BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Fermentations
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
  Electron transport
  Chemiosmotic theory: Phosphorylation

Exam-2 material

Fat Catabolism
  diet
  storage
Fatty acid Catabolism
  FOUR stages in the catabolism of lipids:
    Mobilization from tissues (mostly adipose)
      hormone regulated
      specific lipases
      glycerol
    Activation of fatty acids
      Fatty-acyl CoA Synthetase
    Transport
      carnitine
    Oxidation
      Rationale
      Saturated FA
        β-oxidation
          4 steps:
            dehydrogenation
            hydration
            oxidation
            thiolase
        energetics
      Unsaturated FA
        energetics
      Odd-chain FA
      Ketone Bodies
      Other organelles

Exam-3 material

Protein Degradation (Catabolism)
  Digestion
  Inside of cells
  Protein turnover
    Ubiquitin
    Proteosome
Amino-Acid Degradation
  Dealing with the nitrogen
    Ammonia
      free
      transamination ← know mechanism
      Carbamoyl-phosphate synthetase
  Urea Cycle
    5 Steps
      Ornithine transcarbamylase
      Arginino-succinate synthetase
      Arginino-succinase
      Arginase
    Energetics
    Urea Bi-cycle
  Dealing with the carbon

Amino Acid Catabolism: Urea Cycle

① **Synthesis of Carbamoyl Phosphate**

- The first nitrogen-acquiring reaction of the urea cycle

$$CO_2 + NH_4^+ + 2\,ATP + H_2O \longrightarrow H_2N{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}O{-}\overset{\displaystyle O}{\overset{\|}{P}}(O^-){-}O^- + 2\,ADP + P_i + 3\,H^+$$

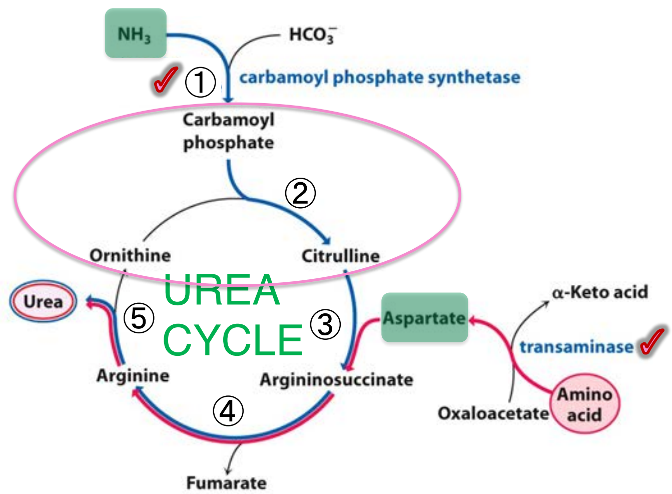
Carbamoyl phosphate

Carbamoyl Phosphate Synthetase I

- Excess CO_2, ATP, and ammonia is present in liver mitochondria. This is where the activation of both waste products occur (the majority of the other urea-cycle reactions occur within the cytosol).
- For step #2, in order to move to the cytosol, carbamoyl phosphate must condense with ornithine to create citrullene. This reaction releases the phosphate of carbamoyl phosphate into the mitochondrial matrix (so it does not deplete the proton motive force). Citrullene can then be transported to the cytosol.

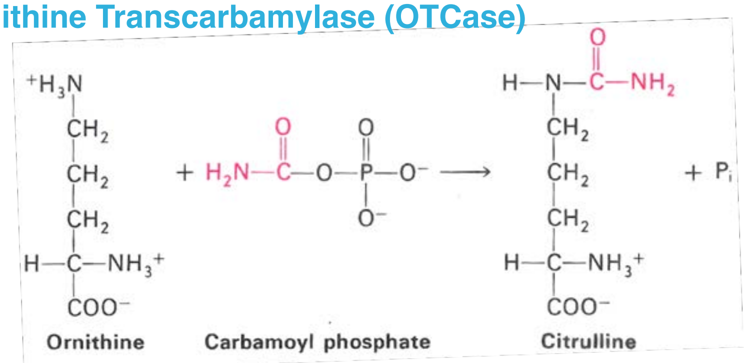
Amino Acid Catabolism: Urea Cycle

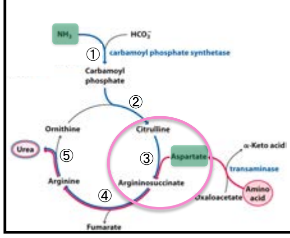
The Urea Cycle



Amino Acid Catabolism: Urea Cycle

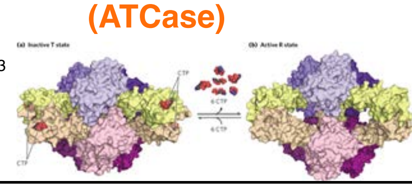
② Ornithine Transcarbamylase (OTCase)





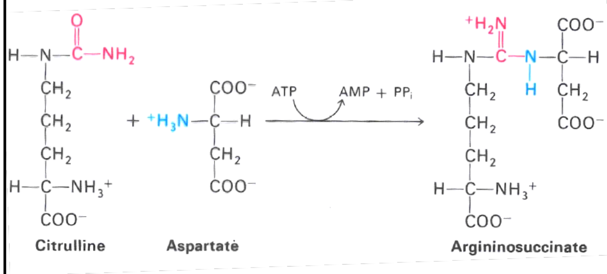
Recall: Aspartate Transcarbamoylase (ATCase)

$(\alpha_3)_2(\beta_2)_3$

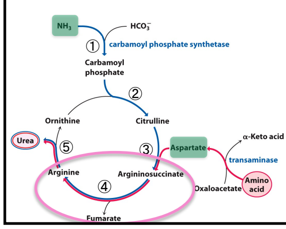


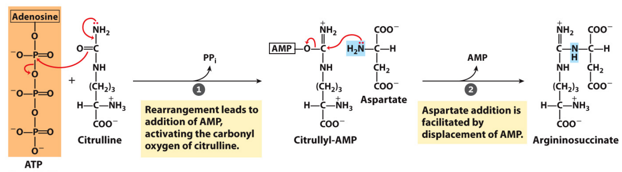
Amino Acid Catabolism: Urea Cycle

③ Argino-succinate Synthetase



- This is the second nitrogen-acquiring reaction.
- The synthetase is in the cytosol
- citrulline reacts with ATP to produce citrullyl-AMP.
- AMP acts as a good leaving group, as aspartate attracts the imide carbon to produce argininosuccinate.
- PP$_i$ product helps drive reaction.



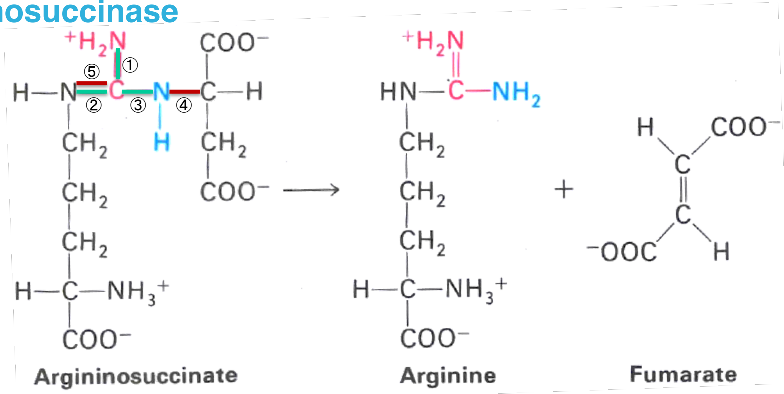


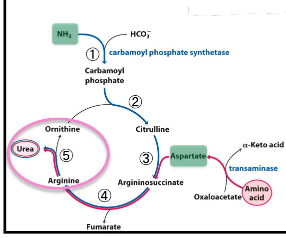
Where have we seen this strategy before?
What drives this reaction?

http://media.saplinglearning.com/priv/he/lehninger/aem/1811b_argininosuccinate.html

Amino Acid Catabolism: Urea Cycle

④ Argininosuccinase

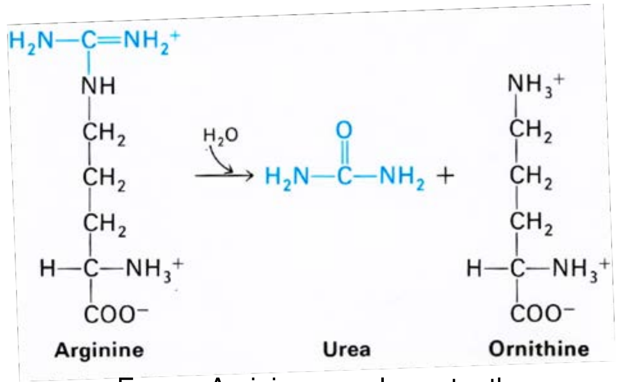




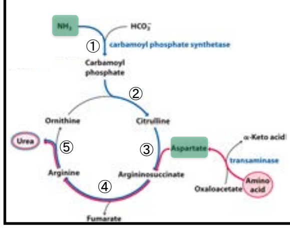
- Argininosuccinate is a good molecule to rationalize the cycle showing were and in what order bonds are made or broken.
- Argininosuccinase cleaves fumarate from argininosuccinate, resulting in arginine.

Amino Acid Catabolism: Urea Cycle

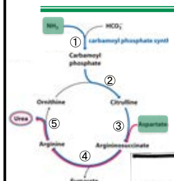
⑤ Arginase



- Excess Arginine can also enter the urea cycle at this point.
- Arginase cleaves both nitrogen atoms added in the urea cycle from ammonia and Asp, resulting in free urea.
- Ornithine is able to serve as a substrate for the next round of the cycle.



Amino Acid Catabolism: Urea Cycle



Enzymes of the Urea Cycle

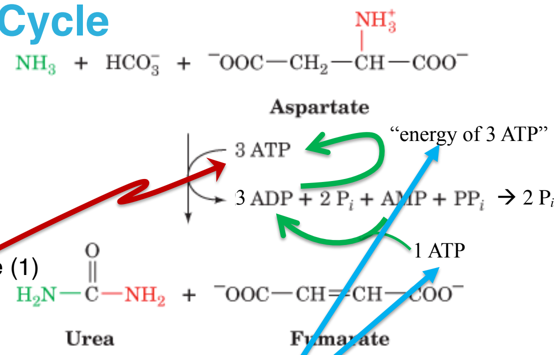
	Enzyme	Compartment	Activity	M_r	pH opt	K_m, mM	Equilibrium Constant	Tissue Distribution
	N-acetyl glutamate synthetase, EC 2.3.11	Mitochondrial matrix	0.30–1.49	200,000	8.5	Glu, 3.0 Ac CoA 0.7 Arg, 0.01	Irreversible	Liver, intestine, kidney (trace), spleen
①	Carbamyl phosphate Synthetase EC 6.3.4.16	Mitochondrial matrix	279†	310,000 dimer	6.8–7.6	NH_4, 0.8 HCO_3, 6.7 Mg ATP, 1.1 NAG, 0.1	Irreversible	Liver, intestine, kidney (trace)
②	Ornithine transcarbamylase, EC 2.1.3.3	Mitochondrial matrix	6600	108,000 trimer	7.7	CP, 0.16 Orn, 0.40	$\frac{(Cit)(p)}{(Orn)(CP)} = 10^5$	Liver, intestine, kidney (trace)
③	Argininosuccinic acid synthetase, EC 6.3.4.5	Cytosol	90	185,000 tetramer	8.7	Asp, .03 Cit, .03	$\frac{(ASA)(AMP)(Mg\,PP)(2H)}{(Cit)(Asp)(Mg\,ATP)} = 0.89$‡	Liver, kidney, fibroblasts, brain (trace)
④	Argininosuccinase, EC4.3.2.1	Cytosol	220	173,200 tetramer	7.5	Asp, 0.017 Cit, 0.016 ATP, 0.041	$\frac{(Arg)(fumarate)}{(ASA)} = 11.4 \times 10^{-3}$	Liver, kidney, brain, fibroblasts
⑤	Arginase, EC 3.5.3.1	Cytosol	86,600	107,000 tetramer	9.5	Arg, 10.5	Irreversible	Liver, erythrocytes, kidney, lens, brain (trace)

*Enzyme activity is expressed as micromoles per hour per gram wet weight. Apart from the equilibrium constants, the values described are those of human liver.
†The monomers may have substantial catalytic activity.[11]
‡AT pH = 7.0.
SOURCE: Table assembled from Ratner,[4] Snodgrass,[9] Meijer and Hensgens,[10] Jackson, et al.,[11] Beaudet et al.,[12] Lusty,[13] and Bachman et al.[14]

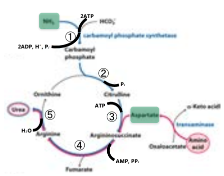
Amino Acid Catabolism: Urea Cycle

Energetics of Urea Cycle

Per 2 nitrogen atoms to make **one** urea molecule:



To make CP (2) and arginosuccinate (1)

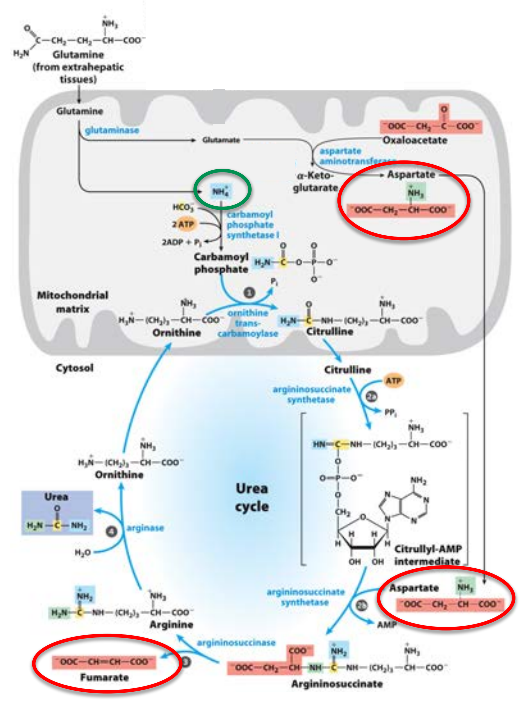


Net is 4 ATP equivalents

[Its not actual ATPs used, its how many it takes to re-cycle the products (2 ADP + 1 AMP)]

- But what about this fumarate?
- What is its fate?
- How do you regenerate Asp to keep the cycle going?

Amino Acid Catabolism: Urea Cycle



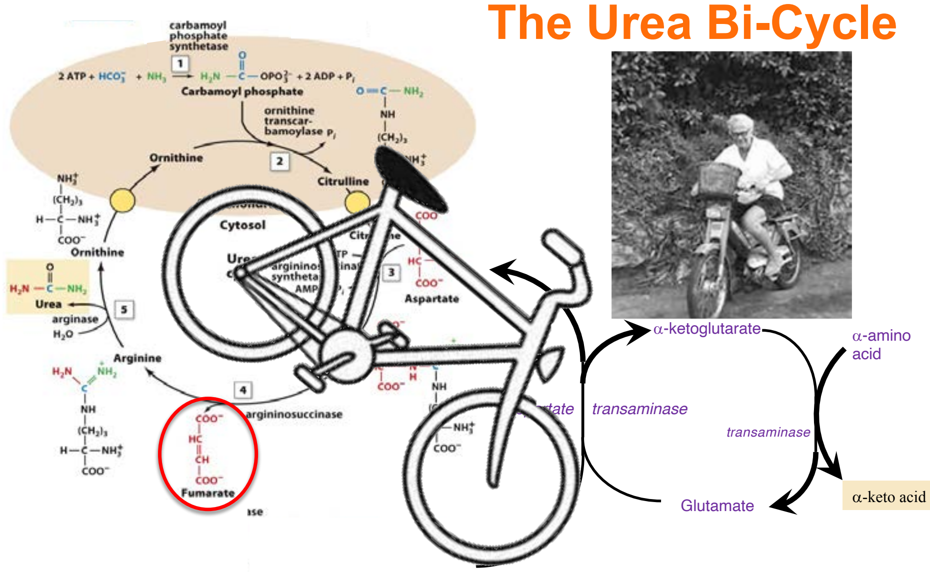
Two issues:

1) What to do with the fumarate?

2) What are the sources of the free ammonia?

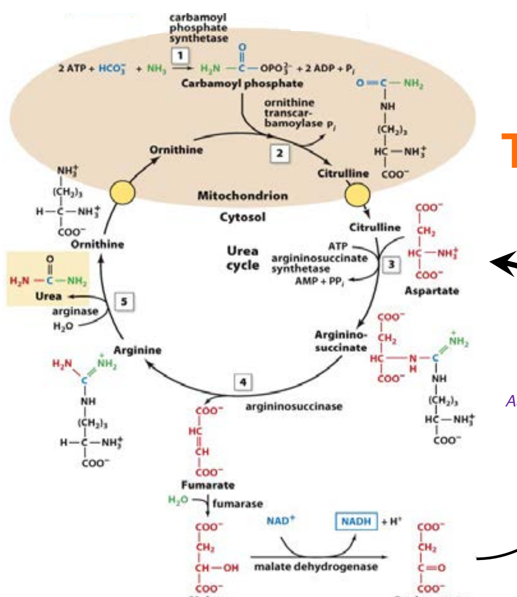
Amino Acid Catabolism: Urea Cycle

The Urea Bi-Cycle



Amino Acid Catabolism: Urea Cycle

The Urea Bi-Cycle



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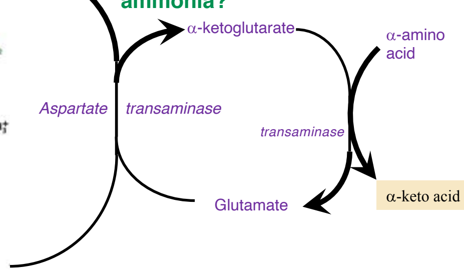
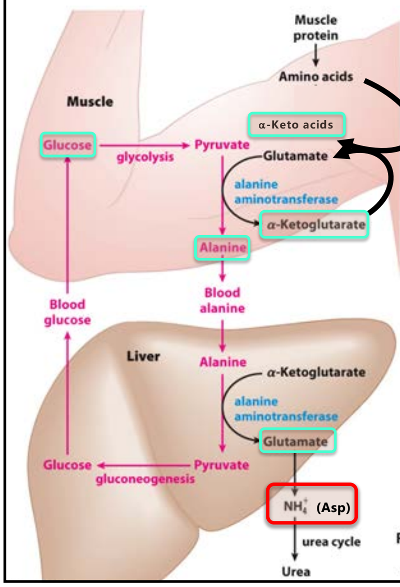


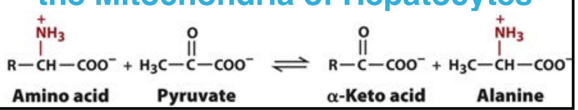
Amino Acid Catabolism: Urea Cycle

The Glucose-Alanine Cycle



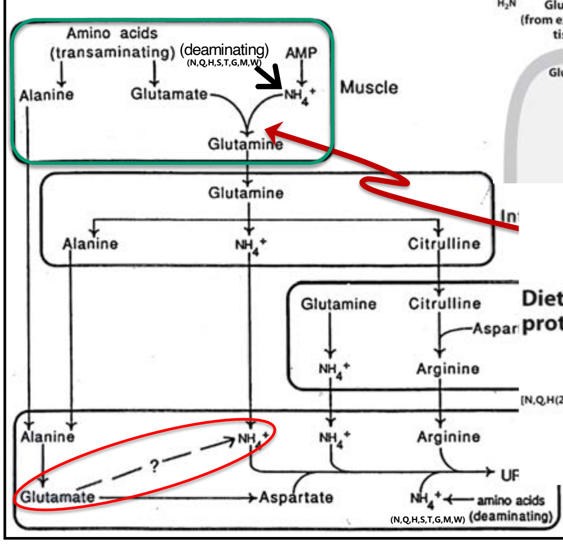
- Vigorously working muscles operate nearly anaerobically and rely on glycolysis for energy.
- Glycolysis yields pyruvate.
  - If not eliminated (converted to acetyl-CoA), lactic acid will build up.
- If amino acids have become a fuel source, this lactate is converted back to pyruvate, then converted to alanine for transport into the liver.

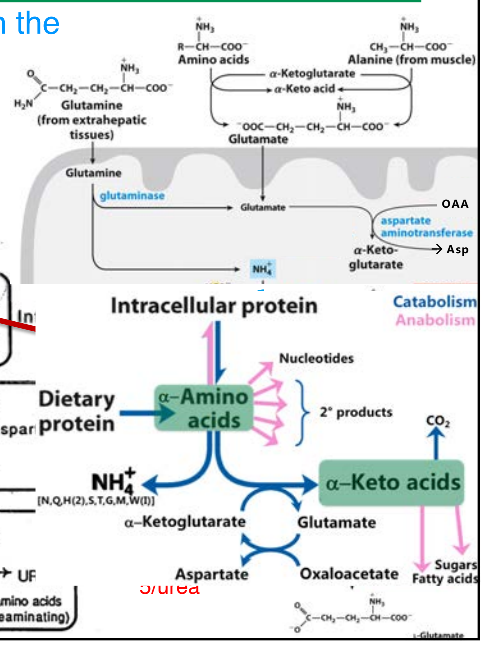
Excess Glutamate is Metabolized in the Mitochondria of Hepatocytes



Amino Acid Catabolism: Urea Cycle

Excess glutamine is processed in the intestines, kidneys, and liver.





Amino Acid Catabolism: Urea Cycle

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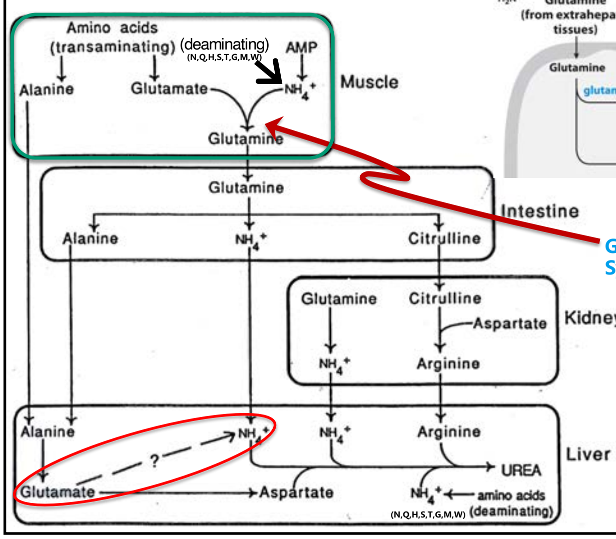
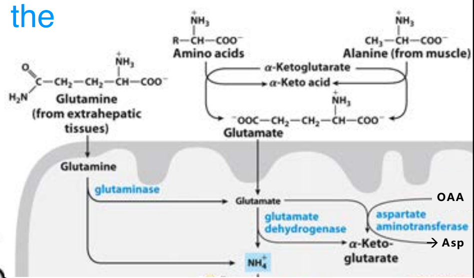
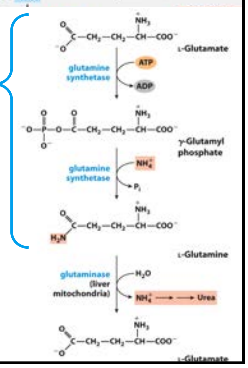




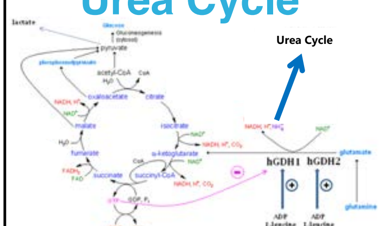
Glutamine Synthetase



This costs another ATP, bringing it closer to 5/urea

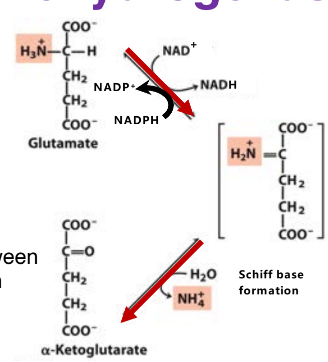
Amino Acid Catabolism: Urea Cycle

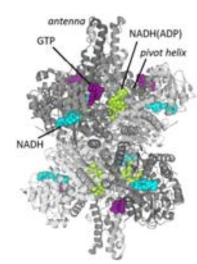
Regulation of the Urea Cycle



- The pool of **Glu** is a critical junction between biosynthesis and catabolism
- The enzyme represents a key link between catabolic and anabolic pathways; when biosynthesis is needed, its off and Glu provides for amino-acid biosynthesis. When energy is needed, its on; the ammonia is pulled off to provide for the urea cycle and oxidation of carbon skeletons.
- Can use either NAD^+ in the catabolic direction (oxidative deamination), or NADPH in the biosynthetic direction.

Glutamate Dehydrogenase





Allosteric inhibitors:
GTP, ATP, Palmitoyl-CoA

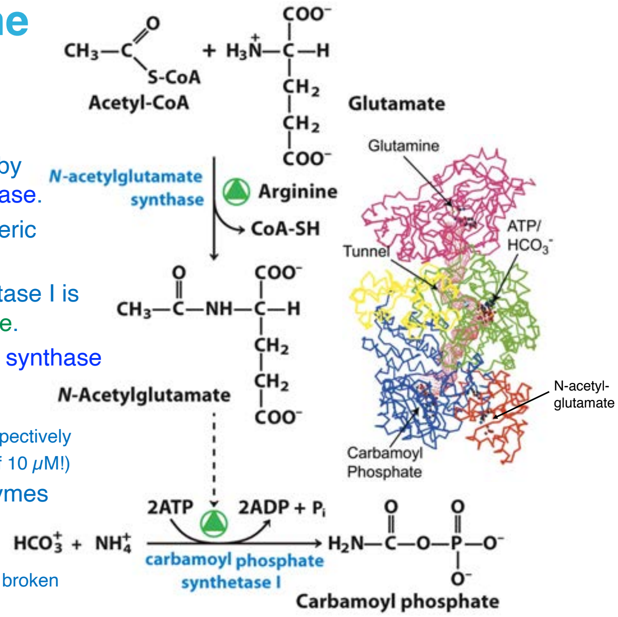
Allosteric Activators:
ADP, Leucine, Isoleucine, Valine

- GluDH has a high K_m value (>1 mM) for ammonia, so it mostly functions to provide the initial material for the urea cycle; ammonia, using NAD^+, which is high when energy charge is low.
- Ammonia is processed into urea for excretion. Carbon skeletons are oxidized for ATP.

Amino Acid Catabolism: Urea Cycle

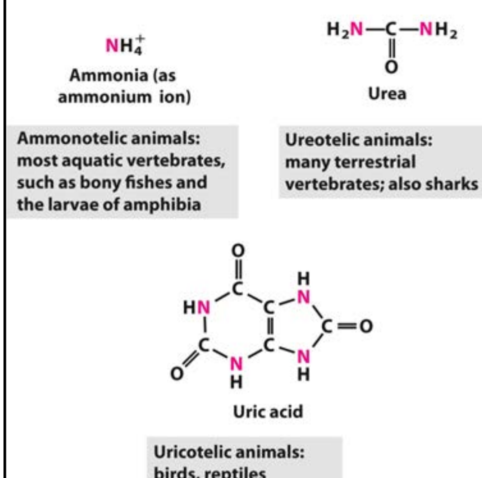
Regulation of the Urea Cycle

- Urea production is controlled by carbamoyl phosphate synthetase.
- *N*-acetylglutamate is an allosteric activator.
- Carbamoyl phosphate synthetase I is activated by *N*-acetylglutamate.
- Formed by *N*-acetylglutamate synthase
  - –when glutamate and acetyl-CoA concentrations are high
  - –K_m values are 1 and 0.7 mM, respectively
  - –activated by arginine (K_a value of 10 μM!)
- Expression of urea cycle enzymes increases when needed.
  - –high-protein diet
  - –starvation, when protein is being broken down for energy



Amino Acid Catabolism: Urea Cycle

Excretory Forms of Nitrogen: Fates of Nitrogen in Organisms



Ure-otelic (urea)

Uric-otelic (uric acid)

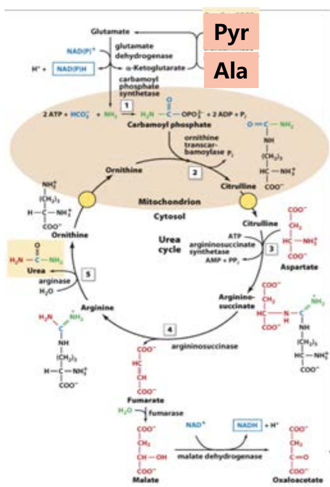
- Plants conserve almost all the nitrogen.
- Many aquatic vertebrates release ammonia to their environment.
  - –passive diffusion from epithelial cells
  - –active transport via gills
- Many terrestrial vertebrates and sharks excrete nitrogen in the form of urea.
  - –Urea is far less toxic that ammonia.
  - –Urea has very high solubility.
  - –Requires lots of water
- Some animals such as birds and reptiles excrete nitrogen as uric acid.
  - –Uric acid is rather insoluble.
  - –Excretion as paste allows the animals to conserve water.
- Humans and great apes excrete both urea (from amino acids) and uric acid (from purines).

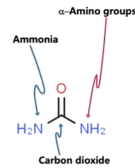
Amino Acid Catabolism: Urea Cycle

Energy from Amino-Acid Degradation & Urea Cycle

In liver

Example: 2 Alanines





$NADP^+ \xrightarrow{GluDH} NADPH$ 2.5

$NAD^+ \xrightarrow{MDH_{cyto}} NADH$ 2.5

5

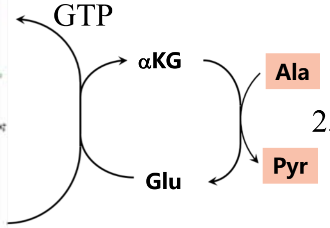
Per Pyruvate in Krebs Cycle:

$4\ NAD^+ \rightarrow 4\ NADH$ 10

$FAD \rightarrow FADH_2$ 1.5

GTP 1

12.5 x 2 = 25



25 + 5 = 30

– 4 (Urea Cycle)

=26/2 = 13 ATP per Ala

Amino Acid Degradation: the carbon "skeletons"

The SEVEN (7) Families

A. Concepts
   1. Convergent
   2. ketogenic/glucogenic
   3. Reactions seen before

B. Transaminase (A,D,E) / Deaminase (N,Q) Family

C. Related to biosynthesis (R,P,H; G,S,C; T,M)
   1. Glu Family
      a. Introduce oxidases/oxygenases
      b. Introduce one-carbon metabolism (1C)
   2. Pyruvate Family
      a. PLP reactions
   3. α–ketobutyric Family
      a. 1-C metabolism

D. Dedicated (F,Y; K,W; V,I,L)
   1. Aromatic Family
      a. oxidases/oxygenases
   2. α–ketoadipic Family
   3. Branched-chain Family

In total there are 29 Nitrogen atoms in the 20 amino acids:
10 are given off as ammonia
17 are taken off in transaminase reactions
2 leave as urea

There are ~75 reactions in total