AMINO ACID METABOLISM

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I. THE HANDOUT

This handout is divided into several parts:

1. a short synopsis of amino acid and nitrogen metabolism (SYNOPSIS OF AMINO ACID AND NITROGEN METABOLISM);

2. a short review of protein digestion in the gut and entry of amino acids into the blood and tissues (PROTEIN DIGESTION AND AMINO ACID ABSORPTION);

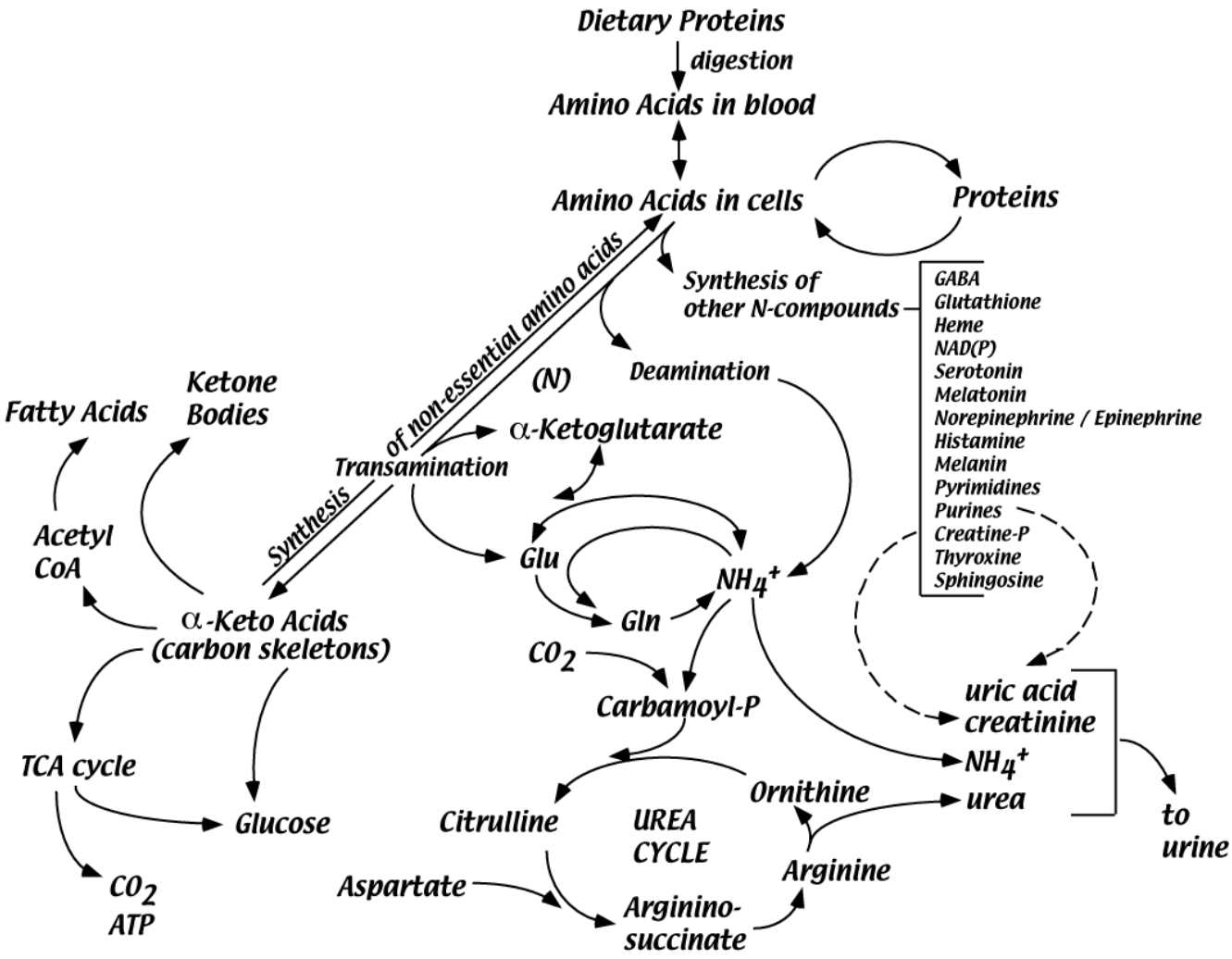
3. a description of the mechanisms the body uses to mobilize nitrogen (AMINO ACID NITROGEN);

4. a description of the mechanism the body uses to dispose of excess nitrogen (THE UREA CYCLE);

5. a description of the synthesis and degradation of selected amino acids, including examples of physiological states that influence the body's amino acid metabolism (SYNTHESIS AND DEGRADATION OF AMINO ACIDS);

6. a description of folate mediated single-carbon metabolism (TETRAHYDROFOLATE, FH_4, AND THE ONE-CARBON POOL)

INTRODUCTION TO AMINO ACID AND NITROGEN METABOLISM



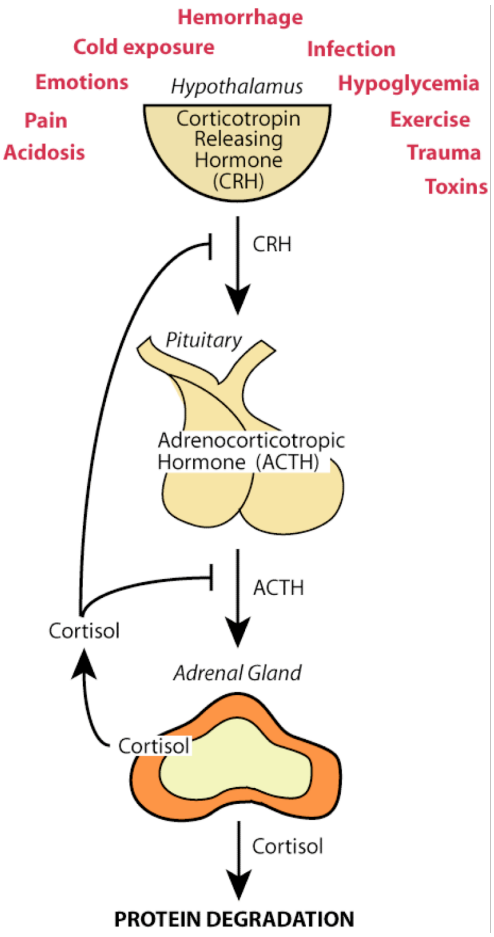
A. Dietary proteins are the primary source of the nitrogen that is metabolized by the body.
   - Average adult humans take in approximately 100 grams of dietary protein per day.
   - Amino acids are produced by digestion of dietary proteins in the intestines, absorbed through the intestinal epithelial cells, and enter the blood.
     - Proteases that digest dietary protein are produced by the stomach (pepsin), pancreas (trypsin, chymotrypsin, elastase, carboxypeptidases), and the intestine (enteropepdidase, aminopeptidases).
   - Various cells take up these amino acids, which enter the cellular amino acid pools.
   - Amino acids are used for the synthesis of proteins and other nitrogen-containing compounds, or they are oxidized for energy.

B. The body maintains a relatively large free amino acid pool in the blood (approximately 35-65 mg / 100 mL), even during fasting; tissues have continuous access to individual amino acids for the synthesis of proteins and essential amino acid derivatives, such as neurotransmitters. The amino acid pool also provides the liver with substrates for gluconeogenesis and ketogenesis. The free amino acid pool is derived from dietary amino acids and the turnover of body proteins.

C. All nitrogen-containing compounds of the body are synthesized from amino acids - cellular proteins, hormones (e.g., thyroxine, epinephrine, insulin), neurotransmitters, creatine phosphate, heme in hemoglobin and cytochromes, melanin, purine and pyrimidine bases.

D. Proteins in the body are constantly synthesized and degraded, partially draining and refilling the cellular amino acid pools.

- In a well fed human adult, approximately 300 - 600 grams of protein are degraded, and approximately 300 - 600 grams of new protein are synthesized each day.
  - Protein turnover allows shifts in the quantities of different proteins produced as physiology requires, and removes modified or damaged proteins.
- In muscle, during fasting, or stress, the synthesis/degradation equilibrium is shifted towards degradation, resulting in loss of muscle mass. The resulting amino acids can be released into the blood for conversion to glucose by the liver to supply metabolic energy for critical tissues (e.g., red blood cells and brain).
  - Insulin promotes protein synthesis by muscle, and decreased blood insulin levels, during fasting for example, result in net proteolysis and release of amino acids from muscle into the blood.
  - Cortisol, the major chronic stress hormone, is a glucocorticoid released in response to various stresses that induces the degradation of proteins — See the diagram of the "Hypothelamic-Pituitary-Adrenal Axis". The resulting amino acids can be used for several different purposes to counteract the effects of particular chronic stresses, e.g., for the synthesis of proteins in the proliferation of cells of the the immune system to respond to infection or for new cells to repair damaged tissues. Alternatively, the amino acids can be degraded to provide carbon skeletons as an energy source or for the synthesis of glucose. In acidosis, the amino and amide groups of glutamine are used by the kidney to buffer excess protons for excretion in the urine.



Chronic stresses induce the hypothalamus to release corticotropin releasing hormone, which acts on the pituitary to cause the release of adrenocorticotropic hormone, which acts on the adrenal gland to cause the secretion of cortisol, the major chronic stress hormone. Chorisol is a steriod hormone, whose action is to induce the transcription of genes that are the target of the glucacorticoid nuclear hormone receptor, some of which cause the degradation of proteins.

E. As a source of energy, amino acid carbon skeletons are directly oxidized, or, in the starved state, converted to glucose and ketone bodies, and then oxidized.

- Nitrogen must be removed before the carbon skeletons of amino acids are oxidized.
- The liver is the major site of amino acid oxidation, but most tissues can oxidize the branched chain amino acids (i.e., leucine, isoleucine, valine).
- Most of the carbons from amino acid degradation are converted to pyruvate, intermediates of the TCA cycle or acetyl CoA. During fasting these carbons are converted to glucose in the liver and kidney, or to ketone bodies in the liver. In the well fed state, they may be used for lipogenesis.

F. Amino acid nitrogen forms ammonia, which is toxic.

G. The liver is the major site of amino acid metabolism in the body and the major site of urea synthesis. The liver is also the major site of amino acid degradation, and partially oxidizes most amino acids, converting the carbon skeleton to glucose, ketone bodies, or CO_2. In liver, the urea cycle converts ammonia and the amino groups from amino acids to urea, which is non-toxic, water-soluble, and easily excreted in the urine.

- Nitrogen derived from amino acid catabolism in other tissues is transported to the liver, in large part, as alanine or glutamine, the major transporters of ammonia in the blood.

H. Certain physiological states trigger protein breakdown to generate amino acids as a source of energy. Skeletal muscle, the largest tissue contributor to the body's amino acid pool derived from protein breakdown, uses branched chain amino acids particularly well as an energy source. Nitrogen derived from these, and other amino acids, in skeletal muscle is converted mainly to alanine and glutamine, which account for approximately 50% of total α-amino nitrogen released by skeletal muscle, as a result of protein breakdown.

I. Alanine, a transamination product of its cognate α-keto acid pyruvate, can donate its amino group via transamination in the liver, and its carbon skeleton can be oxidized for energy derivation, or converted to glucose via the gluconeogenesis pathway for export to the blood and use by other tissues (the so-called "alanine / glucose" cycle).
   - Glucagon enhances alanine transport into the liver. This makes physiological sense because glucagon signals low blood glucose levels, a condition to which skeletal muscle responds by increasing protein breakdown to yield amino acid carbon skeletons as an energy source. Excess nitrogen derived from the increased amino acid pool must be disposed of, first by transport to the liver, in large part as alanine, and then converted, in the liver, to urea for excretion. Increased transport of alanine into the liver, promoted by glucagon, helps the body dispose of the excess nitrogen, and supplies the liver with carbon skeletons for glucose synthesis - the alanine / glucose cycle.

J. Glutamine released from skeletal muscle and other tissues serves several functions:
   - In kidney the nitrogen carried by glutamine is released and excreted into the urine, allowing removal, as NH4+, of protons formed during fuel oxidation, thereby helping maintain the body's pH, especially during metabolic acidosis, when other methods of buffering excess protons may become exceeded.
   - Glutamine provides a fuel source for the kidney.
   - In rapidly dividing cells (e.g., lymphocytes and macrophages), glutamine is used as a fuel, as a nitrogen donor for biosynthetic reactions, and as substrate for protein synthesis. During sepsis, for example, increased numbers of lymphocytes and macrophages are required to subdue infection. Muscle protein breakdown increases to help provide energy and amino acids for the protein synthesis needed to produce these cells.

K. The "non-essential" amino acids
   - Twelve amino acids present in proteins are synthesized in the body - eleven (serine, glycine, cysteine, alanine, aspartate, asparagine, glutamate, glutamine, proline, arginine, histidine) are produced from glucose, one (tyrosine) is produced from phenylalanine.

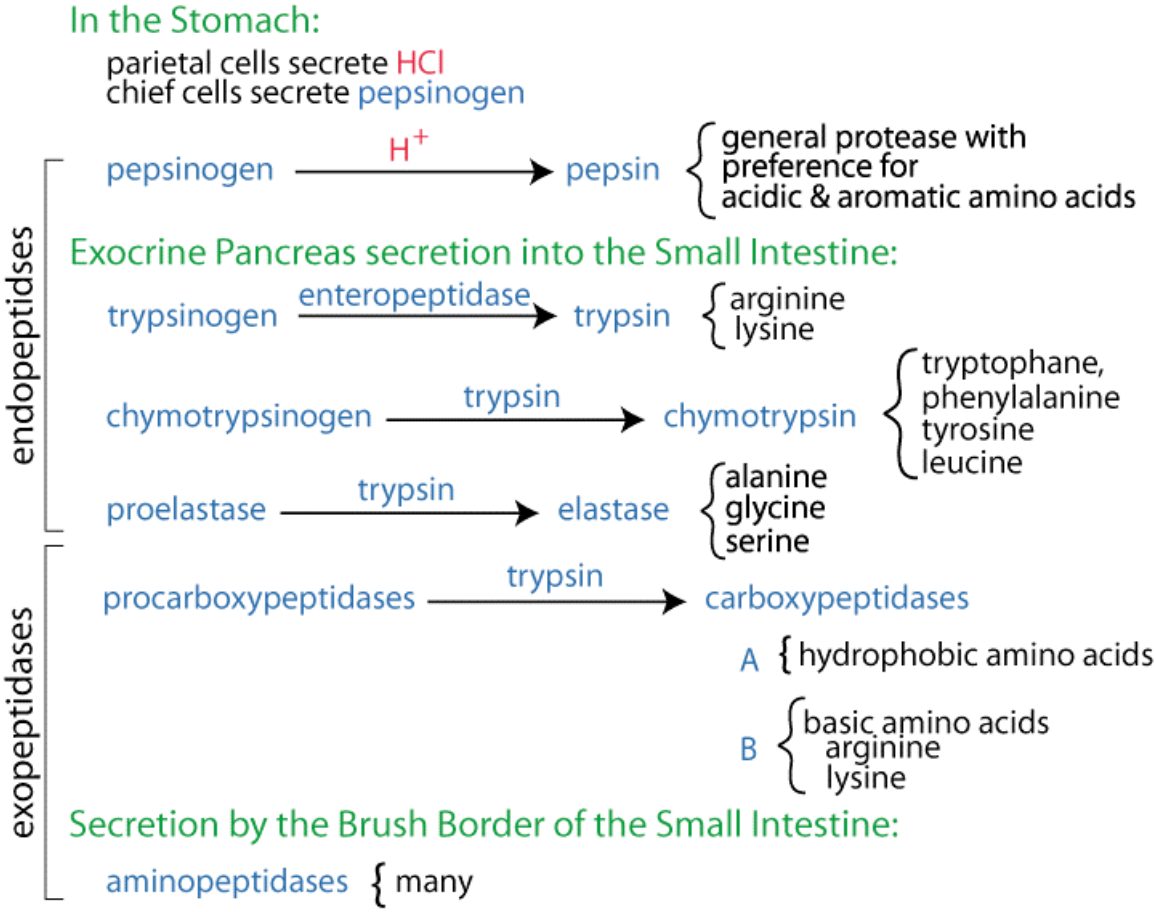
L. The "essential" amino acids
   - Ten amino acids present in proteins (arginine, histidine, isoleucine, leucine, threonine, lysine, methionine, phenylalanine, tryptophan, valine) are required in the diet of a growing human.
   - Arginine, although not required in the diets of adults, is required for growth (children and adolescents), because the amounts that can be synthesized are not sufficient to maintain normal growth rates.
   - Larger amounts of phenylalanine are required if the diet is low in tyrosine because tyrosine is synthesized from phenylalanine. Larger amounts of methionine are required if the diet is low in cysteine because the sulfur of methionine is donated for the synthesis of cysteine.

M. **Nitrogen balance** is the difference between the amount of nitrogen taken into the body (mainly as dietary protein) and the amount lost in urine, sweat, feces.
   - Proteins of the body are constantly being degraded to amino acids and resynthesized. Free amino acids can have two fates: either they are used for synthesis of proteins and other essential nitrogen-containing compounds, or they are oxidized as fuel to yield energy. When amino acids are oxidized their nitrogen atoms are excreted in the urine, principally in the form of urea.
   - Healthy adult humans are in **nitrogen balance** (sometimes referred to as zero nitrogen balance): nitrogen intake = nitrogen excreted (mainly as urea in the urine)
   - **Positive nitrogen balance**: nitrogen intake > nitrogen excreted. Positive nitrogen balance results primarily when new tissue is produced (e.g., during body growth in childhood and adolescence, during pregnancy, and during major wound healing, as after major surgery).
   - **Negative nitrogen balance**: nitrogen intake < nitrogen excreted. Negative nitrogen balance occurs when digestion of body protein exceeds synthesis, and results from several circumstances:

- too little dietary protein
- too little of one or more of the essential amino acids in the diet
  Because all 20 amino acids are required for protein synthesis to proceed, a deficit of any one amino acid reduces or prevents protein synthesis, and the use of the other amino acids for protein synthesis is reduced or abolished. The unused amino acids contributed to the cellular amino acid pools both from protein degradation and dietary input are degraded, resulting in a situation where nitrogen excretion is greater than nitrogen intake.
- Trauma, burns, and septic stress are examples of hypercatabolic states characterized by increased fuel utilization and negative nitrogen balance. In these hypercatabolic states, skeletal muscle protein synthesis decreases and protein degradation increases in an attempt to supply the body with carbon skeletons for energy derivation, or amino acids to repair body damage. The negative nitrogen balance that occurs in these hypercatabolic states results from the accelerated net protein degradation, producing amino acids that must be deaminated before their carbon skeletons can be used as an energy source. The resulting, excess nitrogen is disposed of as urea.
- If negative nitrogen balance persists for too long, body function is impaired because of the net loss of critical proteins.

• The dominant end product of nitrogen metabolism in humans is urea.
- Amino acids in excess of the quantities needed for the synthesis of protein and other nitrogen containing metabolites are neither stored nor excreted. Rather, virtually all amino acid nitrogen is excreted in the form of urea and $NH4^{+}$. On an average diet, an adult human excretes approximately 25 to 30 grams of urea per day, which represents approximately 90% of the total nitrogenous substances in the urine.

PROTEIN DIGESTION AND AMINO ACID ABSORPTION

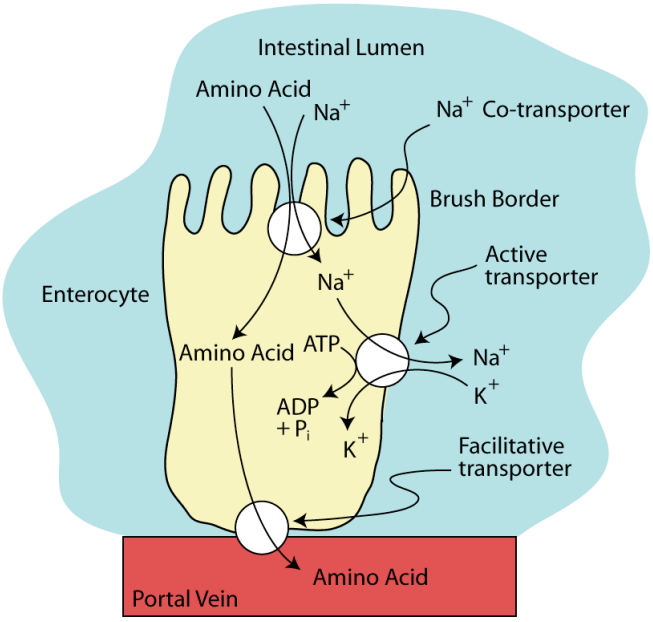


A. Proteolytic enzymes (proteases) degrade dietary proteins into their constituent amino acids in the stomach and intestine.
- Digestive proteases are synthesized as larger, inactive forms (zymogens), which, after secretion, are cleaved to produce active proteases.

B. In the stomach, pepsin begins the digestion of dietary proteins by hydrolysing them to smaller polypeptides.
- Pepsinogen is secreted by chief cells of the stomach, parietal cells secrete HCl. The acid environment alters the conformation of pepsinogen so that it can cleave itself to yield pepsin.
- Pepsin acts as an endopeptidase to cleave dietary proteins with a broad spectrum of specificity, although it prefers to cleave peptide bonds in which the carboxyl group is provided by aromatic or acidic amino acids. The products are smaller peptides and some free amino acids.

C. In the intestine, bicarbonate neutralizes stomach acid, and the pancreas secretes several inactive proenzymes (zymogens), which, when activated, collectively digest peptides to single amino acids.
- Enteropeptidase, secreted by the brush border cells of the small intestine cleaves trypsinogen to yield the active serine protease trypsin.
- Trypsin cleaves inactive chymotrypsinogen to yield active chymotrypsin, inactive proelastase, to yield active elastase, and inactive procarboxypeptidases to yield active carboxypeptidases. Thus, trypsin plays a central role because it cleaves dietary proteins and activates other proteases that cleave dietary protein.
- Each protease exhibits cleavage specificity: trypsin cleaves at the carboxy side of arg and lys; chymotrypsin cleaves at the carboxy side of phe, tyr, trp and leu; elastase cleaves at the carboxy side of ala, gly and ser. Carboxypeptidase A cleaves single amino acids from the carboxyl terminus, with a specificity for hydrophobic and branched side chain amino acids; carboxypeptidase B cleaves single amino acids from the carboxyl terminus, with a specificity for basic (arg and lys) amino acids.
- Aminopeptidases, located on the brush border, cleave one amino acid at a time from the amino end of peptides.
- Intracellular peptidases cleave small peptides absorbed by cells.

D. Amino acids are absorbed by intestinal epithelial cells and released into the blood.
- The sodium-amino acid carrier system involves the uptake by the cell of a sodium ion and an amino acid by the same carrier protein (cotransporter) on the luminal surface of the intestine. There are at least seven different carrier proteins that transport different groups of amino acids. The sodium ion is pumped from the cell on the serosal side (across the basolateral membrane) by the Na^+ - K^+ ATPase in exchange for K^+, providing the driving force for transport of amino acids into the intestinal epithelial cells. The amino acid travels down its concentration gradient into the portal blood, crossing the basal epithelial membrane via a facilitated transporter. Genetic defects in genes encoding the carrier proteins can result in abnormal amino acid uptake from the intestines, leading to amino acid deficiency (e.g. Hartnup disease, in which neutral amino acids are neither transported normally across the intestinal epithelium nor reabsorbed normally from the kidney glomerular filtrate, leading to hyperaminoacidurea; hypercystinurea, high urine cysteine, occurs with a frequency of approximately 1 per 7000 liver births worldwide and may cause renal caliculi - kidney stones).



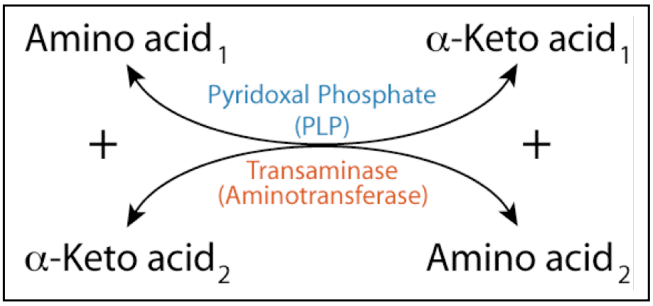
E. Amino acids enter cells from the blood principally by Na^+-dependent cotransporters and, to a lesser extent, by facilitated transporters. The Na^+-dependent transport in liver, muscle, and other tissues allows these

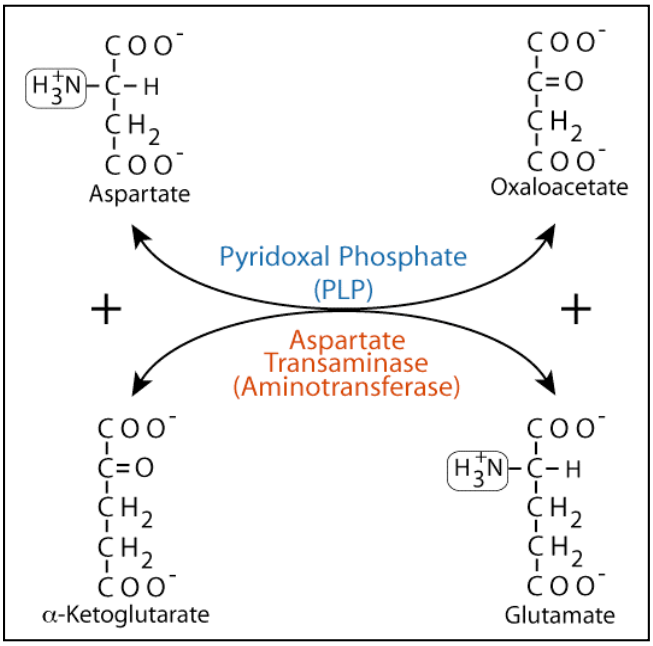
cells to concentrate amino acids from blood. These transport proteins are encoded by different genes, and have different specificities, than those encoded by the genes specifying the luminal membrane amino acid transporters of the intestinal epithelia. They also differ somewhat between tissues (e.g., the transport system for glutamine uptake present in liver is either not present in other tissues or is present as an isoform with different properties).

AMINO ACID NITROGEN

After a meal that contains protein, amino acids released by digestion pass from the gut through the hepatic portal vein to the liver. In a normal diet containing 60 - 100 grams of protein, most of the amino acids are used for the synthesis of proteins in the liver and in other tissues. Carbon skeletons of excess amino acids may be oxidized for energy, converted to fatty acids, or, in some physiological situations, converted to glucose. During fasting, muscle protein is cleaved to amino acids, some of which are partially oxidized to produce energy. Portions of these amino acids are converted to alanine and glutamine, which, along with other amino acids are released into the blood. Glutamine is oxidized by various tissues, including the gut and kidney, which convert some of its carbons and nitrogen to alanine. Alanine and other amino acids travel to the liver, where the carbons are converted to glucose and ketone bodies and the nitrogen is converted to urea, which is excreted by the kidneys. Several enzymes are important in the process of interconverting amino acids and in removing nitrogen so that the carbon skeletons can be utilized. These include transaminases, glutamate dehydrogenase and deaminases. Because reactions catalyzed by transaminases and glutamate dehydrogenase are reversible, they can supply amino groups for the synthesis of non-essential amino acids.

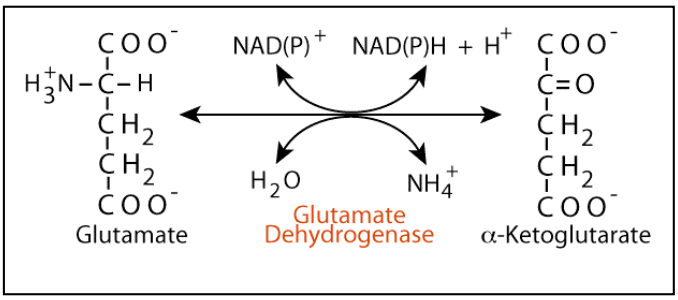
A. Transamination is the major process for removing nitrogen from amino acids.
   - transfer of an amino group from one amino acid (which is converted to its corresponding α-keto acid) to another α-keto acid (which is converted to its corresponding α-amino acid) by **Transaminase (aminotransferase)**. The nitrogen from one amino acid thus appears in another amino acid.
   - α-ketoglutarate and glutamate comprise one α-keto acid / α-amino acid pair.
   - EXAMPLE: the amino acid aspartate can be transaminated to form its corresponding α-keto acid oxaloacetate. The amino group of aspartate is transferred to α-ketoglutarate by the enzyme aspartate transaminase (aminotransferase).
   - All amino acids except lysine and threonine can undergo transamination reactions.
   - Different transaminases recognize different amino acids, but they all use α-ketoglutarate and glutamate as one α-keto acid/α-amino acid pair. α-ketoglutarate and glutamate, therefore play a pivotal role in amino acid nitrogen metabolism.
   - Pyridoxal phosphate (PLP), a derivative of vitamin B_6 is a required cofactor.
   - Because transamination reactions are reversible they can be used to remove nitrogen from amino acids or to transfer nitrogen to α-keto acids to form amino acids. They participate both in amino acid degradation and in amino acid synthesis.





B. **Glutamate dehydrogenase** catalyzes the oxidative deamination of glutamate.

- NH_4^+ released, α-ketoglutarate formed
- NAD^+ or $NADP^+$ required
- reversible
- in mitochondria of most cells

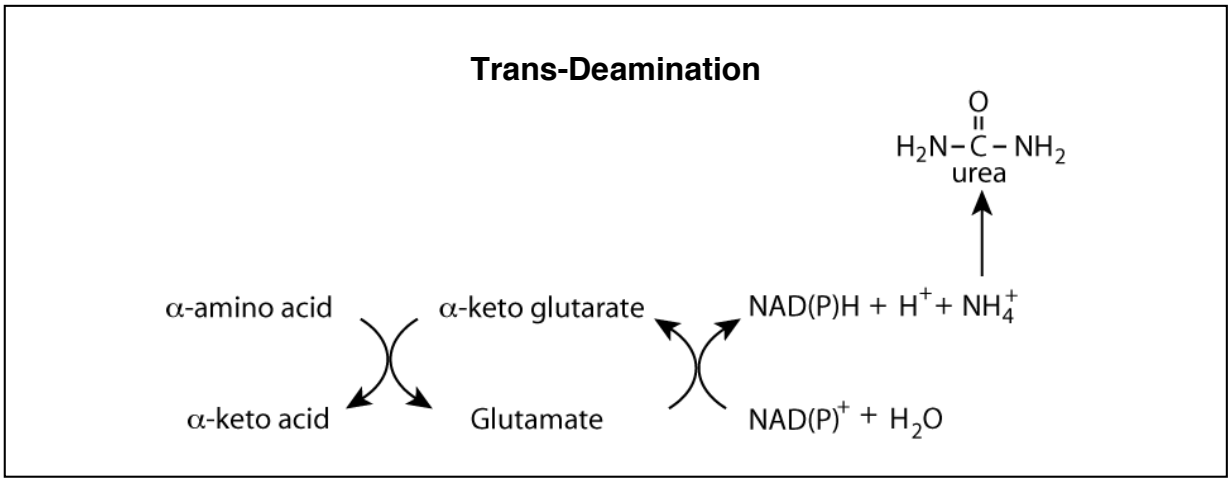


C. A number of other amino acids release their nitrogen as NH_4^+

- Deamination by dehydration
  - serine (enzyme = serine dehydratase; yields pyruvate + NH_4^+)
  - threonine (enzyme = threonine dehydratase; yields α-keto butyrate + NH_4^+)
- Direct deamination
  - histidine (enzyme = histidase; yields urocanate + NH_4^+)
- Hydrolytic deamination (uses water)
  - asparagine (enzyme = asparaginase; yields aspartate + NH_4^+)
  - glutamine (enzyme = glutaminase; yields glutamate + NH_4^+)
  - $NH_4^+ \leftrightarrows NH_3 + H^+$ At physiological pH NH_4^+ / NH_3 = 100. However it is important to note that NH_3 can cross cell membranes, allowing, for example, NH_3 to pass into the urine from kidney tubule cells to decrease the acidity of the urine by binding protons to form ammonium ions (NH_4^+). This is an important mechanism for maintaining normal pH, allowing excess proton excretion by providing a proton buffer; particularly important during acidosis. The kidney uses glutamine, in particular, as a source of NH_3 to buffer excess protons.
- Methionine degradation yields free ammonium ion (see below)

D. Summing up: **The pivotal role of glutamate**

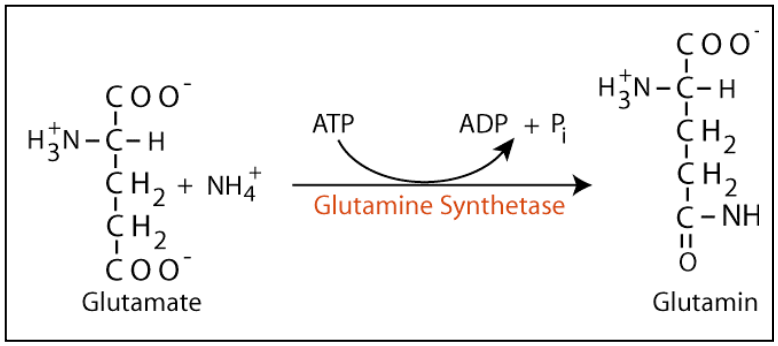
- Removing nitrogen from amino acids
  - Glutamate can collect nitrogen from other amino acids as a consequence of transamination reactions.
  - Glutamate nitrogen may be released as NH_4^+ via the glutamate dehydrogenase reaction.



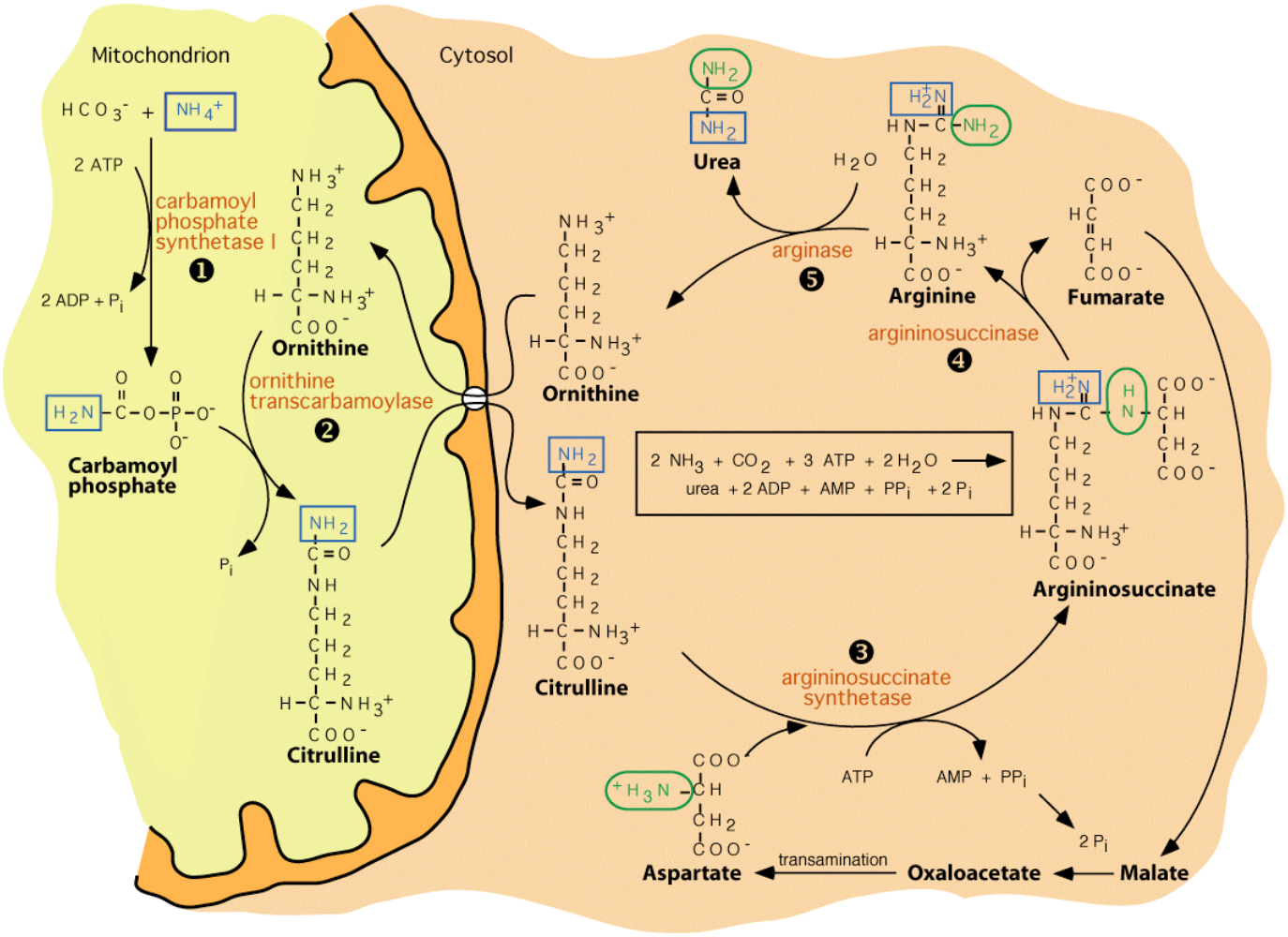
  - NH_4^+ and aspartate (which may be produced by transamination of oxaloacetate, with glutamate as the amino group donor) provide nitrogen for urea synthesis by the urea cycle (see below) for elimination of nitrogen from the body in the urine.
- Providing nitrogen for amino acid synthesis
  - NH_4^+ + α-ketoglutarate $\leftrightarrows$ glutamate (enzyme = glutamate dehydrogenase)
  - glutamate may transfer nitrogen by transamination reactions to α-keto acids to form the corresponding amino acids; the mechanism by which non-essential amino acids obtain their amino group

THE UREA CYCLE

Normally the adult human is in nitrogen balance. The amount of nitrogen ingested each day, mainly in the form of dietary protein, is equal to the amount of nitrogen excreted. The major nitrogenous excretory product is urea, which is produced in the liver, and exits the body in the urine. Ammonia, produced from the α-amino group of amino acids is toxic, particularly to neural tissue, and must, therefore, be transported to the liver for conversion to urea, a non-toxic compound. Alanine and glutamine are the major transporters of nitrogen in the blood. Alanine is produced in a single biochemical step by the transamination of pyruvate, glutamine is produced from glutamate by the addition of nitrogen to the carboxyl group at the γ position by an ATP-dependent reaction catalyzed by **Glutamine Synthetase**. NH_4^+ and aspartate, the forms in which nitrogen enters the urea cycle, are produced from amino acids in the liver by a series of transamination and deamination reactions. Glutamate dehydrogenase is a key enzyme in the process because it generates the free $NH4^+$ previously transferred to α-ketoglutarate from many amino acids by transaminases. As dietary protein increases (a protein-rich diet) the concentration of the enzymes of the urea cycle increase, suggesting a regulated response to meet the increased need for nitrogen disposal.



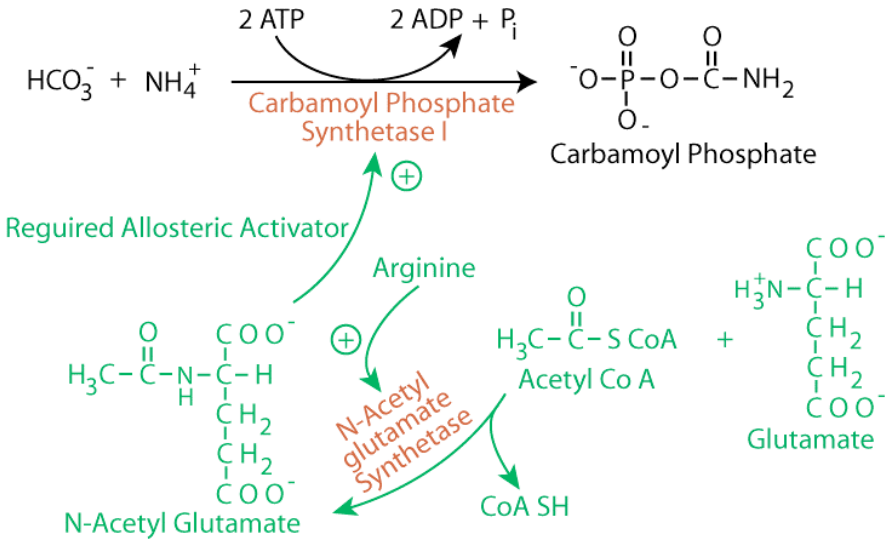
Reactions of the Urea Cycle



Two nitrogen atoms enter the urea cycle as NH_4^+ and aspartate. The first steps of the cycle take place in liver mitochondria, where NH_4^+ combines with HCO_3^- to form carbamoyl phosphate. Carbamoyl phosphate reacts with ornithine, a compound both required as input to, and regenerated by the cycle, to produce citrulline, which, exits the mitochondria to the cytosol, where the remaining reactions of the cycle occur. The amino acid arginine is synthesized as a product of the urea cycle. Fumarate, another product, links the urea cycle with the TCA cycle. The two entering nitrogen atoms exit the cycle as urea, which the liver releases into the blood for disposal, in urine, by the kidneys.

❶ Synthesis of carbamoyl phosphate by **Carbamoyl Phosphate Synthetase I**

- in mitochondria of the liver
- NH_4^+, CO_2 (as bicarbonate) and 2 ATP react to form carbamoyl phosphate.
- 2 ATP molecules provide the energy to create the phosphoanhydride and N-C bonds of carbamoyl phosphate; inorganic phosphate and 2 ADP produced.
- stimulated by N-acetyl-glutamate (a required allosteric activator), which is synthesized from acetyl CoA and glutamate; the synthesis of N-acetyl-glutamate is stimulated by arginine, the immediate precursor of urea in the urea cycle. Increased levels of amino acids, signalled by increased arginine levels, therefore, stimulate urea production by the urea cycle.
- NOTE: Carbamoyl phosphate synthetase I is present in liver mitochondria and uses NH_4^+ as a source of nitrogen; carbamoyl phosphate synthetase II is present in the cytosol of many cells, uses glutamine as a source of nitrogen, and produces carbamoyl phosphate for pyrimidine biosynthesis.



❷ Synthesis of citrulline from carbamoyl phosphate and ornithine by **Ornithine Transcarbamoylase**

- X-linked gene
- in mitochondria; ornithine transported into mitochondria
- carbamoyl phosphate is the carbamoyl donor which has a high transfer potential because of its phosphoanhydride bond
- inorganic phosphate released
- citrulline produced, which is transported from the mitochondria to the cytosol where the remaining reactions of the urea cycle occur

❸ Synthesis of argininosuccinate by condensation of citrulline and aspartate by **Argininosuccinate Synthetase**

- driven by the cleavage of ATP; AMP and inorganic pyrophosphate produced; inorganic pyrophosphate cleaved by cellular pyrophosphatases to inorganic phosphate

❹ Argininosuccinate cleaved by **Argininosuccinase** to produce fumarate and arginine

- NOTE: The carbon skeleton of aspartate is conserved as fumarate, with transfer of the aspartate amino group to arginine. Recall that fumarate is a TCA cycle intermediate, and can be hydrated to form malate. In the fed state malate may be converted by malic enzyme to pyruvate, which serves as a source for the synthesis of fatty acids. It may also be oxidized to oxaloacetate. Oxaloacetate can have several fates. It can be transaminated to aspartate (aspartate transaminase), combine with acetyl CoA to enter the TCA cycle or, in the starved state, be converted to phosphoenolpyruvate for gluconeogenesis.

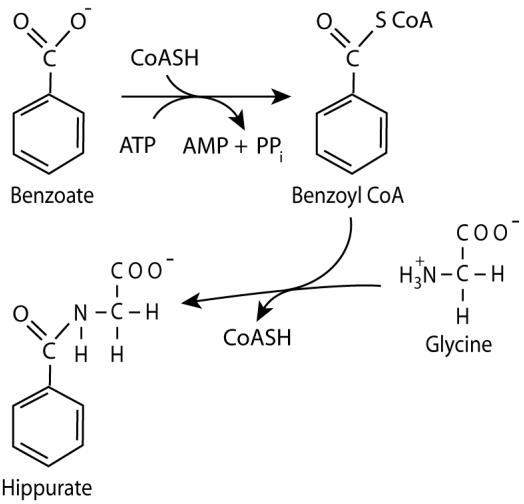
❺ Urea production and the regeneration of ornithine from arginine by **Arginase**

- urea passes into the blood and is eliminated by the kidneys
- urea accounts for approx. 90% of all bodily nitrogenous excretory products.
- ornithine is synthesized from glucose; arginine is synthesized from ornithine by the urea cycle

Generally, substrate availability regulates the rate of the urea cycle; the higher the rate of ammonia production, the higher the rate of urea formation. N-acetyl-glutamate is an allosteric activator of carbamoyl phosphate synthetase I, and its synthesis is stimulated by arginine. During conditions of increased protein metabolism following ingestion of a high protein diet, or during fasting, when muscle protein is degraded to supply carbon skeletons for glucose production (gluconeogenesis), the urea cycle operates at an increased rate to eliminate excess nitrogen as urea. As fasting progresses, ketone body synthesis increases, diminishing the need for muscle protein breakdown to supply amino acids as a source of carbon skeletons for gluconeogenesis. This, in turn, decreases the need for increased nitrogen excretion as urea, and the urea cycle slows.

Deficiencies of the Urea Cycle

Deficiencies of the urea cycle are a threat to health because of the accumulation of ammonia, which is a neurotoxin. Normally free ammonia is fixed into either α-keto glutatate by glutamate dehydrogenase or glutamine by glutamne synthetase. The glutamine can be used by a variety of tissues to donate its amide nitrogen for the synthesis of nitrogen-containing compounds. The resulting glutamate donates its amino group, by transamination, primarily to pyruvate to form alanine, which carries the nitrogen to the liver. In the liver the nitrogen is removed from its carriers and fixed to carbamoyl phosphate by carbamoyl phosphate synthetase I, the first enzyme of the urea cycle.



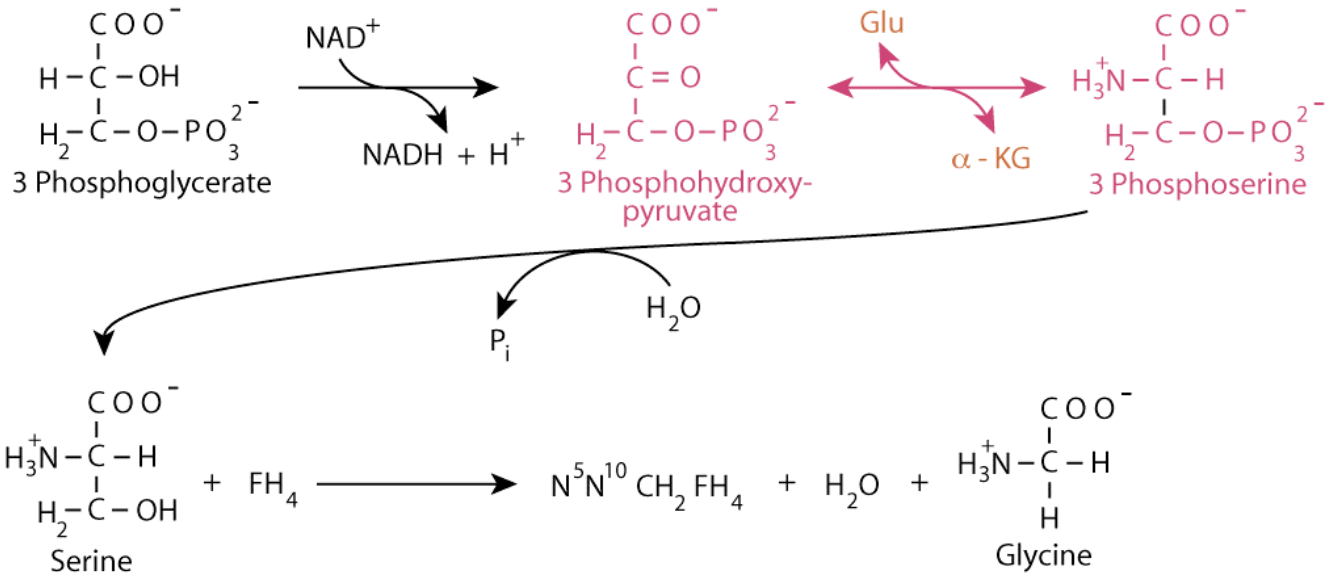
A urea cycle deficiency causes glutamine levels to increase, and because α-ketoglutarate is not regenerated by the removal of nitrogen from glutamine, the α-ketoglutarate level becomes too low to fix more free ammonia, which accumulates in the circulation.

The major clinical problem in treating patients with urea cycle deficiencies is to reduce the effects of excess ammonia on the nervous system, because high levels of ammonia are toxic to neurons, and cause irreversible neuronal damage.

The extent to which the elevation occurs depends on which enzyme of the urea cycle is deficient, and the key to treating a urea cycle deficiency is to identify the deficient enzyme.

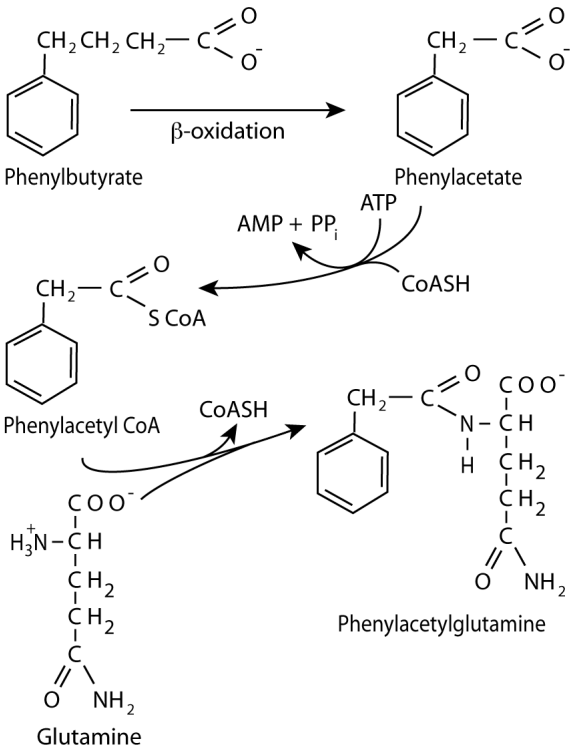
The most common urea cycle deficiency is in ornithine transcarbamoylase (OTC), which is an X-linked disorder. It occurs with a frequency of 1/20,00 - 1/80,000 live births. The variation occurs because there is a late-onset form of OTC deficiency that may be underrepresented in the data used to determine the frequency of the deficiency in the population. Whatever the cause, a diet low in protein is essential to reduce the potential for excessive amino acid degradation with its associated generation of ammonia (ammonium ion).

If the deficiency occurs before the synthesis of argininosuccinate, drugs that form conjugcates with amino acids can be used for treatment. Benzoate (given as benzoic acid), after activation to benzoyl CoA, reacts with glycine to form hippurate, which is excreted. As a result, glycine is depleted, causing the body to synthesize more from 3 phosphoglycerate. In doing so it uses glutamate as a nitrogen donor in a transamination reaction, yielding α-ketoglutarate, which can then accept another nitrogen and continue in the synthesis of another molecule of glycine, which is conjugated to another molecule of benzoyl CoA for excretion as hippurate ...



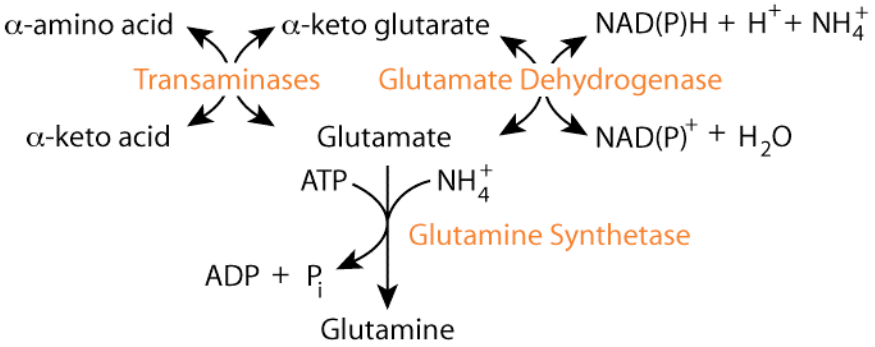
As glycine converted to hippurate, which is excreted, the level of glycine in the body decreases. As a result, more glycine, a non-essential amino acid, is synthesized from 3 phosphoglycerate, requiring input of nitrogen as ammonia. [FH_4= tetrahydrofolate]

Glutamate donates the ammonia to 3 Phosphohydroxypyruvate via a transamination reaction, yielding 3 Phosphoserine and α-ketoglutarate. The α -ketoglutarate can then react, via either glutamate dehydrogenase or another transamination reaction to acquire another ammonia group, which it, in turn, can donate to another molecule of 3 Phosphohydroxypyruvate for the synthesis of another molecule of glycine, which can be eliminated from the body as hippurate.



In addition, or alternatively, phenylbutyrate, can be given. It is converted to phenylacetate, the active compound, — phenylacetate has a bad odor, making it difficult to take orally — which conjugates with glutamine to form phenylacetylglutamine, which is excreted. Each molecule of phenylacetylglutamine excreted removes two nitrogens. As glutamine is depleted, the body synthesis more from glucose, first by synthesizing α-ketoglytarate and then converting it to glutamate by either transamination or the glutamate dehydrogenase reaction, and subsequently adding another nitrogen to the glutamate with glutamine synthetase, thereby using two nitrogens.

If the deficiency occurs after the synthesis of argininosuccinate large amounts of arginine may be beneficial. Once argininosuccinate has been synthesized, the two nitrogens destined for excreation have been incorporated in the substrate and the problem is that ornithine is not regenerated, causing it to be limiting. Ingesting large quantities of arginine leads to ornithine production by the arginase reaction and nitrogen excretion via argininosuccinate is enhanced.



α-ketoglutarate can be converted to glutamate either by transamination, or by glutamate dehydrogenase. The glutamate dehydrogenase reaction fixes free ammonia (ammonium ion) and transamination reactions transfer ammonia from an amino acid. The resulting glutmate can donate its nitrogen to another α-keto acid by transamination, as in the formation of glycine.

In addition, glutamate can be converted to glutamine by glutamine synthetase, thereby using another free ammonia (ammonium ion).

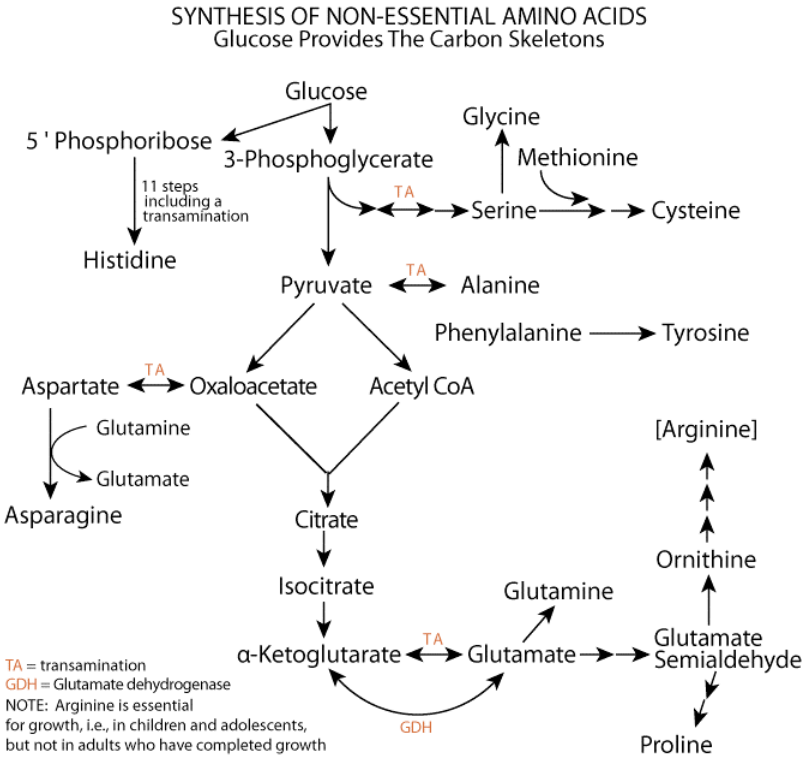
The severe hyperammonema resulting from other urea cycle deficiencies rarely occurs in patients with arginase deficiency for at least two identifiable reasons: arginine can be released from the hepatocyte and excreted in urine as a second inducible type II isozyme occurs in peripheral tissues, which can hydrolyse the arginine released by the hepatocyte to produce urea and ornithine. The ornithine returns to the liver for use in the urea cycle, while the urea is excreted.

Because only a single tissue is involved, the liver, deficiencies in the urea cycle are good candidates for treatment by gene therapy since only one cell type, the hepatocyte, must be targeted by the vector that carries the replacement gene. Gene therapy experiments were carried out on individuals with ornithine transcarbamoylase deficiency, but were halted because one of the patients died of a severe immunologic reaction to the vector used to deliver the gene.

SYNTHESIS AND DEGRADATION OF AMINO ACIDS

A. The liver is the only tissue which has all the pathways of amino acid synthesis and degradation. During fasting, the carbon skeletons of amino acids produce glucose, ketone bodies, and CO_2; in the fed state the liver can convert intermediates of amino acid metabolism to triacylglycerols; the fate of amino acid carbon skeletons, thus, parallels that of glucose and fatty acids.

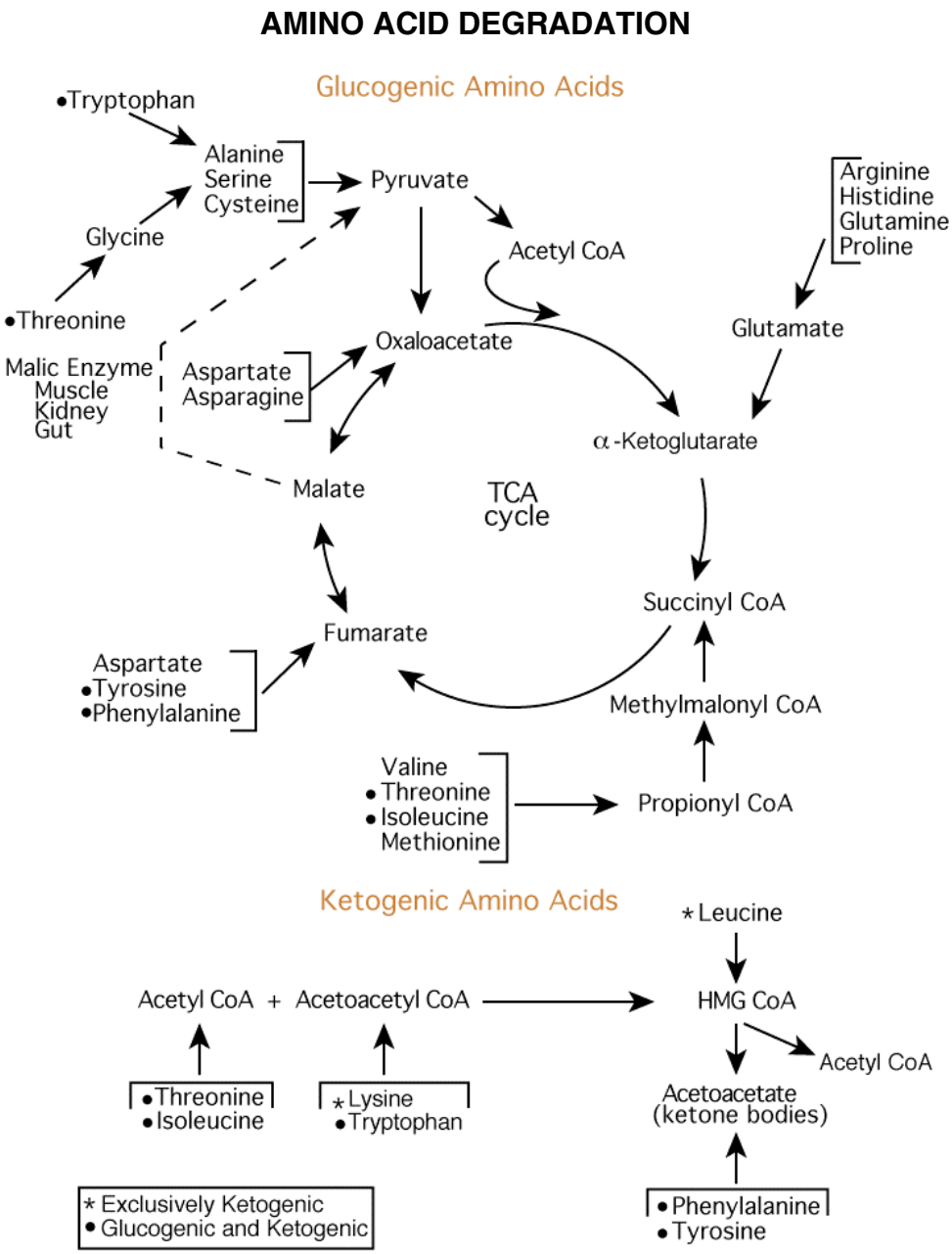
B. Synthesis of the twelve non-essential amino acids
   - Carbon skeletons of eleven of the twelve non-essential amino acids (adult humans) are produced from intermediates of glycolysis and the TCA cycle; four (serine, cysteine, glycine, alanine) from glycolytic intermediates, five (aspartate, asparagine, glutamic acid, glutamine, proline) from TCA cycle intermediates. Histidine is derived from glucose via the pentose phosphate pathway. Arginine is produced from ornithine by the urea cycle. Tyrosine, the twelfth non-essential amino acid, is derived from the essential amino acid phenylalanine.



- Nitrogen is supplied as ammonia via transamination, using glutamic acid as the ammonia donor or, in the case of glutamic acid synthesis, by the reaction catalyzed by glutamate dehydrogenase.

C. Amino acid degradation

- Most amino acids are deaminated as described above (see AMINO ACID NITROGEN) to produce α-keto acids. In the fed state these α-keto acids can be used to synthesize triacylglycerols. In the fasted state they produce glucose, ketone bodies and CO_2.
- In the fasted state, amino acids become a major source of energy. Muscle protein degradation supplies these amino acids, which the liver uses to synthesize the glucose and ketone bodies required to sustain life.
- Amino acids are considered to be glucogenic if their carbon skeletons can be converted, in net amounts, to glucose, and ketogenic if their carbon skeletons are converted directly to acetyl CoA or acetoacetate. Some amino acids are both glucogenic and ketogenic.
  - 13 amino acids are exclusively glucogenic (alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, methionine, proline, serine, valine)
  - Two amino acids, leucine and lysine, are exclusively ketogenic
  - Isoleucine, threonine and the aromatic amino acids (phenylalanine, tryptophan, tyrosine) are both glucogenic and ketogenic.



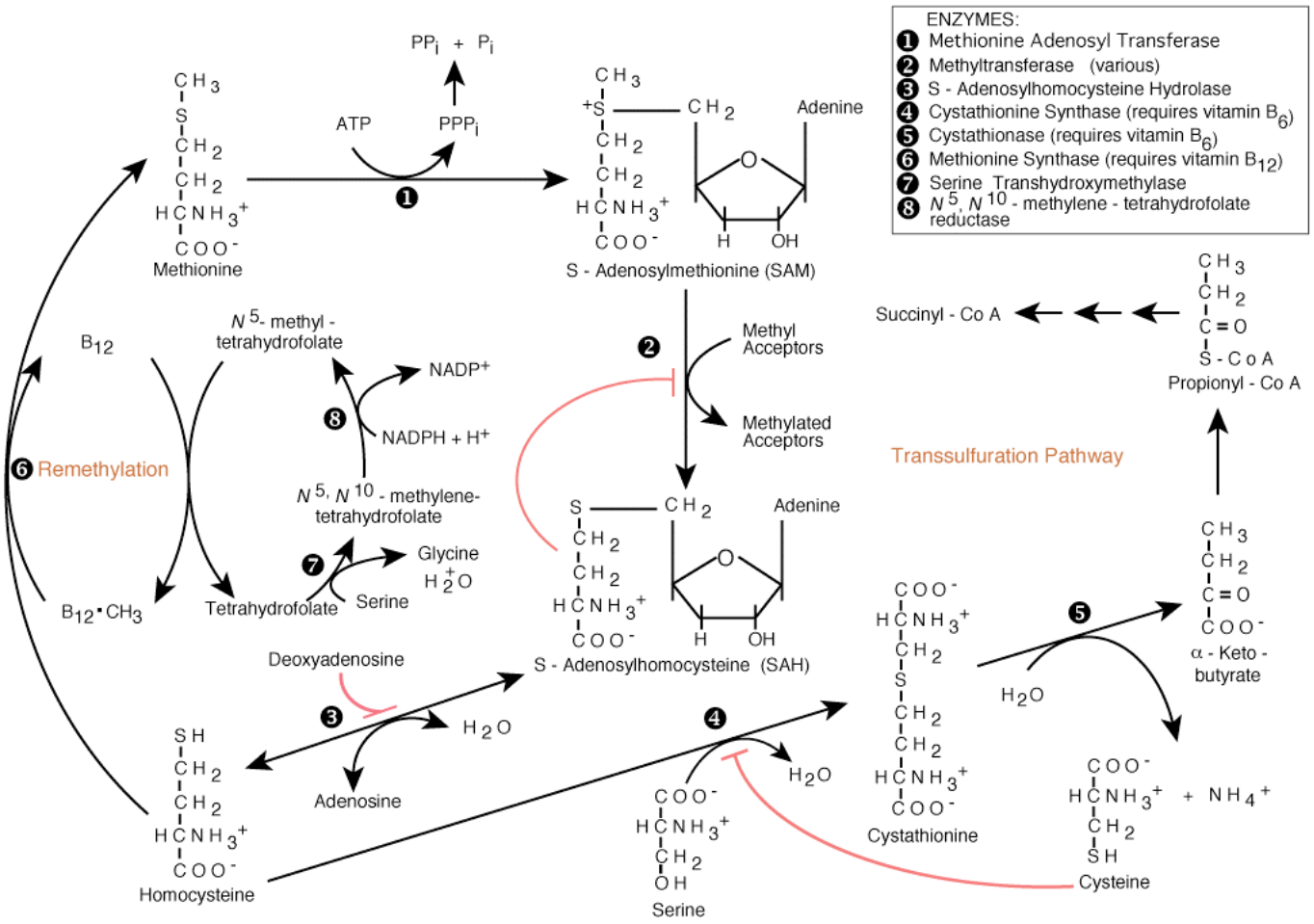
D. Methionine degradation

Methionine is an important source of methyl groups and of the sulfur atom for the synthesis of the non essential amino acid cysteine.

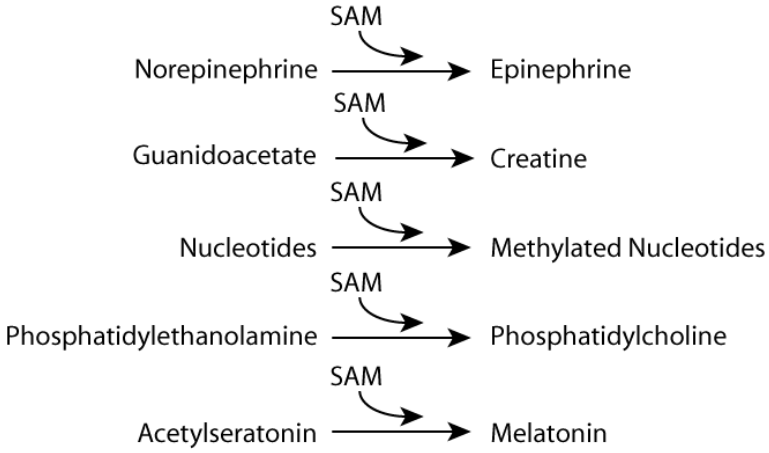
❶ conversion of methionine and ATP to S-adenosylmethionine (SAM) by **Methionine Adenosyl Transferase**

- PPPi formed, which is converted to PPi and Pi; PPi converted to Pi by strong cellular pyrophosphatase

Methionine Degradation



Some Specific Reactions Requiring SAM



❷ SAM is the CH_3 donor for the biosynthesis of many important biological molecules; **Methyltransferases** transfer the methyl group from SAM to various acceptor molecules producing methylated acceptors and S-adenosylhomocysteine (SAH).

❸ SAH conversion to homocysteine by **S-Adenylhomocysteine Hydrolase**
- Adenosine released
- inhibited by deoxyadenosine

❹ Condensation of homocysteine with serine to form cystathionine by **Cystathionine Synthase**
- requires vitamin B_6

❺ Hydrolysis of cystathionine to yield cysteine and α- ketobutarate by **Cystathionase**
- the cysteine produced derives its carbon skeleton from the serine utilized in the previous reaction (cystathionine synthase), and its sulfur from methionine
- NH_4^+ released
- α-ketobutyrate converted to propionyl CoA, which can be converted, in three steps, to succinyl CoA (recall that β-oxidation of odd-chain fatty acids yields propionyl CoA, which can be converted to succinyl CoA via methylmalonyl CoA by a vitamin B_{12} requiring pathway - refer to your notes from Lipid Metabolism for the conversion pathway)

E. Regeneration of methionine

❻ from homocysteine by **Methionine Synthase**
- requires methylated vitamin B_{12} as the CH_3 donor
- *N* 5 - methyltetrahydrofolate supplies CH_3 to maintain vitamin B_{12} in the methylated state (folate and single carbon metabolism will be discussed below).
- *N* 5 - methyltetrahydrofolate production from *N* 5, *N* 10-methylenetetrahydrofolate (❽), with serine as the major supplier of a methyl group (❼), will be discussed in greater detail in the section of Tetrahydrofolate (below).

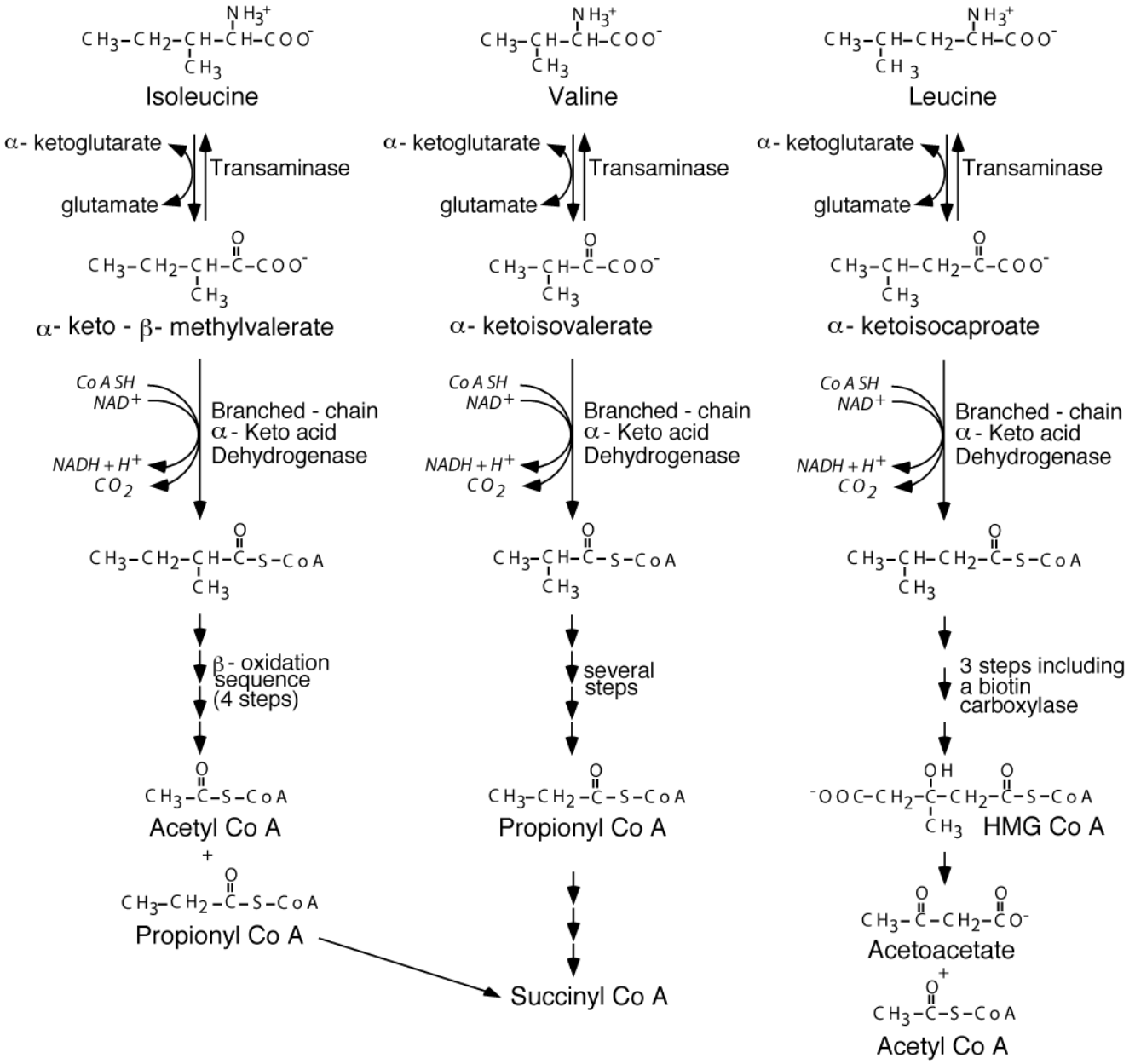
NOTE: Hyperhomocysteinemia (increased levels of homocysteine) has been shown to be a risk factor for cardiovascular disease. Men with plasma homocysteine concentrations 12 % above the upper limit of normal were determined to have approximately a threefold increase in the risk of myocardial infarction, as compared with those with lower levels. Treatment of hyperhomocysteinemia varies with the underlying cause. However, vitamin supplementation (folic acid, vitamin B_6 and vitamin B_{12}) is generally effective in reducing homocysteine concentrations. In most patients,1 to 5 mg./day of folate rapidly decreases homocysteine concentrations. Folic acid alone, folic acid combined with vitamins B_{12} and B_6, and vitamins B_6 and B_{12} have all been shown to reduce homocysteine concentrations. The reduction in mortality from cardiovascular causes since 1960 has been correlated with the increase in vitamin B_6 supplementation in the food supply. Genetic factors, including deficiencies in enzymes for methionine synthase, cystathionine synthase, cystathionase, enzymes involved in folate metabolism, and proteins required for folate, vitamin B_6 or vitamin B_{12} (e.g.,intrinsic factor) absorption can all contribute to hyperhomocysteinemia, and to an increased risk of cardiovascular disease. The deficiencies of methyl tetrahydrofolate, or of methyl B_{12} are due either to an inadequate dietary intake of folate or B_{12}, or to defective enzymes involved in joining methyl groups to tetrahydrofolate, transferring methyl groups from methyl tetrahydrofolate to B_{12}, or passing them from B_{12} to homocysteine to form methionine.

Branched Chain Amino Acids

Unlike most other amino acids, which are degraded mainly in the liver, the branched chain amino acids – isoleucine, leucine and valine – are degraded predominantly in extra-hepatic tissues, mainly in muscle, because extra-hepatic tissues have higher activites of the transaminases for the branched chain amino acids and of the enzyme branch chain keto acid dehydrogenase, the second enzyme in the degradation pathways for branched chain amino acid degradation, than does the liver. Normally, the nitrogen from branched chain amino acids is transported to the liver for removal as urea, but in some physiological states it is diverted for use by other tissues. The degradadtion of isoleucine and valine provides a source of energy and a source of precursors to replenish TCA cycle intermediates (anaplerosis). Both amino acids degrade to produce succinyl CoA, a TCA cycle intermediate, and isoleucine degradation also yields acetyl CoA . Leucine degradation yields acetoacetate, a ketone body, and acetyl CoA, both of which are a source of energy.

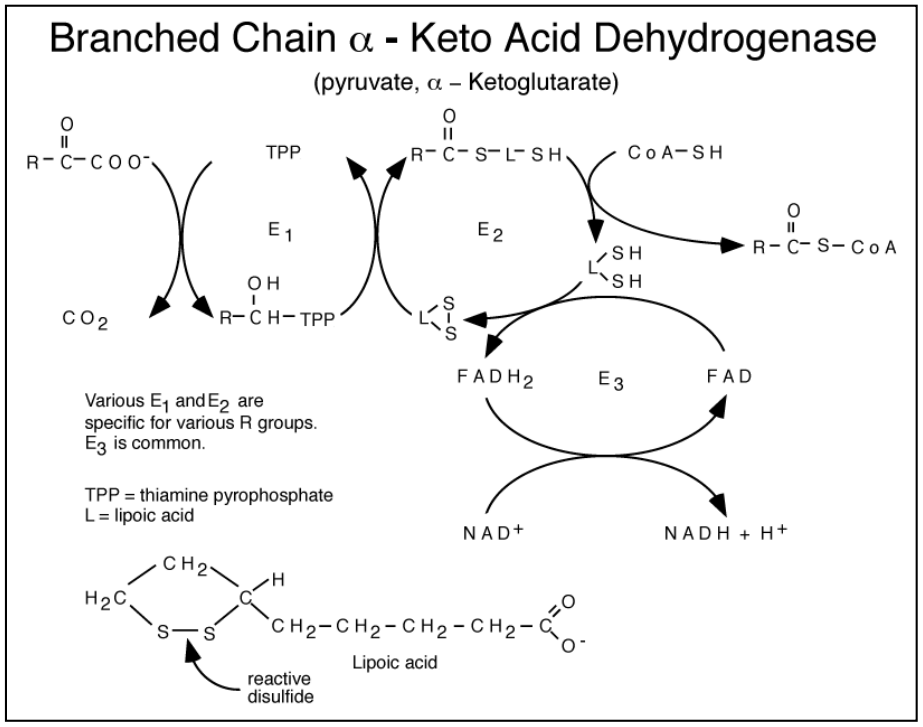
F. Degradation of branched-chain amino acids :
- transamination of the branched-chain amino acids isoleucine, valine and leucine, by **Transaminases** specific for each, to form the cognate α-ketoacids, α-keto-β-methylvalerate, α-ketoisovalerate and α-ketoisocaproate, respectively
  - the branched-chain amino acids play a special role in muscle and most other tissues because they are the major amino acids which can be oxidized in tissues other than liver; brain, heart, kidney and skeletal muscles have high activity of branched-chain amino acid transaminase relative to liver
- oxidative decarboxylation of each resulting α-ketoacid by a single enzyme complex, **Branched-chain α-ketoacid dehydrogenase** in mitochondria

Degradation of Branched Chain Amino Acids



- branched-chain α-ketoacid dehydrogenase generates the CoA thiol ester derivative from each of the branched-chain α-ketoacids, which then follow specific oxidative (NADH- and $FADH_2$ yielding) pathways to yield their ultimate CoA thiol ester derivatives. Isoleucine yields acetyl CoA, which is a ketone body precursor, and propionyl CoA, which is glucogenic via succinyl CoA. Valine yields propionyl CoA, which is glucogenic. Leucine yields acetoacetate, which is a ketone body, and acetyl CoA, which is ketogenic. The conversion pathway from propionyl CoA to succinyl CoA requires vitamin B_{12} and biotin, and was described in the Lipid Metabolism lectures.

NOTE: three different α- ketoacid dehydrogenases have a similar subunit structure. However E_1 and E_2 are substrate-specific, i.e., they recognize different R groups. Pyruvate dehydrogenase recognizes pyruvate, converting it to acetyl CoA; α-ketoglutarate dehydrogenase recognizes α-ketoglutarate, converting it to succinyl CoA; branched-chain α-ketoacid dehydrogenase recognizes all three branched-chain α- ketoacids generated from the three branched-chain amino acids by their respective, specific transaminases. A deficiency in the E_3 component of the enzyme complexes will affect pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched-chain α-ketoacid dehydrogenase because it is shared among them, while deficiencies in either the E_1 or the E_2 component of the complexes will affect only the pathways for which they are specific, i.e., either pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, or branched-chain α-ketoacid dehydrogenase.



- Skeletal muscle branch chain α-ketoacid dehydrogenase activity is increased by cortisol, or when amino acids concentrations are high as, for instance, following a high protein meal.

Maple Suryp Urine Discease (MUSD) is caused by a deficiency of branched chain α-keto acid dehydrogenase, leading to a buildup of branched chain amino acids and their toxic by-products in the blood and urine. The disease is characterized in an infant by the presence of sweet-smelling urine, with an odor similar to that of maple syrup. Infants with this disease seem healthy at birth but if left untreated suffer severe brain damage and eventually die.

Several classes of MUSD have been identified that can be characterized by the percentage of normal activity of the enzyme present. In the **classic** type there is 0-2% normal enzymatic activity. A well-known example exists in the Mennonite community of Lancaster County, PA. in which the disease exists in 1 in 178 live births — the world-wide incidence is 1/185,000 live births! The Mennonite mutation is a single nucleotide substitution of an A for a T in the gene encoding the E1 α subunit of branched chain α-keto acid dehydrogenase that changes a Tyrosine to an Asparagine.

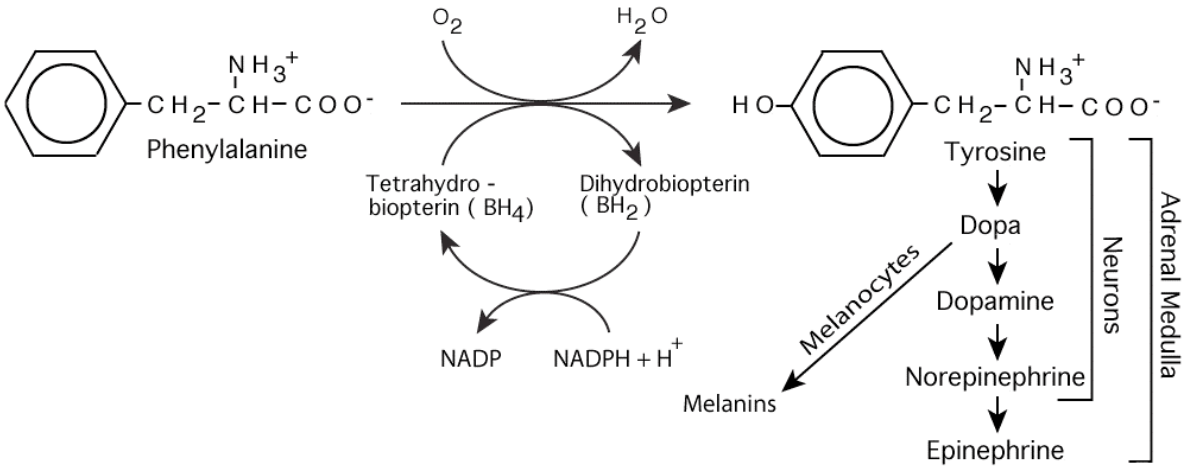
Other classes are **intermediate**, with 3-30% normal enzymatic activity, **intermittent**, with 5-20% normal enzymatic activity, **thymine-responsive**, with 2-40% normal enzymatic activity, **and lipoamide dehydrogenase deficiency**, with 0-25% normal enzymatic activity – this is a deficience of the E3 subunit, which also affects pyruvate dehydrogenase and α ketoglutarate dehydrogenase because the E3 subunit is shared with those enzymes.

The **intermittent** class is particularly interesting because symptoms appear under conditions of chronic stress or when the diet supplies more branched chain amino acids than can be accommodated by the existing branched chain α keto acid dehydrogenase enzymatic activity. In chronic stress, cortisol induces the breakdown of tissue (mainly muscle) protein, increasing the amount of available amino acids, including the branched chain amino acids, which are metabolized prediminanly in extra-hepatic tissues.

Phenylalanine and Tyrosine

G. Tyrosine (a non-essential amino acid) is produced from phenylalanine (an essential amino acid) by **Phenylalanine Hydroxylase,** a mixed function oxygenase.

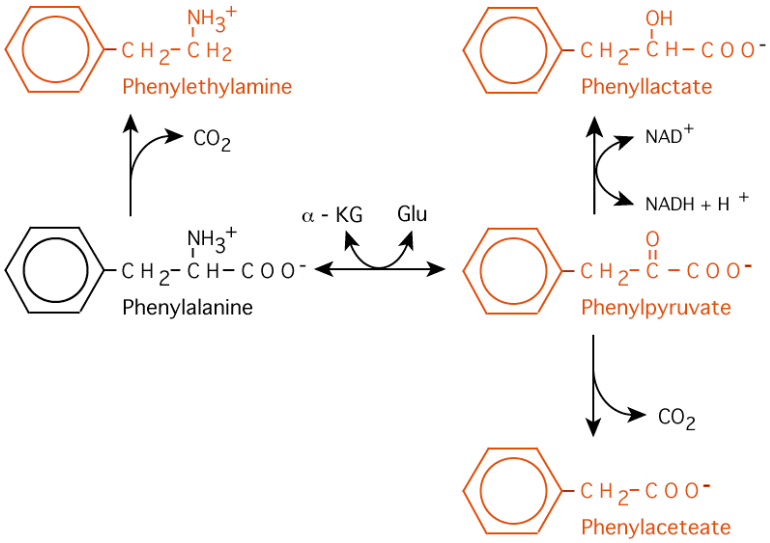
Phenylalanine Hydroxylase



- O_2 consumed, one oxygen atom donated to the hydroxyl group of tyrosine, the other donated to form water
- tetrahydrobiopterin, required as cofactor, donates two hydrogen atoms and is converted to dihydrobiopterin, requiring reconversion to tetrahydrobiopterin for the reaction to continue to produce tyrosine
- tyrosine degraded to fumarate (glucogenic) and acetoacetate (ketone body)
- normally, three quarters of phenylalanine in the body is converted to tyrosine

Phenylalanine Concentrations: Normal, Hyperphenylalaninemia (HPA), Phenylketoneurea (PKU)				
Compound Concentration	Normal	Benign HPA	Variant HPA	Classic HPA / Classic PKU
Phenylalanine	approx. 1 mg/dL (0.061 mM)	4-10 mg/dL (0.242 - 0.605 mM)	10- 20 mg/dL (0.605 - 1.21 mM)	above 20 mg/dL (above 1.21 mM)

- Deficiencies of Phenylalanine Hydroxylase result in increased plasma levels of phenylalanine and several phenyl ketones and other products of phenylalanine metabolism, which are normally minor. The products, which become major, include phenylpyruvate (a phenyl ketone), resulting from transamination of phenylalanine, phenyllactate, resulting from the reduction of phenylpyruvate, phenylethylamine, resulting from the decarboxylation of phenylalanine and phenylacetate, resulting from the deaboxylation of phenylpyruvate or the oxidation of phenylethylamine, and which causes a peculiar odor.



Minor metabolic products of phenylalanie metabolism become major products when phenylalanine hydroxylase is deficient

- Phenylketoneurea (PKU), the major metabolic disease resulting from Phenylalanine Hydroxylase deficiency.
  - autosomal recessive
  - frequency = 1/20,000 live births
  - carriers have reduced phenylalanine hydroxylase
  - Almost all untreated phenylketonurics are severely mentally retarded; about 1% of all patients in mental institutions have phenylketonuria.
  - Brain weight of phenylketonurics is below normal; myelination of nerves is defective; life expectancy is drastically shortened - half are dead by age 20, three quarters are dead by age 30.
  - The biochemical basis of the mental retardation is not known.
  - Treatment is a low phenylalanine-content diet beginning soon after birth to minimize mental retardation along with supplementary tyrosine.

THE POSTABSORPTIVE STATE AND THE ACIDOTIC STATE: EXAMPLES OF AMINO ACID FLUX IN THE BODY

The fasting state and the acidotic state provide examples of the inter-organ flux of amino acids necessary to maintain the free amino acid pool in the blood and supply tissues with their required amino acids, and to maintain physiological pH. During an overnight fast, protein synthesis in the liver and other tissues continues, but at a diminished rate compared to the postprandial state (after eating). Net degradation of labile protein occurs in skeletal muscle, which contains the body's largest protein mass, and in other tissues. The net degradation of protein affects functional proteins, like skeletal muscle myosin, which are sacrificed to meet more urgent demands for amino acids in other tissues, and to provide carbon skeletons for gluconeogenesis, by the liver, to meet the needs for glucose, particularly of brain and red blood cells.

The pattern of inter-organ flux of amino acids is affected by conditions that change the supply of fuels (for example the overnight fast, a mixed meal, a high protein meal), and by conditions that increase the demand for amino acids (metabolic acidosis, surgical stress, traumatic injury, burns, wound healing, and sepsis). The flux of amino acid carbon and nitrogen in these different conditions is dictated by several factors:

1. Ammonia (NH_4^+) is toxic. Consequently, it is transported between tissues as alanine or glutamine. Alanine is the principal carrier of amino acid nitrogen from other tissues back to the liver, where the nitrogen is converted to urea and subsequently excreted into the urine by the kidneys. The amount of urea synthesized is proportional to the amount of amino acid carbon that is oxidized as fuel.

2. The pool of glutamine in the blood serves several essential metabolic functions. It provides ammonia for excretion of protons in the urine as NH_4^+. It serves as a fuel for the gut, the kidney, and the cells of the immune system. Glutamine is also required by the cells of the immune system and other rapidly dividing cells in which its amide group serves as the source of nitrogen for biosynthetic reactions (glutamine is a major donor of nitrogen for biosynthetic reactions). In the brain, the formation of glutamine from glutamate and NH_4^+ provides a means of removing ammonia and of transporting glutamate between cells in the brain. The utilization of blood glutamine is prioritized. During metabolic acidosis the kidney becomes the predominant site of glutamine uptake, at the expense of glutamine utilization in other tissues. On the other hand, during sepsis, cells involved in the immune response (macrophages, hepatocytes) become the preferential sites of glutamine uptake.

3. The branched chain amino acids (valine, leucine, isoleucine) form a significant portion of the average protein, and can be converted to TCA cycle intermediates and utilized as fuels by almost all tissues. They are also the major precursors of glutamine. Except for the branched chain amino acids and alanine, aspartate and glutamine, the catabolism of amino acids occurs principally in the liver.

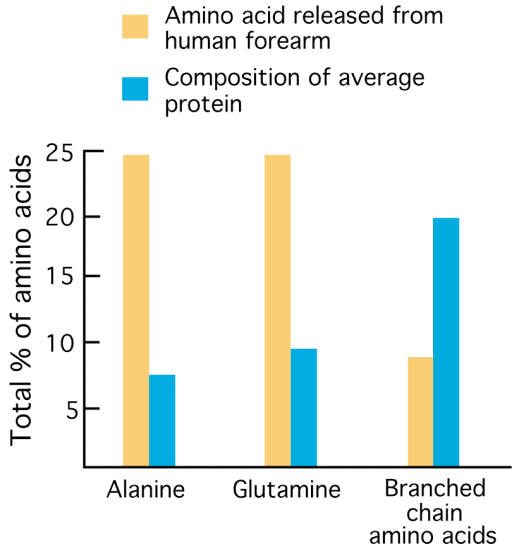
4. Amino acids are major gluconeogenic substrates, and most of the energy obtained from their oxidation is derived from oxidation of the glucose formed from their carbon skeletons. A much smaller percentage of amino acid carbon is converted to acetyl CoA or to ketone bodies and oxidized. The utilization of amino acids for glucose synthesis for the brain and other glucose-requiring tissues is subject to the hormonal regulatory mechanisms of glucose homeostasis.

5. The relative rates of protein synthesis and degradation (protein turnover) determine the size of the free amino acid pools available for the synthesis of new proteins and for other essential functions. For example, the synthesis of new proteins to mount an immune response is supported by the net degradation of the proteins in the body.

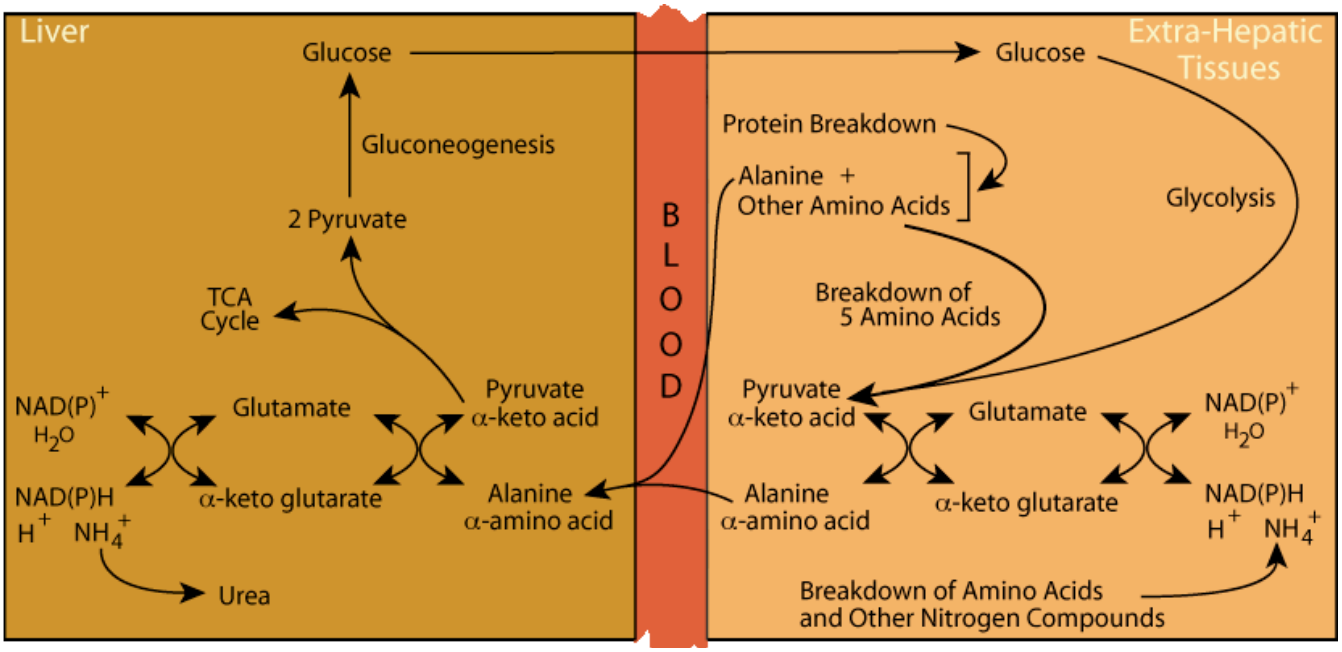
Skeletal Muscle

The release of amino acids from skeletal muscle is stimulated during an overnight fast by the decrease of insulin and increase of glucocorticoid levels in the blood. Insulin promotes uptake of amino acids and the general synthesis of proteins. The fall in blood insulin during an overnight fast results in net proteolysis and release of amino acids, because the equilibrium between protein synthesis and protein degradation is shifted toward degradation.

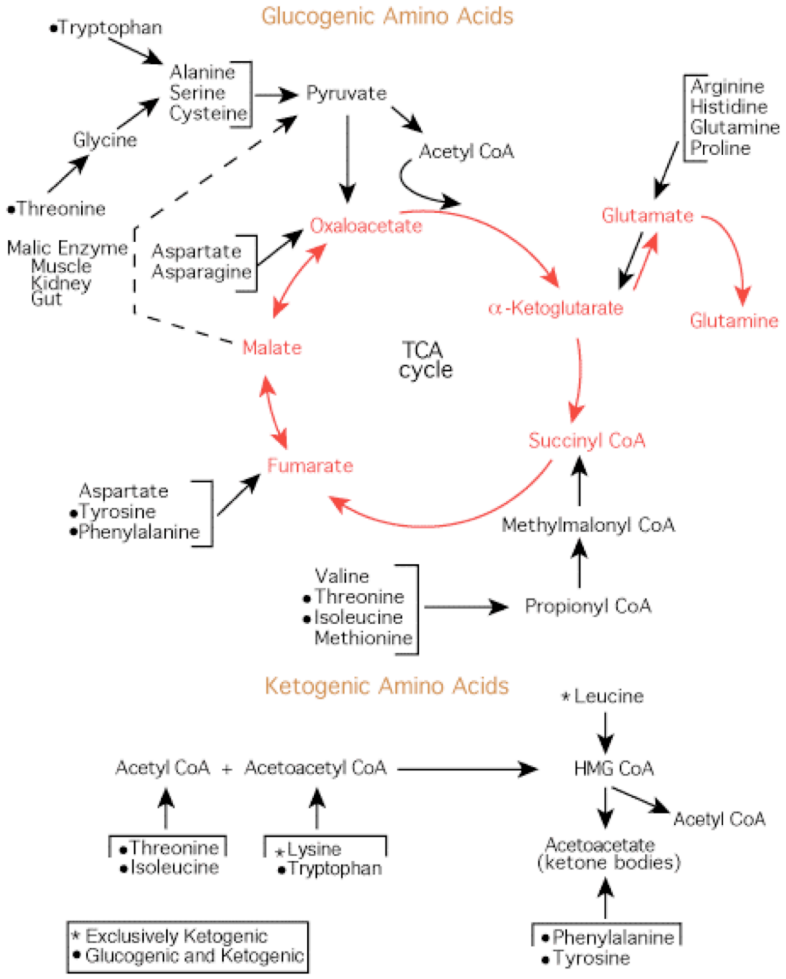
- Because of its large mass, skeletal muscle is a major site of protein synthesis and breakdown.
- Efflux of amino acids from skeletal muscle supports the amino acid pool in the blood.
- Alanine and glutamine account for approximately 50% of all amino acids released from skeletal muscle.
  - The graph at the right shows the difference between alanine, glutamine and branched chain amino acids (leucine, isoleucine, valine) in venus blood leaving the human forearm compared to their percentage representation in average skeletal muscle protein composition. Alanine and glutamine represent a much higher percentage of total nitrogen released than originally present in the degraded proteins, evidence that they are synthesized in the muscle. The branched chain amino acids are released in a much lower percentage than their representation in the degraded protein, evidence that they are catabolized.



ALANINE CYCLE



- The branched chain amino acids, aspartate and glutamate supply the amino groups for alanine and glutamine production.
- Alanine is a major transporter of ammonia (a neurotoxin) from extra-hepatic tissues to the liver (the "Alanine Cycle") for disposal as urea. Its carbon skeleton is used for energy production by the liver or for glucose synthesis in response to hypoglycemia. Alanine is produced by transamintion of pyruvate, a relatively abundant α keto acid.
- The major fate of branched-chain amino acids is to provide carbon skeletons for glutamine formation.
  - oxidation of the branched chain amino acids (valine, leucine, isoleucine) to produce energy, first by removal of the α-amino nitrogen by transamination and then by supplying the carbon skeletons to the TCA cycle; glucose is spared as a result of the use of this alternate energy source during fasting; α-ketoglutarate produced by the TCA cycle is used in the synthesis of glutamine for export
  - entry of carbon skeletons to the TCA cycle at succinyl CoA and acetyl CoA
  - exit of carbon skeletons from the TCA cycle as α-ketoglutarate
  - transamination of α-ketoglutarate to glutamate or glutamate formation from α-ketoglutarate and ammonia via glutamate dehydrogenase
- glutamine production by addition of the amide nitrogen to glutamate (glutamine synthetase)
- Chronic stresses induce the breakdown of tissue protein, – mainly in muscle –leading to the increase in the level of amino acids. Most amino acids are metabolized in the liver, but the branched chain amino acid are metabolized mainly in extra-hepatic tissues, where the carbon skeletons of isoleucine and valine are largely converted to glutamine, which is a nitrogen donor for the synthesis of many biological molecules, or is excreted by the kidney.

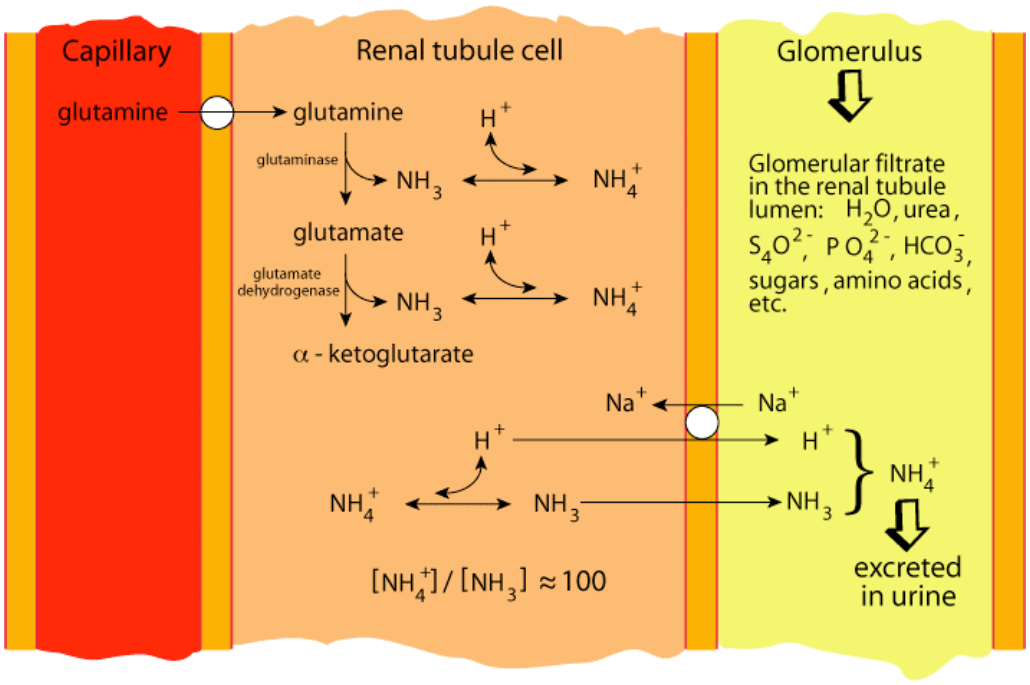


- Depending on the particular stress, different tissues have different needs for glutamine or its amide nitrogen. For example, serious infections cause a proliferation of cells of the immune system to counteract the infections agent, requiring increased immune cell division and cell growth. Nitrogen for the synthesis of several biological molecules, such as ribonucleotides and deoxyribonucleotides is donated by glutamine, and, therefore, the immune cells get most of the glutamine.
- In acidosis (and other stresses) cortisol stimulates glutamine synthetase activity in muscle, leading to increased synthesis and export of glutamine, which the kidney uses as a source of ammonia to buffer excess protons in the urine, thereby promoting their excretion from the body.

Kidney

One of the primary roles of amino acid nitrogen is to provide ammonia in the kidney for the excretion of protons in the urine. The rate of uptake of glutamine, the ammonia donor, from the blood and its utilization by the kidney depends mainly on the amount of acid that must be excreted to maintain a normal pH in the blood.

- During acidosis excretion of NH_4^+ increases several fold.
  - glutamine increases proton excretion by providing a buffer for protons which are transported into the renal tubular fluid, and subsequently into the urine
  - glutamine provides about two thirds of the NH_4^+ excreted
  - uptake of glutamine by the kidney increases during metabolic acidosis to provide more NH_3 to buffer excess protons for excretion in the urine as NH_4^+
  - renal glutamine utilization for proton excretion takes precedence over the requirement of other tissues for glutamine
  - glutaminase releases the amide nitrogen, glutamate dehydrogenase releases the α-amino nitrogen
  - NH_4^+ does not cross the renal tubule cell membrane into the renal tubule because it is charged, but it is in equilibrium – K_{eq}= 100 – with NH_3 , which is uncharged and does cross the membrane
  - In chronic metabolic acidosis the activities of renal glutaminase, glutamate dehydrogenase, phosphoenolpyruvate carboxykinase and mitochondrial glutamine transporter increase and correlate with increased urinary excretion of ammonium ions and increased renal gluconeogenesis from amino acids. The liver participates in this process by synthesizing less urea, which makes more glutamine available for the kidney.



Excretion of Compounds in the Urine		
Component	Gms / 24 hours	Nitrogen (mMol)
H_2O	1,000	-
SO_4^{2-}	2-5	-
PO_4^{2-}	2-5	-
K^+	1-2	-
Urea	12-20	400-650
Creatinine	1-1.8	25-50
Uric Acid	0.2-0.8	4-16
NH_4^+	0.2-1 (up to 10 in acidosis)	11-55 (up to 550 in acidosis)

Major Fuel Sources for the Kidney			
% of Total CO_2 Formed in Different Physiologic States			
Fuel	Normal	Acidosis	Fasted
Lactate	45	20	15
Glucose*	25	20	0
Fatty Acids	15	20	60
Glutamine	15	40	25

* Glucose used in the renal medulla is produced in the renal cortex

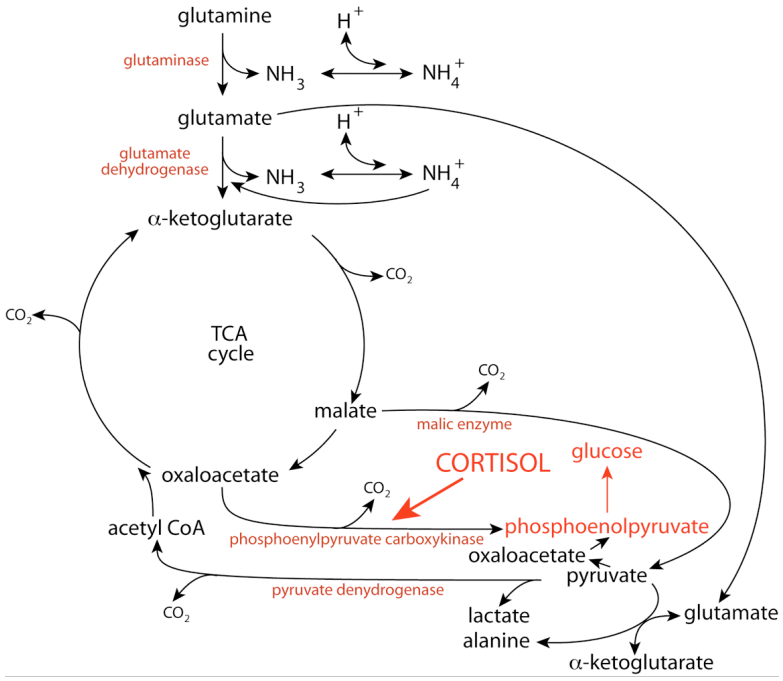
Note the difference in NH_4^+ excretion and the use of glutamine by the kidney between the normal state and acidosis

- Glutamine is used as a fuel by the kidney in the normal fed state, and to a greater extent during fasting and metabolic acidosis.
  - after deamination by glutaminase and glutamine dehydrogenase the resulting α-ketoglutarate can be used as a fuel by the kidney and is oxidized to CO_2, converted to glucose for use in cells in the renal medulla, or converted to alanine to return ammonia to the liver for urea synthesis and excretion.

NOTE: cells of the renal medulla have a relatively high dependence on anaerobic glycolysis due to their lower oxygen supply and mitochondrial capacity ; the lactate released from anaerobic glycolysis in these cells is taken up and oxidized in the renal cortical cells, which have a higher mitochondrial capacity and greater blood supply.

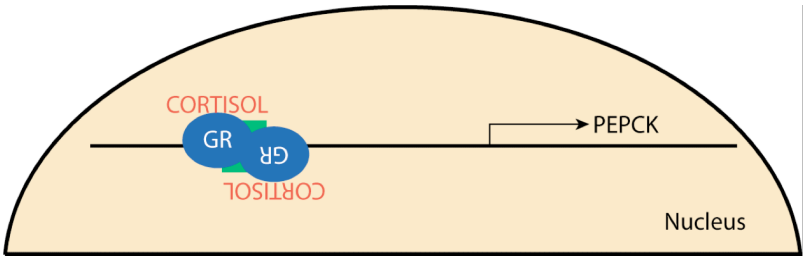
Liver

Liver is the major site of amino acid metabolism. It is the major site of amino acid catabolism, and converts most of the carbon in amino acids to intermediatesof the TCA cycle or pyruvate. Therefore, it can use carbon skeletons derived from amino acids for the generation of energy, or, during fasting, to synthesize glucose. During fasting muscle protein (and protein in other tissues of the body) undergoes net degradation to supply carbon skeletons for glucose production by the liver. Glucagon stimulates uptake of alanine by the liver, which converts it, by transamination, to pyruvate. The pyruvate is then used as a source of carbon skeletons for glucose synthesis. Amino acid nitrogen is converted to urea. In this way, alanine and other amino acids, produced in other tissues as a result of protein breakdown, are used to supply gluconeogenic precursors to the liver; their carbon skeletons are used for gluconeogenesis and their amino groups



Use of glutamine by the kidney

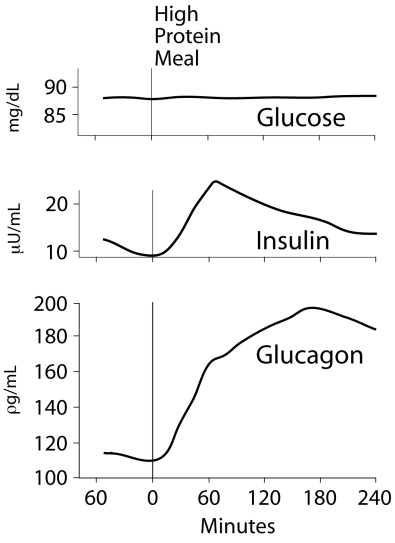
are used for urea synthesis for excretion. In acidosis, glutaminase of the periportal hepatocytes is less active and much of the blood glutamine escapes hydrolysis in the liver for use by kidney, which increases its activity of glutaminase, glutamate dehydrogenase, phosphophenolpyruvate carboxykinsae and mitochondrial glutamine transport to bring more glutamine into the kidney to supply NH_3 to buffer excess protons for excretion in the urine.



The glucocorticoid receptor (GR) receptor bind cortisol to become active, translocates to the nucleus and binds to the glucocorticoid response element in the phosphoenoylpyruvate carboxykinase gene and activates its transcription.

A High Protein Meal

Following ingestion of a high protein meal, the gut and the liver utilize most of the absorbed amino acids. Glutamate and aspartate are utilized as fuels by the gut, and very little enters the protal vein. The gut may also use some branched chain amino acids. The liver takes up 60 - 70% of the amino acids present in the portal vein. These amino acids, for the most part, are converted to glucose.



After a pure protein meal, the increased levels of dietary amino acids reaching the pancreas stimulate the release of glucagon above fasting levels, thereby increasing amino acid uptake into the liver.

- glucagon causes increased expression of amino acid transporters on the liver cell surface
- amino acids are deaminated in the liver and carbon skeletons are used for gluconeogenesis

- urea production increases to eliminate the increased nitrogen
  - arginine is a positive regulator of the first enzyme of the urea cycle, carbamoyl phosphate synthetase I by stimulating the synthesis of N-acetyl glutamate, which in turn, stimulates carbamoyl phosphate synthetase I.

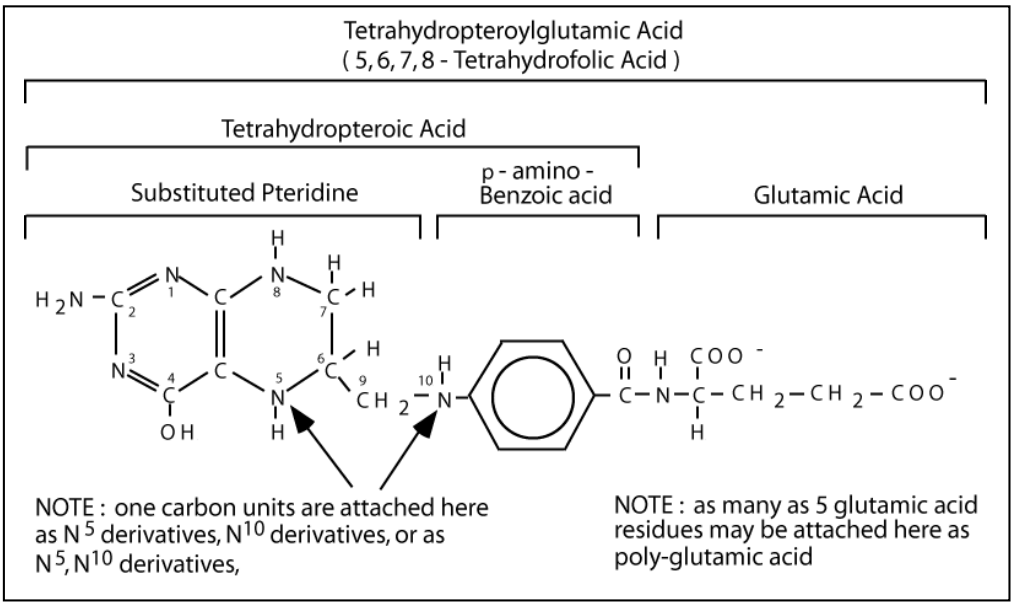
Insulin release is also stimulated, but not nearly to the levels found after a high carbohydrate meal (see figure at the right). In general, the insulin released after a high protein meal is sufficiently high that net protein synthesis is stimulated, but gluconeogenesis in the liver is not inhibited. The higher the carbohydrate content of the meal, the higher the insulin/glucagon ratio and the greater the shift of amino acids away from gluconeogenesis into biosynthetic pathways in the liver, such as the synthesis of plasma proteins.

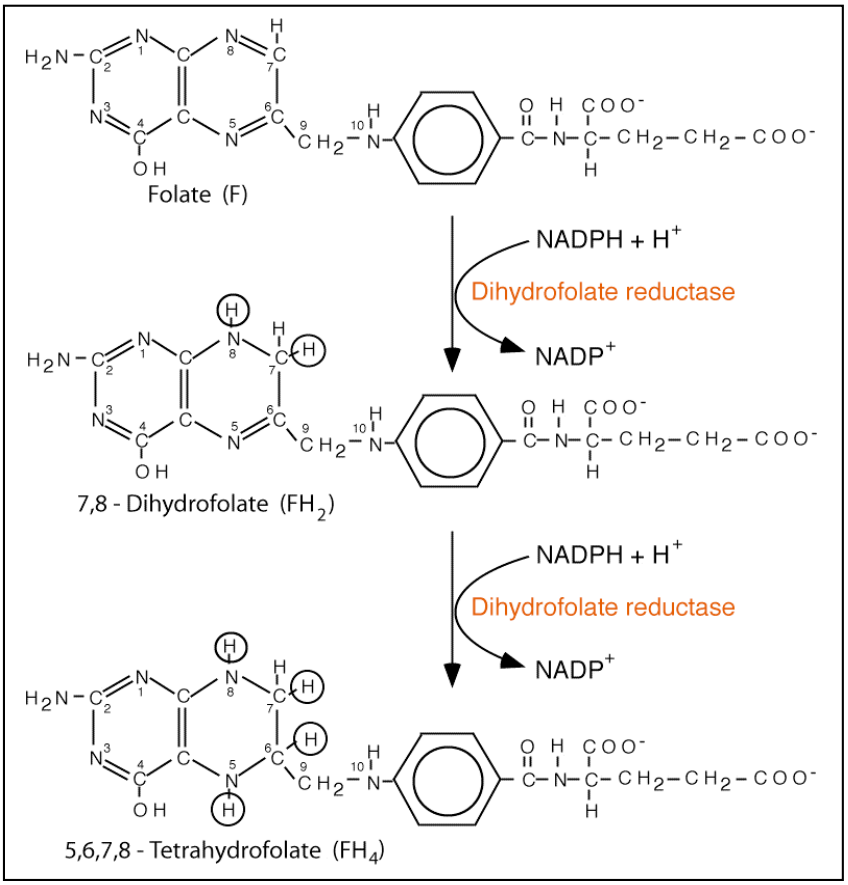
Most of the amino acid nitrogen entering the peripheral circulation after a high protein meal or a mixed meal is present as the branched chain amino acids (leucine, isoleucine, valine). Because the liver has low levels of transaminases for these amino acids, it cannot oxidize them to a significant extent and they enter the systemic circulation. The branched chain amino acids are taken up slowly by skeletal muscle and other tissues. These peripheral non-hepatic tissues utilize the amino acids derived from the diet principally for net protein synthesis.

TETRAHYDROFOLATE (FH_4) AND THE FOLATE ONE-CARBON POOL

A. Folic acid (folate) is a vitamin that must be taken in the diet. Humans cannot synthesize folate.

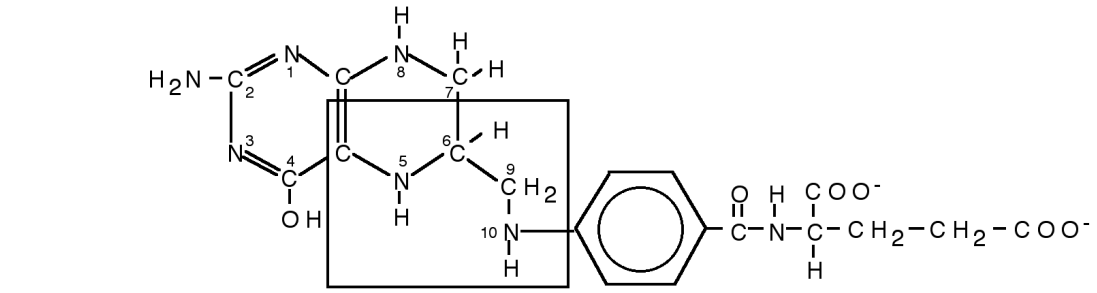
- Bacteria and higher plants can synthesize folate from the bicyclic pteridine ring, *p*-aminobenzoic acid and glutamate.
- Sulfa drugs, which are used to treat certain bacterial infections, are analogues of *p* aminobenzoic acid. They prevent bacterial growth and cell division by interfering with folate synthesis. Because human cells don't synthesize folate, certain sulfa drugs can be used to treat some infections bacteria, which must synthesize folate because they cannot uptake folate from their environment, without affecting folate levels in human cells. Sulfanilamide, a *p*-amino benzoic acid analogue is bacteriostatic. It inhibits bacterial folate synthesis, which, in turn, reduces bacterial DNA synthesis, (nucleotide synthesis requires folate as a one-carbon donor), allowing the immune system time to overtake the bacterial infection.
- **Dihydrofolate Reductase** reduces folate to give, first,7,8-dihydrofolate, and then, by a second reduction, 5,6,7,8 - tetrahydrofolate (FH_4), also known as tetrahydropteroylglutamic acid.
  - NADPH is the source of electrons for both reduction steps

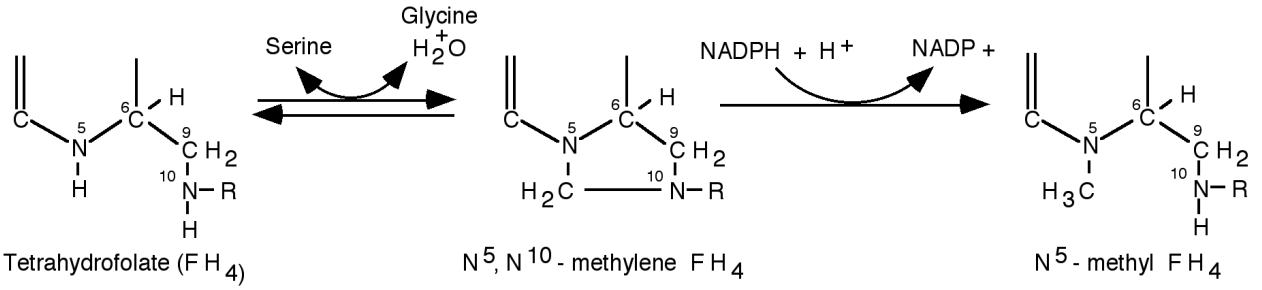


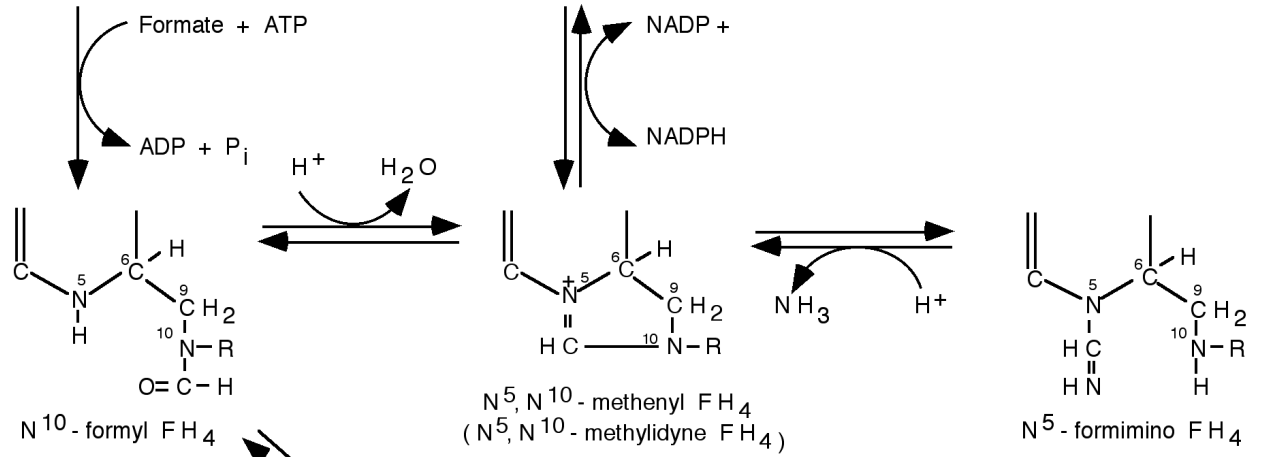


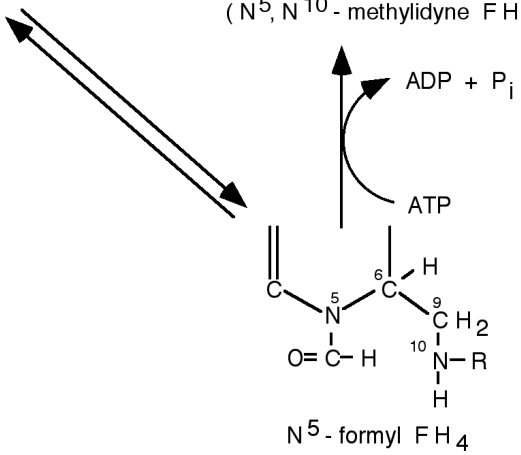
B. One-carbon units are attached either to nitrogen N^5 or N^{10} or they form a bridge between N^5 and N^{10} .The collection of one-carbon groups attached to FH4 is known as the folate one-carbon pool.

STRUCTURES OF ONE-CARBON DERIVATIVES OF FH_4



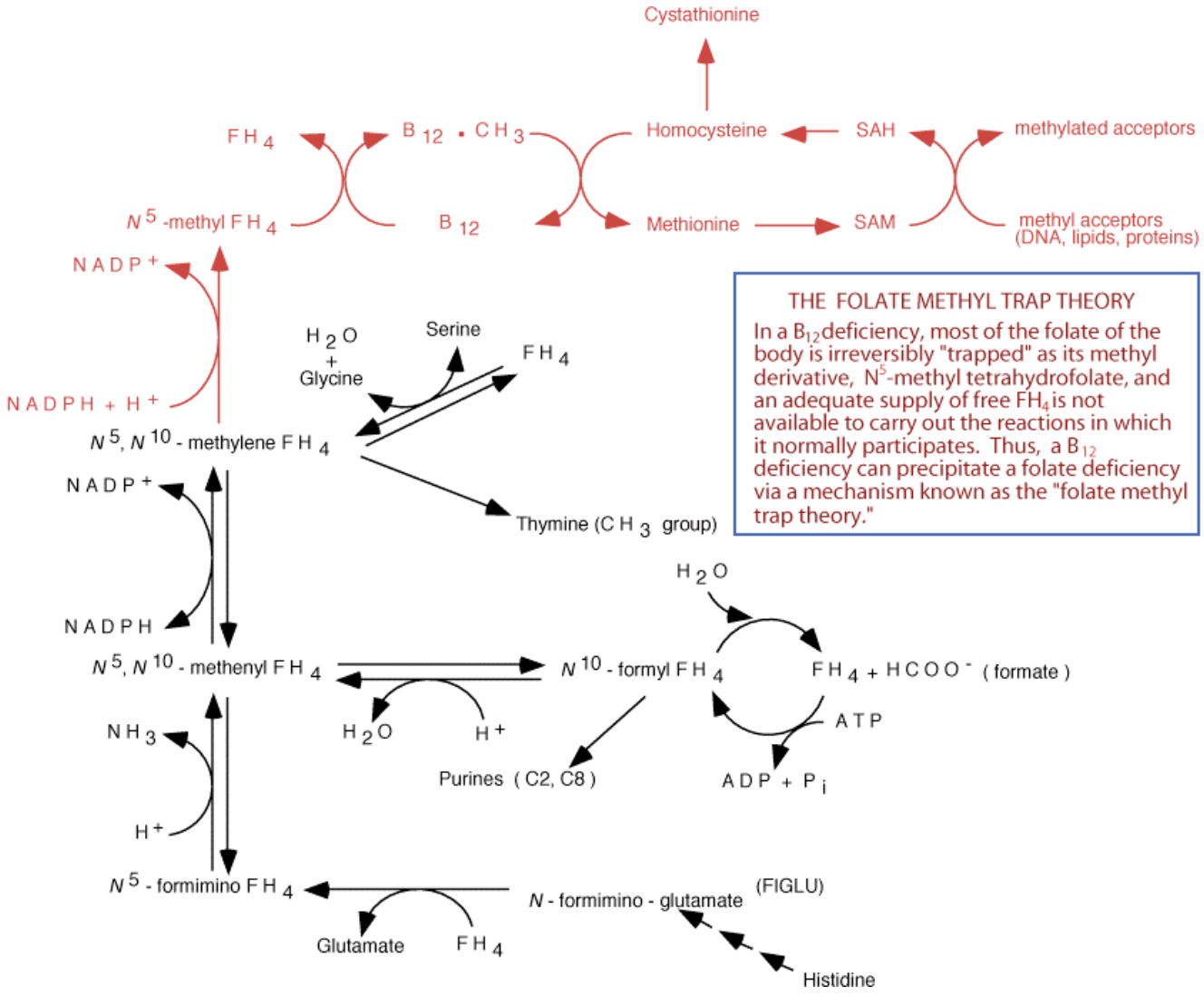






Oxidation state		*Group*
Most reduced (= methanol)	- CH_3	Methyl
Intermediate (= formaldehyde)	- CH_2 -	Methylene
Most oxidized (= formic acid)	- CHO	Formyl
	- CHNH	Formimino
	- CH=	Methenyl

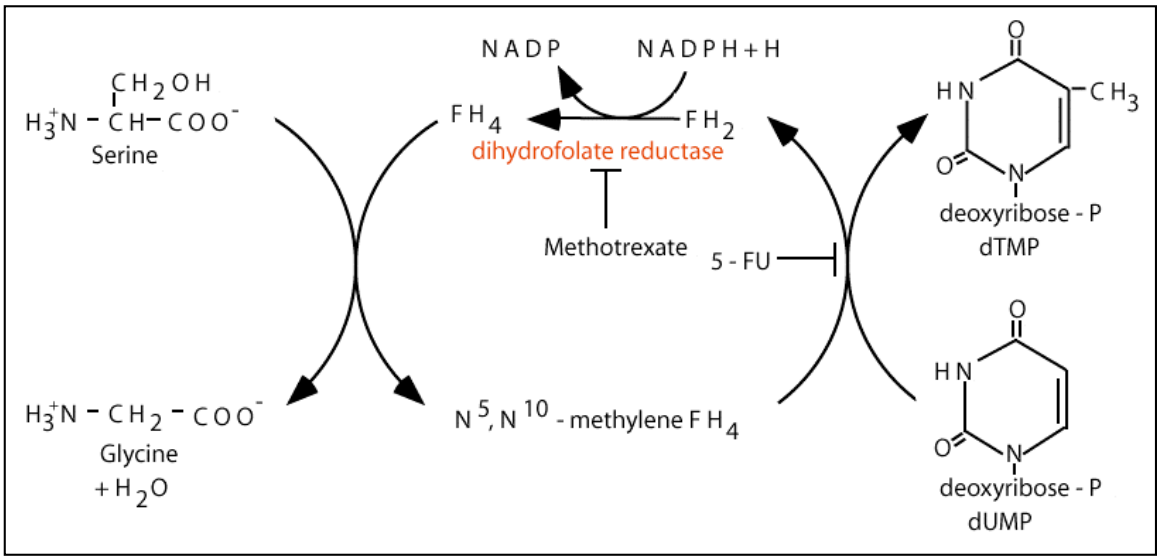
- Serine is the major source of one-carbon units donated to FH_4. Because serine can be synthesized from glucose, dietary carbohydrate serves as a major source of carbon for the one-carbon pool.
- Some other one-carbon donors are glycine, which along with serine and formaldehyde (from, for example, epinephrine and choline breakdown), yield N^5, N^{10}-methylene FH_4, histidine, which yields N^5, N^{10}-methenyl FH_4 via the intermediates *N*-formiminoglutamate and N^5 - forminino FH_4, and tryptophan, which donates a formate group during breakdown, to yield N^{10} - formyl FH_4.
- While attached to FH_4 the one-carbon units are oxidized and reduced.
  - allows one-unit carbons to be accepted and donated in different oxidative states, as required by individual biochemical reactions
- Acceptors of one-carbon units from FH_4 compounds include glycine to yield serine, the dTMP precursor deoxy-uridine monophosphate, to yield dTMP, purine precursors, which derive C_2 and C_8 from N^{10} - formyl FH_4 , and vitamin B_{12}, which accepts a methyl group from N^5-methyl FH_4 to yield methyl-B_{12},which subsequently donates the methyl group to remethylate homocysteine in the production of methionine. Recall that 5' deoxyadenosyl vitamin B_{12} is required for the action of methylmalonyl CoA mutase activity in the conversion of propionyl CoA to succinyl CoA. When B_{12} is deficient, L-methylmalonyl CoA is not readily converted to succinyl CoA, and methylmalonic acid is excreted in the urine.



C. The Folate Methyl Trap Theory

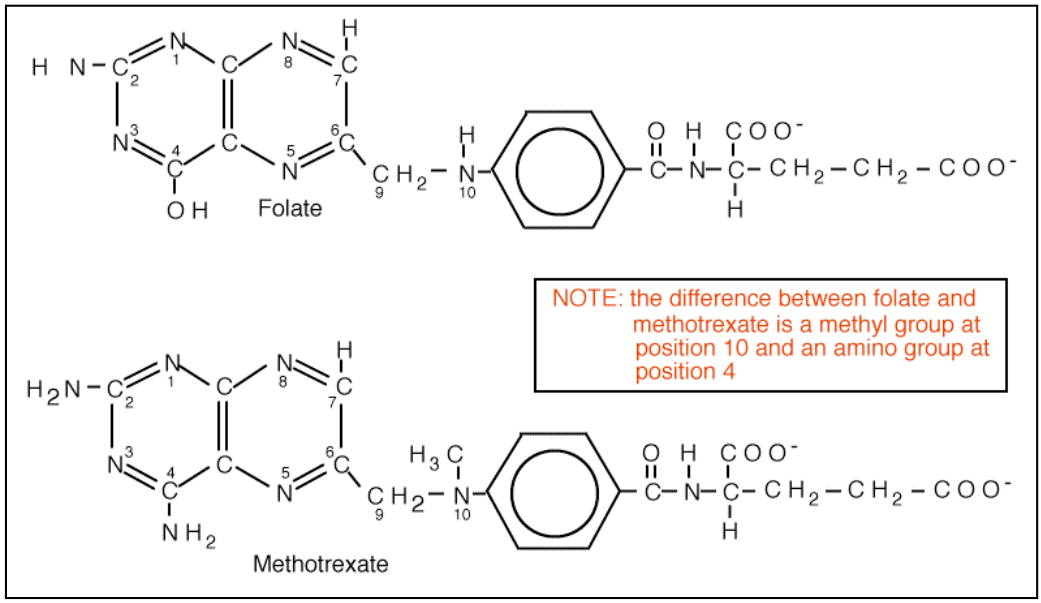
- Vitamin B_{12} is obtained in small amounts from intestinal bacteria, but mainly in the diet from meats, eggs, dairy products, fish, poultry and seafood. Animals that serve as a source of B_{12} obtain it mainly from bacteria in their food supply.
- Deficiencies of vitamin B_{12} trap tetrahydrofolate as N^5-methyl FH_4 .
  - reduction of N^5, N^{10}-methylene FH_4 to N^5-methyl FH_4 by NADPH + H+ is **irreversible**
  - deficiencies of vitamin B_{12} slow or prevent donation of the methyl group from N^5-methyl FH_4, thereby preventing regeneration of FH_4
  - N^5-methyl FH_4 donates its methyl group in only one reaction in the body – the methylation of B_{12}
  - all, or almost all tetrahydrofolate accumulates as N^5-methyl FH_4 thereby diminishing or eliminating the tetrahydrofolate pool available for other reactions that require it
  - RESULT: The B_{12} deficiency causes an apparent folate deficiency, because all, or almost all the folate is "trapped" as the N^5-methyl FH_4 derivative; patient may present with symptoms of folate deficiency, when, in fact, he/she has a B_{12} deficiency. Appropriate tests must be done to distinguish a B_{12} deficiency from a folate deficiency.

D. A folate analog, methotrexate, is an anti-tumor, chemotherapeutic agent that acts by inhibiting dihydrofolate reductase.



- Deoxythymidine monophosphate (dTMP) is synthesized by the addition of a methyl group to deoxyuridine monophosphate (dUMP). dTMP is subsequently phosphorylated to the triphosphate form (dTTP) and used for DNA synthesis. N^5, N^{10} - methylene FH_4 is the methyl group donor, and becomes oxidized to FH_2 during the reaction (the methylene group is reduced to a methyl group for donation to dUMP to produce dTMP). The resulting FH_2 must then be re-reduced by the enzyme dihydrofolate reductase before it can participate in subsequent methyl acceptor / methyl donor function.
- Methotrexate, a folate analogue (an anti-folate) inhibits dihydrofolate reductase, thereby starving cells of FH_4.
- Cells starved of FH_4 are unable to carry out reactions that require or produce folate derivatives, particularly the synthesis of dTMP and purines, and their more highly phosphorylated forms required for DNA synthesis; without them DNA synthesis halts.
- All cells undergoing cell division, and therefore requiring DNA synthesis, are targets of the anti-folate, methotrexate. Because tumor cells divide rapidly and incessantly, they are the prime target for killing, although other cells of the body that normally undergo continuous cell division, e.g. cells of the hair follicles, intestinal epithelium, cells of the immune system and male germ cells, are also targets, and killing those cells results in some of the side-affects of the drug. Normal cells, because of so-called "check points" in the cell cycle don't divide if DNA synthesis does not proceed to completion.

However, if DNA synthesis is prevented for a long period of time, they do die by undergoing apoptosis (programmed cell death). Tumor cells, however, have lost the cell cycle "check points" and continue to divide even though DNA synthesis has not completed successfully. As a result, they undergo so-called "mitotic catastrophy", i.e., their daughter cells have less than the normal amount of DNA and, consequently die. This difference between normal and tumor cells is a central factor in the logic of anti-folate treatment to differentially kill tumor cells. Anti-folates are poisons; the logic is to use them to kill the tumor before they kill the patient!



- A second commonly-used anti-tumor agent is 5-fluorouracil (5-FU), which inhibits the enzyme thymidylate synthase, i.e., the conversion of dUMP to dTMP (see diagram at the bottom of the previous page), and which will be described in more detail later in this course.

A REVIEW OF ONE-CARBON TRANSFERS

Groups containing a single carbon atom can be transferred from one compound to another by different one-carbon "carrier" systems.

- Biotin transfers one-carbon units in the most oxidized form, as CO_2. (For example, the enzymes pyruvate carboxylase and acetyl CoA carboxylase add CO_2 to their respective substrates pyruvate and acetyl CoA and require biotin as the CO_2 donor.)
- S-adenosyl methionine (SAM), tetrahydrofolate, and vitamin B_{12} transfer one-carbon units at oxidation levels lower than CO_2.
  - SAM donates the methyl group derived from methionine to several recipients at the methanol level of oxidation (CH_3).
  - Tetrahydrofolate, which is produced from the vitamin folate, obtains one-carbon units form serine, glycine, histidine, formaldehyde and formate. While attached to FH_4 the one-carbon units undergo oxidation and reduction. Once reduced to the methyl level, however, the one-carbon unit cannot be re-oxidized (the "folate methyl trap"). Some major recipients of one-carbon units from FH_4 are deoxyuridine monophosphate (dUMP) to form deoxythymidine monophosphate (dTMP), the amino acid glycine to form serine, the precursors of the purine bases to produce carbons C_2 and C_8 of the purine ring, and vitamin B_{12}.
  - B_{12} accepts a one-carbon unit from N^5-methyl FH_4 at the methanol level of oxidation (CH_3), and donates it at the same level of oxidation (for example, in the methylation of homocysteine to form methionine).

Folate and Neural Tube Defects

Neural tube defects (NTDs) are common congenital malformations in humans, occurring at a frequency of almost one every 1000 live births in the United States population. The costs are enormous. In California alone, where studies were done, the number of children who are live-born with spina bifida each year generates over $60,000,000 in lifetime medical expenses. In the United Kingdom, NTDs account for 15% of perinatal deaths, second only to cardiac defects among congenital malformation-induced perinatal mortality.

While etiologically heterogeneous, NTDs are, for the most part, multifactoral in their pathogenesis, having both genetic and environmental factors contributing to their development. Several factors have been suggested to influence NTD risk, including genetic predisposition, maternal obesity, and maternal exposure to numerous exogenous agents during early pregnancy, including anticonvulsant drugs. However, periconceptual supplementation of the maternal diet with a multivitamin containing folic acid is the only identified factor that has been definitively shown to have a significant relationship to NTD. Initial efforts demonstrated that first trimester levels of several micronutrients, particularly folate, were significantly lower in mothers of NTD-affected infants than in mothers of healthy infants. Subsequent nonrandomized trials conducted among women who had previously given birth to an infant affected with anencephaly or spina bifida demonstrated that folic acid, or multivitamins taken in the periconceptional period, resulted in a 75% reduction in the recurrence risk for a NTD. This observation has been verified in a double-blind, placebo-controlled, randomized study, which observed a 72% reduction in NTD recurrence risk when the maternal diet was supplemented with 4 mg. of folic acid per day. These studies strongly support the hypothesis that periconceptional supplementation of the maternal diet with folic acid in the dose range of 0.4 to 5 mg. per day is sufficient to overcome the majority of NTD recurrent risk.

Recent evidence suggests that supplementation with multivitamins containing folic acid reduces the occurrence risk for NTDs, just as it reduces the recurrence risk. In one study 0.8 mg. of folic acid taken daily significantly reduced a woman's risk of having an infant with a NTD, supporting a previous study showing supplemental folate reduced, by 60%, the incidence of NTDs. A third study showed a 72% NTD risk reduction among women whose folate intake from both dietary and supplemental sources in early pregnancy exceeded 0.35 mg. per day, compared to women whose intake of folates was less than 0.18 mg. per day. Several other studies corroborate these findings. Women taking the higher dose of 4 mg. per day should do so only under medical supervision, as this higher dose can obscure the signs and symptoms of vitamin B_{12} deficiency. Folate's potential to reduce the risk of neural tube defects is so important that the Food and Drug Administration requires food manufacturers to fortify enriched grain products with folic acid.