



Phenylketonuria

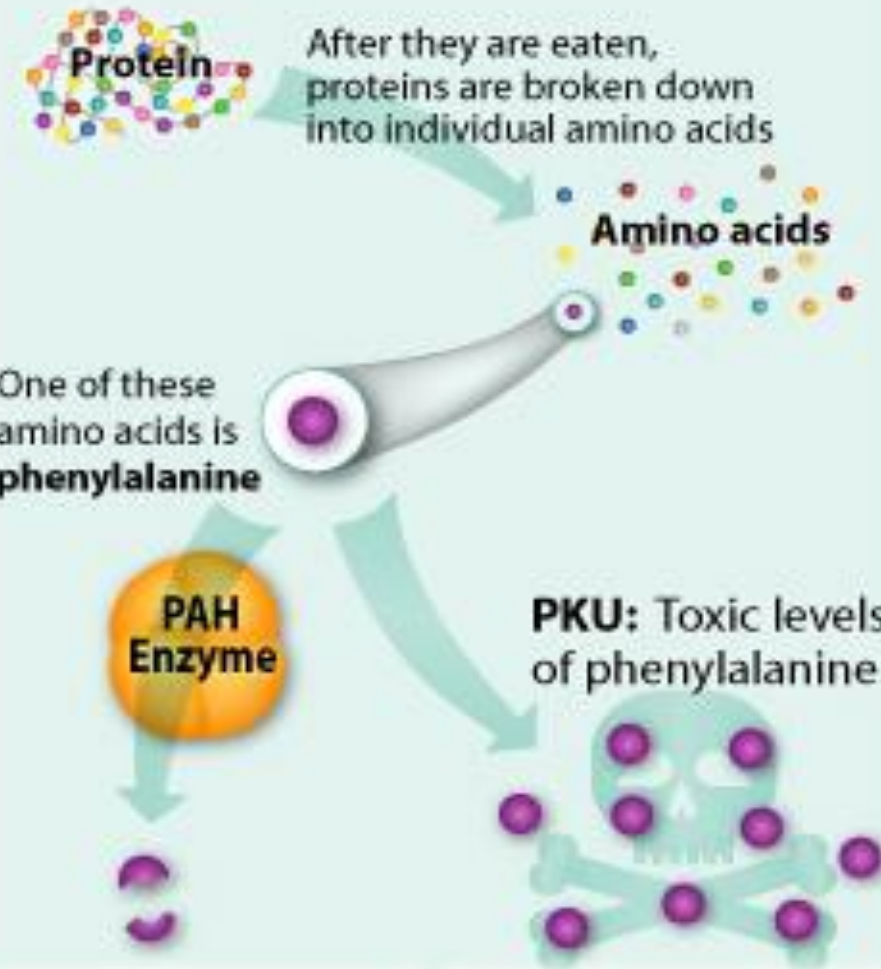
Norwegian doctor Asbjørn Følling (left) discovered phenylpyruvica (later termed phenylketonuria) in 1934 upon discovering that ten mentally retarded patients had phenylpyruvic acid in their urine. The disease was described as an “inborn error of metabolism.” Phenylketonuria was the first inborn error of metabolism proven to affect the mind.

What is phenylketonuria?

Phenylketonuria (PKU) is an **autosomal** recessive genetic disorder that increases the levels of a the amino acid ;phenylalanine in the blood. the body can't metabolize phenylalanine .

phenylalanine is in almost all foods. If the phenylalanine level gets too high, it can **damage** the **brain** and cause severe intellectual disability. All new born babies must now have a screening test for PKU. This makes it easier to diagnose and treat the problem early

People with PKU Have a Defective PAH Enzyme



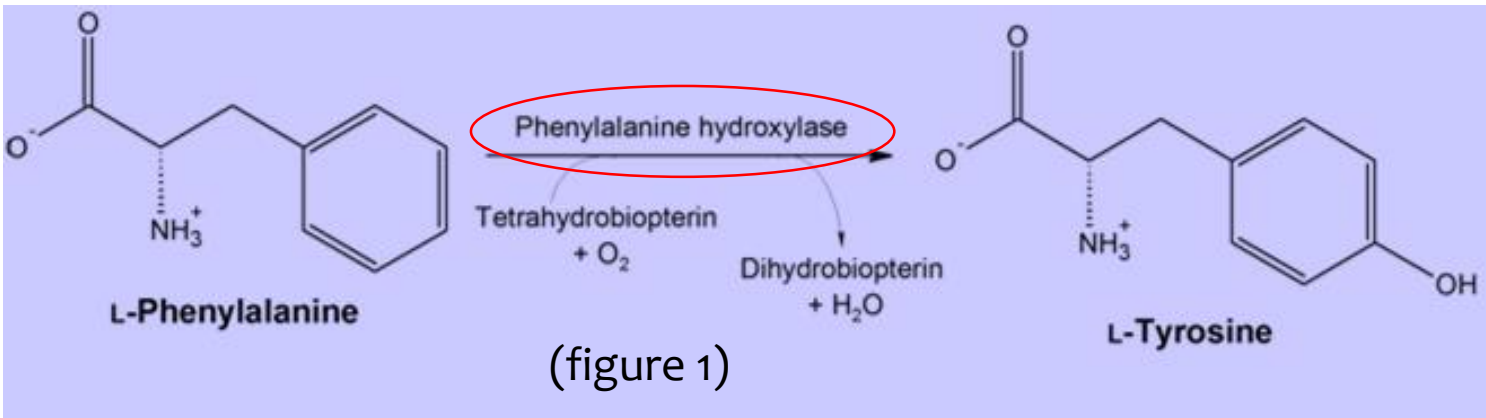
Normally, the PAH enzyme breaks down phenylalanine

People with PKU have a defective PAH enzyme, so toxic levels of phenalalanine build up in their bodies.

Is PKU inherited?

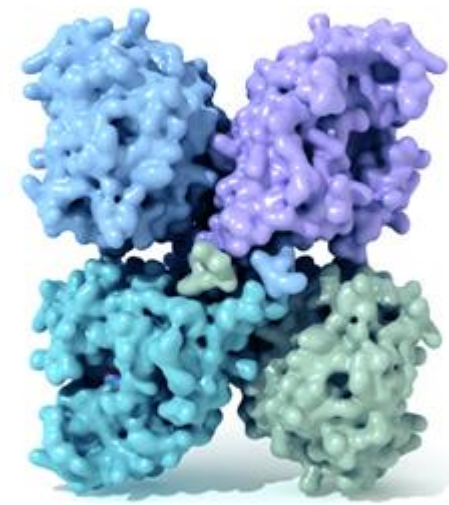
characterized by a mutation in the in chromosome 12 for the **hepatic enzyme phenylalanine hydroxylase (PAH)** rendering it nonfunctional^(figure 2). This enzyme is necessary to metabolize phenylalanine to the amino acid tyrosine. When PAH activity is reduced, phenylalanine accumulates and is converted into **phenylpyruvate**

(also known as **phenylketone**), which can be detected in the blood and urine. ^(figure 1)

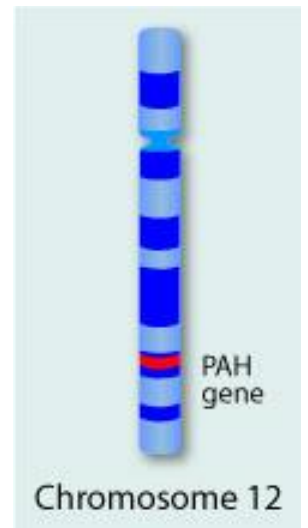


(figure 1)

Phenylalanine hydroxylase



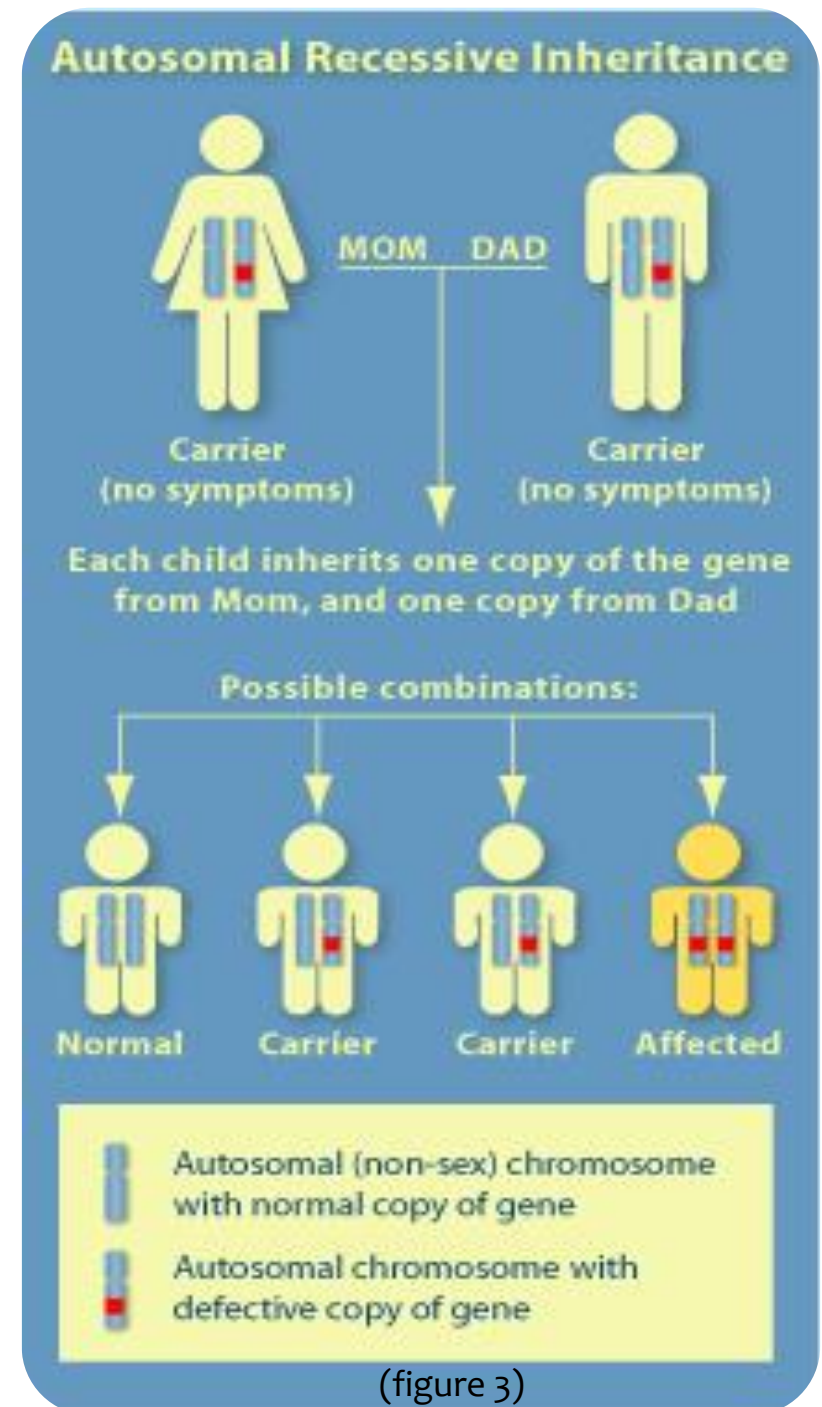
Phenylalanine hydroxylase protein consisting of 4 subunits



Chromosome 12

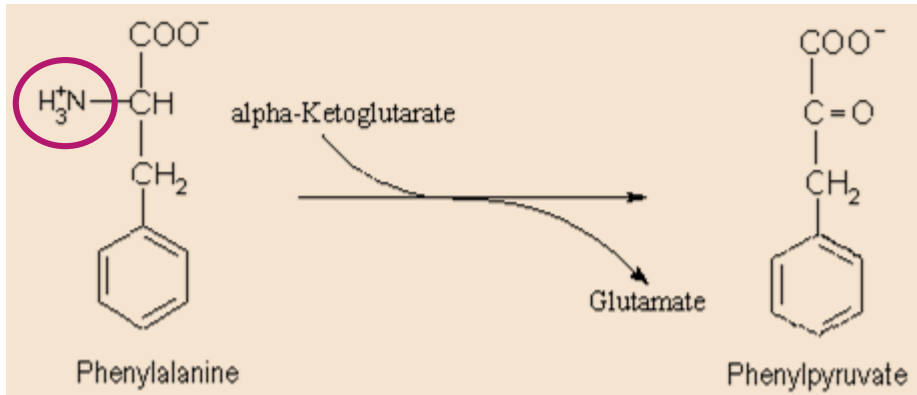
(figure 2)

- The disorder is passed down in a **recessive** pattern, which means that for a child to develop PKU, both parents have to contribute a mutated version of **the PAH gene**.
- If only **one parent carries** the mutated gene, the child **will not** develop PKU.
- Even if **both parents carry** the mutated PAH gene, their child still **may not** develop PKU. This is because a child's parents each carry two versions of the PAH gene, only one of which they will pass on during conception.
- If **both parents are carriers**, there is a **25% chance** that each parent will pass on the normal PAH gene. In this case, the child **will not** have the disorder. Conversely, there also is a **25% chance** that the carrier parents will both pass along the mutated gene, causing the child to **have PKU**.
- However, there is a **50% chance** that a child will inherit one normal gene from one parent and one abnormal one from the other, making the child a **carrier**.(figure 3)

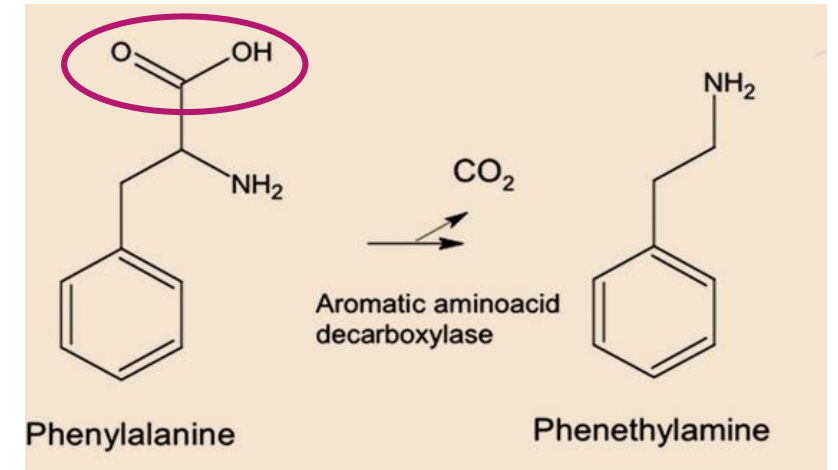


(figure 3)

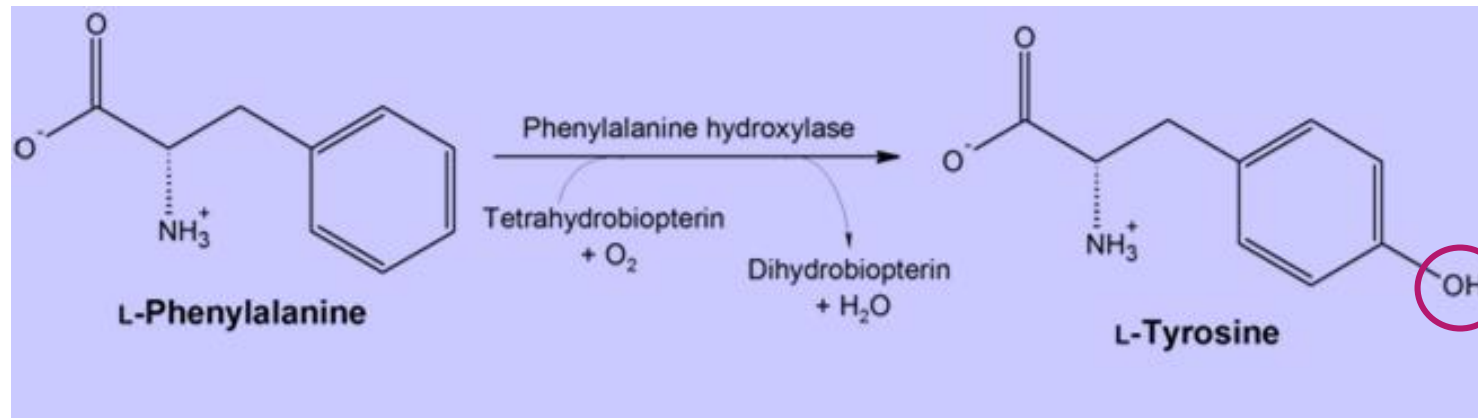
Phenylalanine metabolism pathways



Transamination of phenylalanine



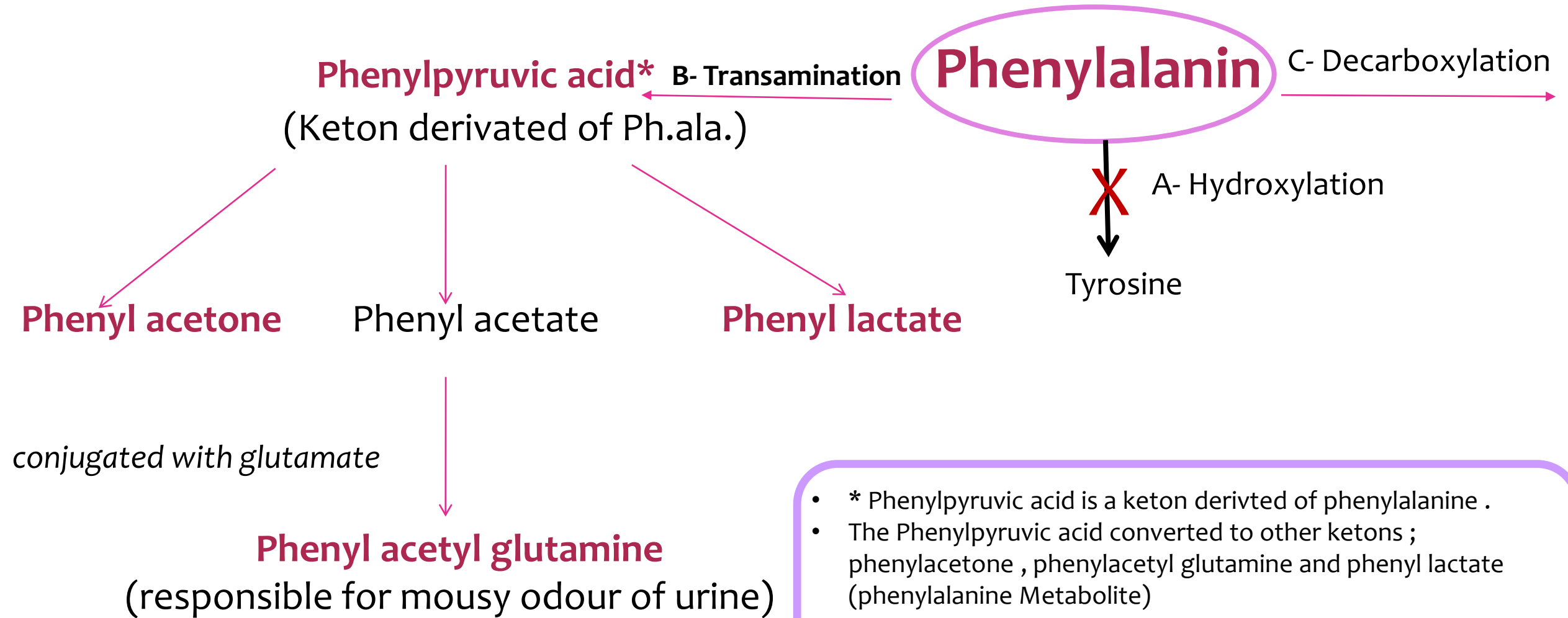
Decarboxylation of phenylalanine



Hydroxylation of phenylalanine

The hydroxylation of phenylalanine is the major pathway while transamination is a minor pathway.

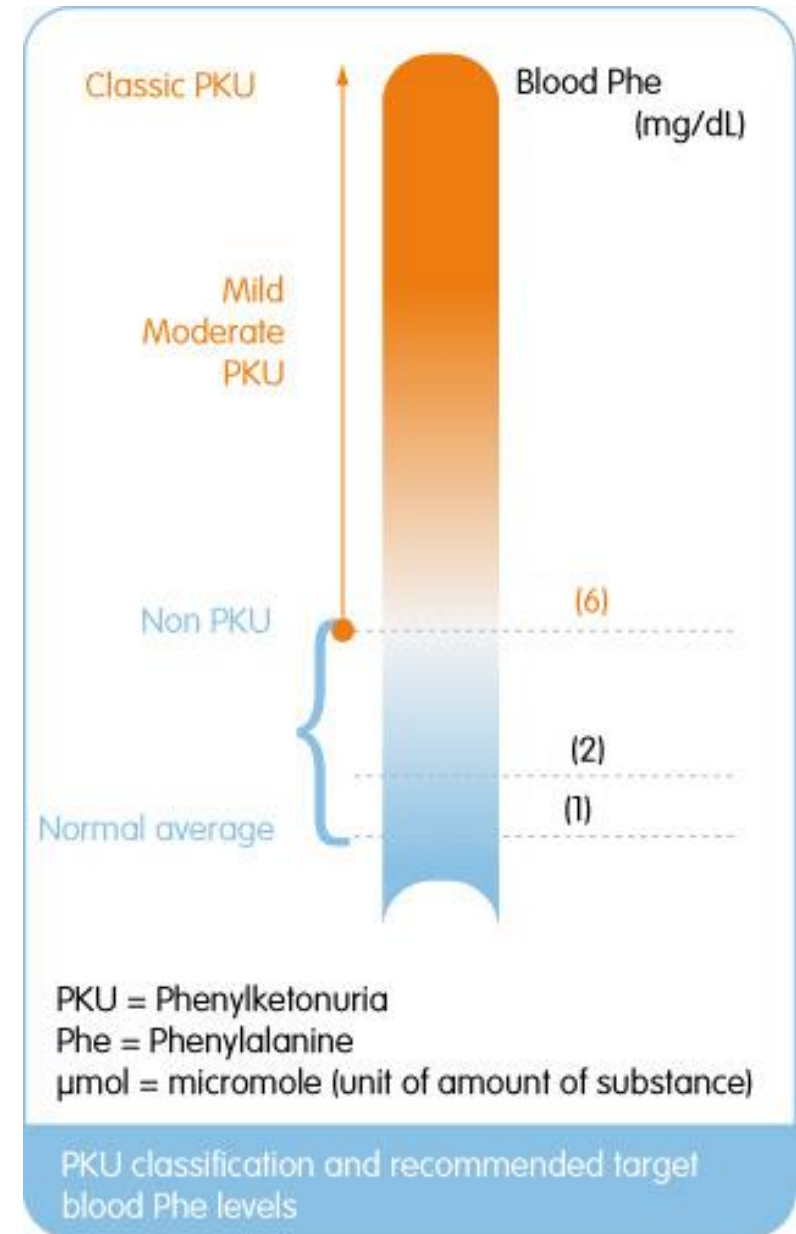
Phenylalanine metabolism pathways



- * Phenylpyruvic acid is a keton derivted of phenylalanine .
- The Phenylpyruvic acid converted to other ketons ; phenylacetone , phenylacetyl glutamine and phenyl lactate (phenylalanine Metabolite)
- In PKU the phenylalanine and its metabolite mainly excreted in urine .

Normal level of phenylalanine

- Normally phenylalanine in blood = 1- 2 mg/dl
- Normally the ratio of **phenylalanine /Tyrosine** = 0.6 -1.5
- In Phenylketonuria (PKU) the level of **phenylalanine** in blood = 15 – 65 mg/dl , also the ration of **phenylalanine /tyrosine** may be reach to 3 or more.



Clinical presentation of PKU :

Infant : they have light skin , hair and eyes

In child :

1- Mentally retardation

2- Hyperactivity

3- Speech problem

The severity of PKU :

excess level of phenylalanine lead to following :

(A)Mental retardation : because the accumulation of ph.ala. Lead to defective of serotonin formation.

(B) High excess of phenylalanine in blood leads to excretion of this amino acid into the intestine. Here it competes with tryptophan for absorption. **Tryptophan** becomes subject to action of intestinal bacteria resulting in formation of **indole** derivatives which are absorbed and excreted in urine.

PKU: the Problem

1 Phe is in most of the foods you eat



2 A defective enzyme (PAH) in the body fails to process the Phe



3 This leads to high Phe levels in the blood



4 This can lead to problems in thinking and behavior



How to diagnose phenylketonuria (PKU)?

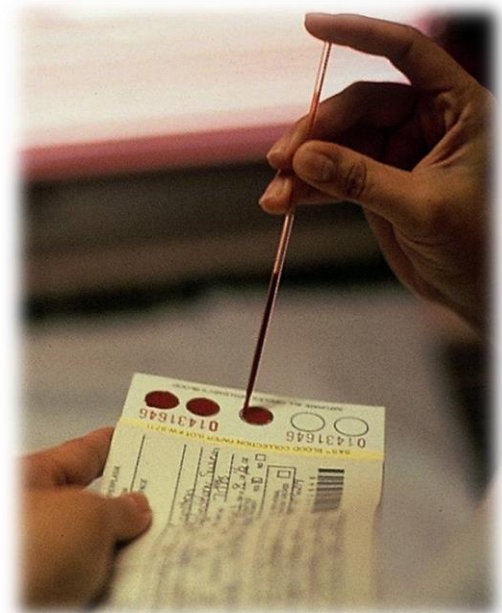
Nearly all cases of PKU are diagnosed through a blood test done on newborns.

Newborn Screening for PKU

- Before screening for PKU was possible, most infants with the disorder developed severe intellectual disabilities. In the 1960s, researchers determined that a test for PKU given to newborns was safe and effective. Since then, PKU has been almost completely eliminated as a cause of intellectual disabilities.

How are newborns tested for PKU?

PKU screening test using a few drops of blood from a newborn's heel. The blood sample, which can be used to screen for other conditions as well, is tested in a laboratory to determine if it has too much phenylalanine in it.



What if a newborn tests positive for PKU?

The child will [need additional tests](#) to confirm that he or she definitely has the disorder. It is very important to follow your health care providers' instructions for further tests. [These tests may be blood or urine tests](#) that may show whether or not the child has PKU. If child does have PKU, getting treatment quickly will help protect your child's health.

health care providers may also suggest [genetic testing](#) to look at the [mutations](#) in genes [that cause PKU](#). This testing is not required to figure out whether your child has PKU, but it will help [identify the specific type of genetic mutation](#) causing the disorder. This information may be useful for determining the best treatment plan going forward.



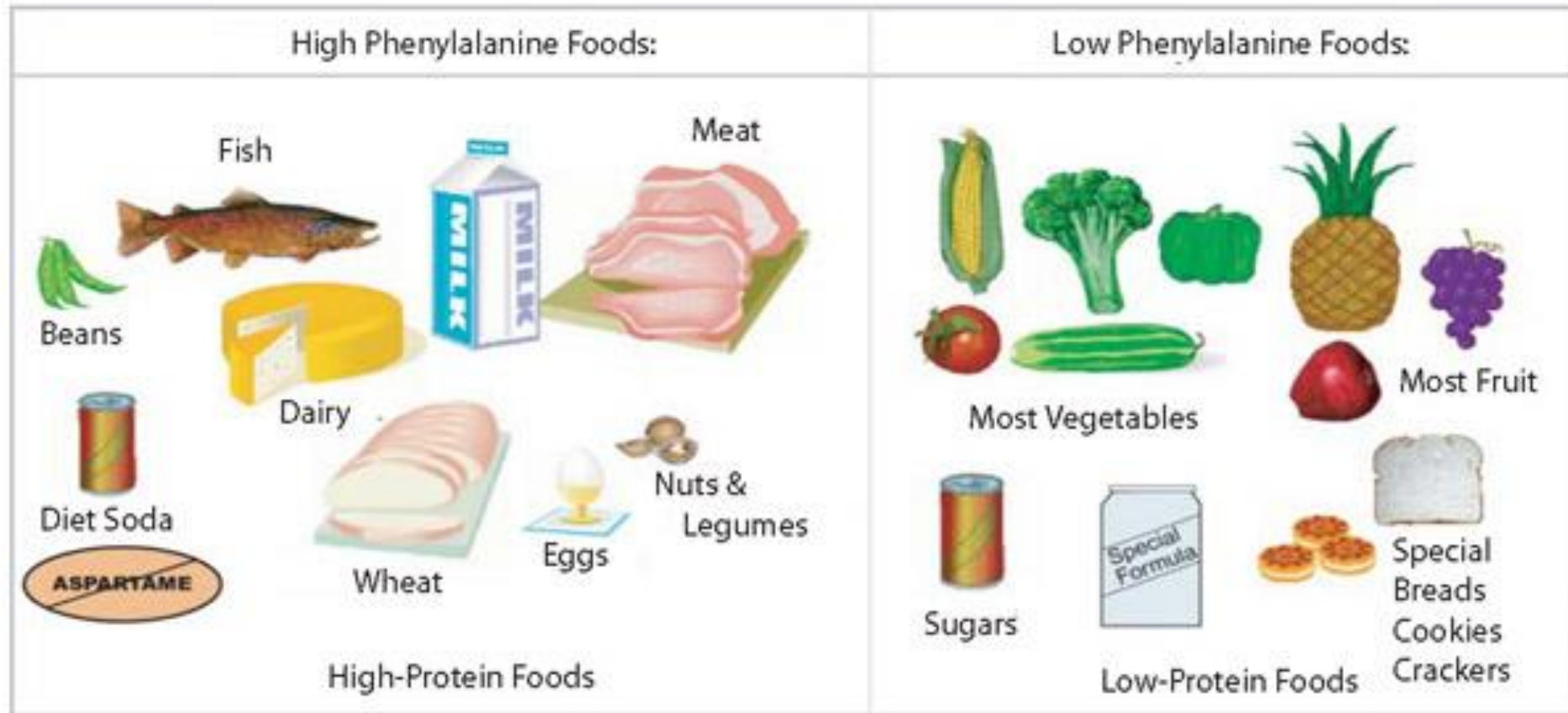
What are treatments for phenylketonuria (PKU)?

- There is no cure for PKU, but treatment can prevent intellectual disabilities and other health problems.

The PKU Diet:

- A newborn with PKU should receive special infant formula. The formula may be mixed with a small amount of breast milk or regular infant formula to make sure the child gets enough phenylalanine for normal development but not enough to cause harm.
- In addition to the formula, fish oil may be recommended to help with fine motor coordination and other aspects of development.
- People with PKU need to follow a diet that limits foods with phenylalanine. The diet should be followed carefully and be started as soon after birth as possible. In the past, experts believed that it was safe for people to stop following the diet as they got older. However, they now recommend that people with PKU stay on the diet throughout their lives for better physical and mental health.

- As long as a person with PKU sticks to a low-protein diet throughout childhood and their phenylalanine levels stay within certain limits, they will remain well and their natural intelligence will be unaffected



Is there any Medication for PKU?

- (FDA) has approved the drug **sapropterin dihydrochloride (Kuvan®)** for the treatment of PKU. Kuvan® is an enzyme cofactor and oral form of tetrahydrobiopterin. **Tetrahydrobiopterin (BH₄)** works with phenylalanine hydroxylase to metabolize phenylalanine .
- **Kuvan®** is specifically indicated to reduce blood phenylalanine levels in patients with hyperphenylalaninemia due to tetrahydrobiopterin- (BH₄-) responsive Phenylketonuria . Kuvan is to be used in conjunction with a Phe-restricted diet

However, Kuvan® only helps some people who **having too little BH₄** .

Therefore, **Kuvan® only helps some people** to reduce the phenylalanine in their blood.

Even if the medication helps, it will not decrease the phenylalanine to the desired amount and must be used together with the PKU diet.

Other Treatments for PKU

researchers are exploring additional treatments for PKU. These treatments include:

- large neutral **amino acid supplementation**, which may help prevent phenylalanine from entering the brain
- **Enzyme replacement** therapy, which uses a substance similar to the enzyme that usually breaks down phenylalanine.
- Researchers are also investigating the possibility of using **gene therapy**, which involves injecting new genes to break down phenylalanine. That would result in the breakdown of phenylalanine and decreased blood phenylalanine levels



Objectives:

Primary diagnosis for Phenylketonuria in different urine sample

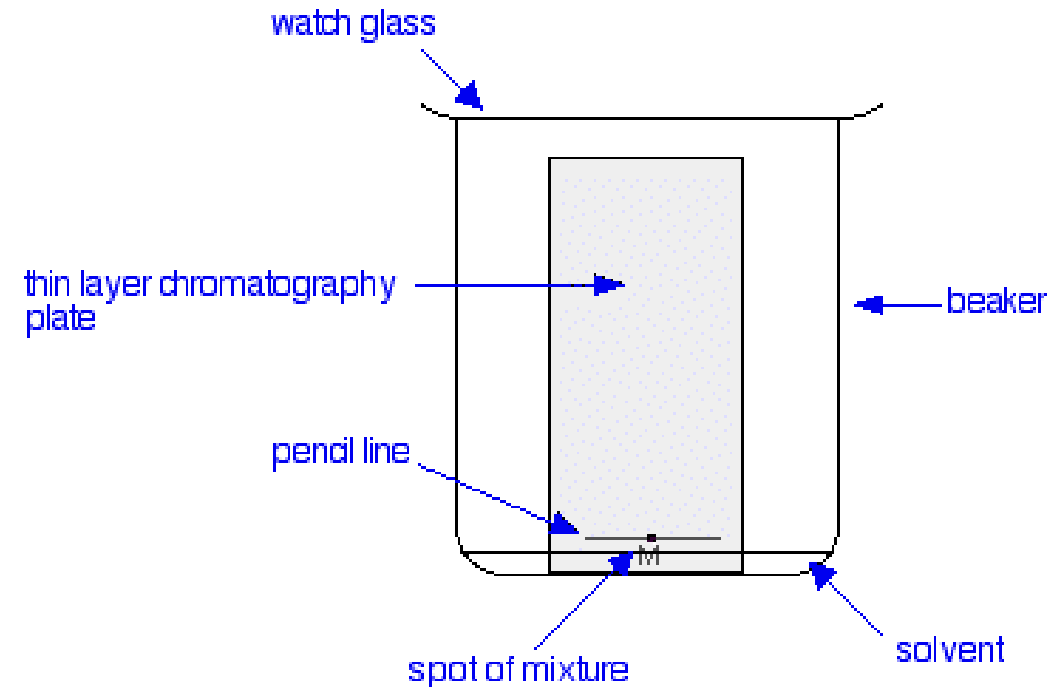
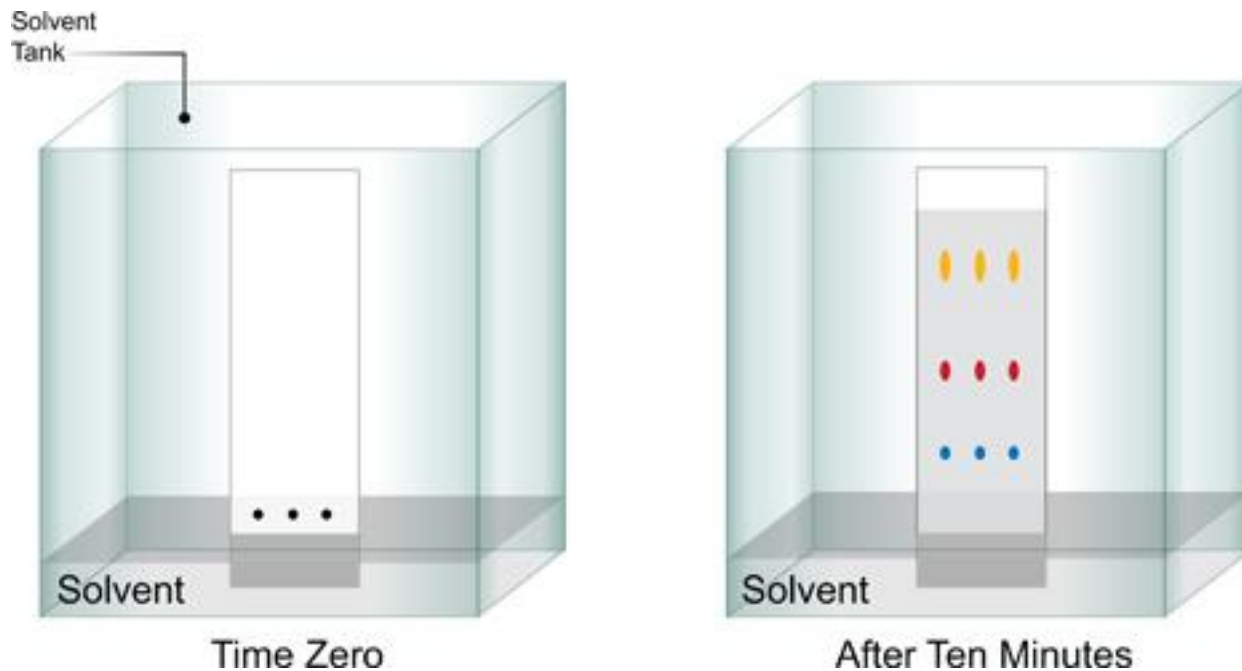
Principle:

Separation of mixture depends on the relative affinity of compounds towards stationary phase and mobile phase using thin layer chromatography.

RF (relative flow) : ratio of the distance moved by compound to that moved by solvent.

Chromatography : a groups of techniques which use in separation of mixture of compounds

Types : Paper , thin layer , Column , Liquid , Gas



Materials:

- Thin layer plates of silica gel
- chamber
- Solvent :
- butanol /acetic acid /H₂O 60/15/25 by volume
- 0.2% ninhydrin in acetone
- Micro-syringe
- Standard phenylalanine solution .
- urine samples

Method:

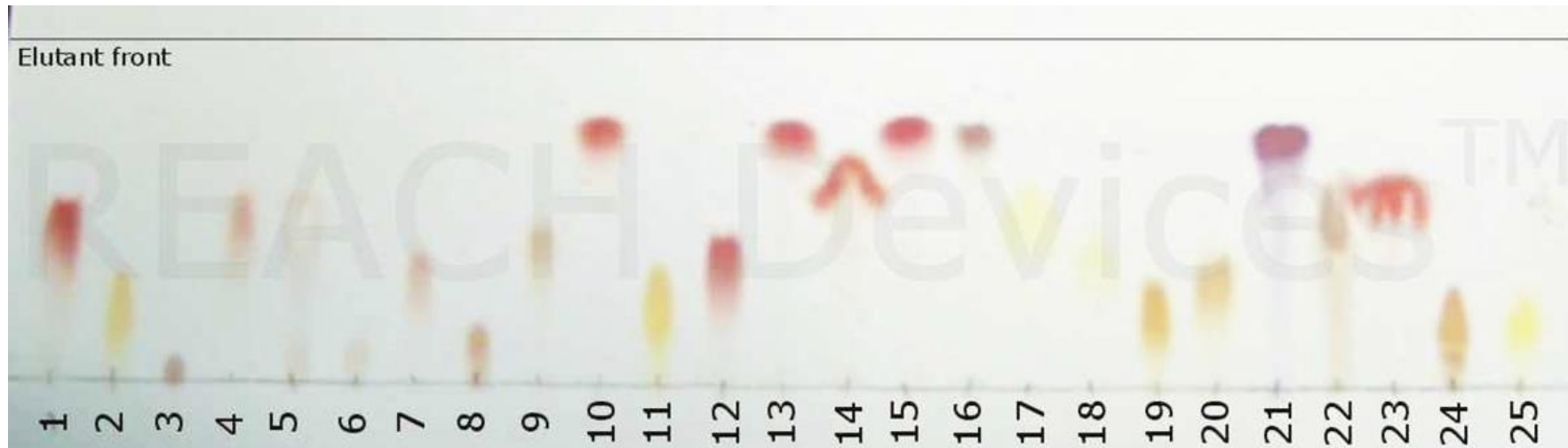
1. You will be provided with a silica gel plate , standard of phenylalanine , and 2 urine samples.
2. With a pencil draw a line about 2 cm . Draw the line gently so as not to break the surface of the gel. At equally spaced intervals mark the line at three different places using the tip of the pencil on the silica gel plate.
3. Carefully spot the standard , urine patient and normal urine with a 5 μ l pipette or spot it by capillary tube .
4. Place the gel coated plate in the chamber.
5. Cover the glass chamber tightly with aluminum foil. The solvent will rise up the silica gel by capillarity.
6. When the solvent front is between 1 and 2 cm from the top of the gel, remove the chromatogram from the chamber and mark the position of the solvent front.
7. Dry the plate in a stream of cold air in the fume cupboard. Locate the spot by spraying the plate with 0.2% ninhydrin and heating the plate briefly at 100°C in an oven.

Results:

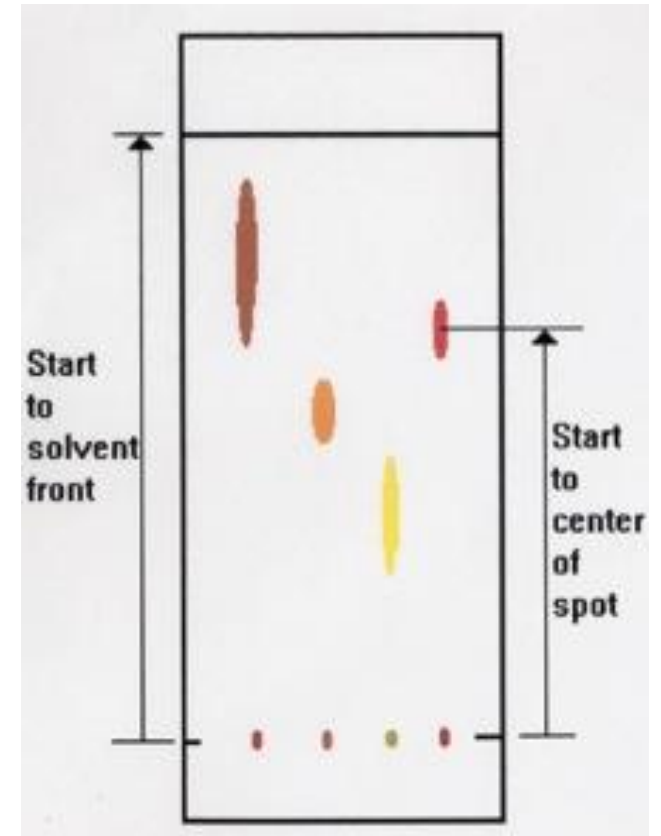
Relative f value is constant for a particular compound, solvent system and insoluble matrix
Calculate the Rf value for each spot .

Relative flow= $\frac{\text{Distance of migration of solute}}{\text{Distance moved by solvent}}$

Sample	Relative flow
standard of phenylalanine	
Urine Sample 1	
Urine Sample 2	



Thin Layer Chromatography of amino acids and short peptides



Discussion:

Measure the Relative flow and discuss your result if there are any abnormalities

Questions:

why should PKU patients avoid aspartame?

References

- <http://epiehonorsbiology.wikispaces.com/Phenylketonuria>
- <http://www.nichd.nih.gov/health/topics/pku/conditioninfo/Pages/causes.aspx>
- 1. Tietz, N. W. (1987). Fundamentals of Clinical Chemistry . W.B. Saunders. Philadelphia. third Edition .
- 2. Chatterjea, MN. and Shinde , R. (2007). Textbook of medical biochemistry. Jaypee Brothers medical publishers (p)LTD. New Delhi
- http://www.reachdevices.com/TLC_ aminoacids.html