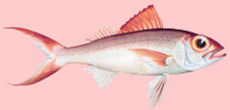

Nitrogen Excretion in Fish

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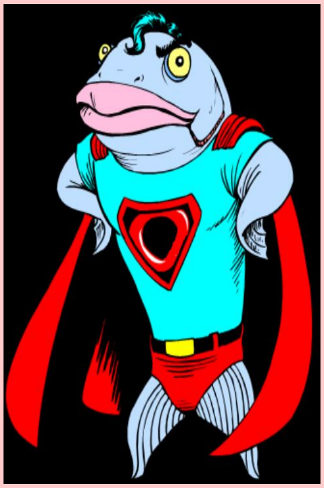
021 Farquharson

Lecture Outline: Nitrogen Excretion In Fish



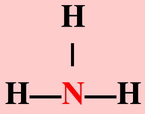
Gulf Toadfish (*Opsanus beta*)

Excretion Strategies
- **Ammoniotelism**
- **Ureotelism**
- **Ammoniotelism to Ureotelism**

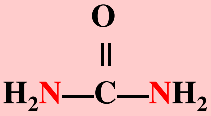
Detoxification Strategies
- **Partial Amino Acid Catabolism**
- **Glutamine Synthesis**

Nitrogen Excretion

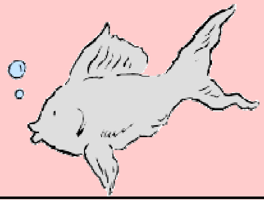
- Most nitrogenous wastes in fish are produced and excreted as ammonia or urea.


$$H-\overset{\overset{\displaystyle H}{|}}{N}-H$$

Ammonia


$$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2$$

Urea



Ammoniotelism: Ammonia Excretion

- Animals that excrete their nitrogenous wastes primarily as ammonia (NH_3) are ammoniotelic.
  - Most fish (including agnathans and most teleosts)
- About 80 to 90% of their nitrogenous wastes are excreted as ammonia and the remainder as urea.

Goldfish
(Carassius auratus)

What is Ammonia (NH_3)?

- **Weak base**
- **Highly soluble**
- **Can diffuse passively across epithelia (e.g. gill)**
  - **In solution, it exists as 2 species:**

$$NH_3 + H_3O^+ \rightleftharpoons NH_4^+ + H_2O$$

However, in fish tissue about 95% of total ammonia exists as NH_4^+.
NH_4^+ <u>cannot</u> diffuse across epithelia.

(side note: pK of NH_3 = 9-10; fish blood = pH 7.4)

- **Highly toxic at high concentrations**

Why is it toxic?

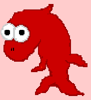
- **increases internal pH**
- **can inhibit key enzymes required for energy generation (destabilizes proteins)**
- **NH_4^+ substitutes for K^+ in ion transporters, (e.g. Na^+-K^+-ATPases) disrupting electrochemical gradients.**

Ammonia Resistance

- In general, fish are much more resistant to build-up of internal ammonia than terrestrial vertebrates.

For example,

Fish → 100 - 200 µM (up to 1000 µM)
Humans → 40 µM (up to 80 µM)



Ammonia Production

- Ammonia is generally produced by two catabolic processes:

1) Amino Acid Catabolism (mainly)

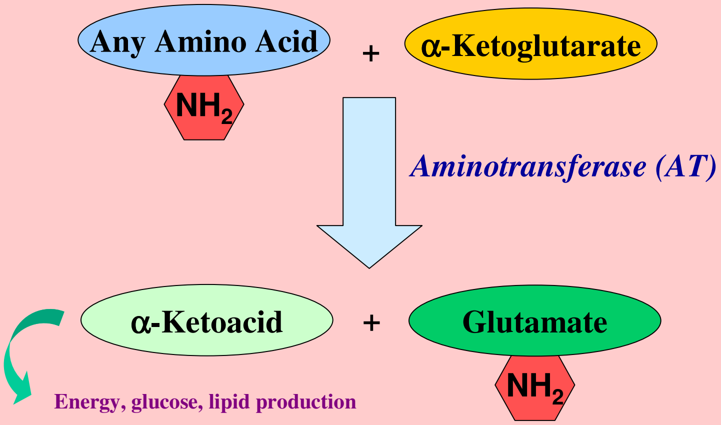
2) Purine Catabolism (trace)

Amino Acid Catabolism

- Majority of ammonia in fish is produced by the catabolism of amino acids
- Requires little to no energy
- α-Ketoacids (e.g. pyruvate) generated can be used for:
  - Production of energy (e.g. in Krebs Cycle)
  - Gluconeogenesis
  - Lipogenesis
- The primary mechanism for amino acid catabolism in fish is transdeamination.

Amino Acid Catabolism: Transdeamination

Step 1: ***Transamination***:



Any Amino Acid + α-Ketoglutarate

NH_2

Aminotransferase (AT)

α-Ketoacid + Glutamate

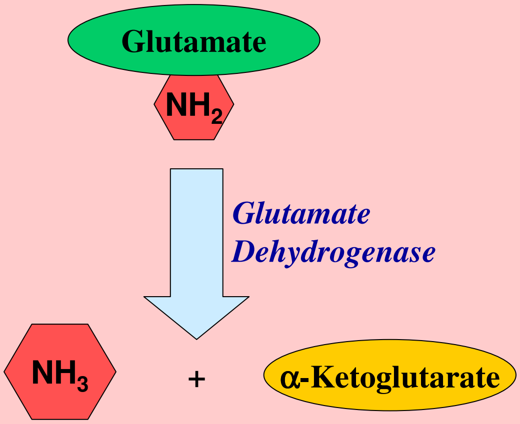
NH_2

Energy, glucose, lipid production

- Amino group (NH_2) of any amino acid is transferred to α-ketoglutarate to form glutamate.

Amino Acid Catabolism: Transdeamination

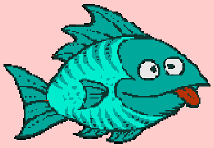
Step 2: *Deamination*



- Amino group of glutamate is released as ammonia.

Amino Acid Catabolism: Transdeamination

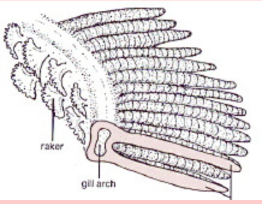
- Typically, 50 to 70% (up to 99% in goldfish) of ammonia produced by transdeamination occurs in the liver.
- The rest originates in the kidney, muscle gill and intestine.



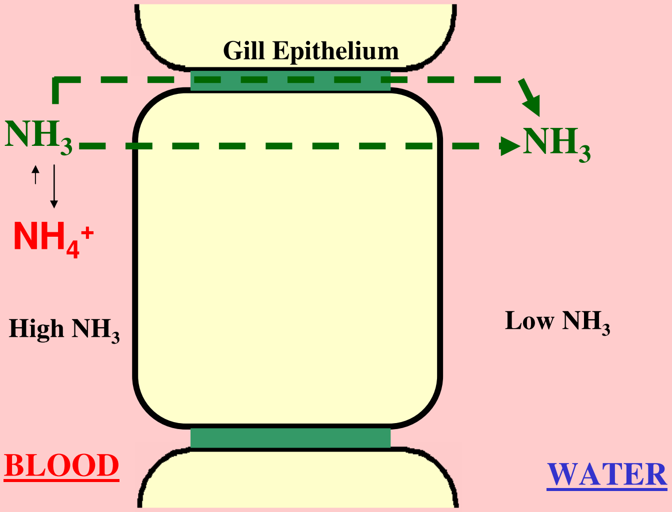
How is Ammonia Excreted???

How is Ammonia Excreted?

- Unlike most vertebrates, >80% of nitrogenous wastes are excreted by the gills, with only trace amounts excreted by the kidney as urine.



Ammonia Excretion in FW Fish Gill



Gill Epithelium

NH_3 ⇅ NH_4^+ → NH_3

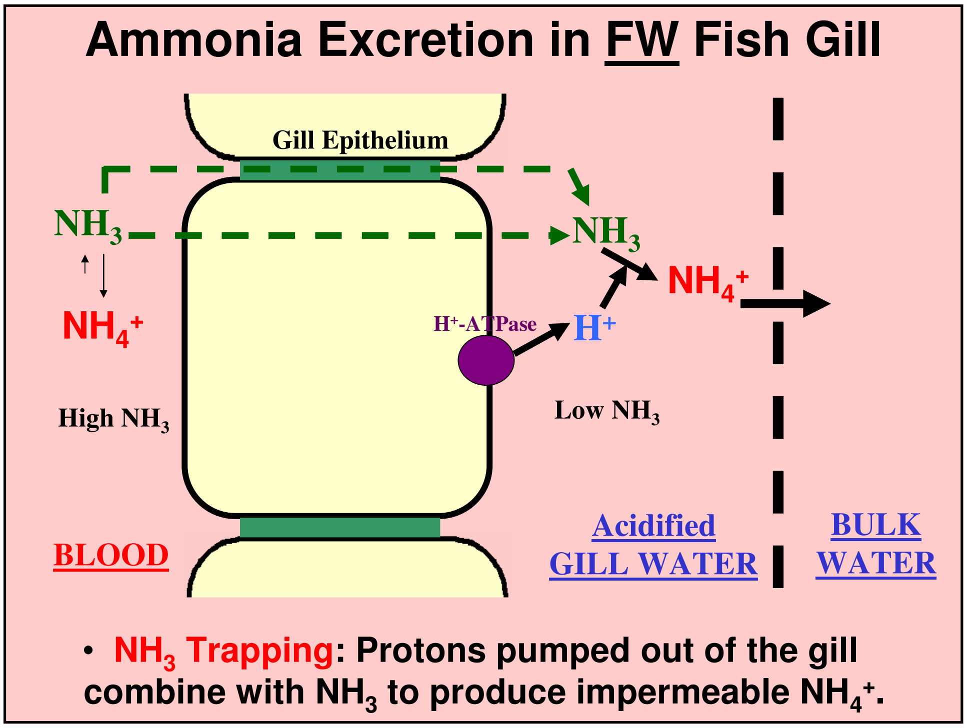
High NH_3

Low NH_3

BLOOD

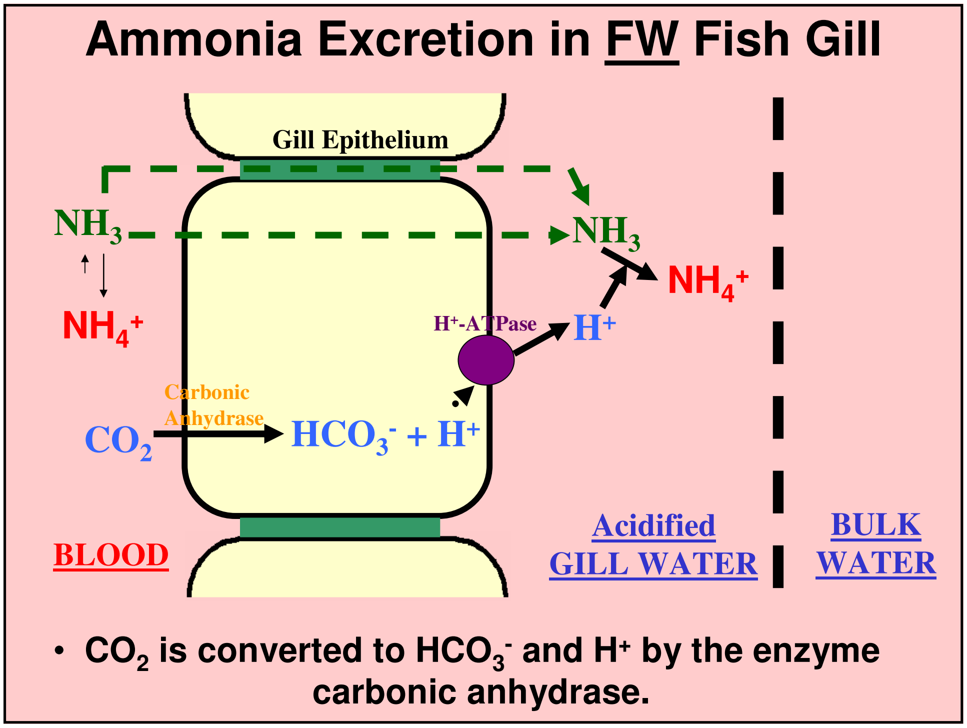
WATER

- Passive diffusion of NH_3 into water (transcellularly or paracellularly)



Ammonia Excretion in FW Fish Gill

- **NH_3 Trapping**: Protons pumped out of the gill combine with NH_3 to produce impermeable NH_4^+.



Ammonia Excretion in FW Fish Gill

- CO_2 is converted to HCO_3^- and H^+ by the enzyme carbonic anhydrase.

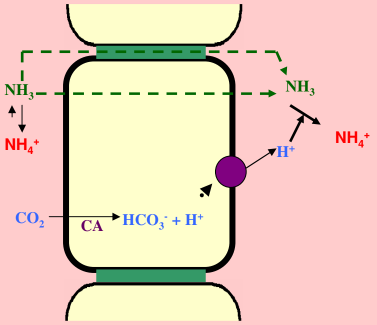
Ammonia Excretion in <u>FW</u> Fish Gill

Review

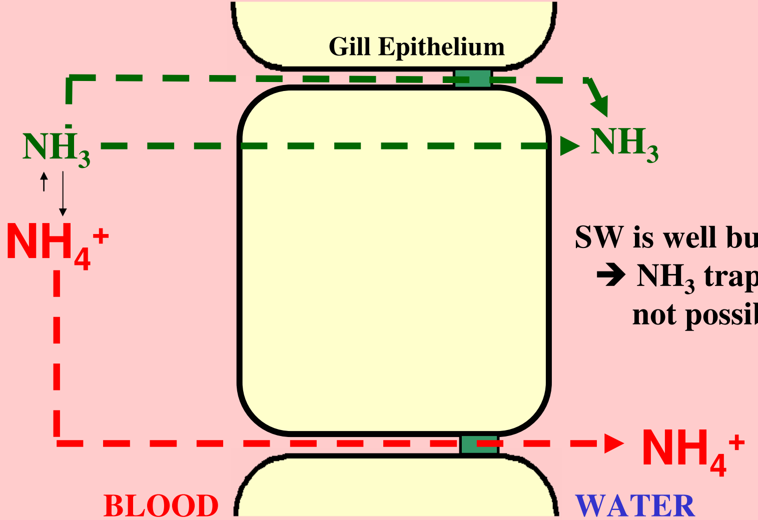
① **Passive diffusion** of NH_3 into water (trancellularly or paracellularly)

② **NH_3 Trapping**: Gill water is acidified by protons pumped out of the gill by an H^+-ATPase. Protons combine with NH_3 to produce impermeable NH_4^+ and maintain NH_3 gradient. (High NH_3 in blood, low in water)

③ **Protons** are produced by carbonic anhydrase from CO_2.



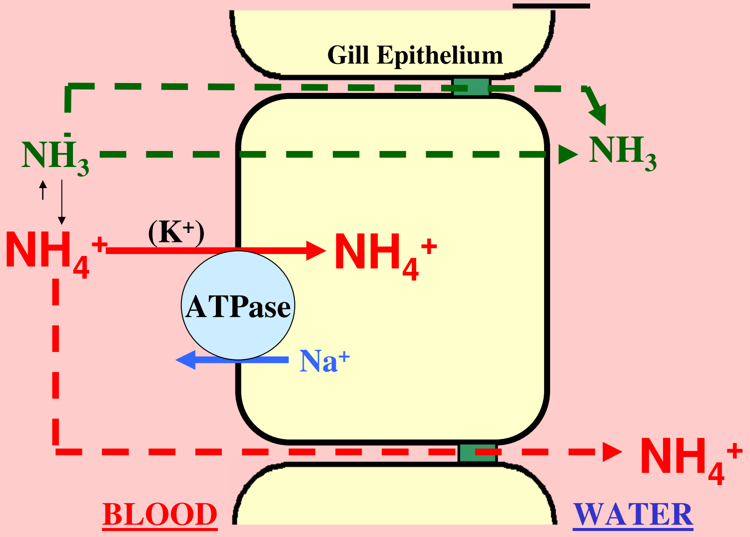
Ammonia Excretion in <u>SW</u> Fish Gill



SW is well buffered
➔ NH_3 trapping
not possible

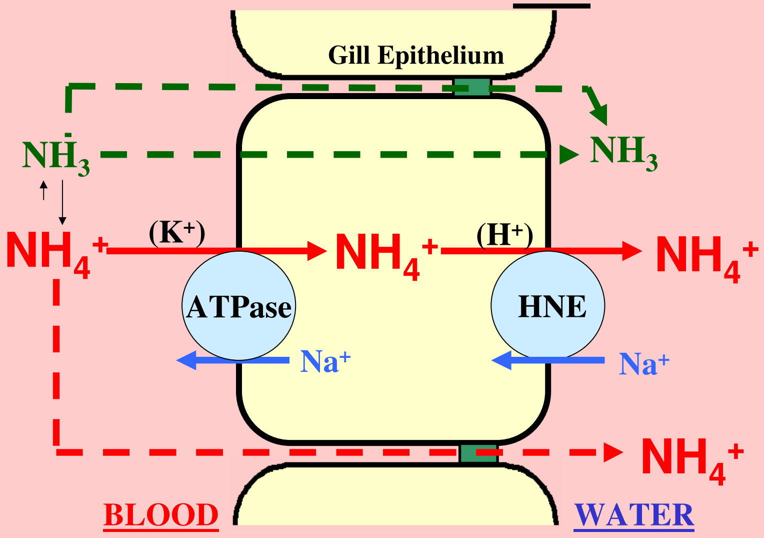
- **Passive diffusion** of NH_3 into water (trancellularly and paracellularly) and **passive diffusion** of NH_4^+ (paracellularly via “leaky” junctions).

Ammonia Excretion in SW Fish Gill



- NH_4^+ is pumped into gill by substituting for K^+ at a basolateral Na^+-K^+-ATPase.

Ammonia Excretion in SW Fish Gill



- NH_4^+ is pumped out of the gill by substituting for H^+ at an apical H^+-Na^+ exchanger (HNE).

Ammonia Excretion in <u>SW</u> Fish Gill

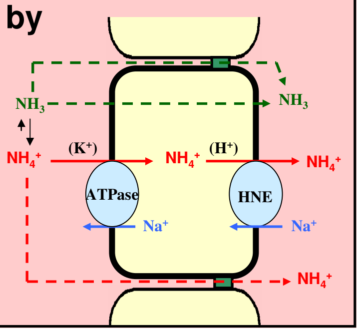
Review

① **Passive diffusion** of NH_3 into water (trancellularly or paracellularly) and **passive diffusion** of NH_4^+ (paracellularly via "leaky" junctions).

Remember: SW is well buffered → NH_3 trapping not possible

② **Active transport** of NH_4^+ into the gill by replacing K^+ in Na^+-K^+-ATPases

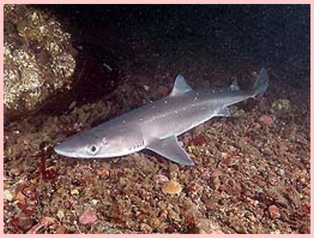
③ **Active transport** of NH_4^+ into the water by replacing H^+ in HNEs.



Ureotelism: Urea Excretion

- **Animals that excrete their nitrogenous wastes primarily as urea are ureotelic.**
  - **Elasmobranchs, coelacanths and a few teleosts.**

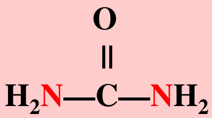
Coelacanth
(Latimeria chalumnae)



Dogfish
(*Squalus acanthias*)

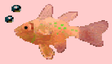
What is Urea?

- Highly soluble
- Ability to diffuse across epithelia (e.g. gill) depends on the species
  - e.g. elasmobranchs
    - – high cholesterol:lipid membrane
      → impedes diffusion
- At high concentrations, much less toxic than ammonia



$$H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2$$

Urea



Urea Production

- Urea is generally produced by two processes:

  1) Ornithine-Urea Cycle (OUC)

  2) Uricolysis
     - Most fish (including teleosts)
     - Breakdown of uric acid

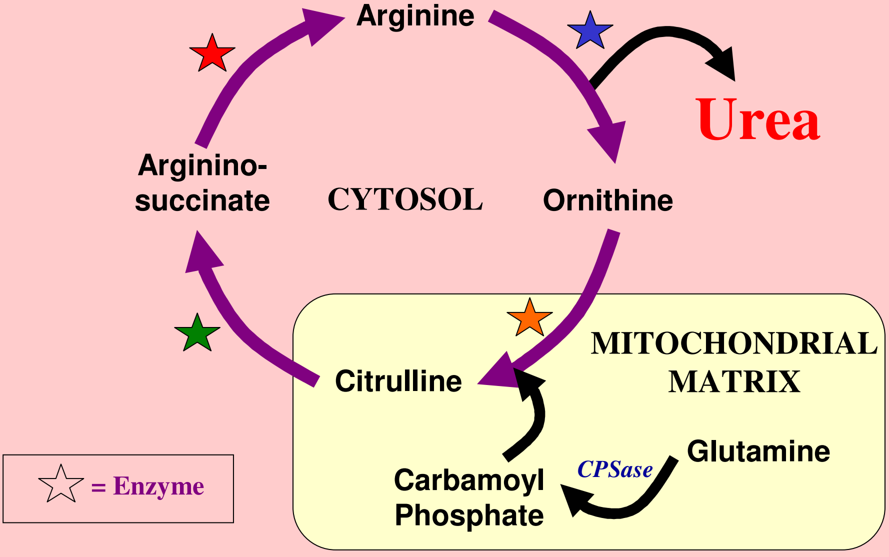
Ornithine-Urea Cycle (OUC)

- Only elasmobranchs, coelacanths and a few teleostean fish that survive periods of air exposure or alkaline environments.
- Assumed that OUC genes encoding enzymes necessary for the cycle have been lost from the genome of most teleosts.
- However, high OUC enzyme activity detected in many teleosts during embryonic stages
  - ➢ OUC genes are silenced in adult stages.

Ornithine-Urea Cycle (OUC)

- Carbamoyl phosphate synthase (CPSase) converts glutamine to carbamoyl phosphate, which is the first substrate fed into the OUC to produce urea.
- Requires energy.
- It occurs primarily in the liver.

OUC in the Liver



Arginine

Urea

Arginino-
succinate

CYTOSOL

Ornithine

MITOCHONDRIAL
MATRIX

Citrulline

Carbamoyl
Phosphate

CPSase

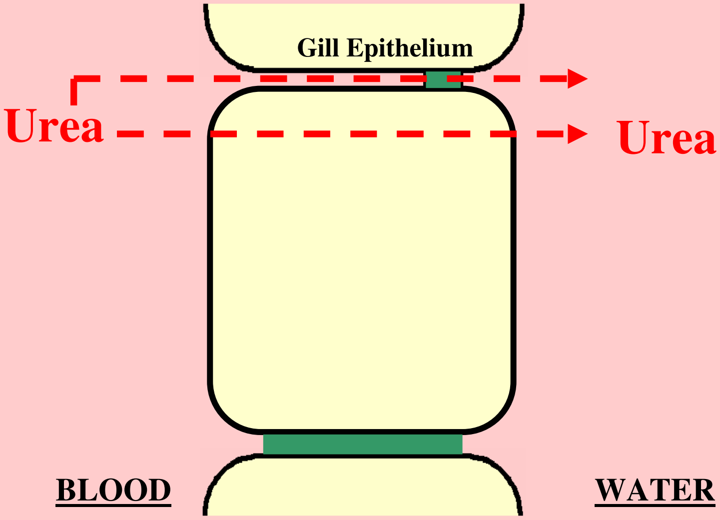
Glutamine

☆ = Enzyme

Urea Excretion and Retention at the Gill

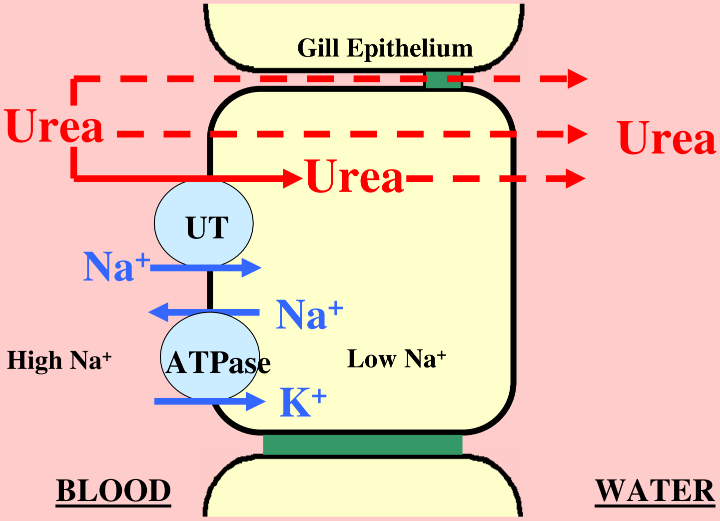
- **Like ammonia, urea excretion occurs at the gill.**
- **However, urea retention also occurs at the gill for marine fish that retain urea as an osmolyte to increase body osmolarity e.g. elasmobranchs.**

Urea Excretion in FW and SW Fish Gill



- Passive diffusion of urea into water transcellularly and paracellularly in marine fish via “leaky” junctions.

Urea Excretion in FW and SW Fish Gill



- Active transport of urea out of the gill by an Na+ dependent, secondary active urea transporter (UT).

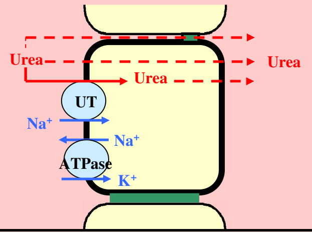
Urea Excretion in FW and SW Fish Gill

Review

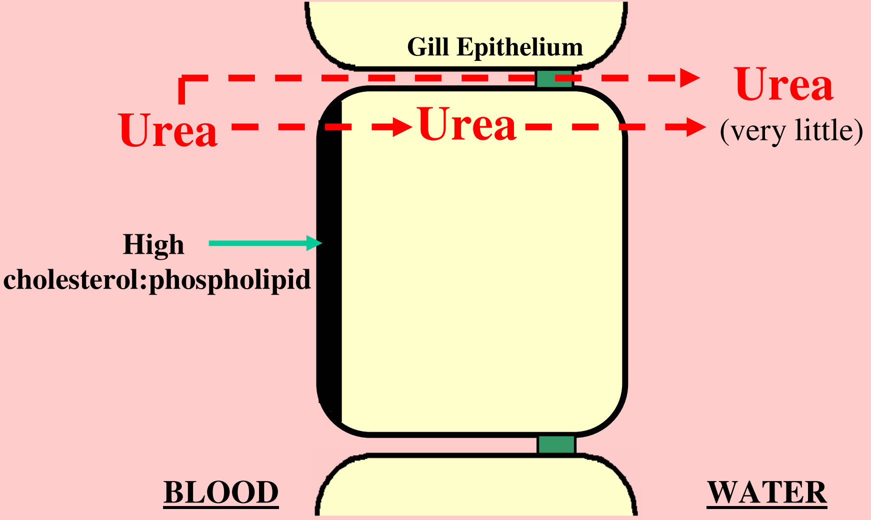
① Passive diffusion of urea into water trancellularly and paracellularly in marine fish via "leaky" junctions only.

② Basolateral Na^+-K^+-ATPases create a gradient of low Na^+ in the gill epithelium and high Na^+ in the blood.

③ Na^+ diffuses down its concentration gradient via a urea transporter (UT) taking urea with it. Urea then diffuses out of the gill.

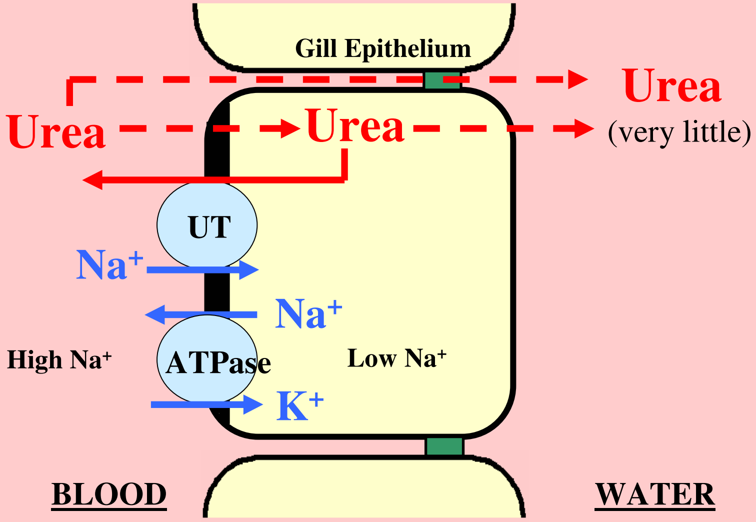


Urea Retention in Elasmobranch Gill



- Passive diffusion of urea into water transcellularly and paracellularly via "leaky" junctions.

Urea Retention in Elasmobranch Gill



- Active transport of urea back into the blood by an Na+ dependent, secondary active urea transporter (UT).

Urea Retention in Elasmobranch Gill

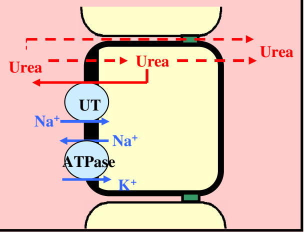
Review

① Very little passive diffusion of urea into water trancellularly due to high cholesterol:phospholipid basolateral membrane.

- Some excretion paracellularly via “leaky” junctions only.

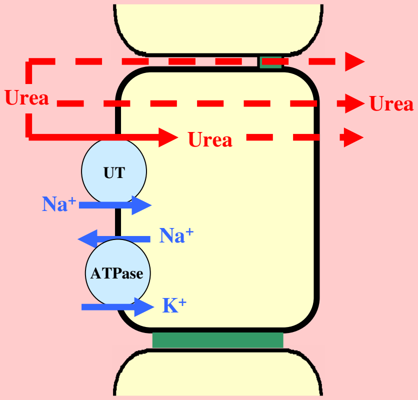
② Basolateral Na+-K+-ATPases create a gradient of low Na+ in the gill epithelium and high Na+ in the blood.

③ Na+ diffuses down its concentration gradient via a urea transporter (UT) transporting urea back into the blood



Comparison at the Gill

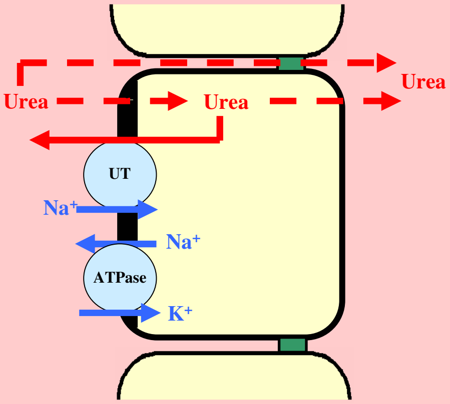
Urea Excretion



FW and SW Fish Gill

UT = Symporter

Urea Retention



Elasmobranch Gill

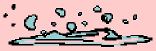
UT = Antiporter

Ureotelism vs. Ammoniotelism

1) Ammonia takes little energy to produce/excrete;
   Urea production/excretion is energy expensive.

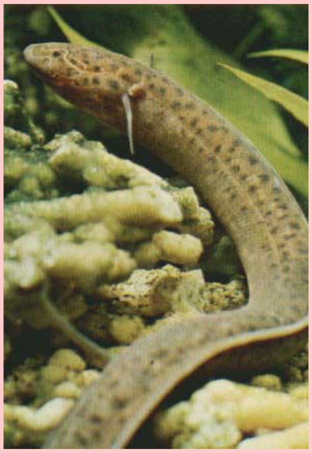
2) Ammonia requires a large volume of water for excretion since it occurs by diffusion;
   Urea requires less water for excretion
   - about 10x less water

Therefore, ureotelism is better suited for air-breathing fish, e.g. African Lungfish, which can live on land for extended periods and have limited access to water.



African Lungfish: Ammoniotelism to Ureotelism

- Can live for extended periods out of water in dried mucous cocoons, relying entirely on aerial respiration.
- In the water, it is ammoniotelic.
- On land, it shifts to ureotelism.
- Lack of water makes it impossible for the diffusion of ammonia from the gills.
- Uses OUC to convert toxic ammonia to urea for safer storage of nitrogenous wastes.
  - → Increases levels of active OUC enzymes



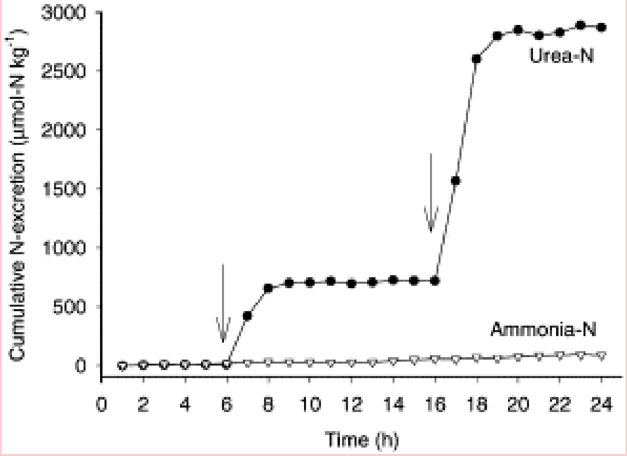
African Lungfish
(*Protopterus dolloi*)

Gulf Toadfish

- Under normal conditions → Ammoniotelic
- Under stressful conditions (e.g. crowding, confinement to a small volume of water)
  - → Intermittently ureotelic – excretes pulses of urea
- Transition is accompanied by an upregulation of active OUC enzymes in the liver.
- Stress hormone, cortisol, believed to be involved in regulating the transcription of enzymes required for the initiation of the OU cycle.

Gulf Toadfish
(*Opsanus beta*)

Gulf Toadfish



- **Intermittent pulses of urea excretion (arrows) following confinement of a Gulf Toadfish (at time = 0).**
  - **Note the negligible ammonia excretion.**

(Wood et. al., 2003)

Other Strategies to Defend Against Ammonia Toxicity on Land

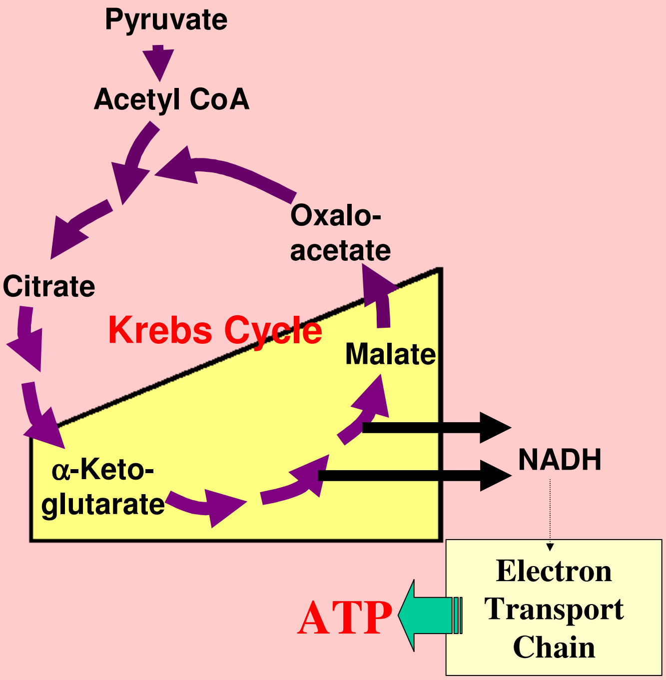
1) **Partial Amino Acid Catabolism**
   - **Giant Mudskipper**
2) **Glutamine Synthesis**
   - **Four-Eyed Sleeper**

Giant Mudskipper:
Partial Amino Acid Catabolism

- **Air-breathing ammoniotelic teleost fish that can live in mud burrows.**
- **When exposed to air it does not switch to ureotelism**
- **Uses partial amino acid catabolism to generate energy, yet reduce production of ammonia.**

- **Prerequisites: 1) Alanine Aminotransferase (ALT)**
  2) Malic Enzyme

Giant Mudskipper
(*Periophthalmodon schlosseri*)



Pyruvate

Acetyl CoA

Oxalo-acetate

Citrate

Krebs Cycle

Malate

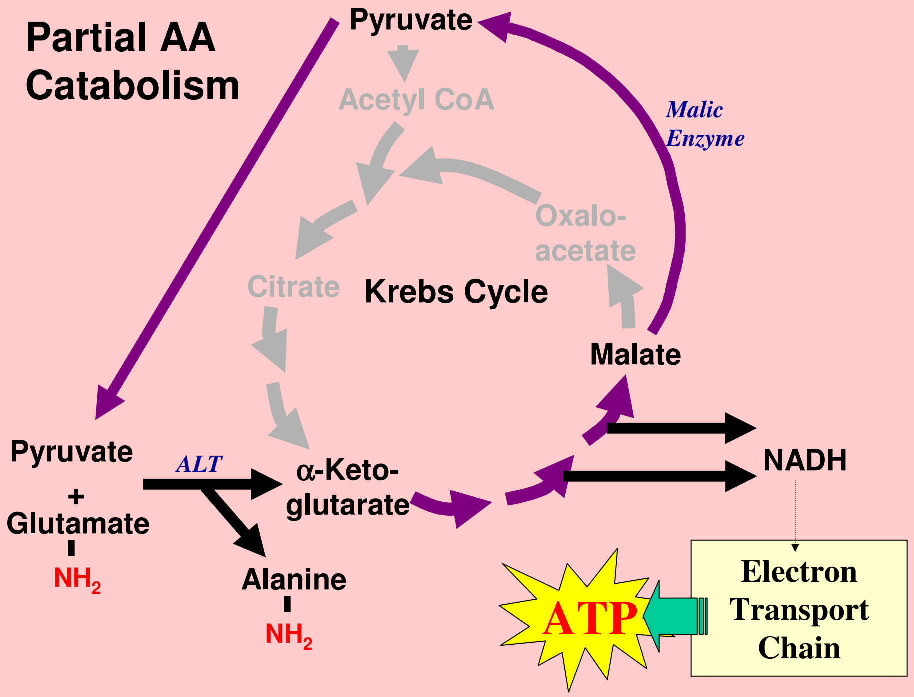
NADH

α-Keto-glutarate

Electron Transport Chain

ATP

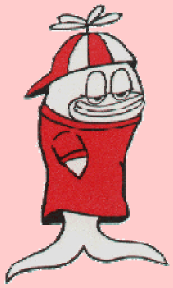
Partial AA Catabolism



Partial Amino Acid Catabolism

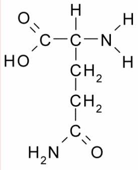
Main point:

- Allows amino acids to be used as an energy source while on land without producing toxic ammonia, which would be difficult to excrete due to a lack of external water.



Four-Eyed Sleeper: Glutamine Synthesis

- Air-breathing ammoniotelic teleost fish that can live in crevices above river mouths
- When exposed to air it does not switch to ureotelism
- Uses the enzyme glutamine synthetase to combined NH_3 with glutamate forming glutamine.
- Glutamine → safer ammonia storage than urea
- Cost-effective:
  - only 2 mol ATP per NH_3 incorporated in glutamine
  Vs. 2.5 mol ATP per NH_3 incorporated in urea



Glutamine

Four-Eyed Sleeper
(*Bostrichyths sinensis*)

The End