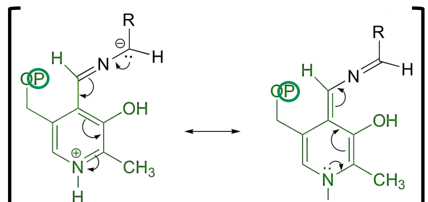
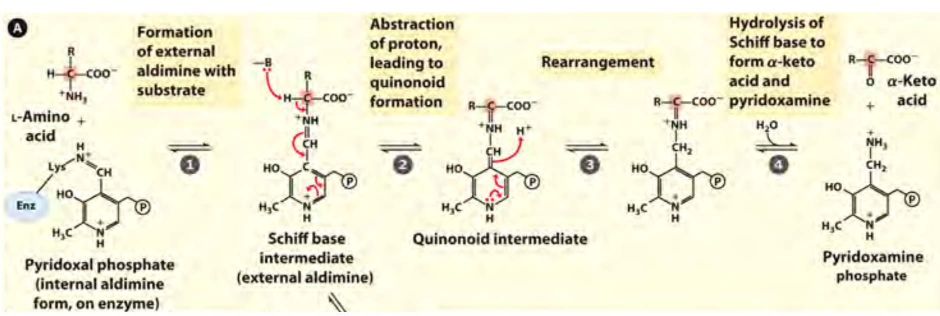
Amino Acid Catabolism: Transamination

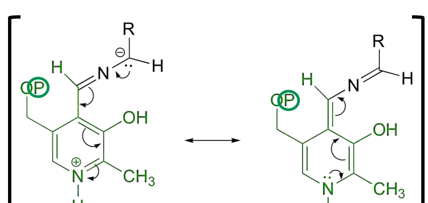


quinonoid intermediate

Amino Acid Catabolism: Transamination

See Sapling animated Figure(a)



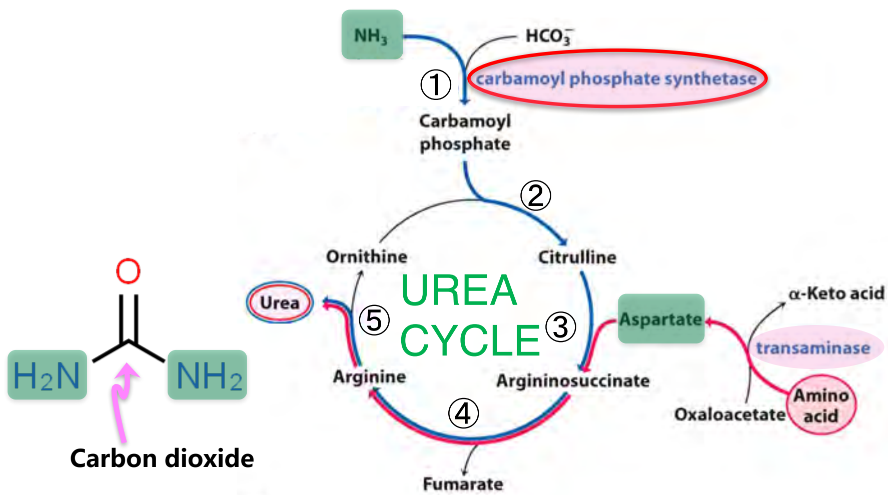


quinonoid intermediate

http://media.saplinglearning.com/priv/he/lehninger/aem/1806a_pyridoxal_phosphate.html

Amino Acid Catabolism: Urea Cycle

Synthesis of Carbamoyl Phosphate



Amino Acid Catabolism: Urea Cycle

① Synthesis of Carbamoyl Phosphate

- The first nitrogen-acquiring reaction of the urea cycle

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

Carbamoyl phosphate

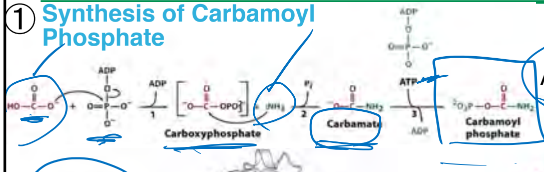
Carbamoyl Phosphate Synthetase I

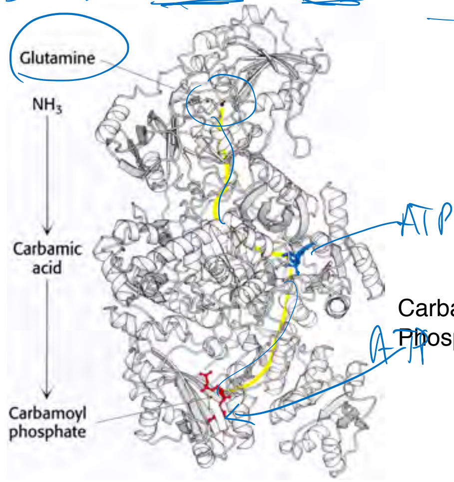
- Excess CO_2, ATP, and ammonia is present in liver mitochondria. This is where the activation of both waste products occur (the majority of the other urea-cycle reactions occur within the cytosol).
- For step #2, in order to move to the cytosol, carbamoyl phosphate must condense with ornithine to create citrullene. This reaction releases the phosphate of carbamoyl phosphate into the mitochondrial matrix. Citrullene can then be transported to the cytosol.

Amino Acid Catabolism: Urea Cycle

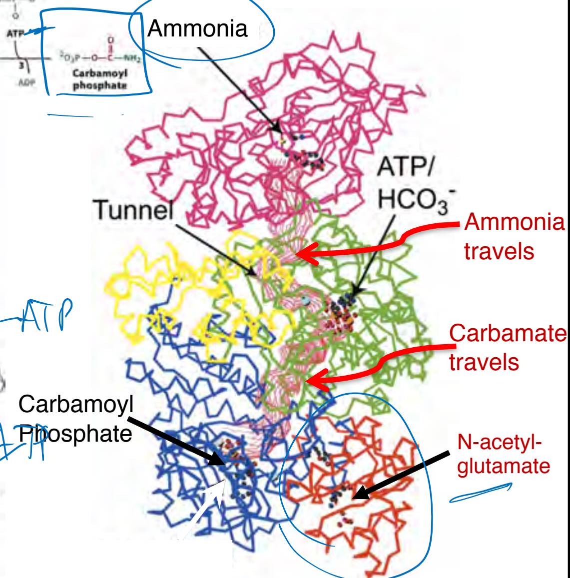
① **Synthesis of Carbamoyl Phosphate**

See Sapling animated Figure





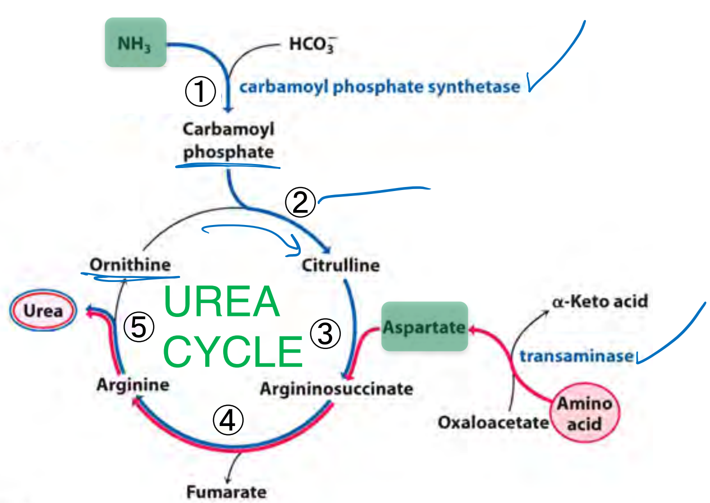
Carbamoyl Phosphate Synthetase II



Carbamoyl Phosphate Synthetase I

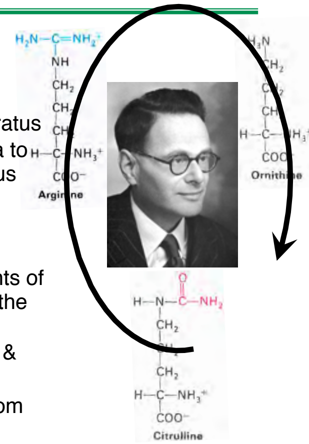
Amino Acid Catabolism: Urea Cycle

The Urea Cycle

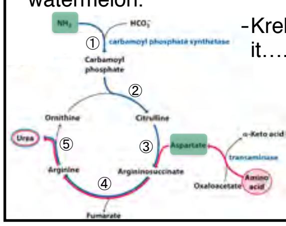


Amino Acid Catabolism: Urea Cycle

The Urea Cycle: Evidence for a cycle



- Already known that Arg gives rise to Urea and Orn ⑤
- How do CO_2 and NH_3 get into Arg?
- Enter H. Krebs, who studies metabolism using the Warburg apparatus
- Urease (recently purified by Sumner in 1925) can hydrolyzed urea to CO_2 + 2 NH_3; Could measure CO_2 from urea by Warburg apparatus
- Using liver slices, which amino acids gave rise to urea?
- ….. Most gave some
- Then as a control, he tried Orn
- Added Orn to prep: extraordinary occurrence of “catalytic” amounts of urea!! Orn, which is the product, will give rise to more urea than the Orn added at a rate of 7-30x more: “catalytic”
- In the library, Krebs looked for intermediates that might have CO_2 & NH_3 stuck to Orn: citrulline
- Dr. Wada in Japan had just published to purification of citrulline from watermelon.



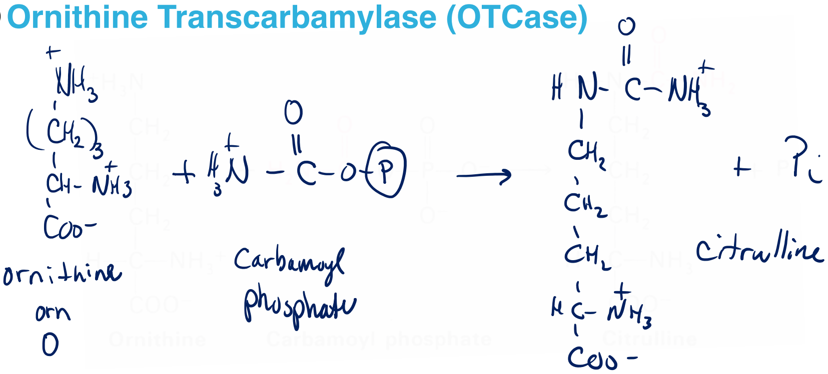
- Krebs attained 10 mg citrulline and added it……same catalytic phenomenon!!

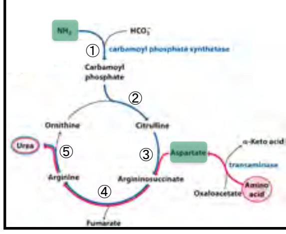
First cyclic process!
……lead to lots of notariety

Dr. Kornberg: **Lecture 03.15.17 (23:00-31:15)- Krebs (8 min)**

Amino Acid Catabolism: Urea Cycle

② Ornithine Transcarbamylase (OTCase)



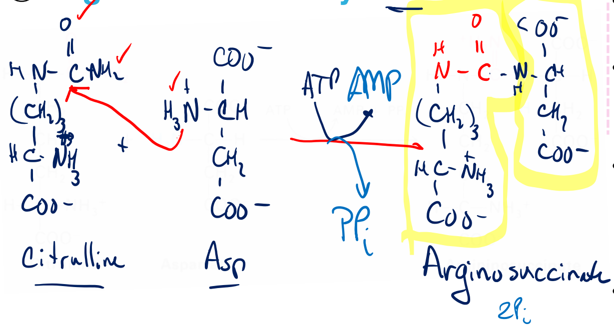


Aspartate Transcarbamoylase (ATCase)

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

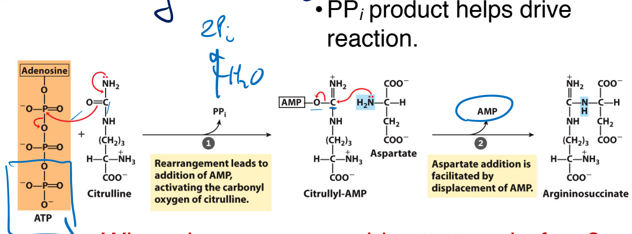
Amino Acid Catabolism: Urea Cycle

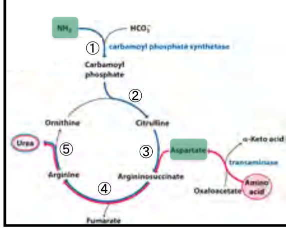
③ Argino-succinate Synthetase



See Sapling animated Figure

- This is the second nitrogen-acquiring reaction.
- 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.



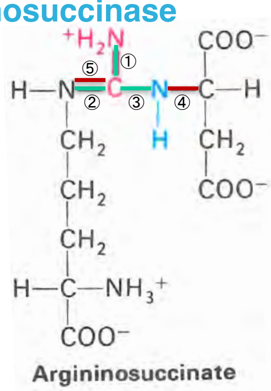


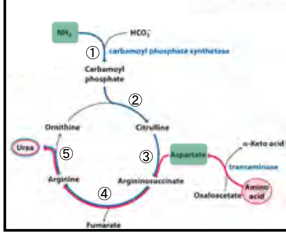
Where have we seen this strategy before?

What drives this reaction?

Amino Acid Catabolism: Urea Cycle

④ Argininosuccinase

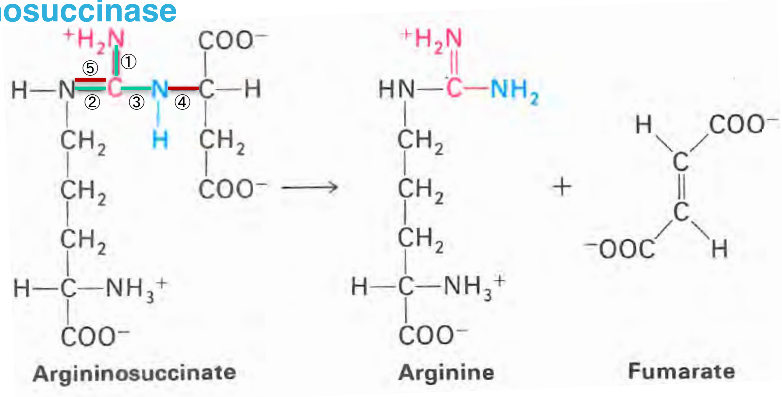


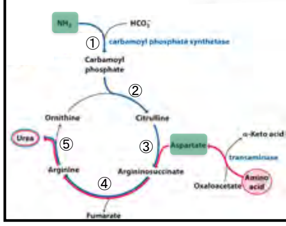


- Argininosuccinate is a good molecule to rationalize the cycle showing were and in what order bonds are made or broken.
- Argininosuccinase cleaves fumarate from argininosuccinate, resulting in arginine.

Amino Acid Catabolism: Urea Cycle

④ Argininosuccinase





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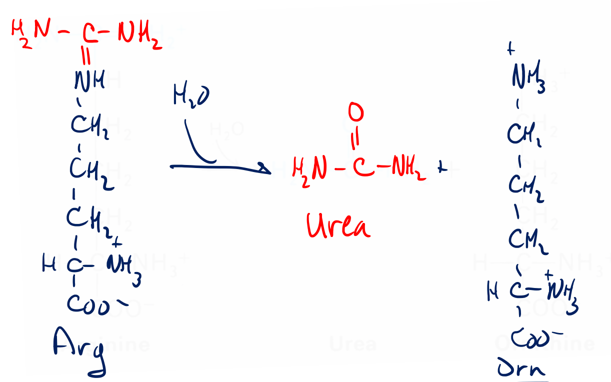
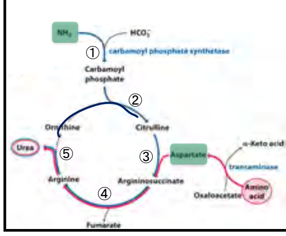
Amino Acid Catabolism: Urea Cycle

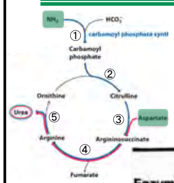
⑤ Arginase





- 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]