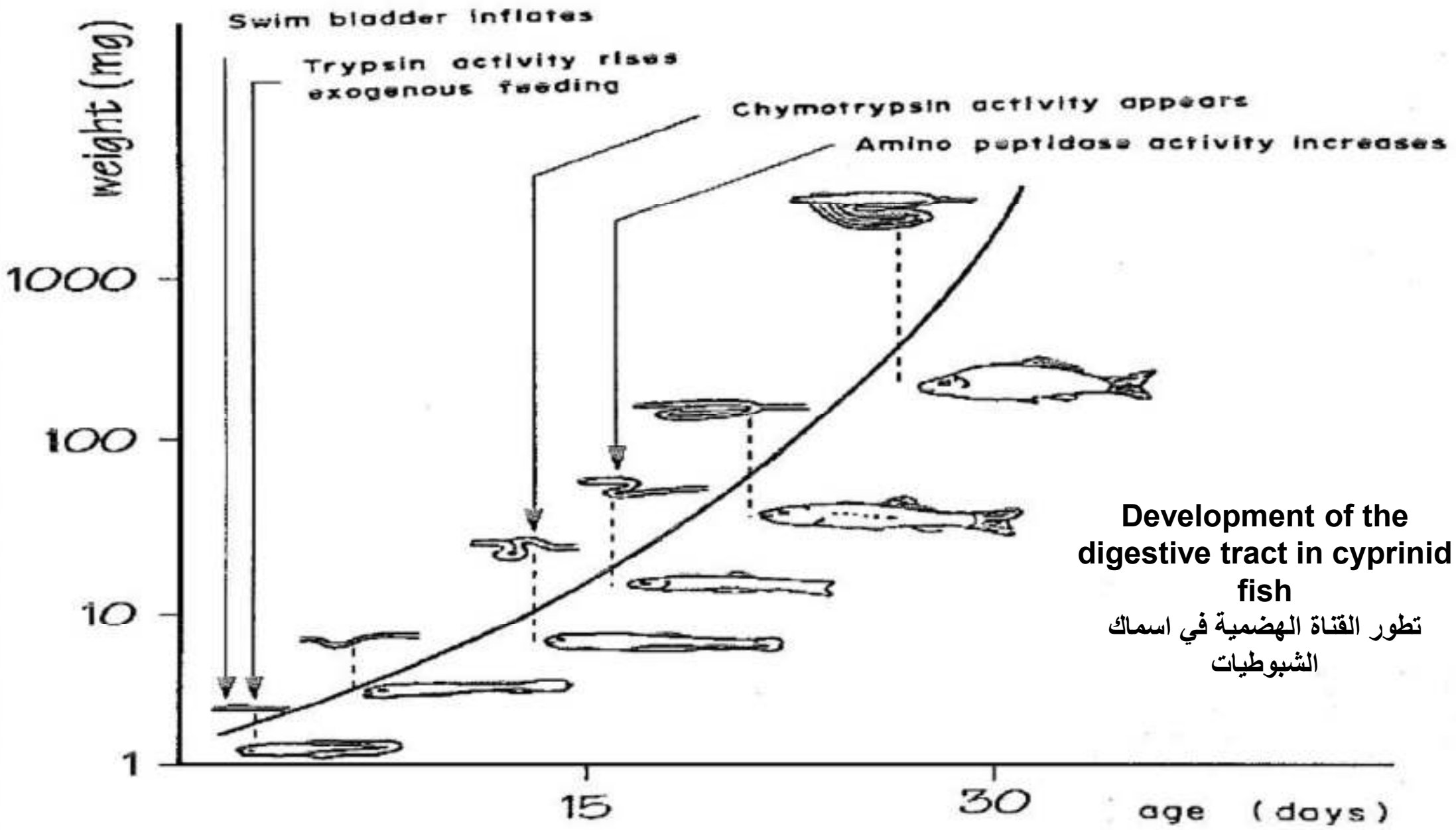
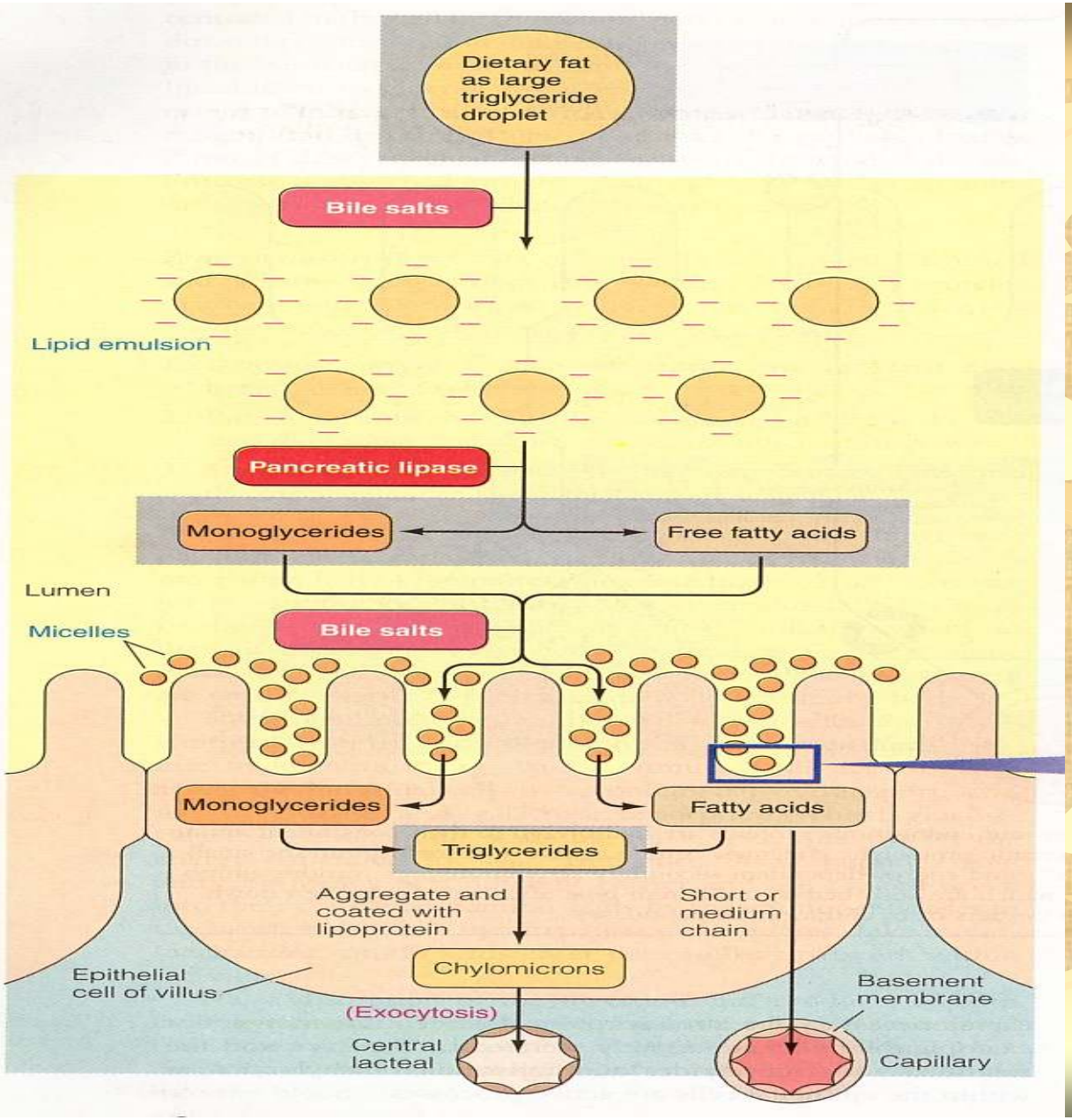


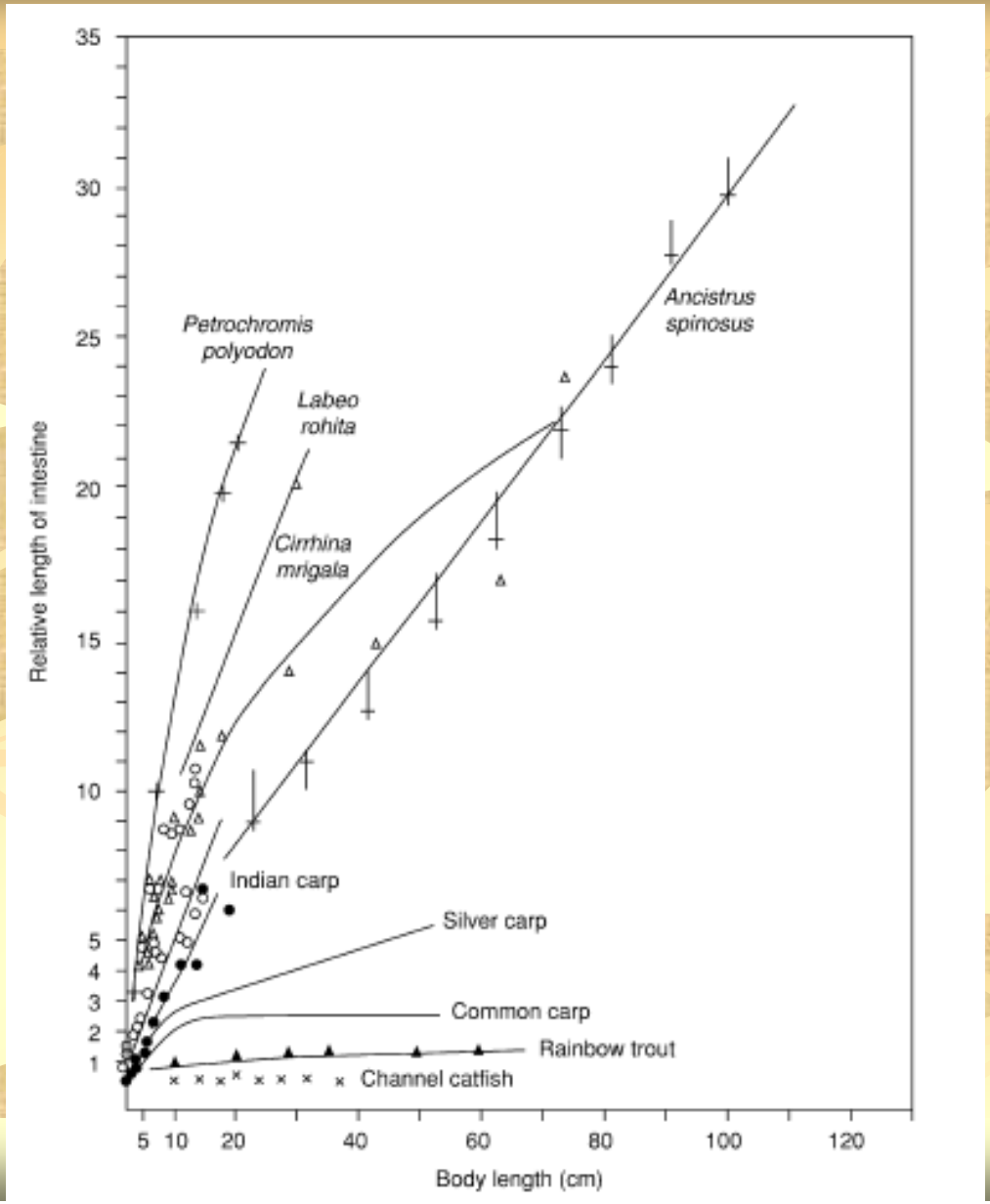
Figure 2.2-A. Formation of a peptide bond, showing the structure of the dipeptide valylalanine.



Development of the digestive tract in cyprinid fish

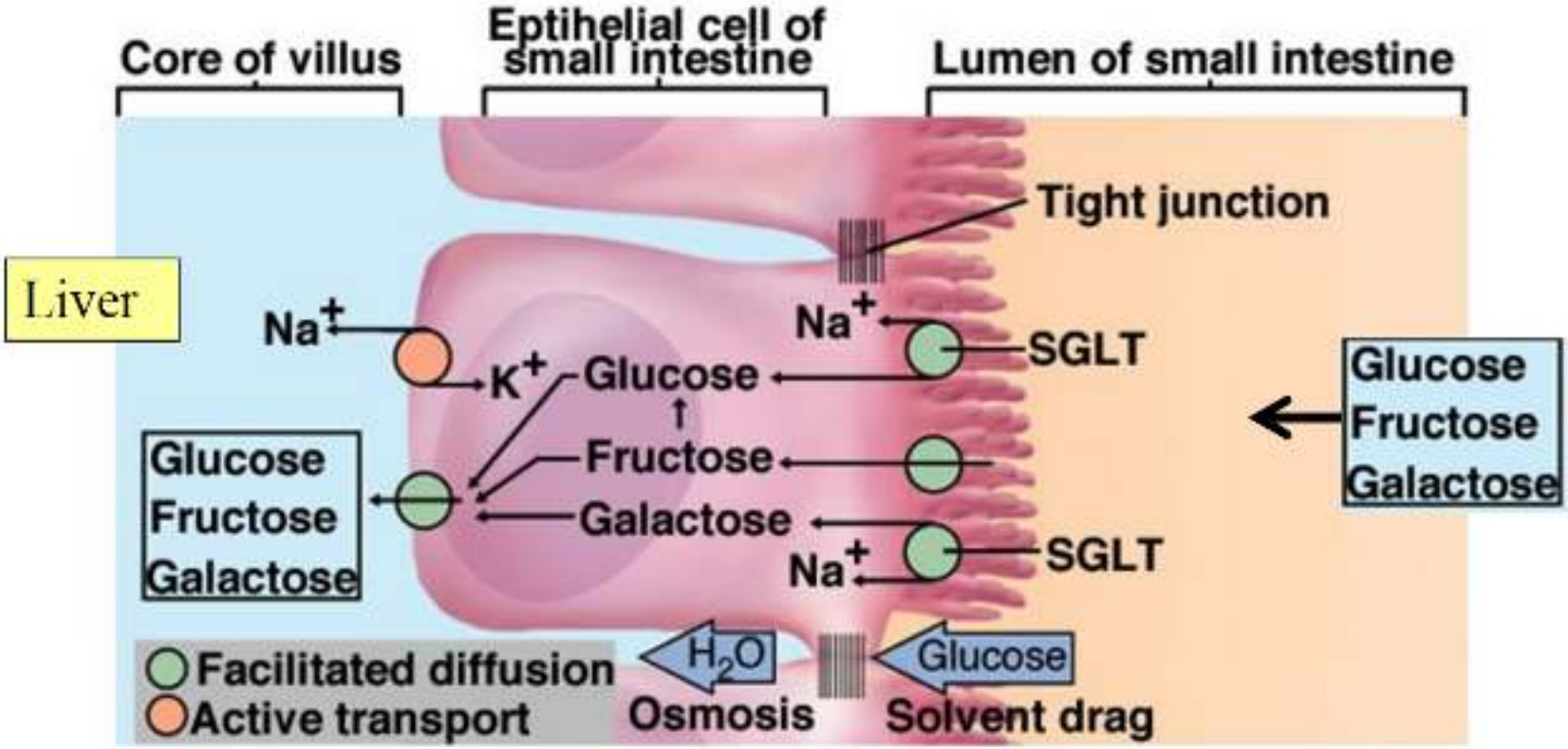
تطور القناة الهضمية في اسماك الشبوطيات


Dietary fat as large triglyceride droplet
Bile salts
Lipid emulsion
Pancreatic lipase
Monoglycerides
Free fatty acids
Lumen
Micelles
Bile salts
Monoglycerides
Fatty acids
Triglycerides
Aggregate and coated with lipoprotein
Short or medium chain
Chylomicrons
Epithelial cell of villus
(Exocytosis)
Basement membrane
Central lacteal
Capillary

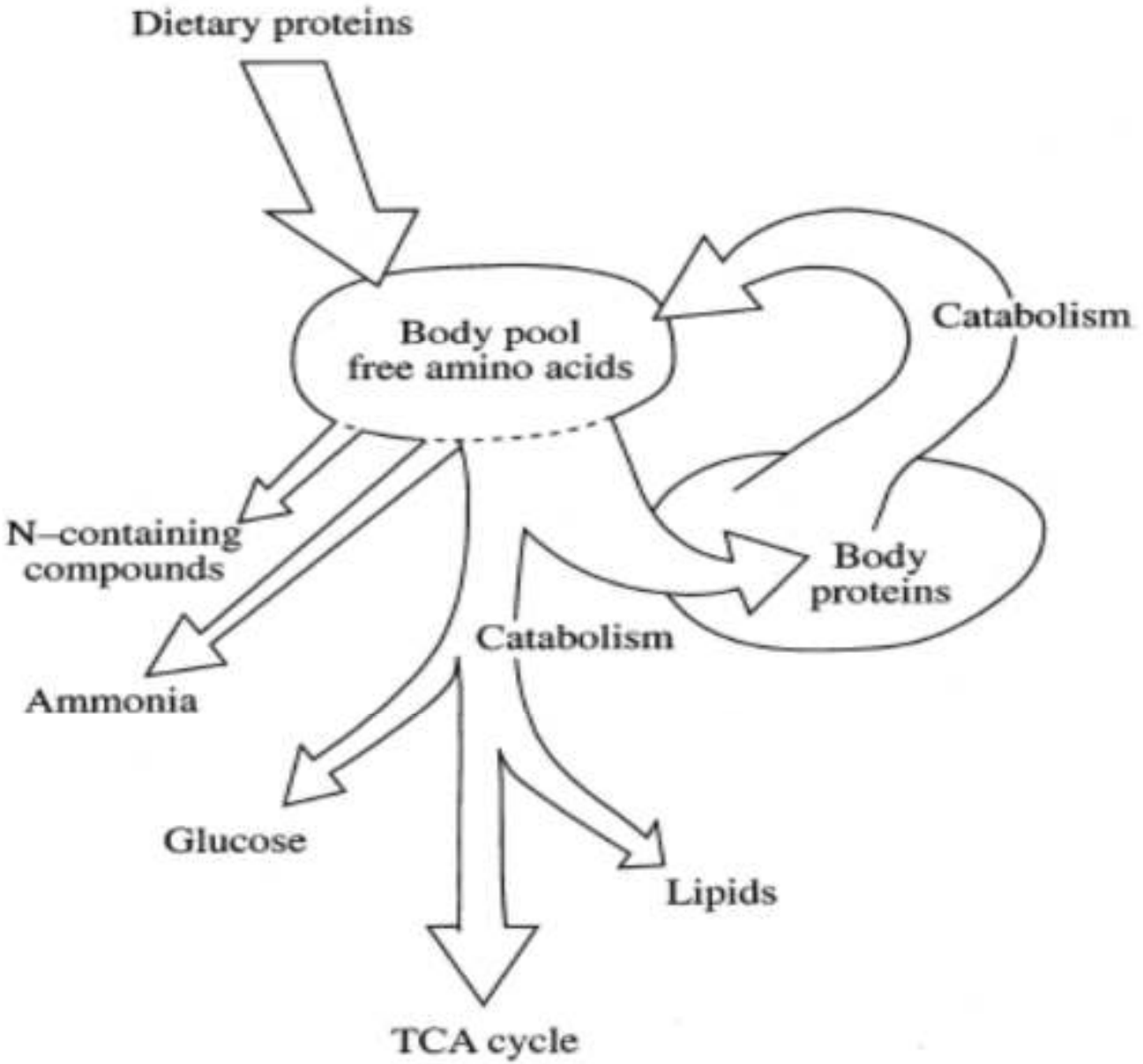


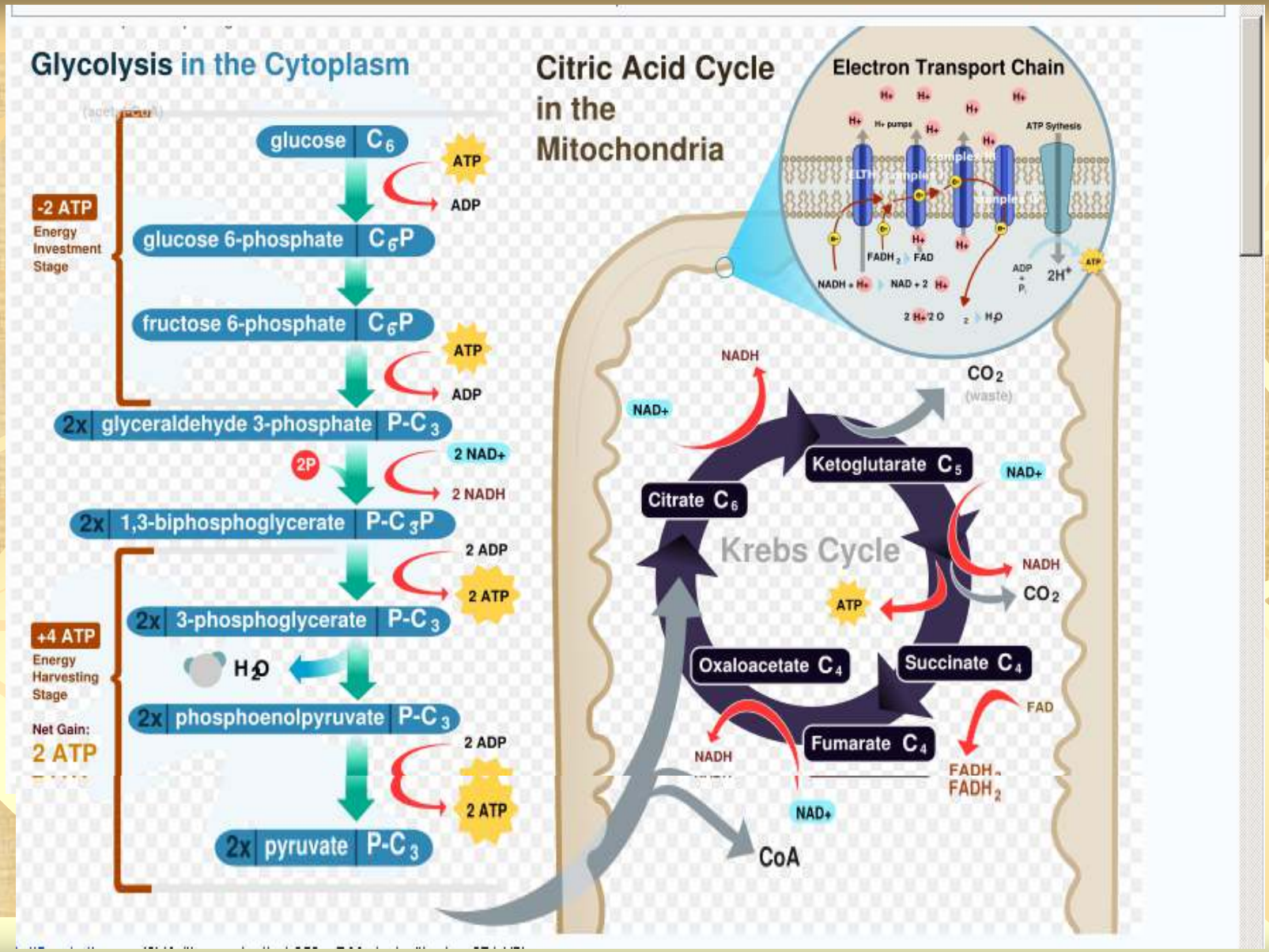
Changes in the relative length of intestine (expressed in body lengths) in several fish species

Carbohydrate Absorption



- Sodium-glucose transport proteins (SGLT) in membrane help absorb glucose & galactose
- Fructose absorbed by facilitated diffusion then converted to glucose inside the cell


Dietary proteins
Body pool
free amino acids
Catabolism
N–containing
compounds
Body
proteins
Catabolism
Ammonia
Glucose
Lipids
TCA cycle


Glycolysis in the Cytoplasm
glucose C6
ATP
ADP
-2 ATP
Energy Investment Stage
glucose 6-phosphate C6P
fructose 6-phosphate C6P
ATP
ADP
2x glyceraldehyde 3-phosphate P-C3
2P
2 NAD+
2 NADH
2x 1,3-biphosphoglycerate P-C3P
2 ADP
2 ATP
2x 3-phosphoglycerate P-C3
+4 ATP
Energy Harvesting Stage
H2O
2x phosphoenolpyruvate P-C3
Net Gain:
2 ATP
2 ADP
2 ATP
2x pyruvate P-C3
Citric Acid Cycle in the Mitochondria
Electron Transport Chain
H+ pumps
ATP Sythesis
FADH2
FAD
NADH + H+
NAD + 2 H+
ADP + Pi
2H+
ATP
2 H+ 2 O
H2O
NADH
CO2
(waste)
NAD+
Ketoglutarate C5
NAD+
Citrate C6
Krebs Cycle
NADH
CO2
ATP
Oxaloacetate C4
Succinate C4
FAD
NADH
Fumarate C4
FADH2
FADH2
NAD+
CoA