

## CELL STRUCTURE AND FUNCTION 2002-2003

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**FRI., DEC. 13, 10:00 A.M.**  
**MON., DEC. 16, 9:00 A.M.**  
**MON., DEC. 16, 10:00 A.M.**  
**TUES., DEC. 17, 8:00 A.M.**  
**DR. BUDDY ULLMAN**

**AMINO ACID METABOLISM**  
**I, II, III, AND IV**

**READING ASSIGNMENT:** *Principles of Medical Biochemistry*, Meisenberg & Simmons, pp. 281, 283, 421-451

### **LEARNING OBJECTIVES**

1. Be familiar with the avenue by which atmospheric nitrogen is converted into a useable form of nitrogen in the biosphere. Know the key nitrogen fixation reaction.
2. Know the three major functions for which amino acids serve in human cells and tissues.
3. Know the essential and nonessential amino acids and the basis for their definition as either category.
4. Know the disease states associated with a failure to ingest or obtain adequate nitrogen nutrition.
5. Know the routes of amino acid entry into cells and tissues and the names of the genetic deficiencies in amino acid permeation.
6. Know the two major enzymatic steps involved in ammonium ion assimilation in human cells.
7. Know how amino groups are shuttled from one amino acid to another via transamination reactions. Know the names of two diagnostic transaminases.
8. Know the role that pyridoxal phosphate plays in transamination reactions.
9. Know the routes by which nonessential amino acids are synthesized from glycolytic or TCA cycle intermediates.
10. Be familiar with the role played by tetrahydrofolate in one carbon metabolism and identify therapeutically relevant inhibitors of tetrahydrofolate metabolism.
11. Know how nonessential amino acids are synthesized from essential amino acids.
12. Know the enzymatic deficiencies that lead to homocystinuria, cystathioninuria, and phenylketonuria.
13. Know how amino groups are removed from amino acids during amino acid catabolism.
14. Know the urea cycle pathway and the inborn errors of metabolism associated with genetic defects in each of the urea cycle enzymes.
15. Know how the carbon skeletons of amino acids are catabolized to energy producing metabolic intermediates of amino acids. Know the terms ketogenic and glucogenic, and be able to identify alcaptonuria and maple syrup urine disease as two disorders of amino acid catabolism.

16. Know how 4-aminobutyrate, histamine, serotonin, catecholamines, creatine and creatine phosphate, S-adenosylmethionine, and polyamines are synthesized from precursor amino acids.

## **GENERAL OUTLINE**

- I OVERVIEW OF NITROGEN METABOLISM**
- II THE NITROGEN CYCLE**
- III FUNCTIONS OF AMINO ACIDS**
- IV ESSENTIAL VERSUS NONESSENTIAL AMINO ACIDS**
- V OVERVIEW OF AMINO ACID METABOLISM**
- VI BODY AND DIETARY PROTEIN**
- VII AMMONIA ASSIMILATION BY MAMMALIAN CELLS AND TISSUES**
- VIII TRANSAMINATION REACTIONS**
- IX SYNTHESIS OF NONESSENTIAL AMINO ACIDS FROM METABOLIC INTERMEDIATES**
- X SYNTHESIS OF NONESSENTIAL AMINO ACIDS FROM ESSENTIAL INTERMEDIATES**
- XI AMINO ACID CATABOLISM**
- XII SYNTHESIS OF AMINO ACID DERIVATIVES**

## **OUTLINE**

### **I OVERVIEW OF NITROGEN METABOLISM**

The next eight lectures will cover nitrogen metabolism. Specifically, we will cover the pathways for:

1. amino acid metabolism
2. porphyrin and heme metabolism
3. purine and pyrimidine metabolism

The first and third of these groups of low molecular weight compounds have the unique property of serving as monomers which can undergo polymerization reactions to form molecules which are informational, i.e. nucleic acids and proteins. In this section, we will devote ourselves exclusively to the metabolism of the monomeric precursors of these macromolecules, as well as to the metabolism of heme.

The pathways by which humans synthesize nitrogen containing compounds are numerous and complex. Rather than attempt to memorize these pathways in detail, metabolic principles will be emphasized. Useable nitrogen compounds are rather scarce in the environment and are used economically. Thus, the metabolic fluxes through the amino acid and nucleotide pathways are much less than those through the carbohydrate or lipid pathways. However, the syntheses of these

compounds are tightly regulated, since adequate and balanced amounts of these compounds must be made available to the macromolecular machinery of the cell. The regulation of these pathways will be considered in detail.

Since these monomers exist within cells they must interact with other cellular molecules as well. Indeed, most of the nitrogenous compounds possess carbon skeletons which are generated from and/or are interconvertible with these other cellular molecules. Particular emphasis on understanding these interactions and interconversions will be placed in these lectures.

There is a vast quantity of new material to be learned in these lectures, much of which has great scientific and clinical significance. Genetic (inborn errors of metabolism), pharmacologic, and dietary aberrations of amino acid, purine and pyrimidine, and porphyrin metabolism are common clinical entities, and the consequences of some of these metabolic abnormalities will be discussed. A fundamental understanding of the biochemical bases for the pathogenesis of these disease states will be valuable.

## **II THE NITROGEN CYCLE**

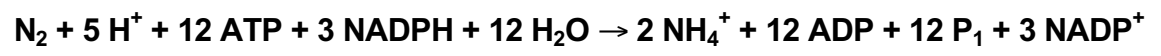
### **A. NITROGEN CYCLE**

1. Key step is nitrogen fixation reaction
2. Portions of nitrogen cycle occur in microbes and plants
3. Nitrogen cycle does not occur in humans.

### **B. NITROGEN FIXATION**

1. Catalyzed by nitrogenase complex that occurs *Rhizobium* bacteria and blue-green algae

### **C. NITROGENASE**



#### D. NITRIFICATION / DENITRIFICATION

1. Nitrification is oxidation of ammonia to nitrite and nitrate.
2. Denitrification is reduction of nitrate to nitrite and ammonia.

### III FUNCTIONS OF AMINO ACIDS

- A. Amino acids serve as the monomeric precursors for peptide, polypeptide, and protein synthesis.
- B. Amino acids are valuable energy substrates.
- C. Amino acids are the precursors in the biosynthesis of a variety of specialized products. Among them are:
  1. Purines and pyrimidines
  2. Porphyrins and hemes
  3. Neurotransmitters
  4. Hormones
  5. Pigments
  6. Creatine
  7. Polyamines
  8. Glutathione
  9. Antibiotics
  10. Aminosugars
  11. Nitric Oxide

### IV ESSENTIAL VERSUS NONESSENTIAL AMINO ACIDS

Humans require certain amino acids in their diets. Those amino acids which humans can synthesize from building blocks are termed nonessential amino acids. These amino acids are synthesized from precursors provided from the glycolytic pathway and TCA cycle. Those amino acids which are required in the diet are designated essential amino acids. A list of the ten nonessential and essential amino acids is provided below.

<u>Essential</u>	<u>Nonessential</u>
Arginine*	Alanine
Histidine	Aspartic acid
Isoleucine	Asparagine
Leucine	Cysteine**
Lysine	Glutamic acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine**

\* Essential for growing mammals but not for adults.

\*\* Derived from essential amino acids. Tyrosine is derived from phenylalanine, cysteine from methionine.

Given an abundance of other amino acids, the  $\alpha$ -keto acids corresponding to the nutritionally essential amino acids can substitute for these amino acids in the diet. Thus, the classification of amino acids as essential or nonessential refers to the capacity of the organism to synthesize the carbon skeletons of these amino acids and is not due to an inability to incorporate nitrogen.

## **V OVERVIEW OF AMINO ACID METABOLISM**

Amino acids can be generated from the turnover of body protein, from the breakdown of dietary protein (digestion), and from glycolytic or TCA intermediates. In turn, amino acids can be resynthesized into body protein, converted to a variety of non-protein derivatives, or converted to utilizable energy substrates. This is diagrammed schematically on the next page.

## **VI BODY AND DIETARY PROTEIN**

### **A. TURNOVER OF BODY PROTEIN**

1. 1-2 % of total body protein is degraded to amino acids per day.
2. 75-80 % of catabolized amino acids are reutilized.
3. 1 g N  $\cong$  6.25 g protein.

## **B. DIETARY PROTEIN**

1. Dietary protein is required to replenish excess catabolized amino acids
2. Kwashiorkor - malignant malnutrition disease caused by dietary deficiency of protein intake
3. Marasmus - disease caused by prolonged dietary deficiency of both protein and calories
4. Trypsinogen deficiency - attributed to a failure to digest intact protein
5. Pancreatitis - pancreatic inflammation caused by premature activation of pancreatic enzymes.

## **C. AMINO ACID TRANSPORT**

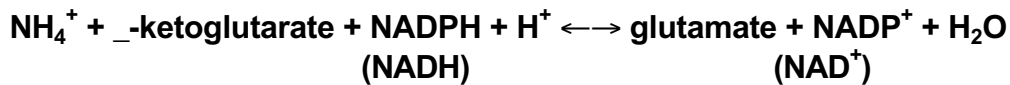
The process of amino acid transport from the intestinal ileum into the intestinal cells and from the blood plasma into tissues is mediated by several relatively specific ATP-dependent and sodium-dependent permeases, one each for basic, neutral, and acidic amino acids, and one for proline and imino acids.

1. Cystinuria - relatively insoluble cystine stones form in the kidney due to a genetic deficiency in basic amino acid transport
2. Hartnup's Disease - a relatively benign condition caused by a genetic deficiency in neutral amino acid transport

## VII AMMONIA ASSIMILATION BY MAMMALIAN CELLS AND TISSUES

Ammonia can be covalently incorporated into metabolites by four separate reactions. These reactions involve the formation of glutamic acid, glutamine, asparagine, and carbamoyl phosphate. Glutamic acid and glutamine play extremely important roles in the assimilation of nitrogen into other molecules in mammalian cells and tissues. The  $\alpha$ -amino group in other amino acids can originate from the  $\alpha$ -amino moiety of glutamate by transamination reactions (see below) while the amido group of glutamine participates (as we will see later) in many biologically important synthetic reactions, e.g. biosynthesis of purines, pyrimidines.

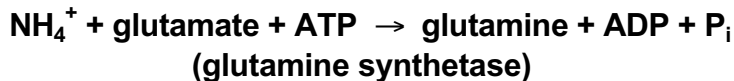
### A. GLUTAMATE DEHYDROGENASE



(glutamate dehydrogenase)

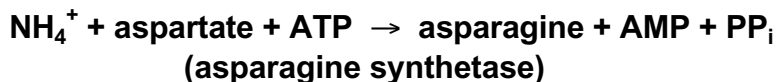
Glutamate is a nitrogen donor in transamination reactions.

### B. GLUTAMINE SYNTHETASE



Ammonium ion is incorporated into glutamine by the action of glutamine synthetase. The amidation reaction requires glutamate as the substrate and ATP

### C. ASPARAGINE SYNTHETASE



Catalyzes the synthesis of asparagine from aspartate

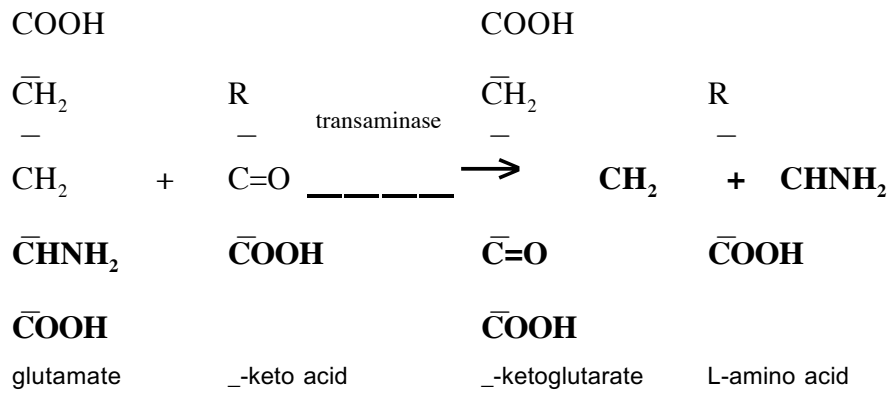
### D. CARBAMOYL PHOSPHATE SYNTHETASE



Initial step in the excretion of excess nitrogen - a component of the urea cycle

### VIII TRANSAMINATION REACTIONS

The transfer of amino groups to the carbon skeletons of virtually all amino acids is carried out by enzymes termed transaminases, also referred to as aminotransferases. These are thermodynamically reversible reactions with a  $\Delta G^\circ = 0$  and  $K_{eq} = 1$ . Thus, they operate in both directions.



#### A. GLUTAMATE-OXALOACETATE TRANSAMINASE AND GLUTAMATE-PYRUVATE TRANSAMINASE

1. These two transaminases, i.e. GOT and GPT, are clinically important enzymes that are useful for diagnosis of liver ailments, such as hepatitis or cirrhosis, and for the detection of myocardial infarction.
2. Glutamate-oxaloacetate transaminase = aspartate aminotransferase
3. Glutamate-pyruvate transaminase = alanine aminotransferase

## B. PYRIDOXAL PHOSPHATE

Pyridoxal phosphate, a derivative of vitamin B<sub>6</sub>, is a required prosthetic group for all the amino acid transaminases. Vitamin B<sub>6</sub> is a composite of three compounds; pyridoxine, pyridoxal, and pyridoxamine. Pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP) are the prosthetic groups and serve as electrophilic catalysts and electron sinks in transamination reactions, as well as in a myriad of other enzymatic reactions.

### 1. Schiff Base

The PLP coenzyme binds to appropriate enzymes via a Schiff base linkage between its aldehyde group and the  $\epsilon$ - amino group of a lysine residue in the active site.

## **IX SYNTHESIS OF NONESSENTIAL AMINO ACIDS FROM METABOLIC INTERMEDIATES**

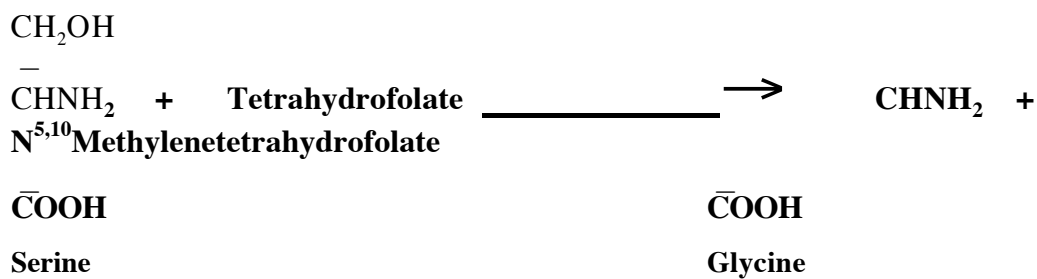
The pathways by which the strictly nonessential amino acids are synthesized are relatively simple. **Their carbon skeletons are derived either from glycolytic or TCA cycle intermediates** as indicated below.

## A. SERINE

1. Serine is generated from 3-phosphoglycerate by two independent pathways, each of which consists of the same 3 enzymes. The difference in the two pathways is in the metabolic sequence.

## B. GLYCINE

1. Glycine is formed from serine and tetrahydrofolate, the latter a carrier of one-carbon compounds and a derivative of the water-soluble B vitamin, folic acid (folate). Reaction is catalyzed by serine transhydroxymethylase.





## 2. Folate and Tetrahydrofolate

- a. Folate is a vitamin, while tetrahydrofolate is the active derivative.
- b. Tetrahydrofolate is a carrier of one-carbon moieties
- c. Folate synthesis in bacteria is targeted by sulfanilamides, which are used in combatting bacterial infections

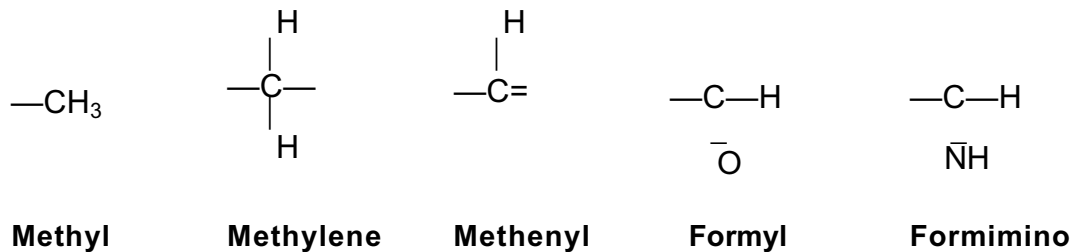
## 3. Dihydrofolate reductase

- a. Site of action of methotrexate, an anticancer agent, and trimethoprim and pyrimethamine, two antimicrobials. Both trimethoprim and pyrimethamine are utilized in conjunction with sulfa drugs.

#### 4. Other Tetrahydrofolate Derivatives

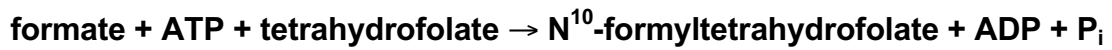
The one carbon fragment carried by tetrahydrofolate is bonded either to the N<sup>5</sup> or N<sup>10</sup> nitrogen. The one carbon fragment carried on tetrahydrofolate can be found in several forms including methyl (-CH<sub>3</sub>), methylene (-CH<sub>2</sub>-), methenyl (-CH=), formyl (-CHO), or formimino (-CH=NH) groups, and all oxidation states are metabolically interconvertible as shown below and on the next page.

#### The 1-carbon groups transferred by enzymes requiring tetrahydrofolate





- a. 5-formyltetrahydrofolate (also known as folinic acid, leukovorin, or citrovorim rescue factor) is a stable tetrahydrofolate derivative that is used in chemotherapeutic protocols to circumvent the toxicity of high dose methotrexate or in conjunction with 5-fluorouracil to treat a variety of solid tumors.
- b. Tetrahydrofolate derivatives are interconvertible.
- c. The one carbon fragments can be attached to tetrahydrofolate described earlier either by the serine transhydroxymethylase reaction or by direct incorporation of formate into N<sup>10</sup>-formyltetrahydrofolate.

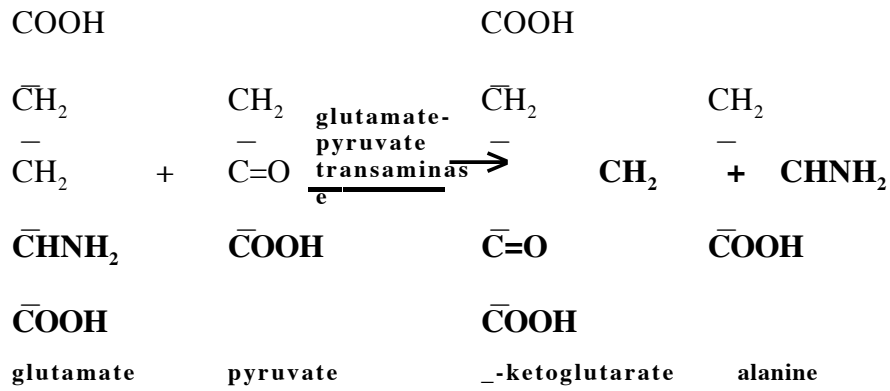


### c. CYSTEINE

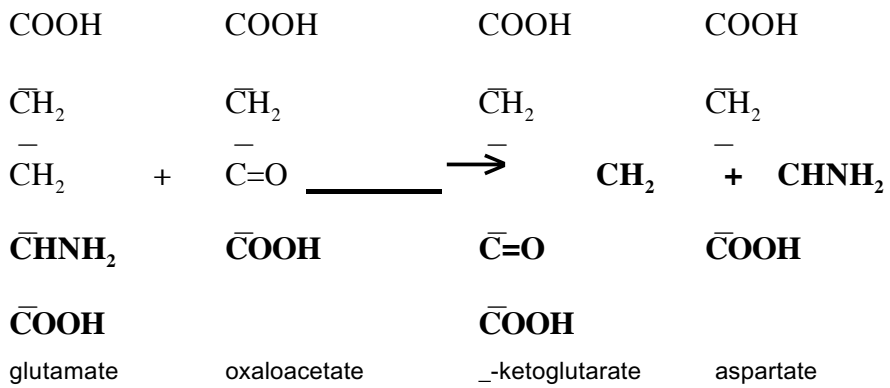
1. Homocystinuria - deficiency in either cystathionine synthase or methionine synthase. Can be either vitamin B<sub>6</sub> or vitamin B<sub>12</sub>/folate responsive.

2. Cystathioninuria - deficiency in cystathionine lyase. Can be vitamin B<sub>6</sub> responsive.

**D. ALANINE**



**E. ASPARTIC ACID**



**F. ASPARAGINE**

**G. GLUTAMIC ACID**

**H. GLUTAMINE**

## **I. PROLINE**

## **J. ARGININE**

Formed as essential intermediate in urea cycle

## X SYNTHESIS OF NONESSENTIAL AMINO ACIDS FROM ESSENTIAL INTERMEDIATES

There are two nonessential amino acids which are generated from essential amino acids. Cysteine is formed from methionine (and serine) by a multienzyme pathway presented above, whereas tyrosine is synthesized from phenylalanine, a reaction catalyzed by phenylalanine hydroxylase. Phenylalanine uses molecular O<sub>2</sub> and requires tetrahydrobiopterin as the electron donor. Dihydrobiopterin is then reconverted to tetrahydrobiopterin by dihydropteridine reductase.

### 1. Phenylketonuria

A frequently occurring inborn error of metabolism is associated with a genetic absence of either phenylalanine hydroxylase (less severe) or dihydropteridine reductase (more severe).

This disease is called phenylketonuria, or PKU, and is characterized by the presence of elevated levels of neurotoxic phenylalanine derivatives.

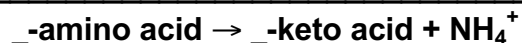
## XI AMINO ACID CATABOLISM

Under conditions of amino acid surplus or during conditions of fasting or diabetes mellitus when carbohydrates are unavailable or unusable, and amino acids are oxidatively degraded and used as metabolic fuel.

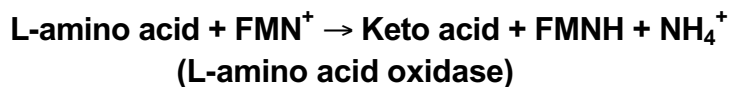
### A. OXIDATIVE REMOVAL OF AMINO GROUPS

#### 1. Glutamate dehydrogenase and transaminases

The most important mechanism for amino acid degradation occurs in the liver. The  $\alpha$ -amino group is removed from most, but not all, amino acids by transamination and transferred to  $\alpha$ -ketoglutarate to form glutamate.



#### 2. Amino acid oxidase - a secondary pathway

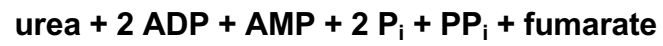
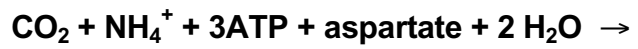


### B. UREA CYCLE

Ammonia is very toxic to the central nervous system. Humans and higher vertebrates have the capacity to convert toxic ammonia to the relatively nontoxic nitrogenous waste produce urea, a highly soluble compound.

Urea is synthesized in the liver by the enzymes of the urea cycle.

The overall chemical equation of the urea cycle is as follows:



## 1. Inborn errors of nitrogen metabolism and hyperammonemia

Inherited disorders associated with partial to virtually complete deficiencies in all five of the urea cycle enzyme activities have been discovered. The common clinical finding in these disorders is elevated ammonium ion levels in the blood stream (hyperammonemia). Ammonia is neurotoxic.

## C. FATES OF CARBON SKELETONS OF AMINO ACIDS

The pathways for amino acid catabolism are long and complex and will not be considered in great detail. The overall strategy of amino acid degradation is to convert amino acids into metabolic fuels. All the amino acid catabolic pathways converge to form seven compounds, acetyl-CoA, acetoacetyl-CoA, pyruvate, oxaloacetate, fumarate, succinyl-CoA, and  $\alpha$ -ketoglutarate. Amino acids that are degraded to either acetyl-CoA or acetoacetyl-CoA are called ketogenic, because they are precursors for the synthesis of ketone bodies and cannot be utilized to form net glucose. The amino acids that are degraded into either a TCA cycle intermediate or pyruvate are called glucogenic, because net synthesis of glucose can occur. Only lysine and leucine are purely ketogenic. Isoleucine, phenylalanine, threonine, tyrosine, and tryptophan are both ketogenic and glucogenic, and the remaining 13 amino acids can be considered to be purely glucogenic.

1. Alkaptonuria - inborn error of tyrosine degradation
2. Maple syrup urine disease - defect in branch chain amino acid catabolism

## **XII SYNTHESIS OF AMINO ACID DERIVATIVES**

A.  $\gamma$ -amino butyric acid (GABA) - an inhibitory neurotransmitter

B. **HISTAMINE - A VASODILATOR**

C. **SEROTONIN - A POTENT VASOCONSTRICTOR AND STIMULATOR OF SMOOTH MUSCLE CONTRACTION**

**D. CATECHOLAMINES - DOPAMINE, NOREPINEPHRINE, AND EPINEPHRINE**

1. Catecholamines are neurotransmitters and hormones.
2. L-Dopa is used to treat Parkinson's disease.

## **E. CREATINE PHOSPHATE AND CREATININE**

1. Creatine phosphate is a muscle energy store.
2. Creatinine is a muscle waste product that is excreted in urine in direct proportion to body muscle mass - clinically useful.

**F. S-ADENOSYLMETHIONINE (SAM)**

1. SAM is the universal methyl donor
2. SAM synthesis is energetically expensive and requires expenditure of three high energy phosphate bonds per molecule

## **G. POLYAMINES - PUTRESCINE, SPERMIDINE, AND SPERMINE**

1. Polyamines are cationic molecules with unknown but essential biochemical functions.
2. Ornithine decarboxylase is a rate-limiting step.
3. Ornithine decarboxylase and S-adenosylmethionine decarboxylase have been targeted in antiparasitic chemotherapies.

## **H. NITRIC OXIDE**

1. A short-lived chemical signal molecule.
2. Synthesized from arginine