
بسم الله الرحمن الرحيم

Laboratory techniques for diagnosis of metabolic disorders through tandem mass spectrometry

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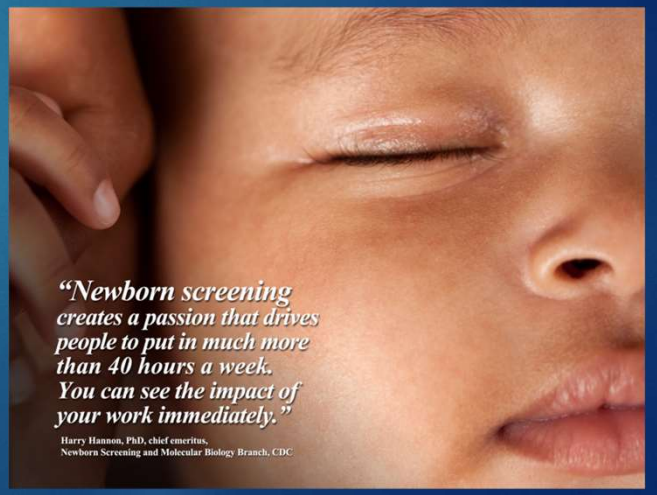
Clinical Biochemistry, Ph.D

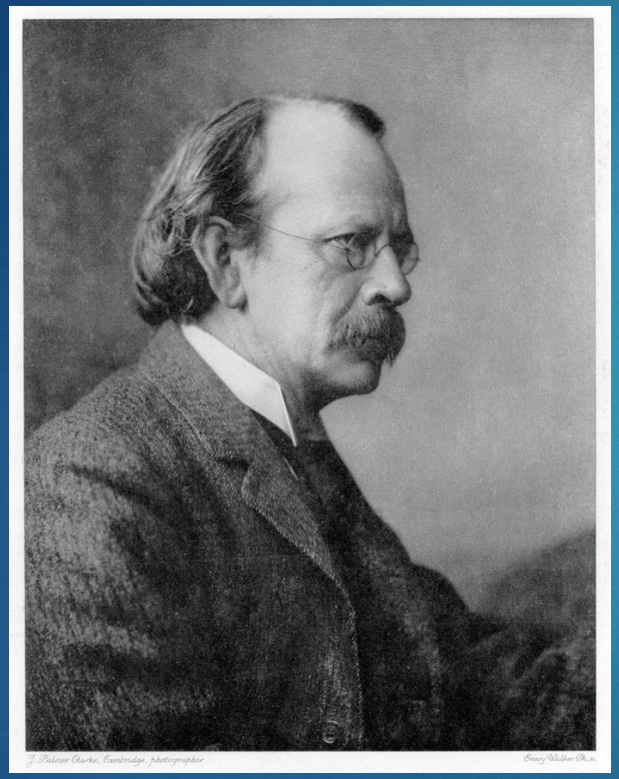


Outline

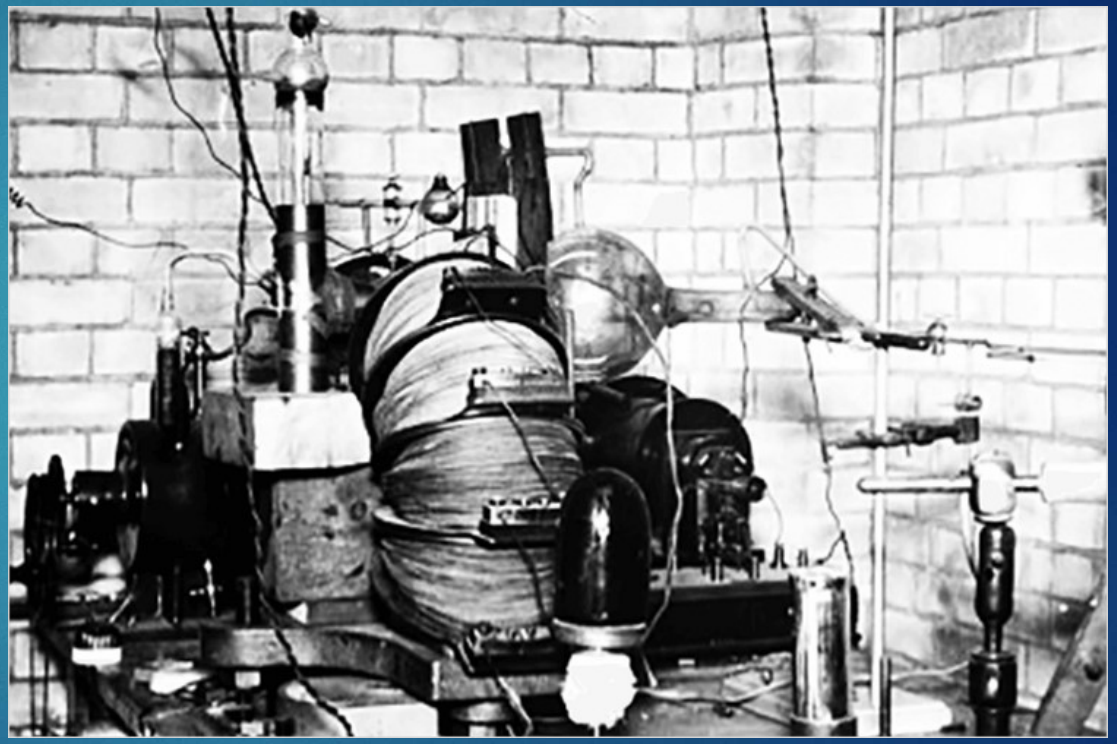
- Basic Mass Spectrometry Concepts & Its Applications In Clinical Lab
- Inborn Errors Of Metabolism
- Newborn Screening for Metabolic Disorders With Mass Spectrometry

Basic Mass Spectrometry Concepts & Its Applications In Clinical Lab

Discovering the electron



Portrait of Thomson



Thomson's positive ray analyzer in the Cavendish Laboratory in Cambridge.

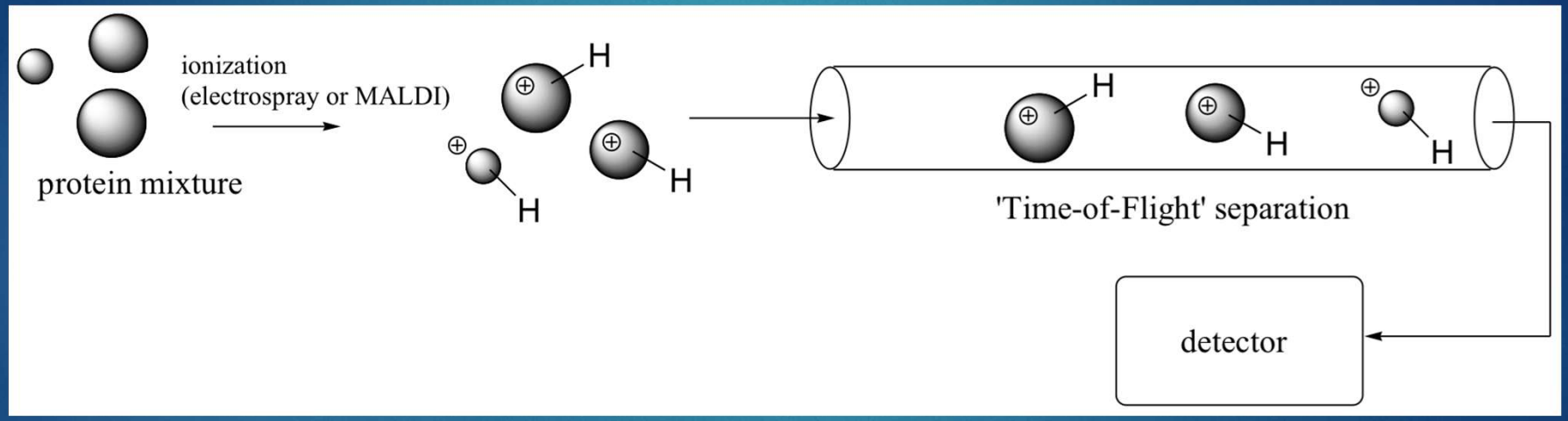
What is a mass spectrometer?

An instrument that essentially weighs molecules

Mass spectrometry application

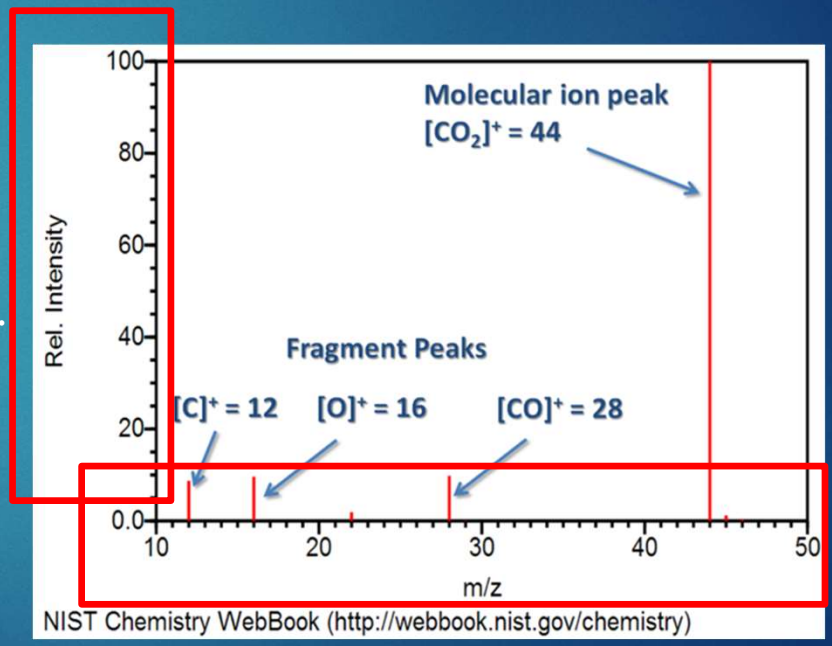
- Identify unknown organic or inorganic compounds
- Determine the structure of complex molecules
- Quantitate extremely low concentrations of known analytes (down to one part in 10^{12})

How to work mass spectrometry?

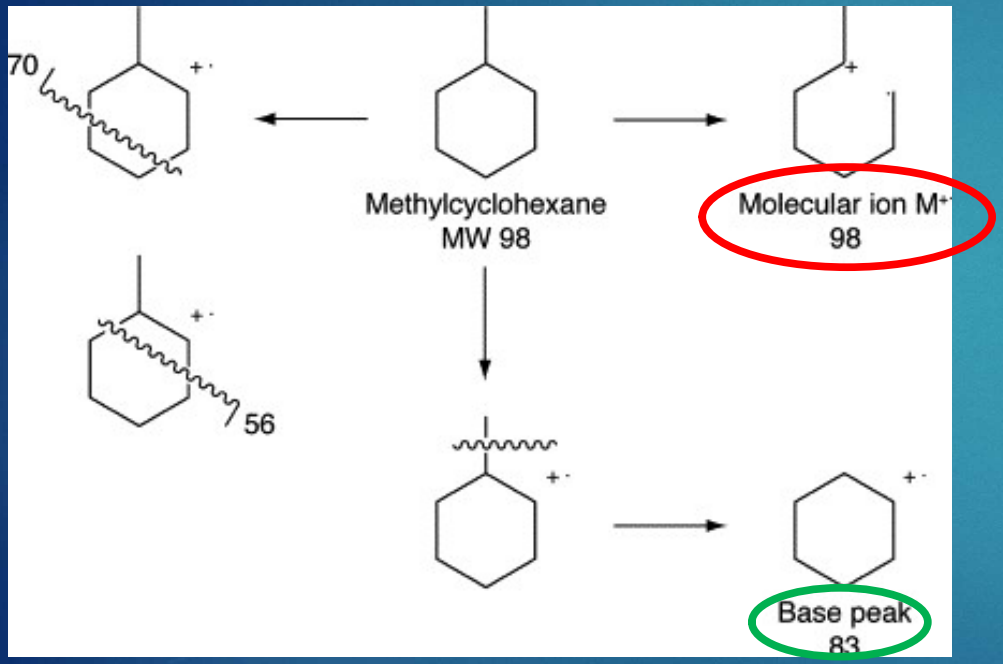


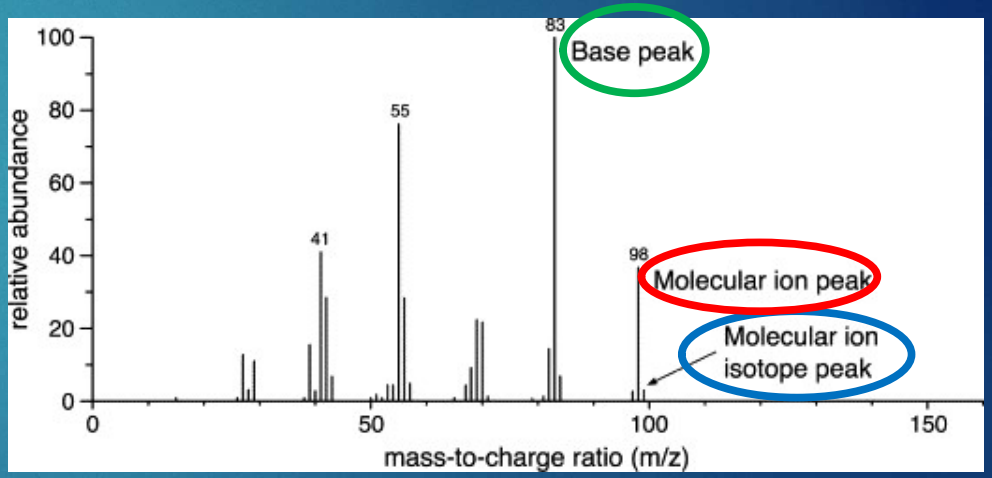
Different terms in mass spectrum

- **Mass spectrum** m/z vs abundance
- **Base peak:** the highest peak or more intense peak in the spectrum.
- **Molecular ion (parent ion, $M^{+\cdot}$):** a positively charged molecule with an unpaired electron.



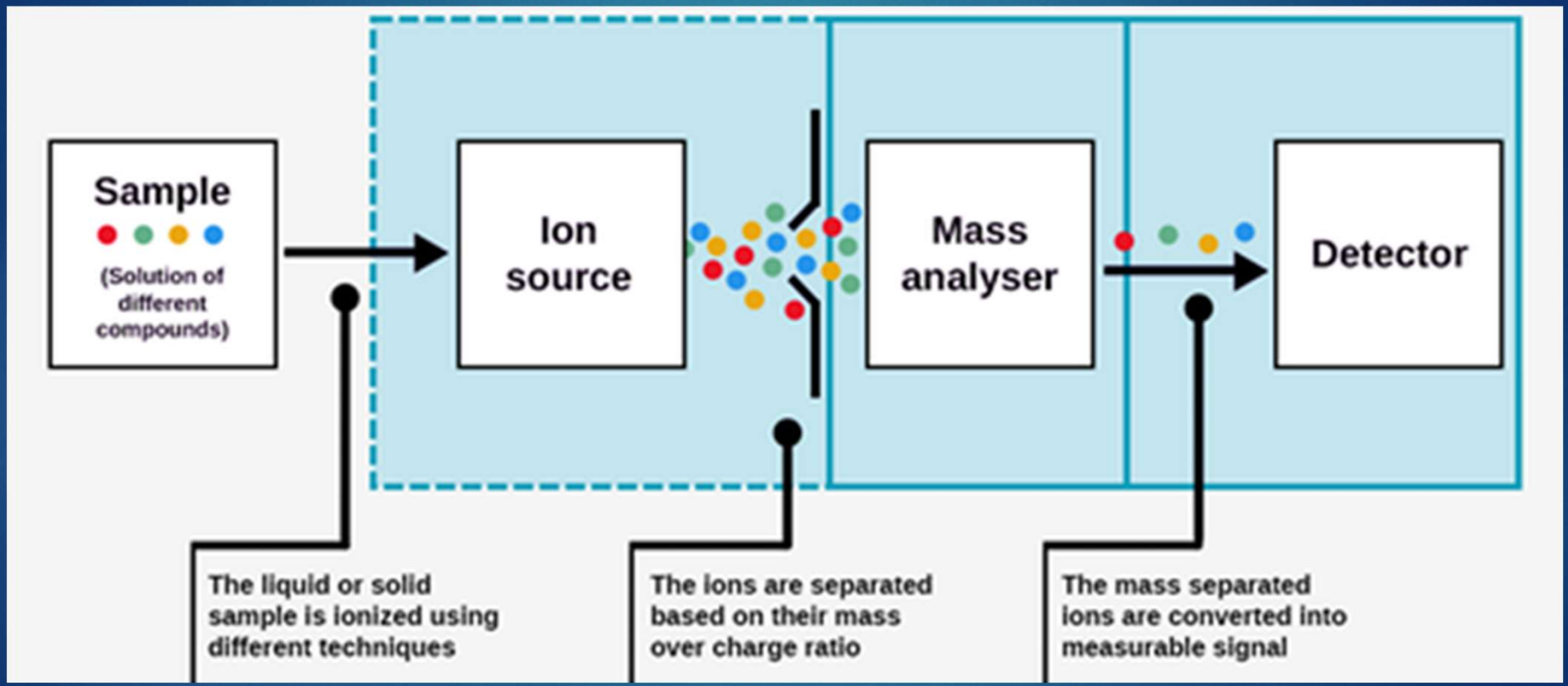
Basic Mass Spectral Interpretation



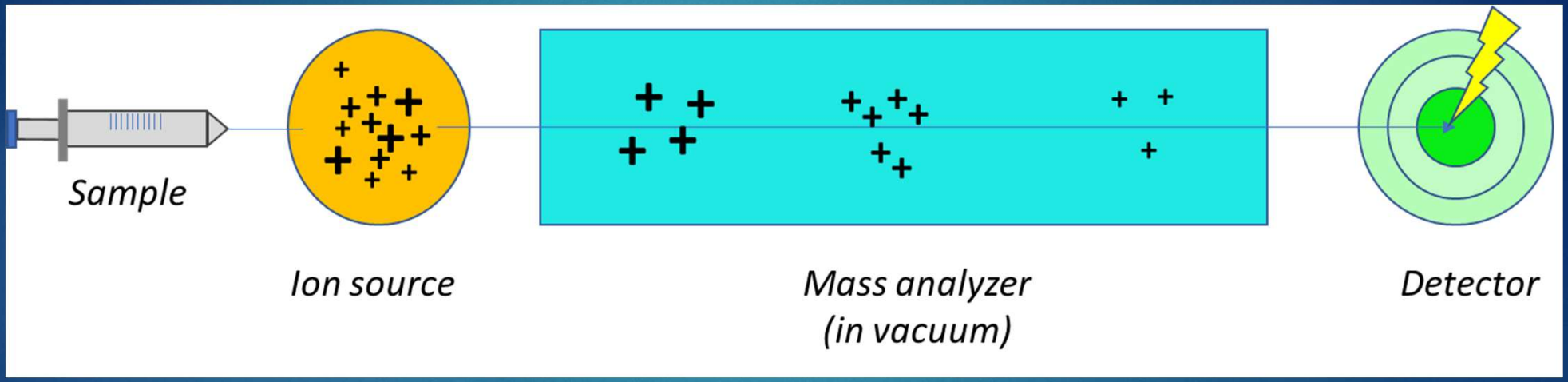


Mass spectrum of methylcyclohexane

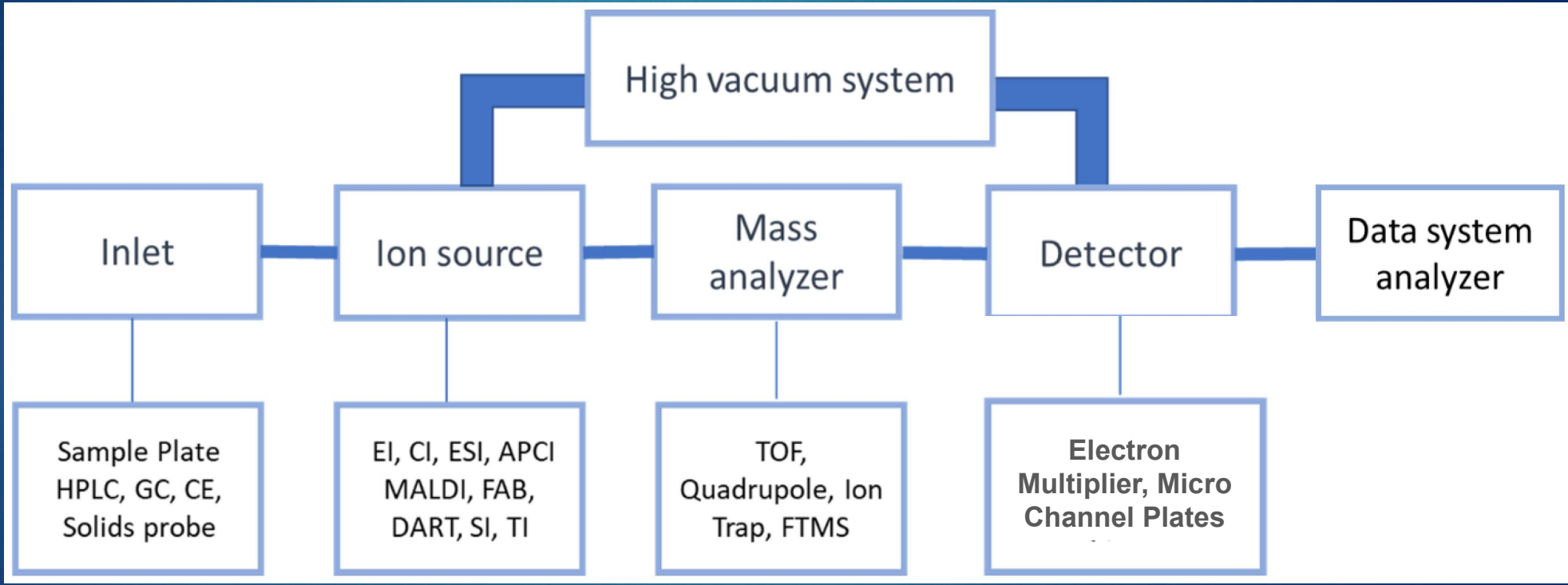
Mass Spectrometer Instrumentation



Essential parts of an MS device

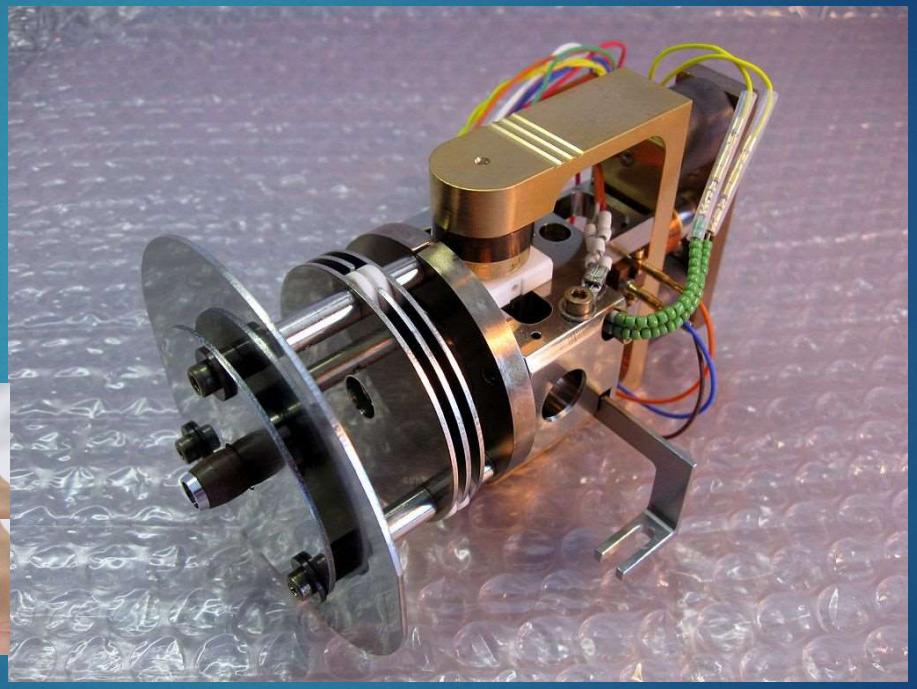


Essential parts of an MS device



Ion source

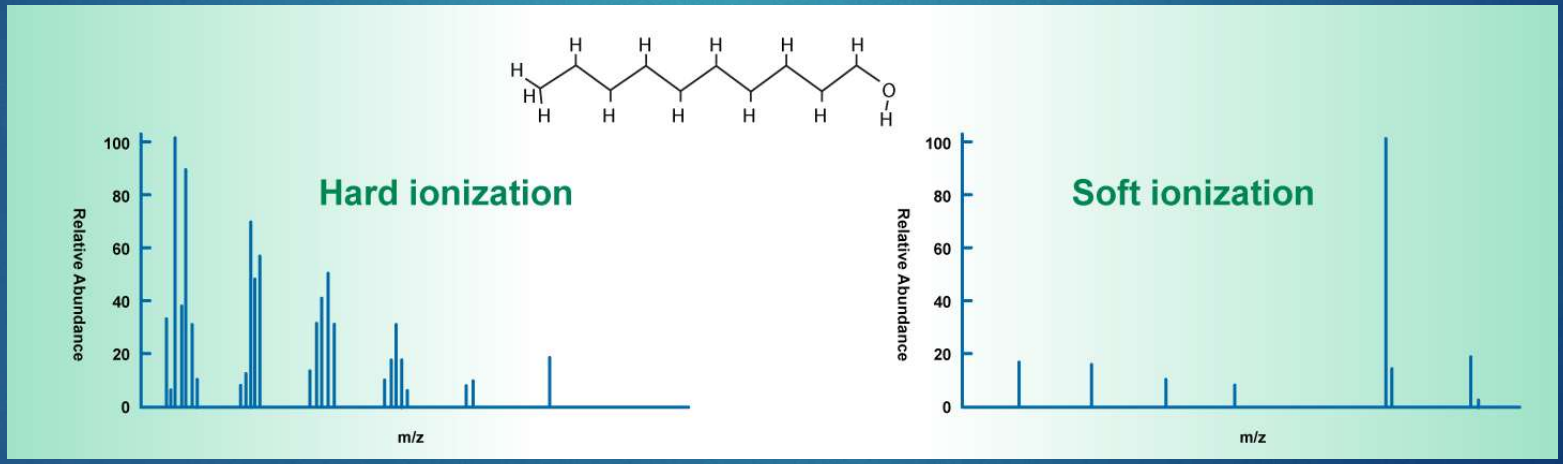
Heart of the mass spectrometer



Ionization Technique types

1) **Hard Ionization Techniques:-**
   High energy will be involved, In that *no. of fragments ion* will be Higher and *no. of Molecular ion* will be low.

2) **Soft Ionization Techniques:-**
   Low energy, low fragmentation, high molecule ion.



Ionization techniques

Gas phase	Desorption	Evaporative
Electron ionization	Field Desorption	Thermospray
Chemical ionization	Fast atom bombardment	Electrospray
	MALDI	Atmospheric pressure Chemical ionization
		Atmospheric pressure photo ionization

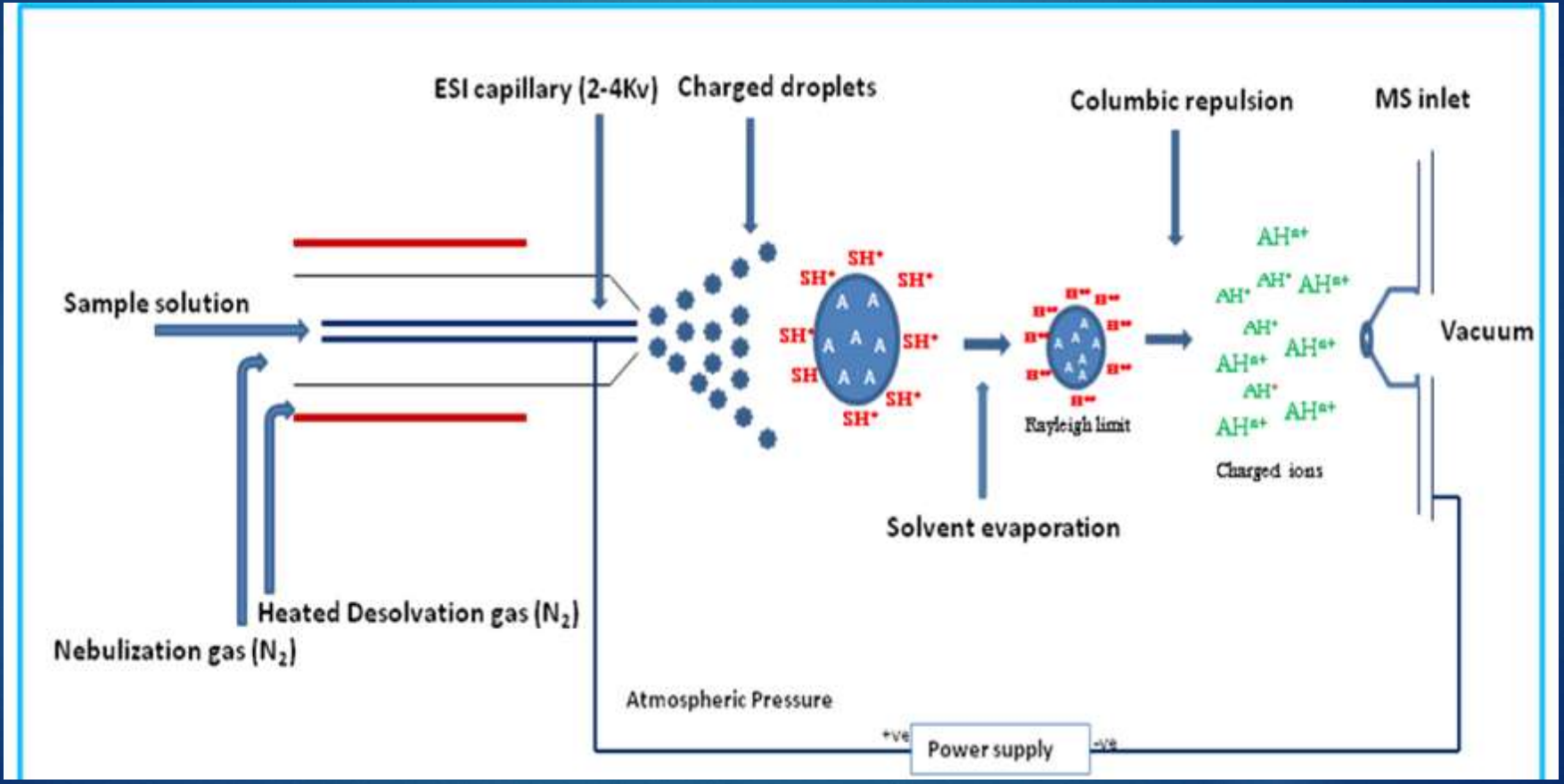
Ionization Technique types

Ionization Method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Electron Impact (EI)	Relatively small. Volatile.	GC or liquid or solid probe	To 1000 Daltons	Hard method. Provides structural info
Chemical Ionization (CI)	Relatively small. Volatile.	GC or liquid or solid probe	To 1000 Daltons	Soft method. Molecular ion peak $[M+H]^+$
Electrospray (ESI)	Peptides/proteins. Non-volatile.	Liquid Chromatography	To 200,000 Daltons	Soft method. Ions often multiply charged.
Matrix Assisted Laser Desorption (MALDI)	Peptides/proteins. Non-volatile.	Sample mixed in solid matrix	To 500,000 Daltons	Soft method. Very high mass range.
Fast Atom Bombardment (FAB)	Carbs/peptides. Non-volatile.	Sample mixed in viscous matrix	To 6000 Daltons	Soft method, but harder than ESI or MALDI

Common atmospheric pressure ion sources

- Electrospray ionization (ESI)
- Atmospheric pressure chemical ionization (APCI)
- Atmospheric pressure photoionization (APPI)

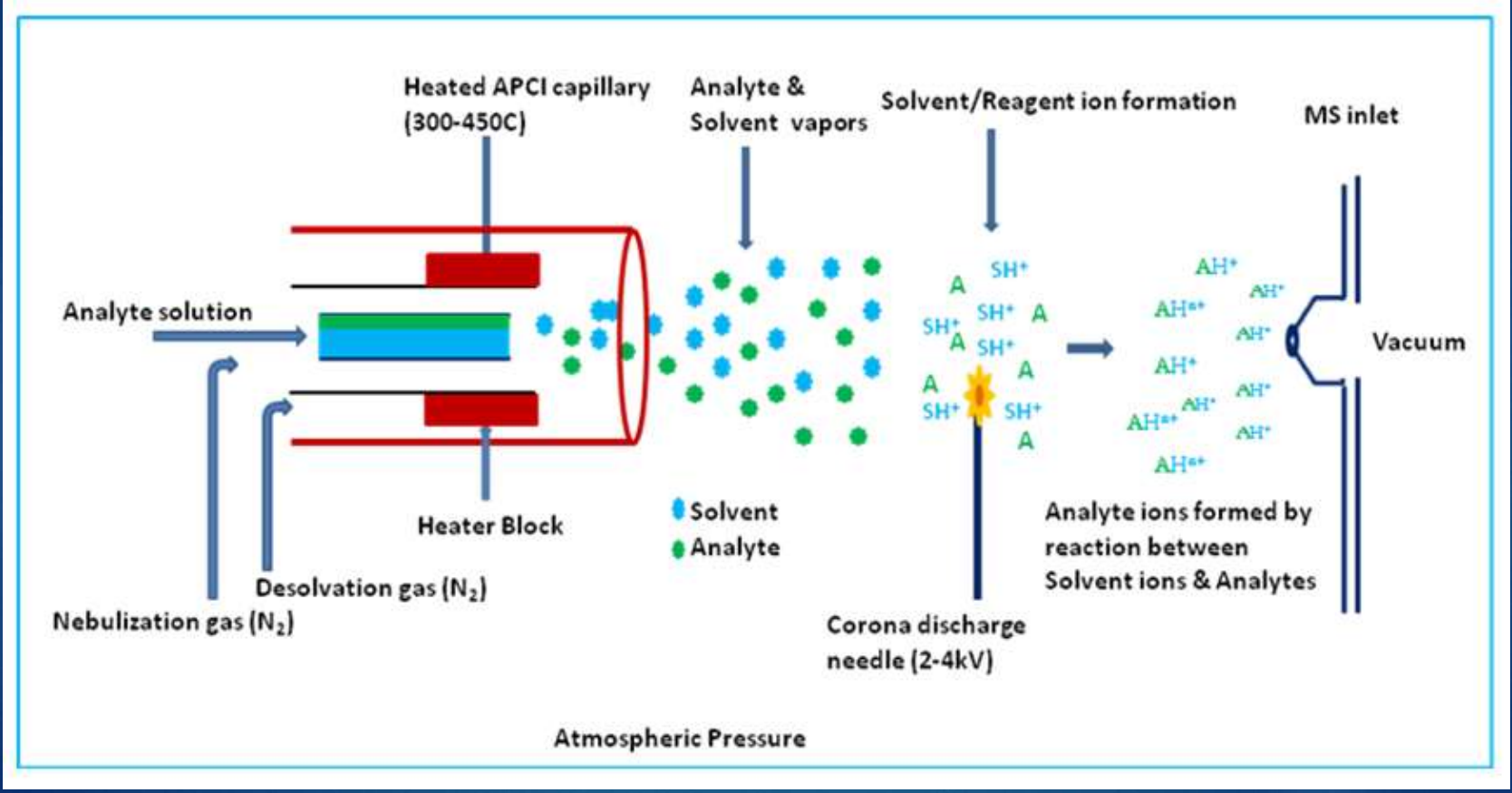
Electrospray ionization (ESI)



ESI characteristics

- Softest ionization method.
- Use: highly polar, least volatile, or thermally unstable compounds, such as natural substances, biological macromolecules, and pharmaceuticals.
  - Biochemical compounds including: peptides and proteins, lipids, oligosaccharides, oligonucleotide, bio-organic compounds, synthetic polymers, and intact non-covalent complexes.
- ESI is an atmospheric pressure process. This makes it easy to use and easy to interface with HPLC and CE separation techniques.
- Is subject to matrix effects, particularly ion suppression.

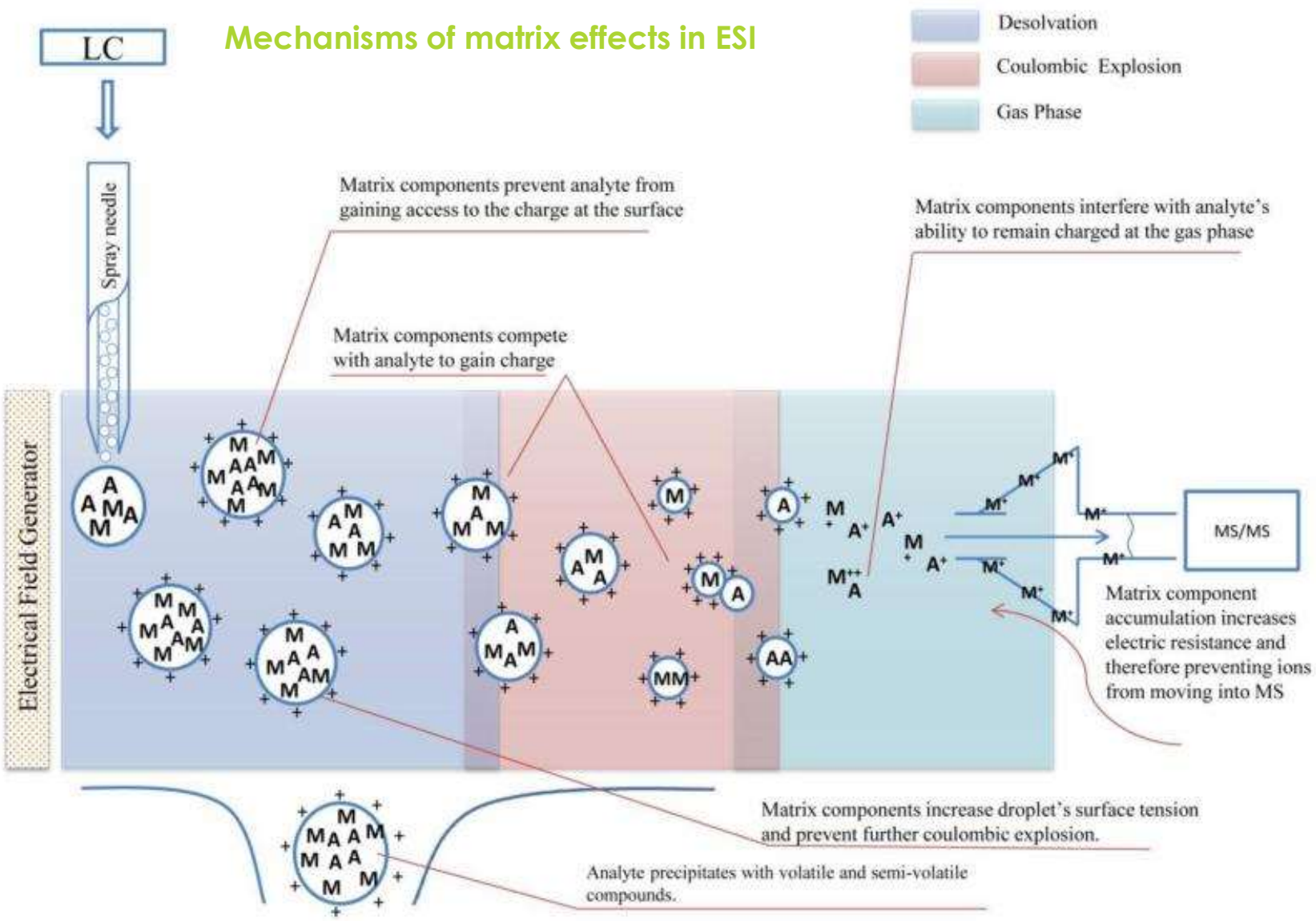
Atmospheric pressure chemical ionization (APCI)



APCI characteristics

- For analysis using APCI, the analytes of interest should be heat stable and volatile for best results.
- Use: highly fat-soluble compounds or compounds that do not ionize in solution and nonpolar analytes.
- APCI is often less susceptible to matrix effects (including ion suppression)

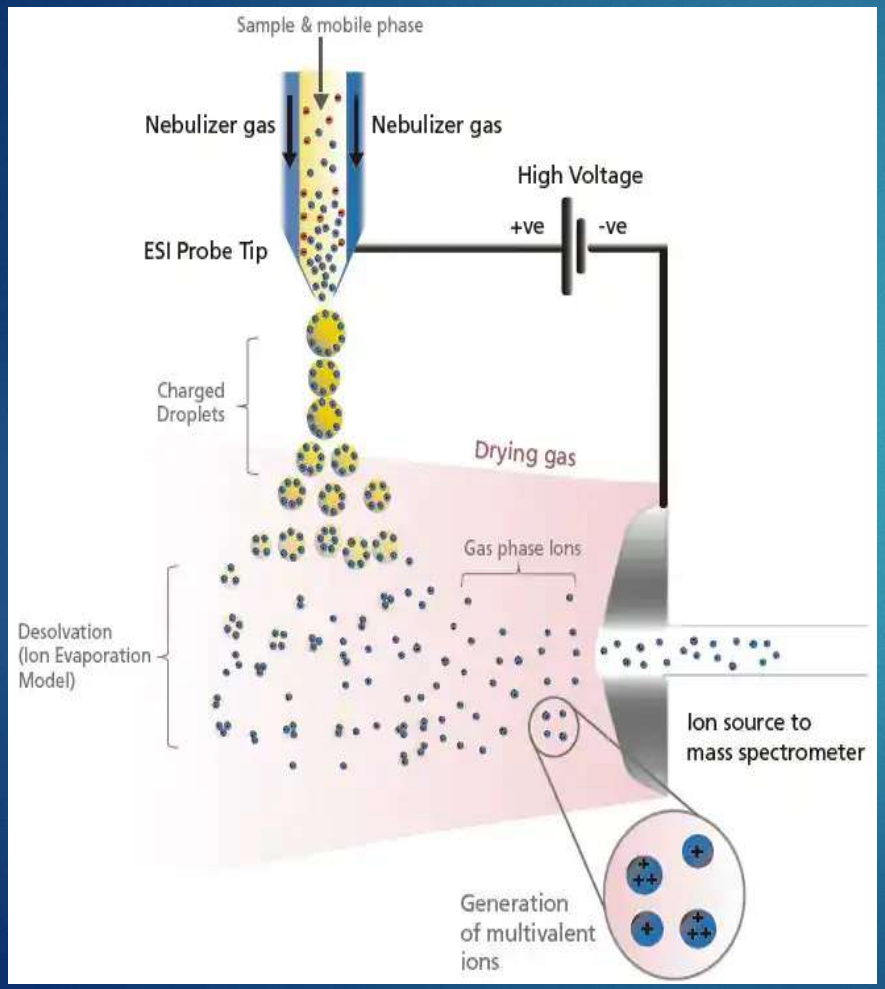
Mechanisms of matrix effects in ESI



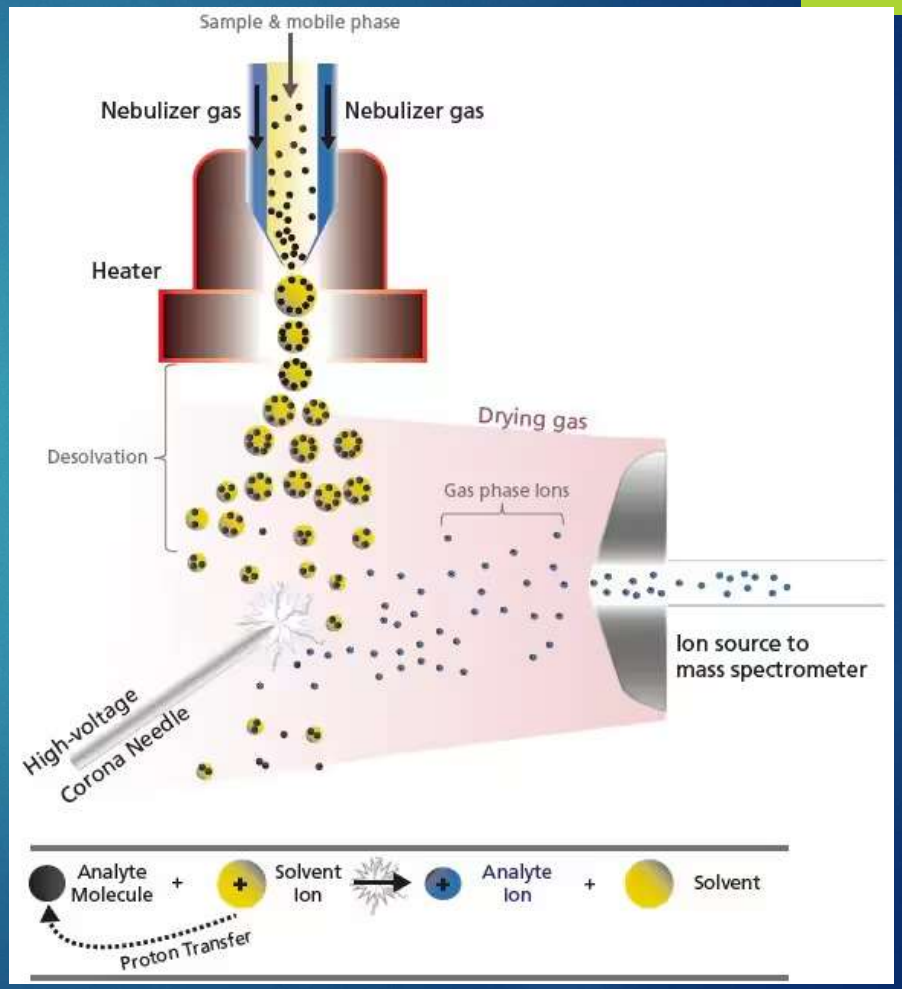
Components	Matrices		
	Plasma/Serum	**Urine**	**Breast Milk**
Cell			Epithelial, Leukocytes, Lymphocytes, Macrophages, Neutrophils
Ions	Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}, HCO_3^-, HPO_4^{2-}, HSO_4^-	Na+, K+, Ca2+, Cl−, Mg?+; NH4+, Sulfates, Phosphates	Bicarbonate, Calcium Chloride Citrate Magnesium Phosphate Potassium Sodium Sulfate Trace minerals Chromium Cobalt Copper Fluoride Iodine Iron Manganese Molybdenum Nickel Selenium Zinc
Organic molecules	Urea, Creatinine, Uric Acid, Amino Acids, Glucose, Bilirubin, Insulin	Urea, Creatinine, Uric Acid, Citrate, DNA, Amino Acids	Lactose, Glucose, Nucleotide Sugars, Creatinine, Glucosamine, Urea, Uric Acid, Carotenoids
Protein	Albumins, Globulins, Fibrinogen, Clotting factors	Immunoglobulins, Albumin	Albumins, Immunoglobulins, Lysozymes, Casiens Thyroxine, Amylaze, Lipase, Glycoproteins
Lipid	Phospholipids, Cholesterol, Triglycerides		Triglycerides, Essential Fatty Acids, Glycolipids, Phospholipids
Others		Water-soluble vitamins	Fat-soluble vitamins (A, D, E, K); Water-soluble vitamins, Biotin, Choline, Folate, Inositol, Niacin, Pantothenic acid, Riboflavin, Thiamin

General composition of selected biological matrices

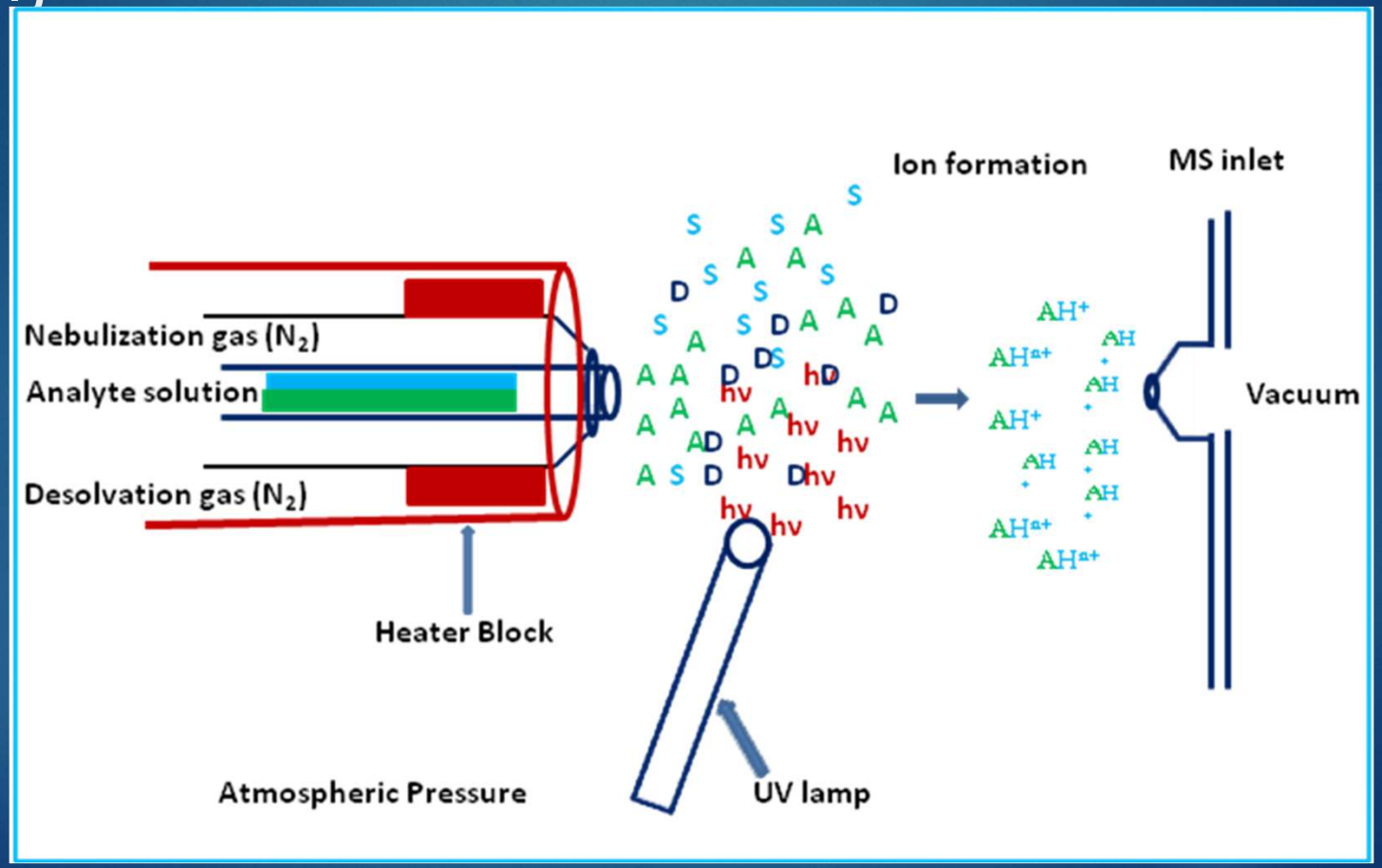
ESI


Sample & mobile phase
Nebulizer gas
Nebulizer gas
High Voltage
+ve
-ve
ESI Probe Tip
Charged Droplets
Drying gas
Gas phase Ions
Desolvation (Ion Evaporation Model)
Ion source to mass spectrometer
Generation of multivalent ions

APCI


Sample & mobile phase
Nebulizer gas
Nebulizer gas
Heater
Desolvation
Drying gas
Gas phase Ions
Ion source to mass spectrometer
High-voltage Corona Needle
Analyte Molecule
+
Solvent Ion
Analyte Ion
+
Solvent
Proton Transfer

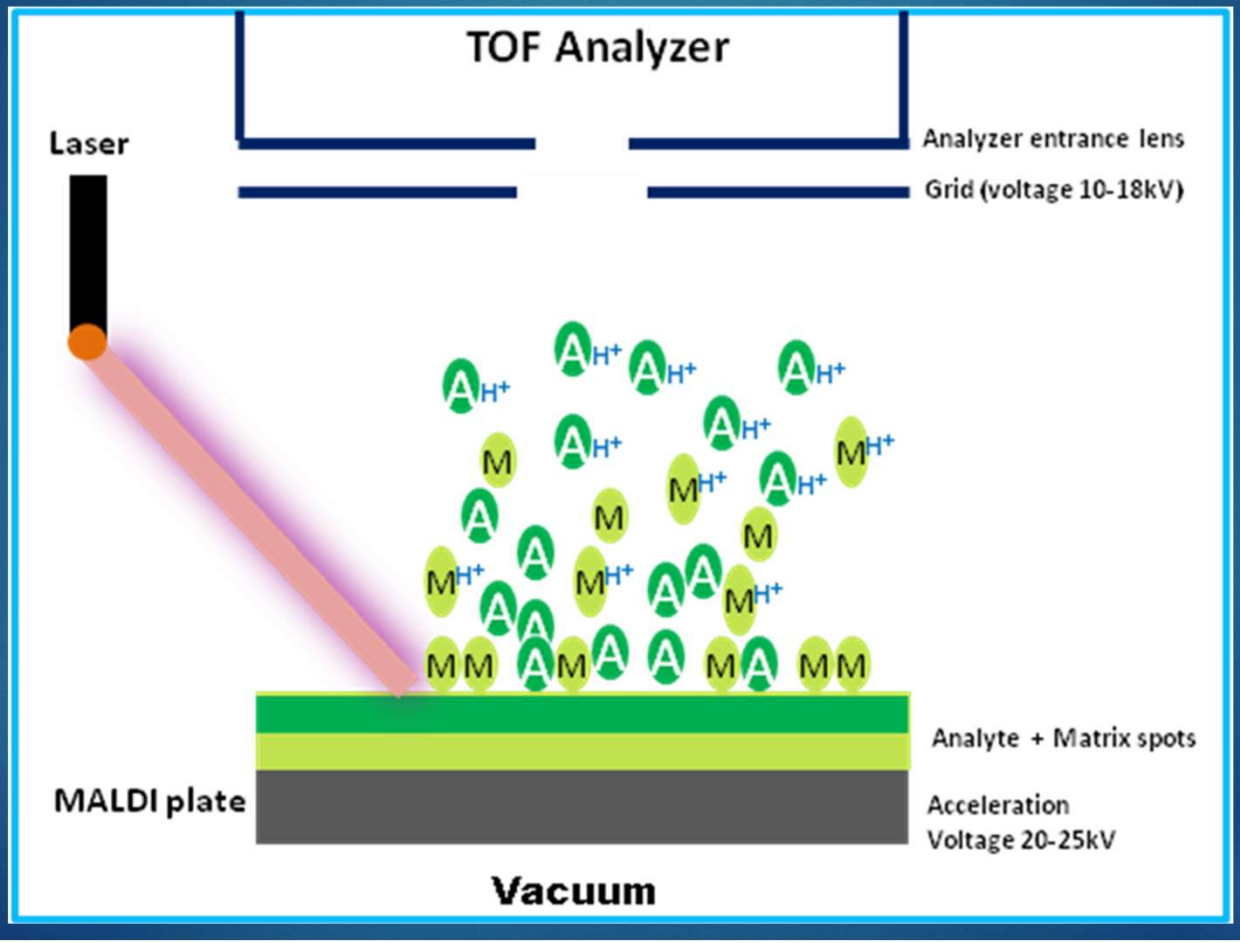
Atmospheric Pressure Photoionization (APPI)



Atmospheric Pressure Photoionization (APPI)

- The application area of APCI is the analysis of drugs, nonpolar lipids, natural compounds, pesticides and various organic compounds.
- Limited use in the analysis of biopolymers, organometallics, ionic compounds and other labile analytes.

Matrix-assisted laser desorption/ionization (MALDI)



Matrix-assisted laser desorption/ionization (MALDI)

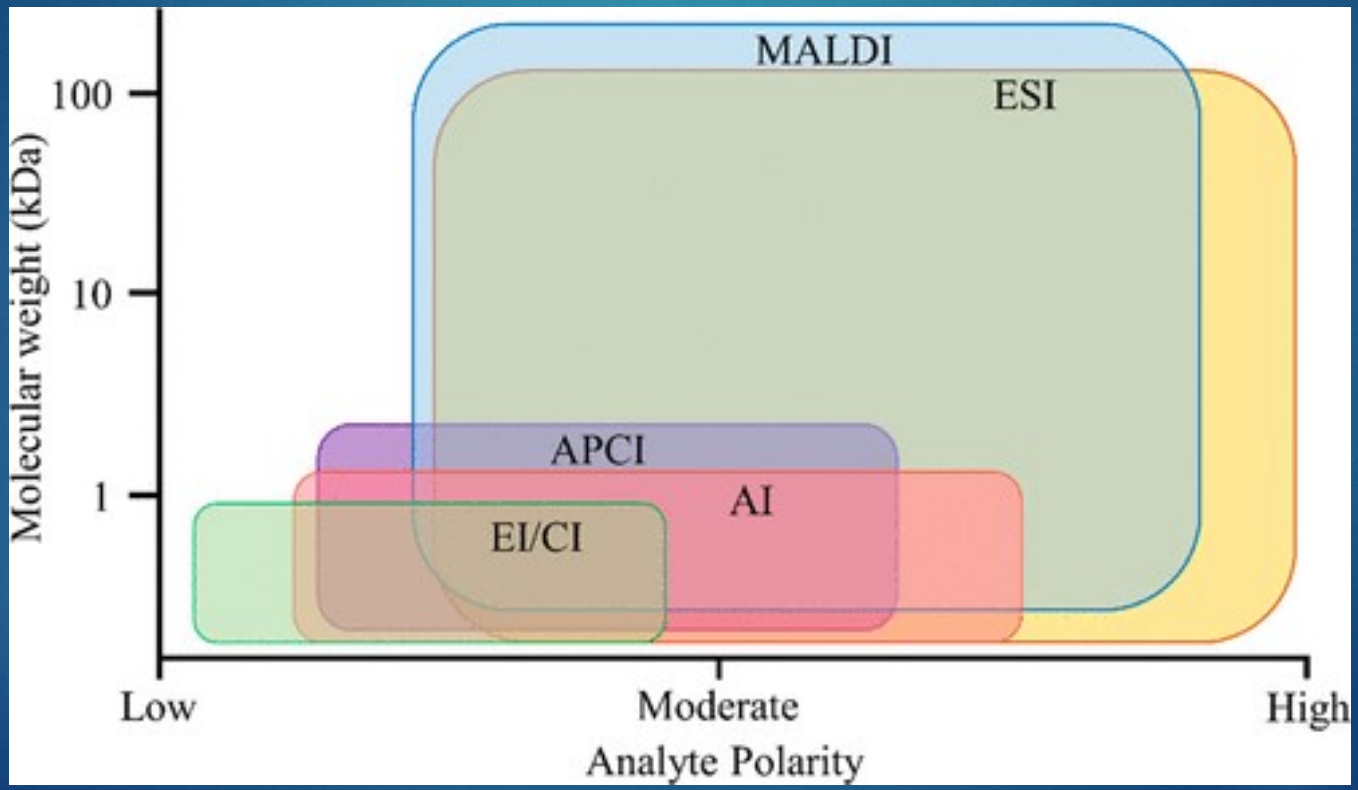
- Use: high molecular weight compounds such as organic macro molecules and labile biomolecules.

Three Ionization Techniques Used in Clinical Mass Spectrometry

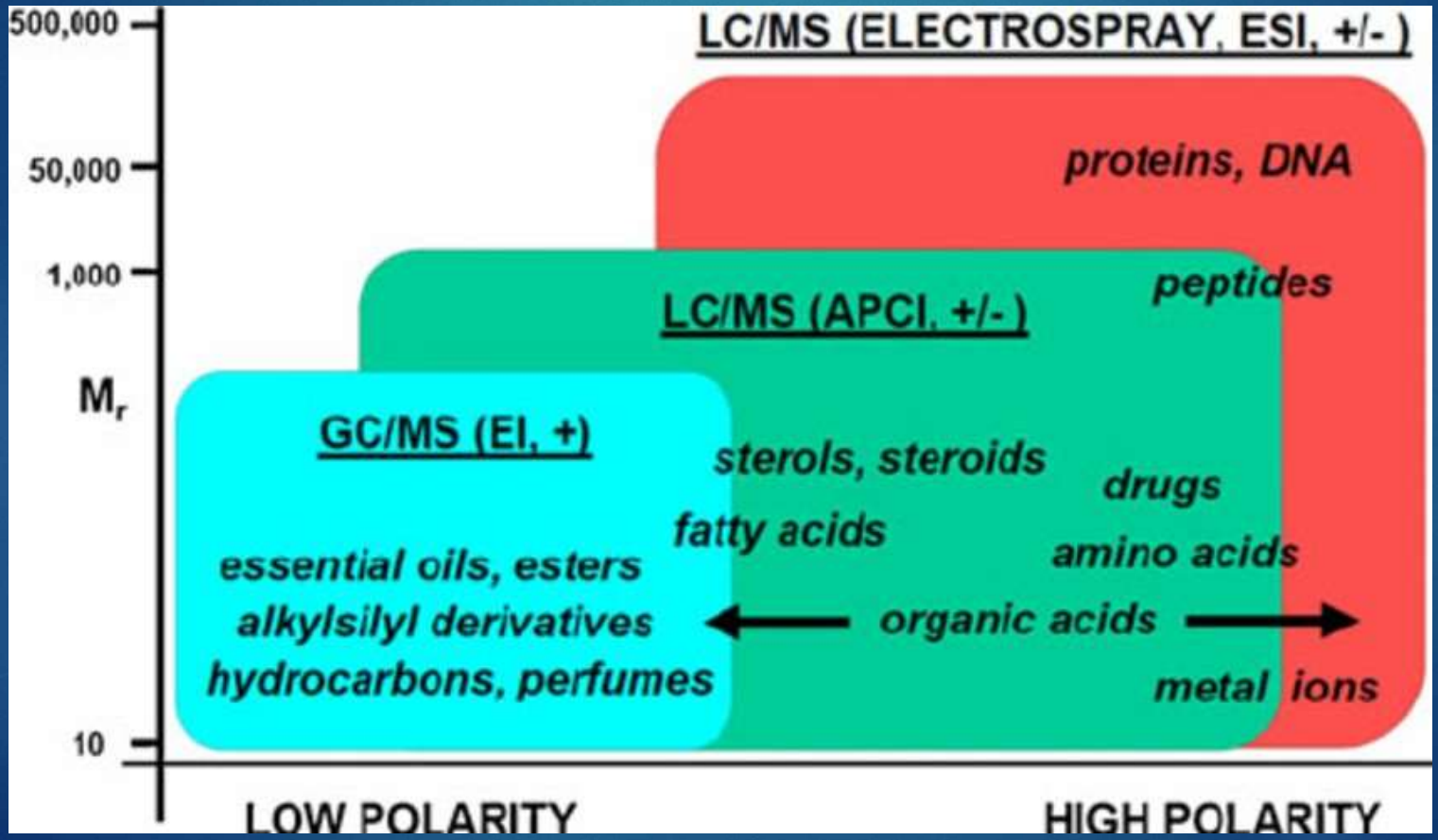
Ionization Technique	Advantages	Limitations
ESI	• Sensitive ionization technique for polar analytes or ions generated in solution • Has broad applicability for relevant analytes in clinical MS • May yield multiply charged ions, which allows for analysis of larger molecules (i.e., > 1000 Da)	• May be more sensitive to matrix effects compared to APCI
APCI	• Typically less sensitive to matrix effects than ESI • May provide better sensitivity for less polar analytes	• Typically only singly charged ions are formed, limiting the effective mass range, • May be unsuitable for thermally labile analytes • May yield less absolute signal relative to ESI
APPI	• Works well with nonpolar analytes • In some cases will ionize analytes that do not ionize by either ESI or APCI.	• Demonstrates limited applicability in clinical MS to date.

APCI, Atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; ESI, electrospray ionization.

Relationship between ionization method and applicable analytes



Relationship between ionization method and applicable analytes



Mass analyzers

Takes ionized masses

Separates them

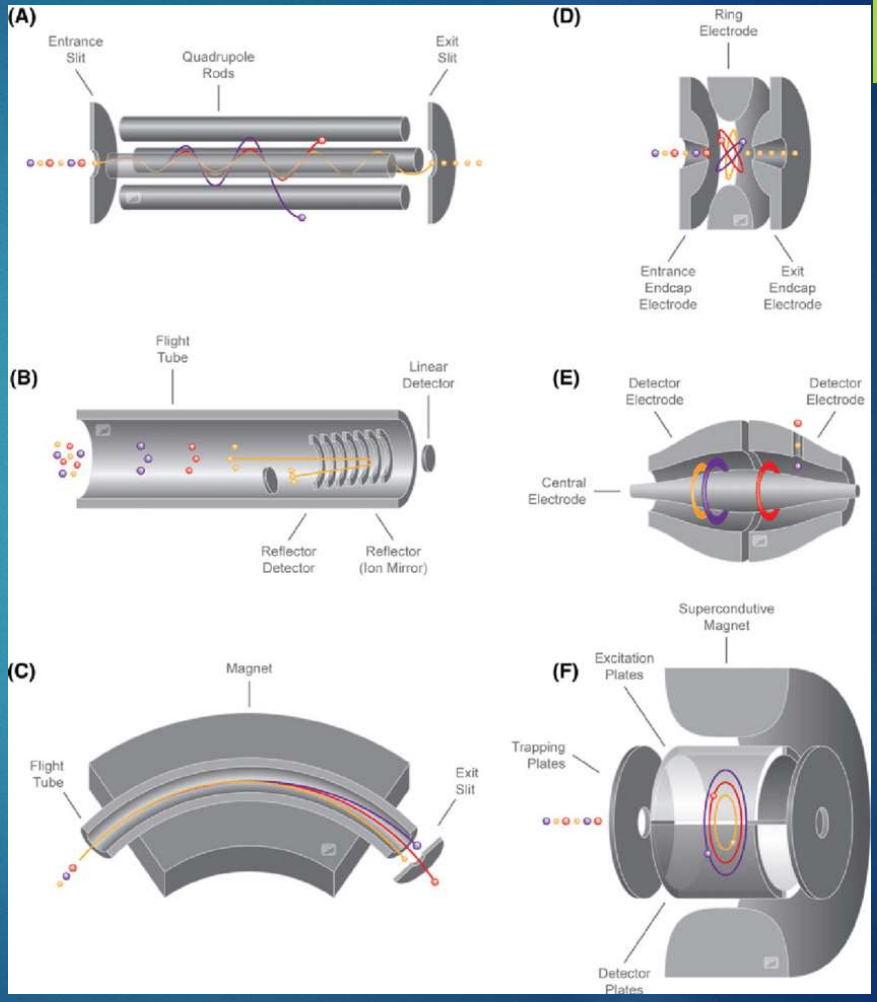
Outputs them to the detector

Mass analyzers

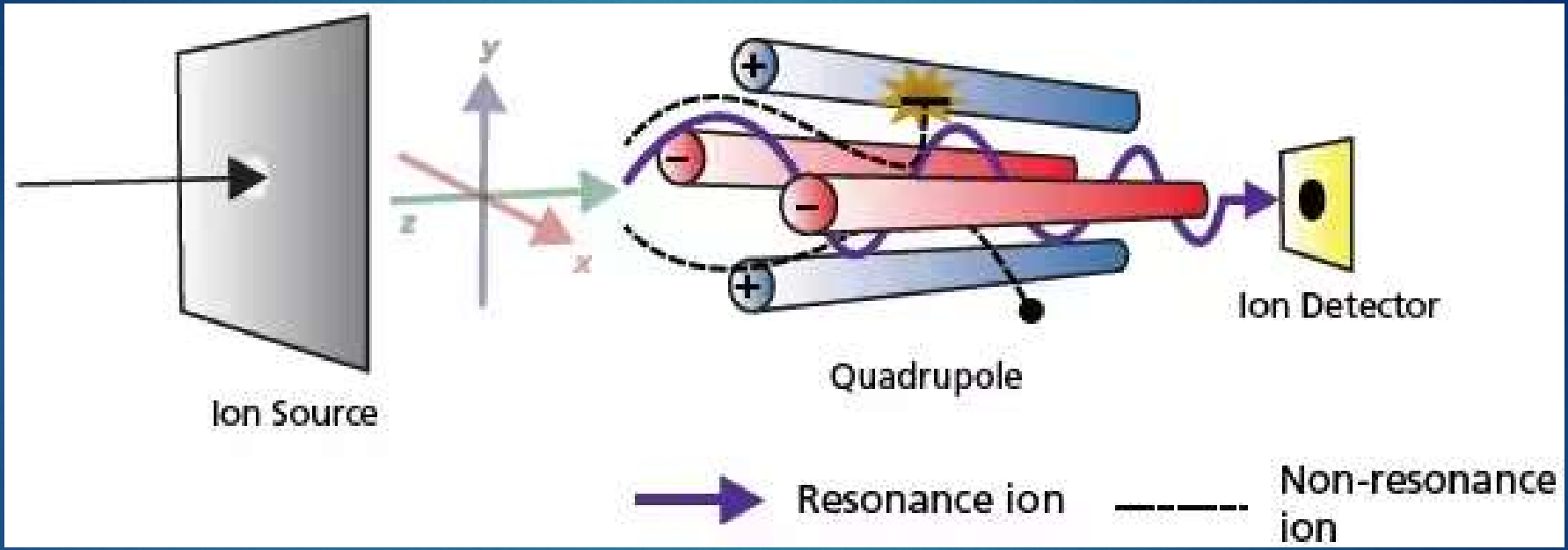
- There are a variety of mass analyzers and they can be classified by how the ions are being introduced:
  - Continuous MS: allows an uninterrupted supply of ions to enter the mass analyzer.
  - Pulsed MS: requires the ions to be introduced only at a specific time point.

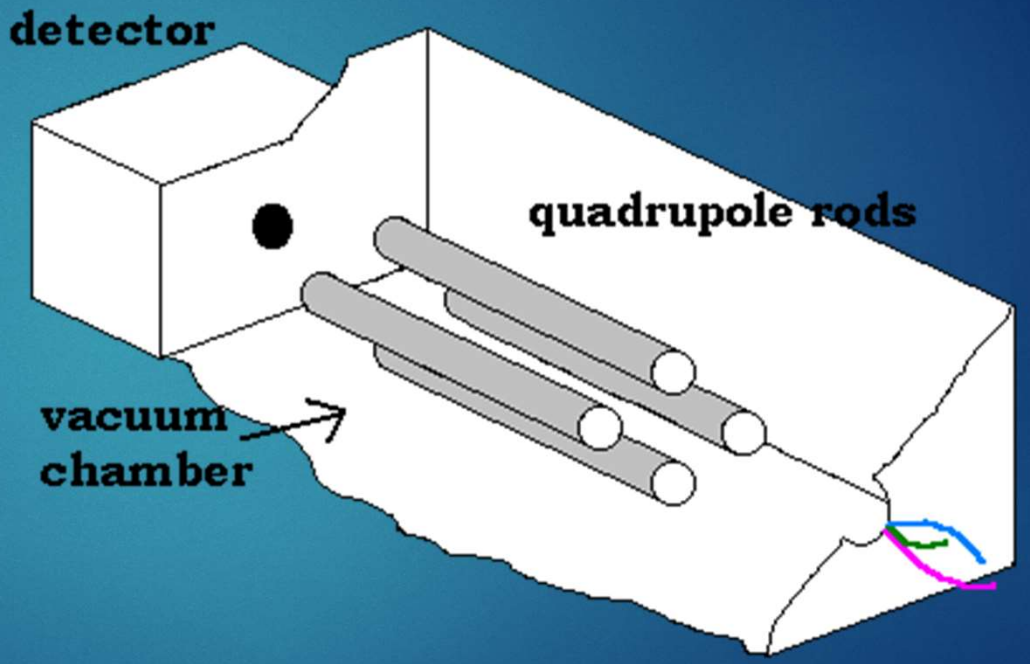
Six mass analyzers

A) Quadrupole (Q)
B) Time-of-flight (TOF)
C) Magnetic Sector (MS)
D) Ion Trap (IT)
E) Orbitrap (OT)
F) Ion Cyclotron Resonance (ICR)

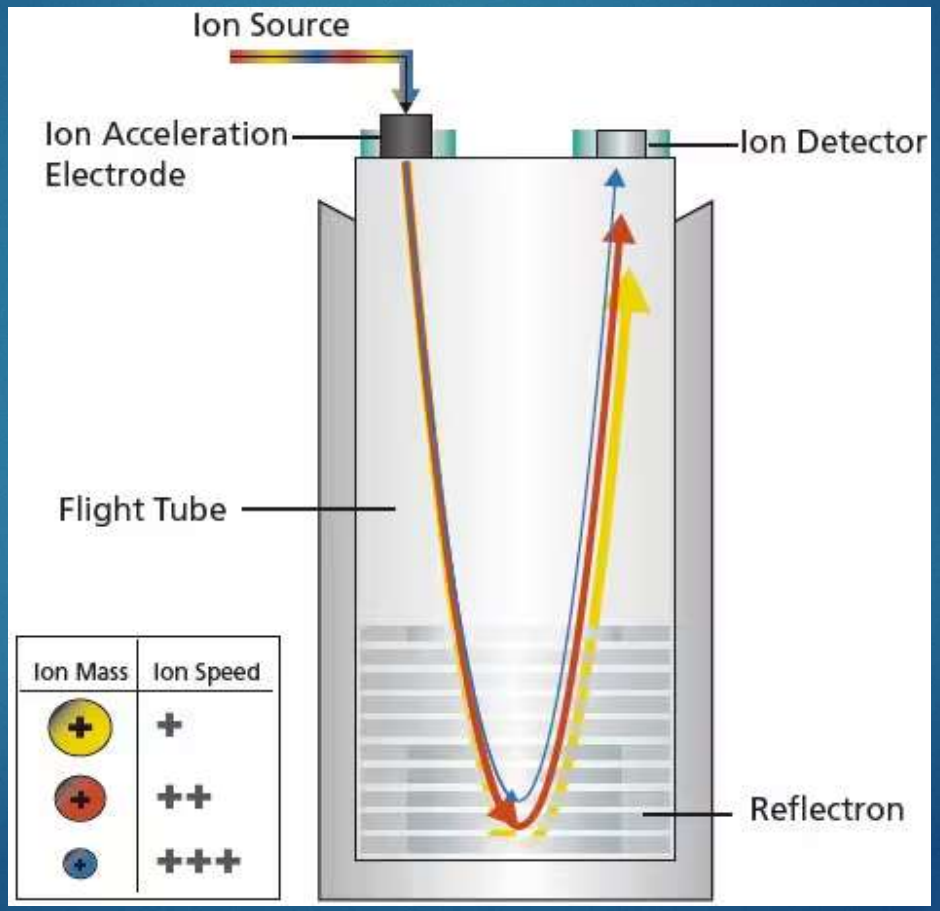


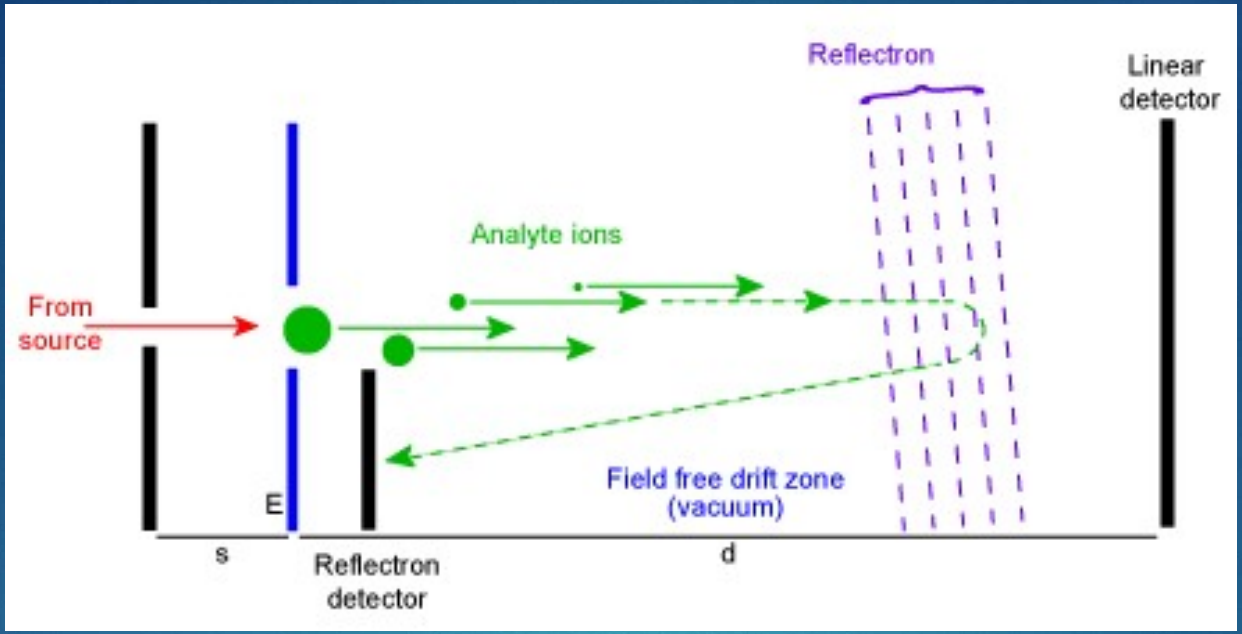
Quadrupole MS

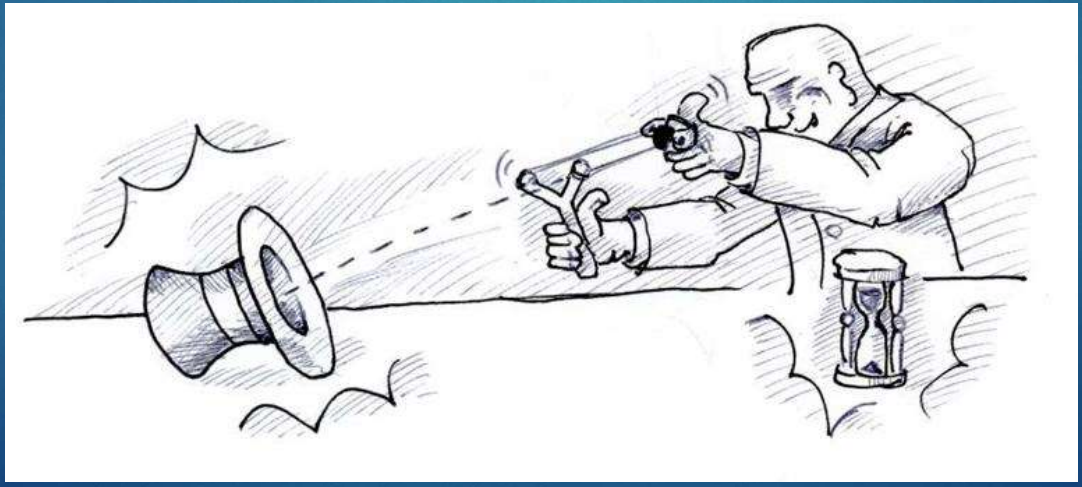



detector
quadrupole rods
vacuum
chamber

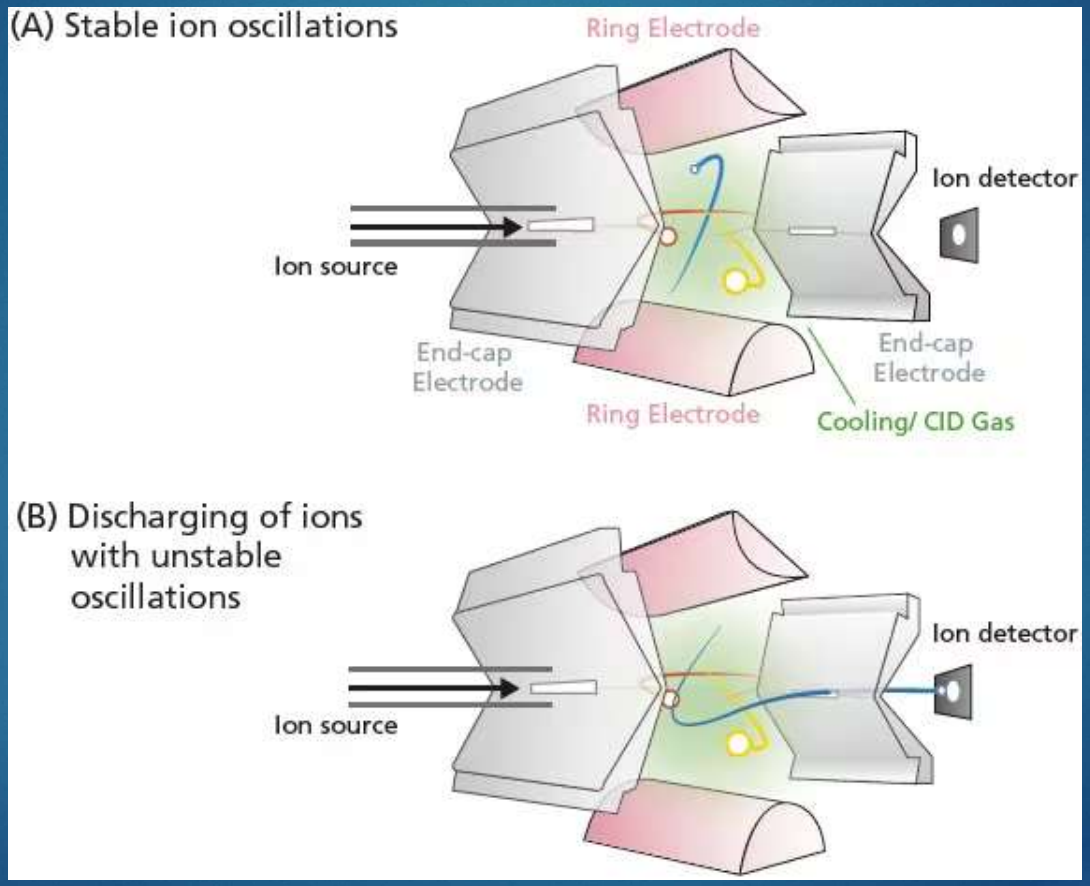
Time-of-Flight (TOF) MS



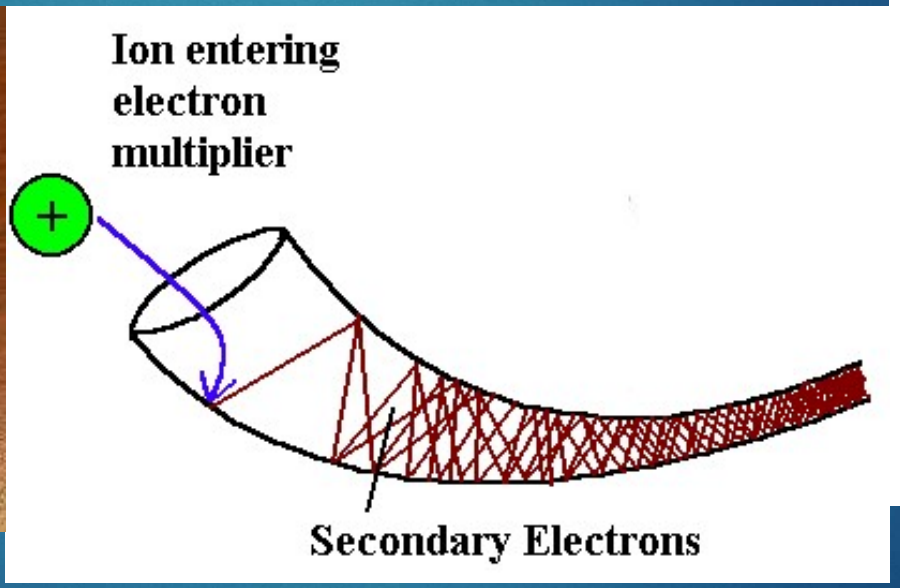

Reflectron
Linear detector
Analyte ions
From source
E
Field free drift zone (vacuum)
s
Reflectron detector
d

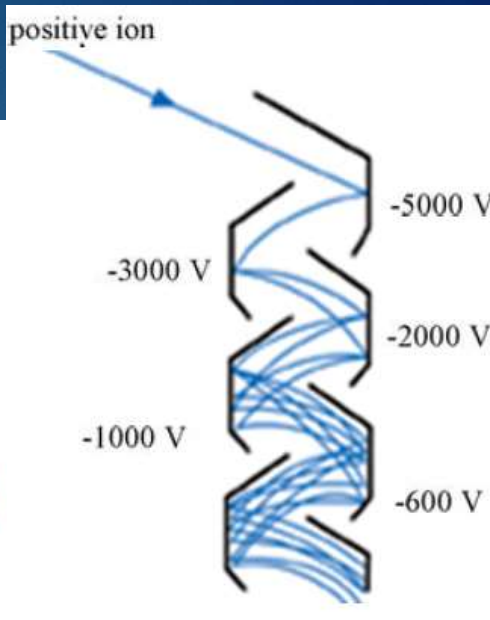


Ion Trap (IT) MS



Detectors: Electron Multiplier





MS APPLICATIONS IN CLINICAL LAB

Nobel Prizes during MS development process

- Mass spectrometry techniques have been awarded 5 Nobel Prizes during their development process.

Nobel Prize winners

Joseph John Thompson — Nobel Prize in Physics in 1909, one of the first users of mass spectrometry

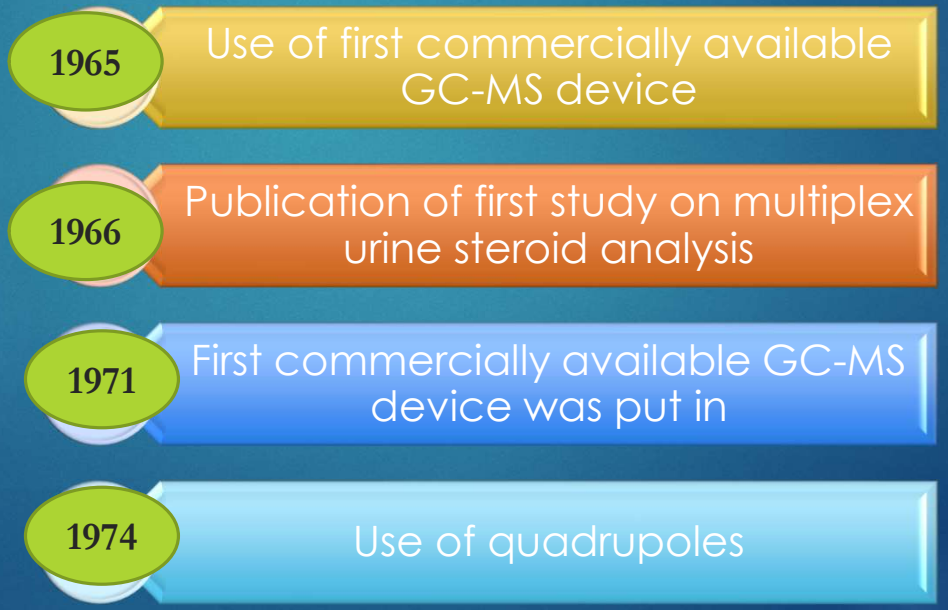
Francis William Aston — Nobel Prize in Chemistry in 1922 for discovering stable isotopes

Wolfgang Paul — Nobel Prize in Physics 1989l, for discovering the ion trapping technique

John Bennett Fenn, Koichi Tanaka — Nobel Prize in Physics in 2002 for introduction the MALDI technique, and discovering the soft electron spray ionization (ESI) technique

The clinical laboratory journey of the MS method

- The MS technique was firstly used in sterol metabolism in the 1930s with the discovery of deuterium.



The clinical laboratory journey of the MS method

- **Between 1980 and 1990:**
  - **Production of silica columns in chromatography technique**
  - **Development of solid-phase extraction method**
  - **Development of thermospray ionization technique**
  - **Introduction of triple quadrupole devices**

The clinical laboratory journey of the MS method

- On May 26, 1981 → accident on the aircraft carrier Nimitz.

↓

Killing 14 and injuring 45

CLINICAL MASS **SPECTROMETRY** MILESTONES

Francis Aston develops the first mass spectrograph that is able to accurately determine the masses of individual atoms.
The measurements supported the existence of isotopes in nonradioactive elements and led the pathway toward modern-day mass spectrometry techniques.

The Nimitz accident, a military jet crash, kills 14 service members.
Immunoassays revealed the presence of marijuana in members of the crew. At a time when immunoassays had a high rate of false positives, this high-profile case sparked the need for mandatory confirmatory studies in clinical toxicology laboratories.

Gas chromatography-mass spectrometry (GC-MS) becomes the gold standard for confirming drug screens across federal and state agencies, workplaces, and other institutions.
The large number of inaccuracies and insensitivity associated with immunoassay and thin-layer chromatography led these tests to be considered preliminary until confirmed by GC-MS.

Millington et al.[1] propose tandem MS of dried blood spots for newborn screening.
The method enabled screening for multiple organic acid and fatty acid oxidation disorders within a single test. Combining this technology with electrospray ionization (ESI) allowed for the detection of even more disorders with a single test.

Introduction of a multiplex electrospray source that interfaces with MS leads to higher sample throughput and cost savings.
Used in conjunction with ultra-high flow rate liquid chromatography-mass spectrometry (LC-MS), the technology made rapid and parallel determination of pharmaceuticals present in plasma achievable, with throughputs reaching 120 samples per hour.

The Nobel Prize in chemistry goes to John B. Fenn and Koichi Tanaka for the development of ESI and "soft" ionization techniques (shared with Kurt Wüthrich for development of NMR).
The research (conducted in the 1980s) allowed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to simplify MS techniques by eliminating the need for volatile analytes and preliminary sample preparation and shifted clinical applications away from GC-MS.

A nonprofit organization committed to the advancement of MS in the clinical laboratory called Mass Spectrometry: Applications to the Clinical Laboratory is incorporated.
The organization began holding an annual conference to provide education and training on MS in the laboratory setting to leaders and practitioners in the field.

MS is increasingly used in metabolomics—the analysis of cellular metabolites during gene alterations or physiological stimuli.
Its use enabled the capture of either a subset of targeted biological molecules or thousands of molecules via an untargeted approach. MS also started to gain traction in lipidomic, proteomic, and other -omics clinical applications.

The intelligent knife (iKnife) is used in cancer surgery.
Rapid evaporative ionization mass spectrometry (REIMS) combined with electrosurgery-enabled real-time evaluation of surgical tissue using lipidomic profiles that highly correlate with histopathological analysis.

Matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) MS is successfully cleared by the FDA for microorganism identification.
MALDI-ToF revolutionized the field by providing rapid and robust results. It displaced more time-intensive and expensive methods such as standard culture techniques and susceptibility testing.

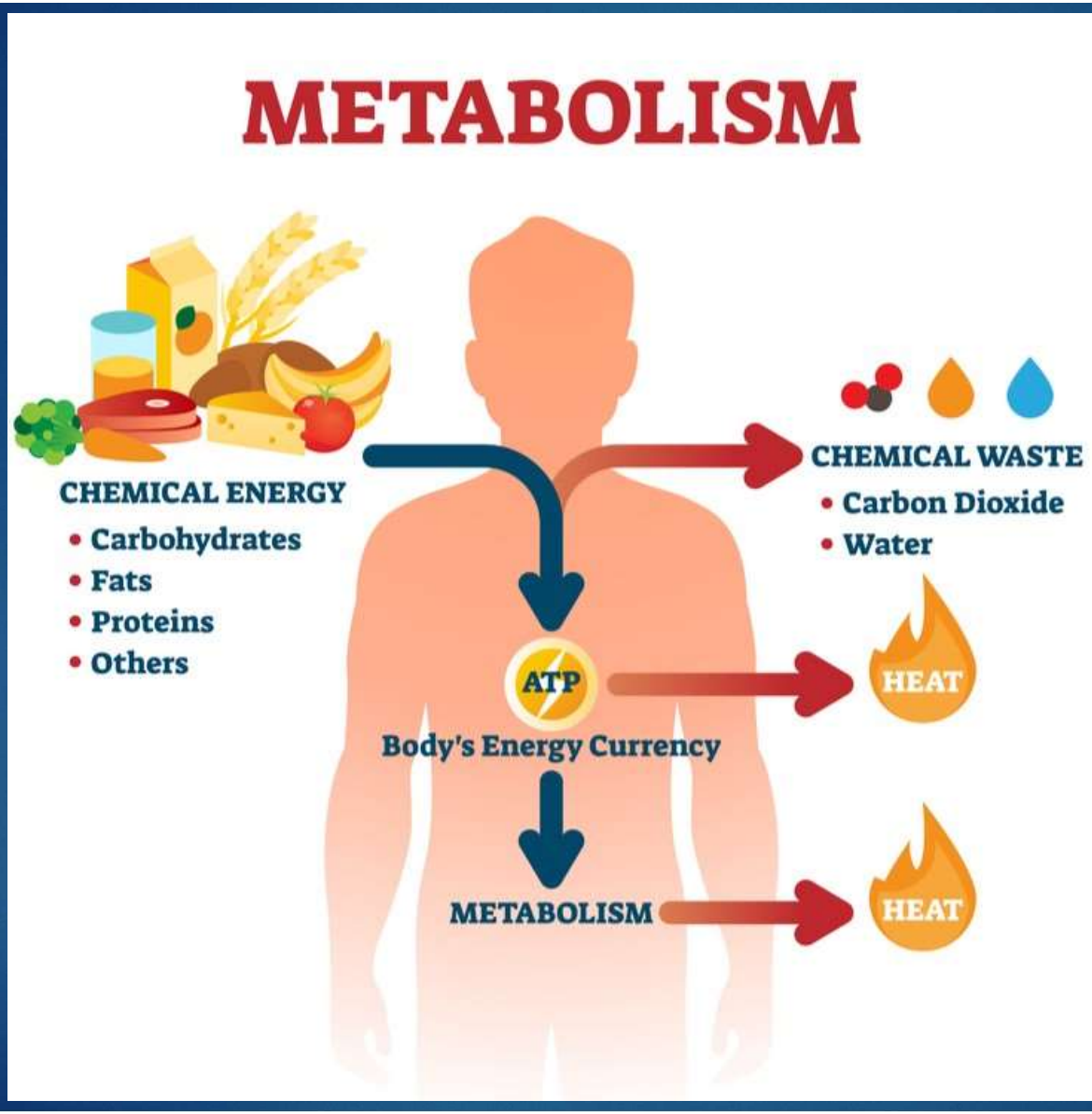
1920 1940 **1960** 1980 **1985** 1990 **1995** 2000 **2003** 2006 **2009** 2012 **2015**

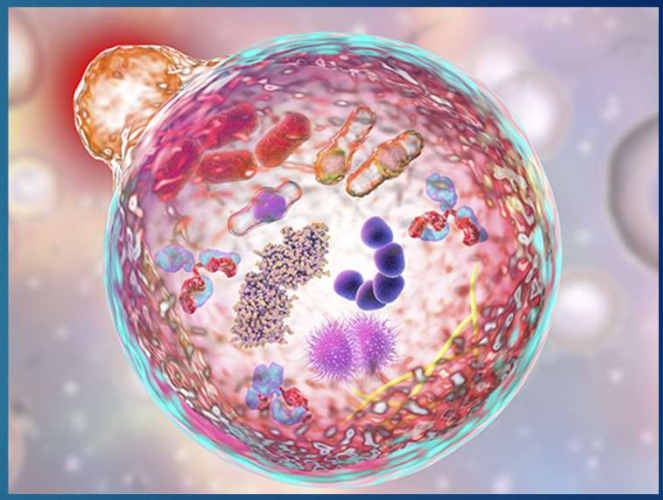
1. Millington DS, Kodo N, Norwood DL, Roe C[illegible] tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism." *Journal of Inherited Metabolic Disease* 13(1990): 321-324.

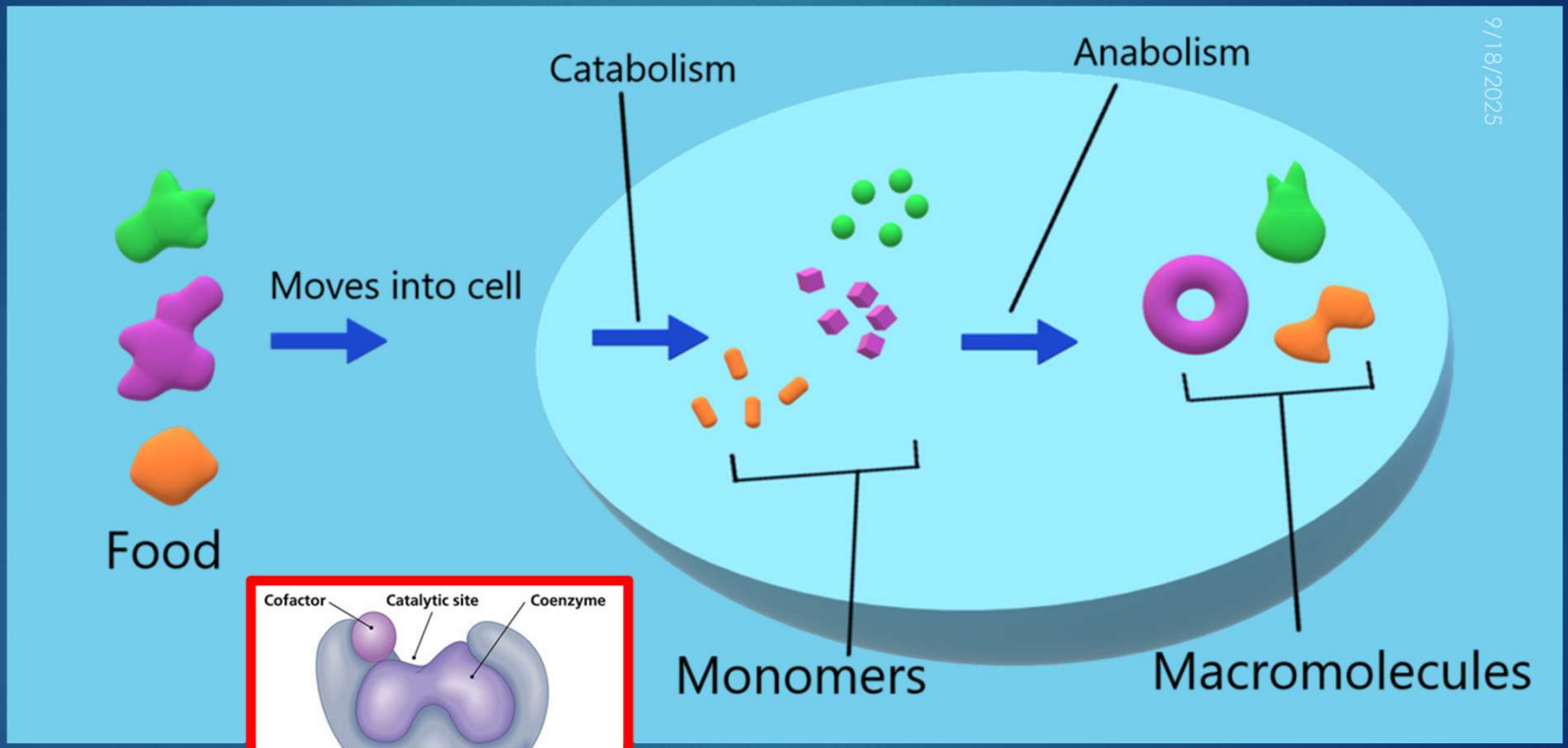
Applications in Clinical Laboratories

- Endocrinology
  - steroid hormones
  - amino acid derivative hormones
  - biological amines
- Toxicology
  - clinical (especially therapeutic drug monitoring)
  - forensic toxicology
- Microbiology
  - classification and identification of bacteria and other microorganisms
- Metabolism
  - hereditary metabolism disorders

Inborn Errors Of Metabolism


METABOLISM
CHEMICAL ENERGY
• Carbohydrates
• Fats
• Proteins
• Others
CHEMICAL WASTE
• Carbon Dioxide
• Water
ATP
Body's Energy Currency
HEAT
METABOLISM
HEAT

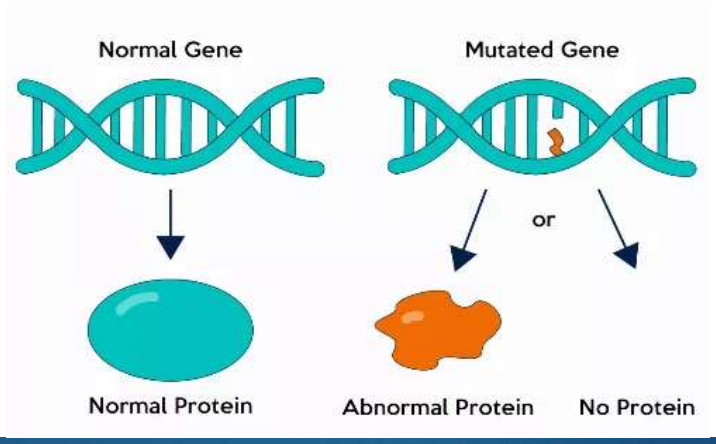



Catabolism
Anabolism
Moves into cell
Food
Monomers
Macromolecules

Cofactor
Catalytic site
Coenzyme
Apoenzyme
Holoenzyme

Inherited metabolic diseases (Inborn Errors Of Metabolism)

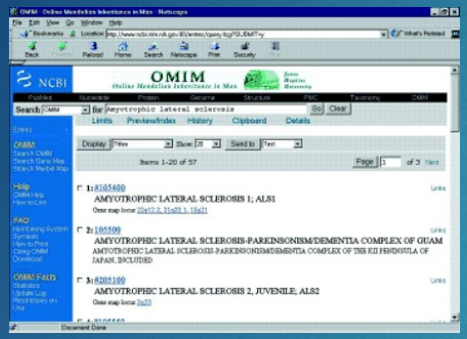
- Inherited conditions that develop as a result of mutations affecting the function of proteins.
- The majority of IMDs are monogenic conditions and the mutant proteins are enzymes, but others involve structural proteins, receptors, hormones or transport proteins.



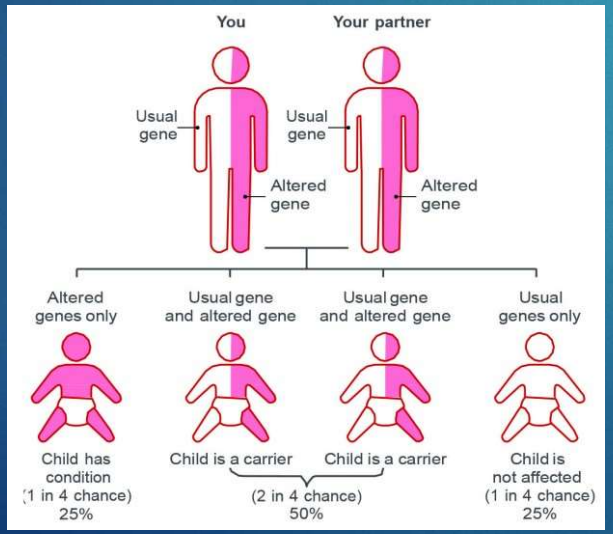
Single-gene defects

- Most congenital metabolic disorders known as inborn errors of metabolism result from single-gene defects.
- A single-gene disorder (or monogenic disorder) is the result of a single mutated gene. Single-gene disorders can be passed on to subsequent generations in several ways:
  - Autosomal dominant
  - Autosomal recessive
  - X-linked dominant
  - X-linked recessive
  - Y-linked
  - Mitochondrial
- Genomic imprinting and uniparental disomy, however, may affect inheritance patterns.

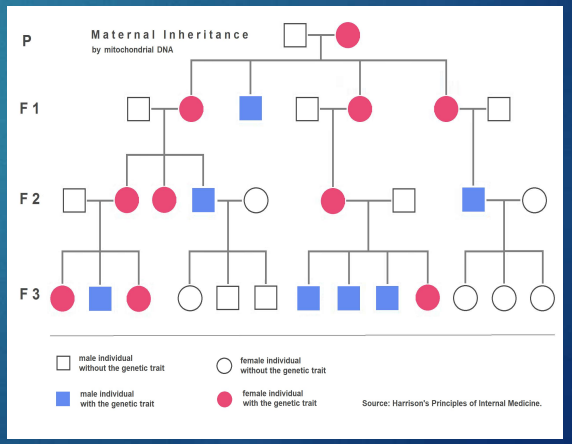
Inherited metabolic diseases (IMDs)



- **OMIM**



- **Controllable**



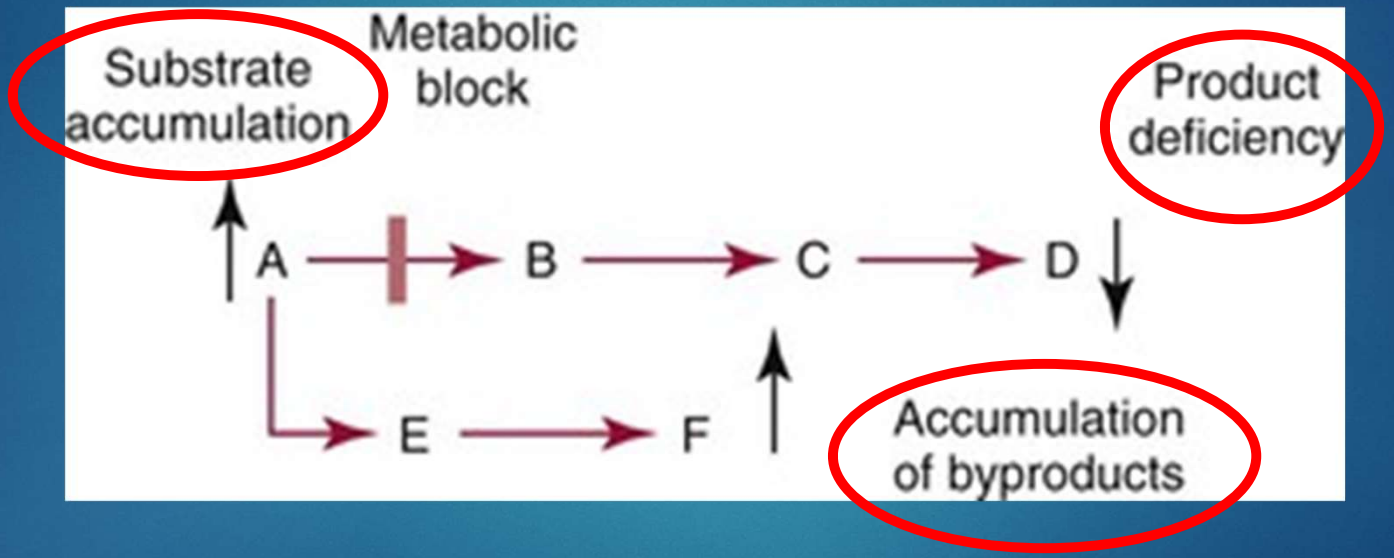
How are genetic disorders identified?

- If there's a family history, DNA testing for genetic disorders can be an important part of starting a family. Options include:
  - Carrier testing: This blood test shows whether you or your partner carry a mutation linked to genetic disorders. This is recommended for everyone considering pregnancy, even if there is no family history.
  - Prenatal screening: This testing usually involves blood testing from a pregnant woman that tells a person how likely it is that an unborn child could have a common chromosome condition.
  - Prenatal diagnostic testing: You can find out whether your unborn child faces a higher risk for certain genetic disorders. Prenatal testing uses a sample of fluid from the womb (amniocentesis).
  - Newborn screening: This test uses a sample of your newborn baby's blood. Detecting genetic disorders early in life can help your child receive timely care if needed.

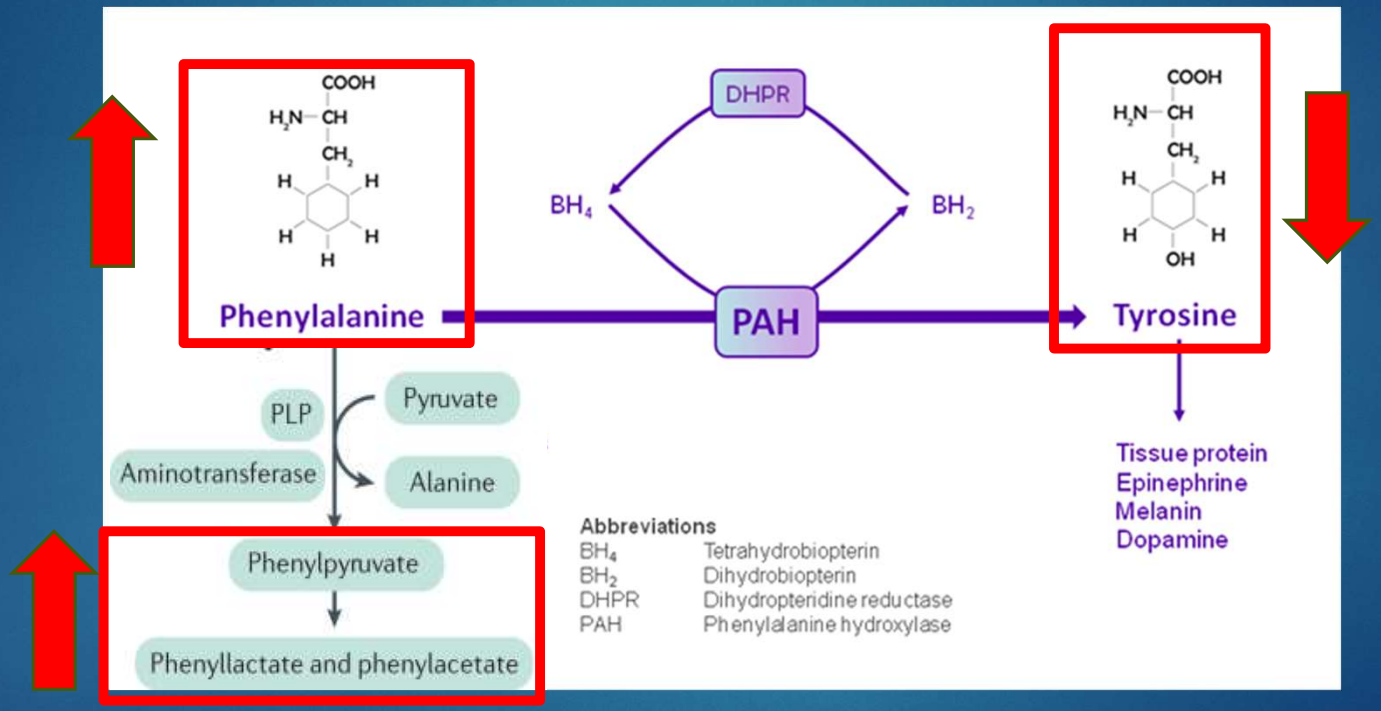
Carrier testing

- The first opportunity to address IEM occurs with testing of asymptomatic future parents. Certain populations have increased carrier rates for IEM, and preconception screening has been shown to decrease disease prevalence.
- first began in the Ashkenazi (Eastern European) Jewish population in the early 1970s with preconception screening for carriers of Tay-Sachs disease.
- With the advent of carrier screening, the incidence of Tay-Sachs disease decreased by 90% between 1970 and 1993 in the Jewish populations of North America.

Metabolic pathway blockage



PKU


Phenylalanine
DHPR
BH4
BH2
PAH
Tyrosine
PLP
Pyruvate
Aminotransferase
Alanine
Phenylpyruvate
Phenyllactate and phenylacetate
Tissue protein
Epinephrine
Melanin
Dopamine
Abbreviations
BH4 Tetrahydrobiopterin
BH2 Dihydrobiopterin
DHPR Dihydropteridine reductase
PAH Phenylalanine hydroxylase

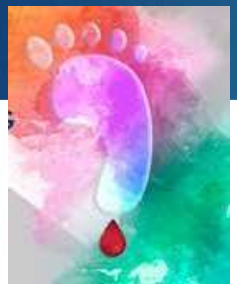
Newborn Screening for Metabolic Disorders With Mass Spectrometry

Screening History

- Mass newborn screening began in the 1960s when Guthrie and Susi developed a method for estimation of phenylalanine in blood samples collected on a filter paper for the detection of phenylketonuria (PKU) using a bacterial inhibition assay.

- Until the early 1990s, few other diseases, “though one at a time,” were added to the newborn screening programs
- In the 1990s, with the introduction of tandem mass spectrometry (MS/MS) into the metabolic screening laboratories, the paradigm of analyzing one analyte per disorder changed. With a single and “2–3min” long analysis of a small blood spot, MS/MS allows the determination of multiple analytes characteristic of several (>40) metabolic disorders.

Newborn screening timeline



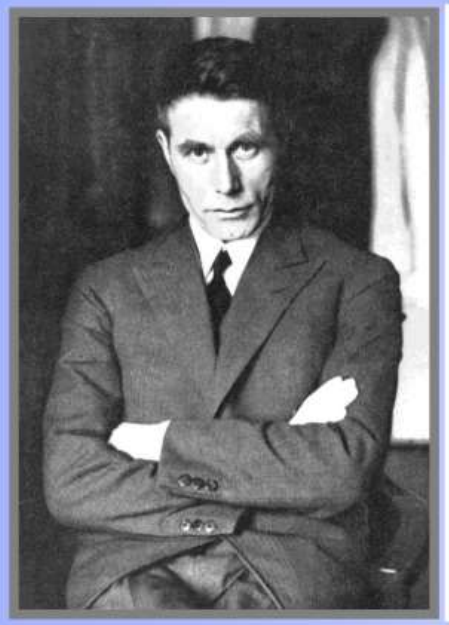
Screening History

"It began with our second child, John," wrote Robert Guthrie, in a medical journal article about the breakthrough he developed that has saved hundreds of thousands of lives.

"He is mentally retarded. John stimulated me to go into research aimed at preventing mental retardation and developmental disabilities."

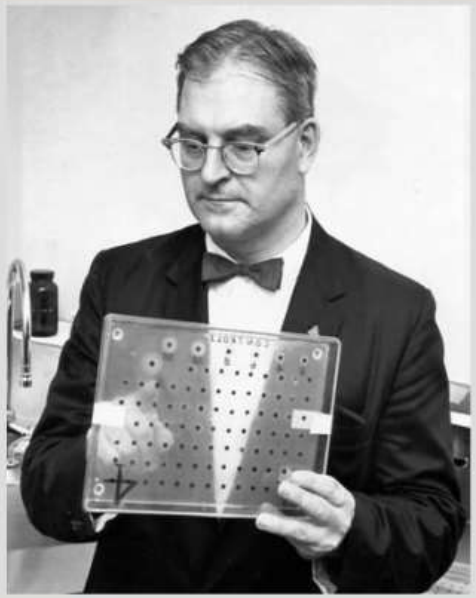
INTERNATIONAL NEONATAL SCREENING DAY
JUNE 28

Early heroes of PKU



Dr. Asbjörn Fölling

Dr. Horst Bickel



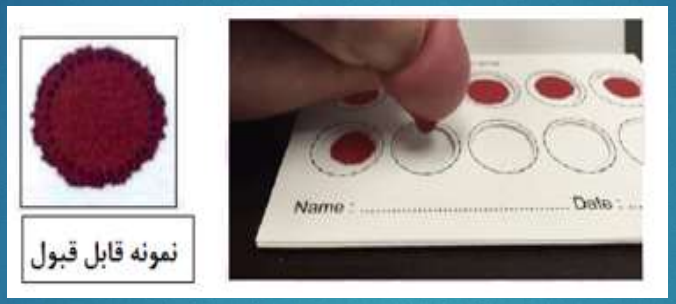
Dr. Robert Guthrie

Diagnosis Treatment Prevention

LC-MS/MS

- Since LC-MS/MS is based on a separation technique, the molecule to be analyzed must be separated from its matrix by pre-treatment.
- Blood, serum or plasma, urine, and cerebrospinal fluid (CSF) have different matrices, and the desired molecule must be separated from these matrices.
- For the separation process, techniques such as extraction, derivatization and often liquid or gas chromatography are used as pre-treatment steps.

Sample preparation



NEWBORN SCREENING TIMELINE

Sample collection within 48-72 hours of life

Shipping of the sample to the Neonatal Screening Lab within 24-48 hours of collection

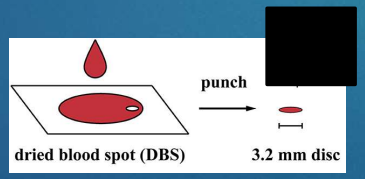
The sample is analyzed by the Neonatal Screening Lab within 24-48 hours of arrival

Medical reporting and recalls where necessary

7 DAYS

Newborn screening with MS/MS

Simultaneous Analysis of Acylcarnitines and Amino Acids in Dried Spot Blood (DBS) with MS/MS



Amino acids	Acylcarnitines	Additional acylcarnitines	
Alanine	Carnitine	C3DC-Carnitine	C16:1-Carnitine
Arginine	C2-Carnitine	C4OH-Carnitine	C16:1OH-Carnitine
Aspartic acid	C3-Carnitine	C4DC-Carnitine	C16OH-Carnitine
Citrulline	C4-Carnitine	C5:1-Carnitine	C18:2-Carnitine
Glutamic acid	C5-Carnitine	C5OH-Carnitine	C18:1-Carnitine
Glycine	C5DC-Carnitine	C6DC-Carnitine	C18:2OH-Carnitine
Leucine	C6-Carnitine	C8:1-Carnitine	C18:1OH-Carnitine
Methionine	C8-Carnitine	C10:2-Carnitine	C18OH-Carnitine
Ornithine	C10-Carnitine	C10:1-Carnitine	
Phenylalanine	C12-Carnitine	C12:1-Carnitine	
Proline	C14-Carnitine	C14:2-Carnitine	
Tyrosine	C16-Carnitine	C14:1-Carnitine	
Valine	C18-Carnitine	C14OH-Carnitine	

Internal Standard IS

- **Amino Acids and Acylcarnitines**

Analytes:

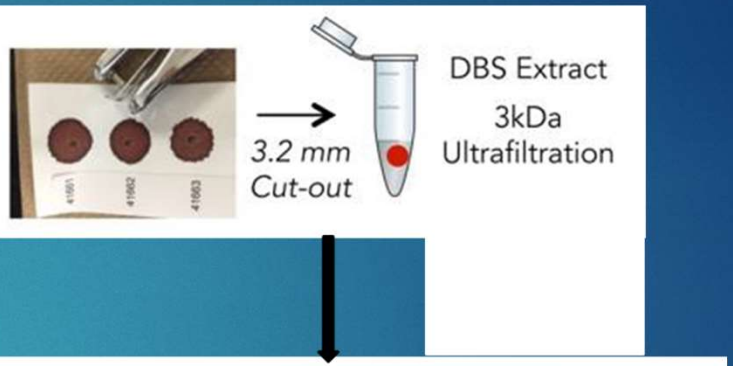
Amino Acids

$^{13}C_3{}^{15}N$-Alanine, $^{13}C_6$-Arginine, $^{13}C_4$-Aspartic Acid, $^{2}H_7$-Citrulline, $^{13}C_5$-Glutamic Acid, 2-$^{13}C^{15}N$-Glycine, $^{2}H_3$-Leucine, $^{2}H_3$-Methionine, $^{2}H_6$-Ornithine, $^{13}C_6$-Phenylalanine, $^{13}C_5$-Proline, $^{13}C_6$-Tyrosine, $^{2}H_8$-Valine

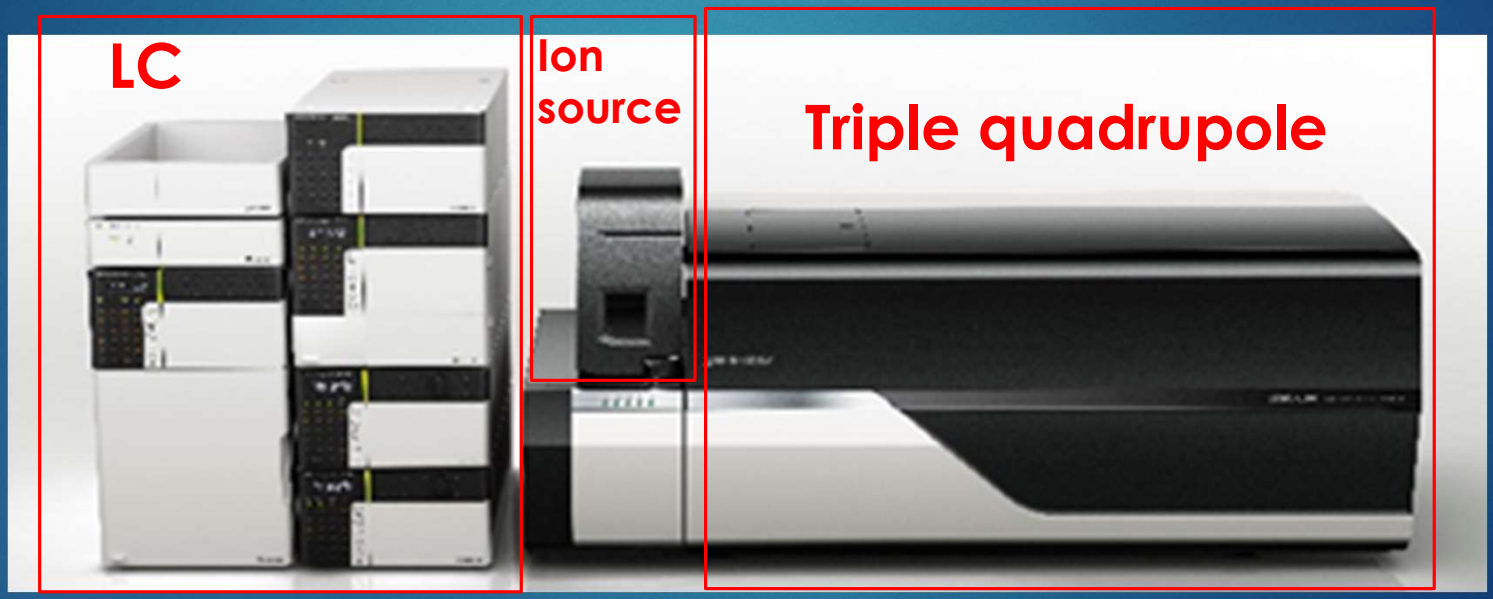
Acylcarnitines

$^{2}H_9$-Carnitine ($^{2}H_9$-C0), $^{2}H_3$-Acetylcarnitine ($^{2}H_3$-C2), $^{2}H_3$-Propionylcarnitine ($^{2}H_3$-C3), $^{2}H_3$-Butyrylcarnitine ($^{2}H_3$-C4), $^{2}H_9$-Isovalerylcarnitine ($^{2}H_9$-C5), $^{2}H_9$-Glutarylcarnitine ($^{2}H_9$-C5DC), $^{2}H_3$-Hexanoylcarnitine ($^{2}H_3$-C6) $^{2}H_3$-Octanoylcarnitine ($^{2}H_3$-C8), $^{2}H_3$-Decanoylcarnitine ($^{2}H_3$-C10), $^{2}H_3$-Dodecanoylcarnitine ($^{2}H_3$-C12), $^{2}H_3$-Tetradecanoylcarnitine ($^{2}H_3$-C14), $^{2}H_3$-Hexadecanoylcarnitine ($^{2}H_3$-C16), $^{2}H_3$-Octadecanoylcarnitine ($^{2}H_3$-C18)

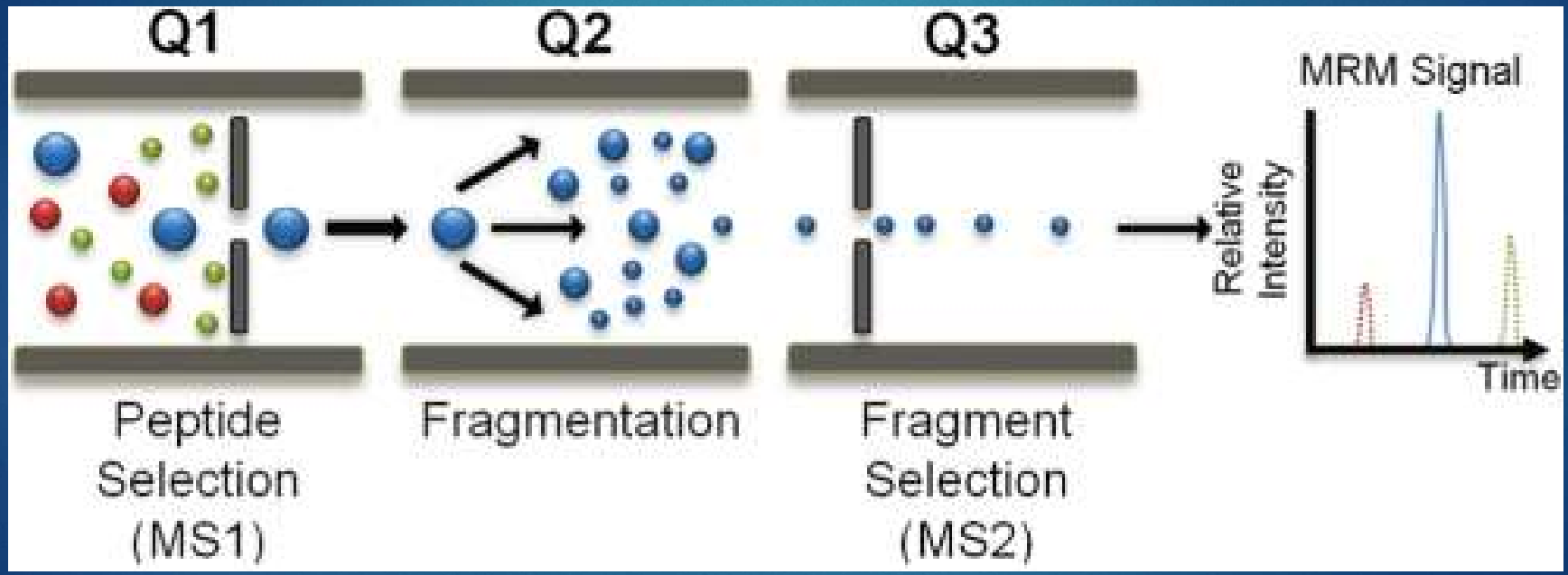
Sample Preparation



Tandem Mass Spectrometer (MS/MS)



Tandem Mass Spectrometry (MS/MS)



	FATTY ACID OXIDATION DISORDERS	ORGANIC ACIDEMIAS	AMINOACIDOPATHIES	UREA CYCLE DISORDERS
Metabolism	**Fat** Defect in β-oxidation of fatty acids.	**Protein** Defect in amino acid breakdown leads to accumulation of organic acid byproducts	**Protein** Defect in amino acid breakdown leads to accumulation of certain intact amino acids	**Protein** Defect in making urea (blood urea nitrogen) from ammonia that results from amino acid breakdown
Disorders	Medium-chain acyl CoA dehydrogenase Long-chain 3-hydroxy acyl CoA dehydrogenase Very long-chain acyl CoA dehydrogenase	Propionic Methylmalonic Isovaleric	Maple syrup urine Phenylketonuria Homocystinuria tyrosinemia	Ornithine transcarbamylase (X-linked) Citrullinemia Arginosuccinic aciduria
Presentation	**Hypoketotic Hypoglycemia** Lethargy, vomiting Sudden infant death syndrome, Reye syndrome Long-chain disorders have cardiomyopathy and rhabdomyolysis	**Metabolic Acidosis With Anion Gap** Neonatal lethargy, vomiting, coma, strokes, death	**No Acidosis or Hyperammonemia** Elevations in specific amino acids See text for clinical features	**Hyperammonemia Without Acidosis** Neonatal lethargy, vomiting, coma, death
Laboratory Tests	Newborn Screen **Plasma acylcarnitines** Hypoglycemia No or inappropriately low ketones	Newborn Screen **Urine organic acids** Plasma acylcarnitines	Newborn Screen **Plasma amino acids**	Newborn Screen (not for ornithine transcarbamylase) **Hyperammonemia** **Plasma amino acids** **Urine orotic acid**

The Recommended Uniform Screening Panel (RUSP) is a national guideline for newborn screening (NBS).

- ✓ **Core conditions**: The HHS Secretary recommends including these in every NBS program. Newborn screening is specifically designed to assess whether your baby might have these conditions.
- ✓ **Secondary conditions**: These may be found while screening for a core condition. Although NBS is not specifically designed to assess whether your baby might have these conditions, it sometimes finds babies likely to have them.

29 core conditions	25 secondary targets
MS/MS-detectable organic acid disorders	
Isovaleric acidemia	Methylmalonic acidemia (Cbl C, D)
Glutaric acidemia type I	Malonic acidemia
3-hydroxy 3-methyl glutaric aciduria	Isobutyryl-CoA dehydrogenase deficiency
Methylmalonic acidemia (mutase deficiency)	2-methyl 3-hydroxy butyric aciduria
3-methylcrotonyl-CoA carboxylase deficiency	2-methylbutyryl-CoA dehydrogenase deficiency
Methylmalonic acidemia (Cbl A, B)	3-methylglutaconic aciduria
Multiple carboxylase deficiency (holocarboxylase synthetase deficiency)	
Propionic acidemia	
β-ketothiolase deficiency	
MS/MS-detectable fatty acid oxidation disorders	
Medium-chain acyl-CoA dehydrogenase deficiency	Short-chain acyl-CoA dehydrogenase deficiency
Very long-chain acyl-CoA dehydrogenase deficiency	Medium/short-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency
Long-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency	Medium-chain ketoacyl-CoA thiolase deficiency
Trifunctional protein deficiency	Carnitine palmitoyltransferase II deficiency
Carnitine uptake defect	Carnitine acylcarnitine translocase deficiency
	Carnitine palmitoyltranferase I deficiency (liver)
	Glutaric acidemia type II (multiple acyl-CoA dehydrogenase deficiency)
	2, 4-dienoyl-CoA reductase deficiency
MS/MS-detectable amino acid disorders	
Phenylketonuria	Benign hyperphenylalaninemia
Maple syrup urine disease	Tyrosinemia type II
Homocystinuria	Tyrosinemia type III
Citrullinemia type I	Defects of biopterin cofactor biosynthesis
Argininosuccinic aciduria	Defects of biopterin cofactor regeneration
Tyrosinemia type I	Argininemia
	Hypermethioninemia
	Citrullinemia type II
Hemoglobinopathies	
Sickle cell anemia (SS-disease)	Variant hemoglobinopathies
Sickle-C disease	
S-β thalassemia	
Other	
Transferase-deficient galactosemia (classical)	Galactokinase deficiency
Primary congenital hypothyroidism	Galactoepimerase deficiency
21-hydroxylase-deficient congenital adrenal hyperplasia	
Biotinidase deficiency	
Hearing screening	
Cystic fibrosis	

MS/MS: Tandem mass spectrometry.
Data taken from [35,36].

Fatty acid oxidation disorders (FAODs)

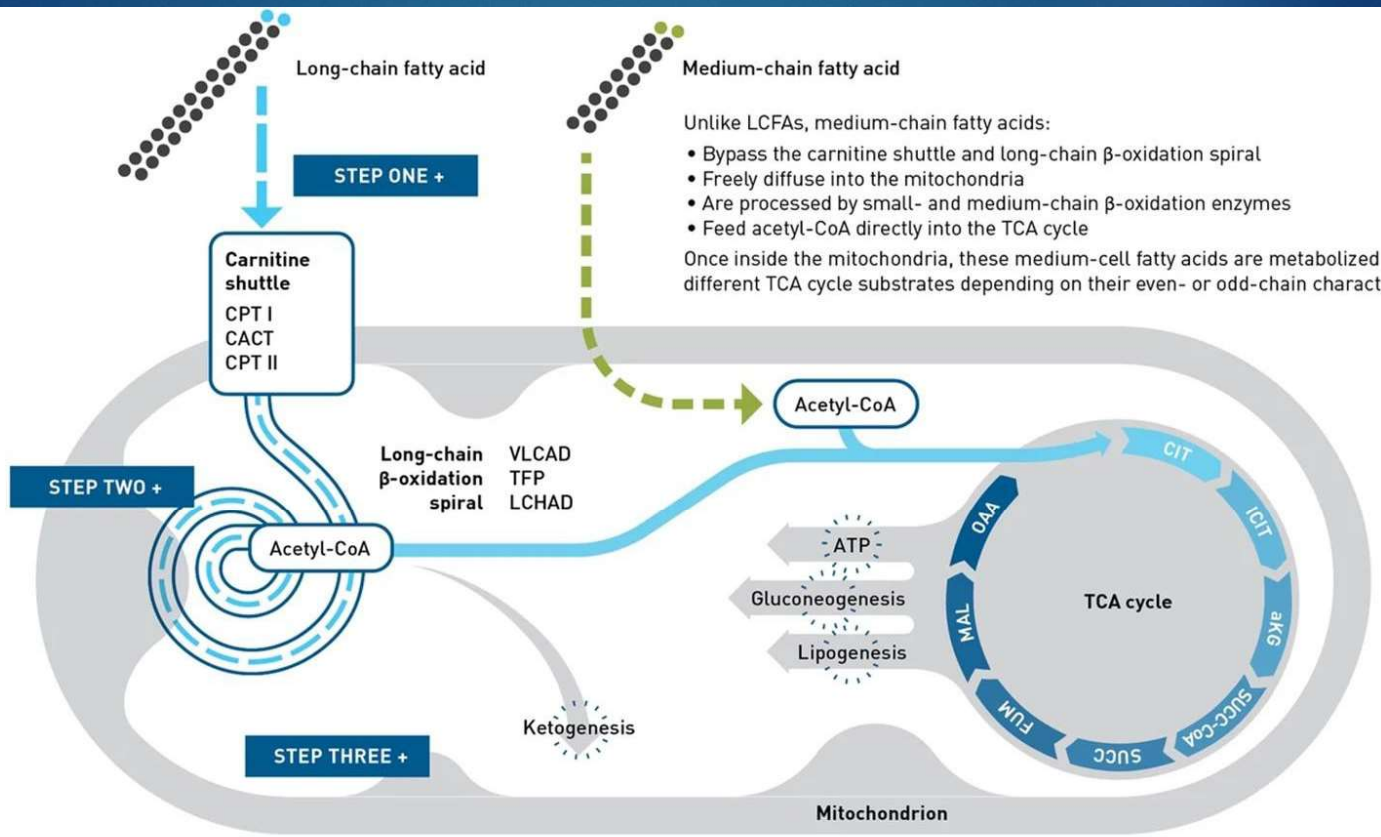
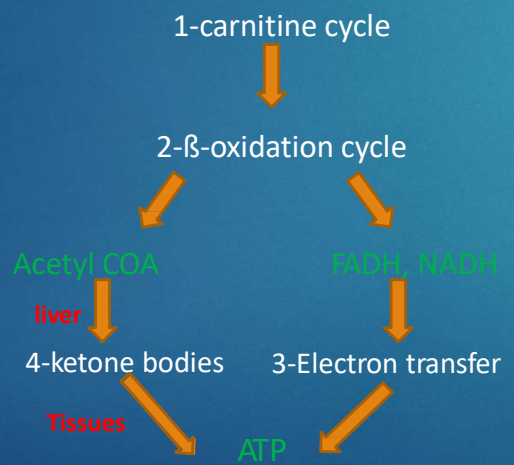


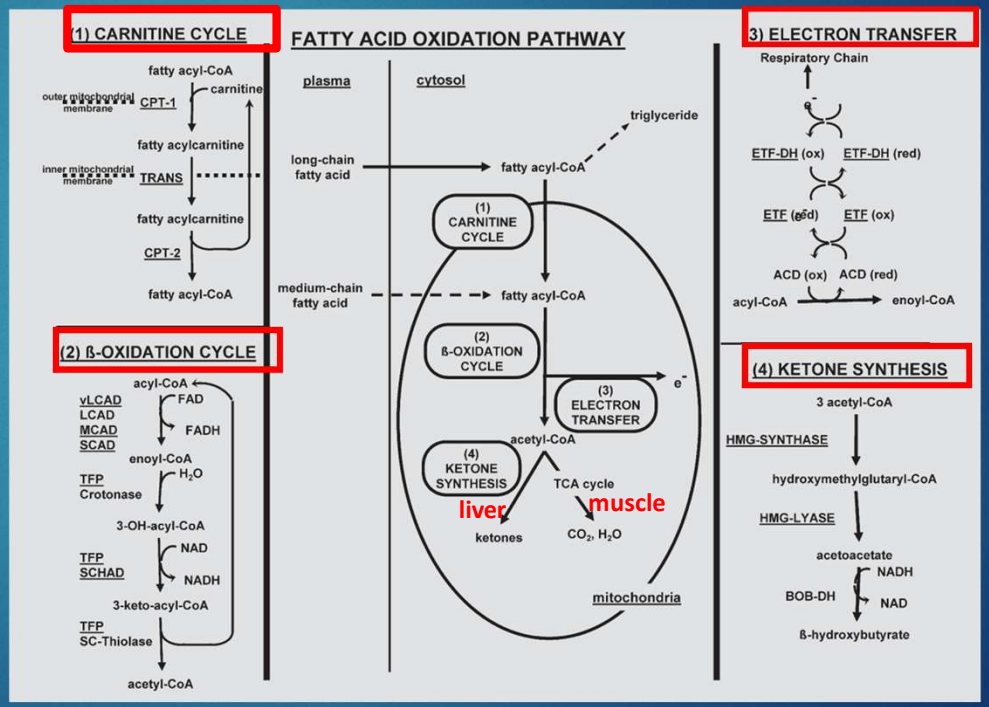
Image obtained from FAOD in Focus website. https://www.faodinfocus.com/hcp/mechanism-of-disease/
Acetyl-CoA, acetyl coenzyme A; ATP, adenosine triphosphate; CACT, carnitine-acylcarnitine translocase deficiency; CPT 1, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; LCFA, long-chain fatty acid; LCHAD, long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency; TCA, tricarboxylic acid; TFP, trifunctional protein deficiency; VLCAD, very-long-chain acyl-CoA dehydrogenase deficiency.

Fatty acid oxidation disorders

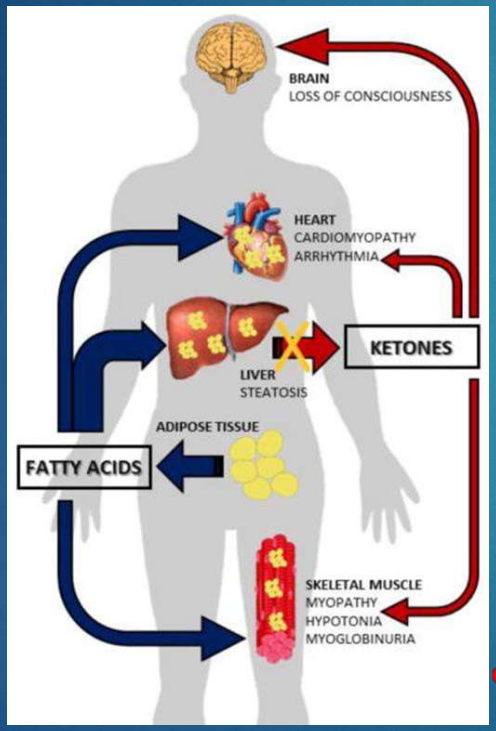
Fatty acid oxidation comprises four components:

1. carnitine cycle
2. ß-oxidation cycle
3. electron-transfer path
4. synthesis of ketone bodies

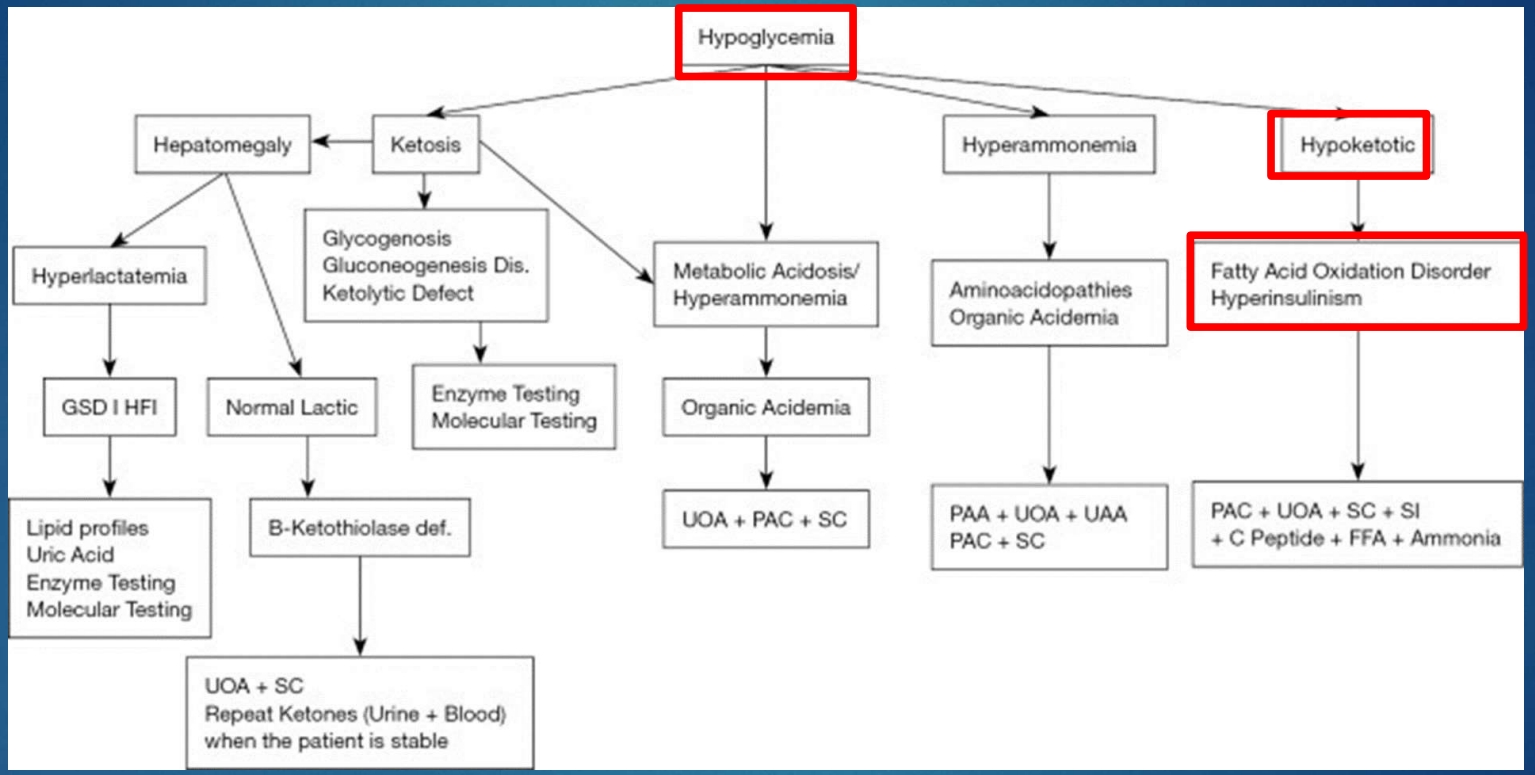




Fatty acid oxidation disorders



Defect	Clinical manifestations of defect			
	Hepatic	Cardiac	Skeletal muscle	
			Acute	Chronic
Carnitine cycle				
CTD	+	+		(+)
CPT-1	+			
Trans	+	+		+
CPT-2	+	+	(+)	+
β-Oxidation cycle				
Acyl-CoA dehydrogenases				
VLCAD	+	+	+	+
MCAD	+			
SCAD				+
3-Hydroxyacyl-CoA dehydrogenases				
LCHAD	+	+	+	
SCHAD			+	+
MCKT			+	+
DER				+
Electron transfer				
ETF	+	+	(+)	+
ETF-DH	+	+	(+)	+
Ketone synthesis				
HMG-CoA synthase	+			
HMG-CoA lyase	+			


Hypoglycemia
Hepatomegaly
Ketosis
Hyperammonemia
Hypoketotic
Hyperlactatemia
Glycogenosis
Gluconeogenesis Dis.
Ketolytic Defect
Metabolic Acidosis/
Hyperammonemia
Aminoacidopathies
Organic Acidemia
Fatty Acid Oxidation Disorder
Hyperinsulinism
GSD I HFI
Normal Lactic
Enzyme Testing
Molecular Testing
Organic Acidemia
Lipid profiles
Uric Acid
Enzyme Testing
Molecular Testing
B-Ketothiolase def.
UOA + PAC + SC
PAA + UOA + UAA
PAC + SC
PAC + UOA + SC + SI
+ C Peptide + FFA + Ammonia
UOA + SC
Repeat Ketones (Urine + Blood)
when the patient is stable

Fatty acid oxidation defects

Disorders	Primary metabolite in MS/MS	Confirmatory tests / follow-up	Findings in confirmatory tests
Carnitine acylcarnitine translocase (CACT) deficiency	↑ C16–C18 acylcarnitines, ↓ Free carnitine	PACP, CK, glucose, NH3	↑ C16–C18 acylcarnitines on PACP; ↓ free carnitine, ↑ CK, ↓ glucose, ↑ NH3 on plasma
Carnitine palmitoyl transferase type 1 (CPT-1) deficiency	↓ C16–C18 acylcarnitines, ↕–↑ free carnitine	PACP, CK, glucose, NH3	↓ C16–C18 acylcarnitines on PACP; ↕–↑ free carnitine, ↑ CK, ↓ glucose, ↑ NH3 on plasma
Carnitine palmitoyl transferase type 2 (CPT-2) deficiency	↑ C16–C18 acylcarnitines, ↓ free carnitine	PACP, CK, glucose, NH3	↑ C16–C18 acylcarnitines on PACP; ↓ free carnitine, ↑ CK, ↓ glucose, ↑ NH3 on plasma
Carnitine uptake/ transporter defect	↓ C16–C18 acylcarnitines, ↓ free carnit	PACP, urine carnitine, CK, glucose, NH3	↓ C16–C18 acylcarnitines on PACP; ↑ urine carnitine; ↓ free carnitine, ↑ CK, ↓ glucose, ↑ NH3 on plasma
3-Hydroxy long chain acyl-CoA dehydrogenase deficiency (LCHAD/MTP)	↑ Long chain 3-hydroxy acylcarnitines	PACP, UOA, CK, glucose, NH3	↑ Long chain 3-hydroxy acylcarnitines on PACP; ↑ 3-OH dicarboxylic acids on UOA; ↑ CK, ↓ Glucose, ↑ NH3 on plasma
Medium chain acyl-CoA dehydrogenase (MCAD) deficiency	↑ C8–C10 acylcarnitines	PACP, UOA, CK, glucose, NH3	↑ C8–C10 acylcarnitines on PACP; ↑ dicarboxylic acids, hexanoylglycine, phenylpropionylglycine and suberylglycine on UOA; ↑ CK, ↓ glucose, ↑ NH3 on plasma
Multiple acyl-CoA dehydrogenase deficiency (MADD) or glutaric acidemia-type 2	↑ Multiple acylcarnitines	PACP, UOA, CK, glucose, NH3	↑ Multiple acylcarnitines on PACP; ↑ glutaric, ethylmalonic, dicarboxylic acids, hexanoylglycine, phenylpropionylglycine and suberylglycine on UOA;↑ CK, ↓ glucose, ↑ NH3 on plasma
Short chain acyl-CoA dehydrogenase deficiency (SCAD)	↑ Butyrylcarnitine (C4)	PACP, UOA	↑ Butyrylcarnitine on PACP; ↑ ethylmalonic, methylsuccinic, butyrylglycine on UOA
Very long chain acyl-CoA dehydrogenase deficiency (VLCAD)	↑ C14, C14:1, C14:2 acylcarnitines, ↓ Free carnitine	PACP, CK, glucose, NH3	↑ Long chain acylcarnitines on PACP; ↑ CK, ↓ glucose, ↑ NH3 on plasma

Amino acid disorders

Amino acid disorders

Collectively affect approximately 1 in 8000 newborns.

Almost all are transmitted as autosomal recessive traits.

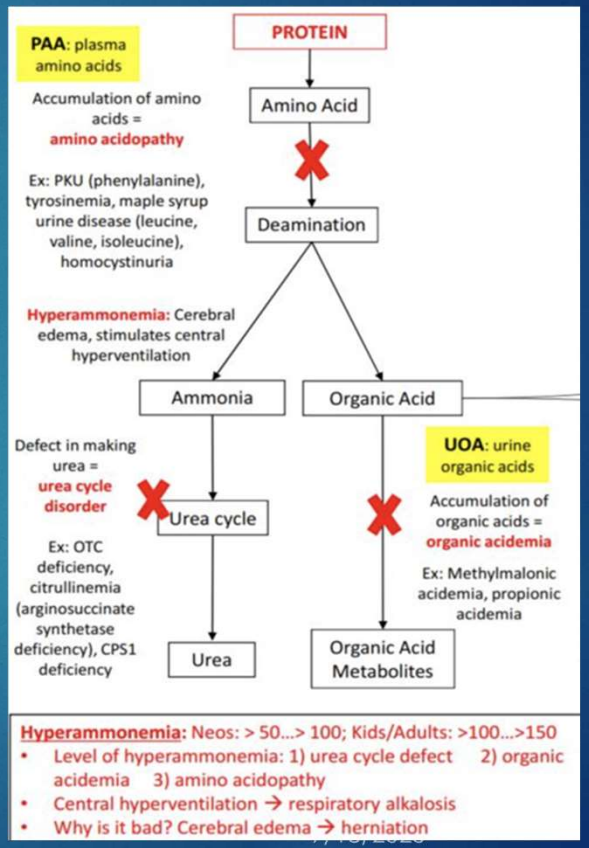
Result from a lack of a specific enzyme in the metabolic pathway of an amino acid.

This leads either to the accumulation of (1) the parent amino acid, (2) its by-products or (3) the catabolic products (organic acids).

Disorders of amino acid metabolism are divided into two groups:

(1) **Aminoacidopathies**, in which the parent amino acid accumulates in excess in blood and spills over into urine.

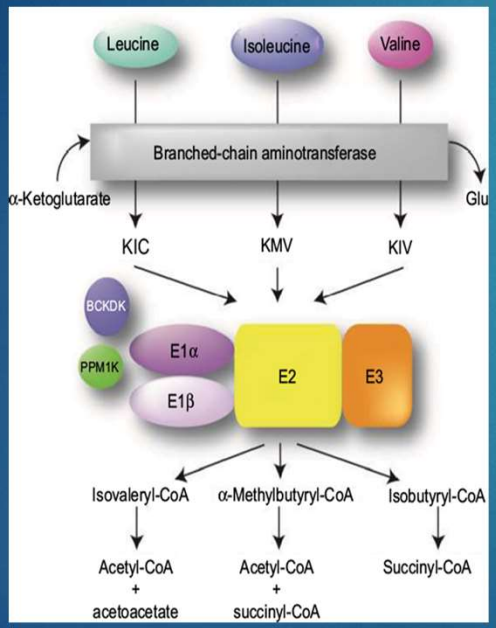
(2) **Organic acidemias**, in which products in the catabolic pathway of certain amino acids accumulate.

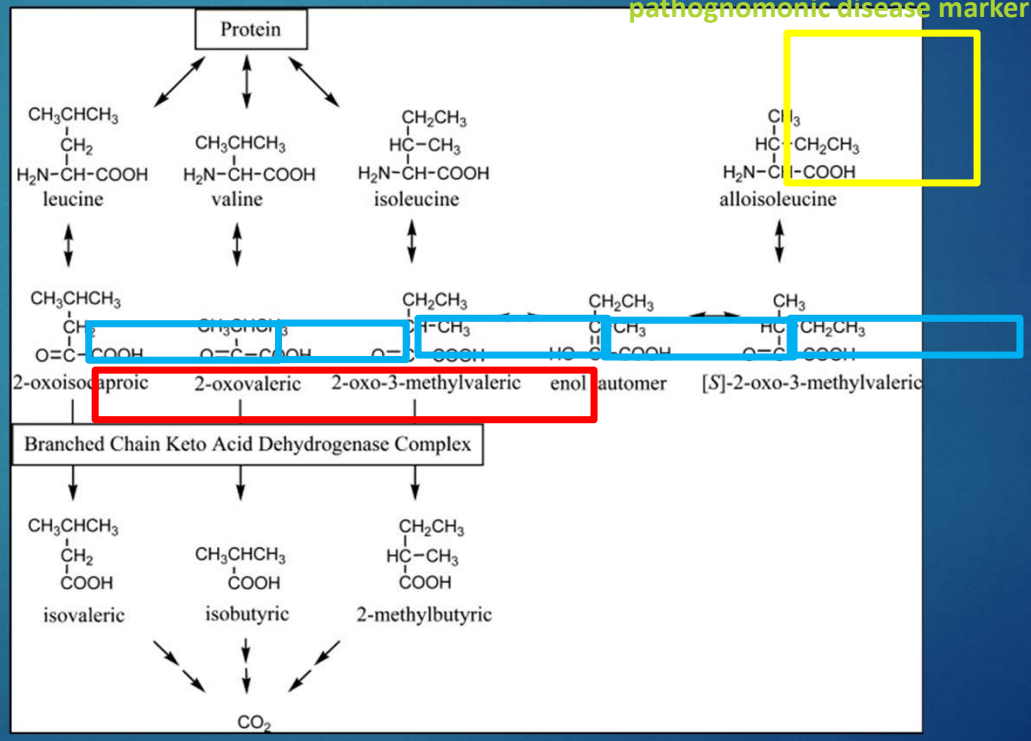


Aminoacidopathies

Disorders	Primary metabolite in MS/MS	Confirmatory tests / follow-up	Findings in confirmatory tests
Argininemia	↑ Arginine	Plasma NH3, PAA, enzyme assay	↑ NH3, ↑ arginine on PAA, ↓ hepatic arginase activity
Argininosuccinic aciduria (ASA)	↑ Citrulline	Plasma NH3, UAA, PAA, enzyme assay	↑ NH3, ↑ argininosuccinic acid on UAA and PAA, ↓ fibroblast/liver ASL activity
Citrullinemia Type 1 "Neonatal" citrullinemia	↑ Citrulline	Plasma NH3, PAA	↑ NH3, ↑ citrulline on PAA, ↓ fibroblast/liver ASS activity
Homocystinuria	↑ Methionine	PAA, Hcy in P, UAA, UOA	↑ Blood and urine homocyst(e)ine on PAA and UAA; ↑ urine methylmalonic acid on UOA in cobalamin C, D, F synthesis defects
Maple syrup urine disease (MSUD)	↑ total "Leucine, isoleucine, alloisoleucine ↑ Valine	PAA, Urine DNPH, UOA	↑ Leucine, isoleucine, alloisoleucine and valine on PAA; positive DNPH; ↑ branched chain a-keto and hydroxyl acids on UOA
Phenylketonuria	↑ Phenylalanine ↑ phenylalanine tyrosine ratio	PAA, urine and/or blood or CSF neopterin and biopterin studies	↑ Phenylalanine on PAA, ↑ phenylalanine/ tyrosine ratio; abnormal urinary and/or blood or CSF pterins in BH4 synthesis defects
Tyrosinemia type 1	↑ Tyrosine	PAA, UOA	↑ Tyrosine and methionine on PAA; ↑ succinylacetone and tyrosine metabolites on UOA
Tyrosinemia type 2 Oculocutaneous tyrosinemia	↑ Tyrosine	PAA, UOA	↑ Tyrosine on PAA; ↑ tyrosine metabolites without increased succinylacetone on UOA

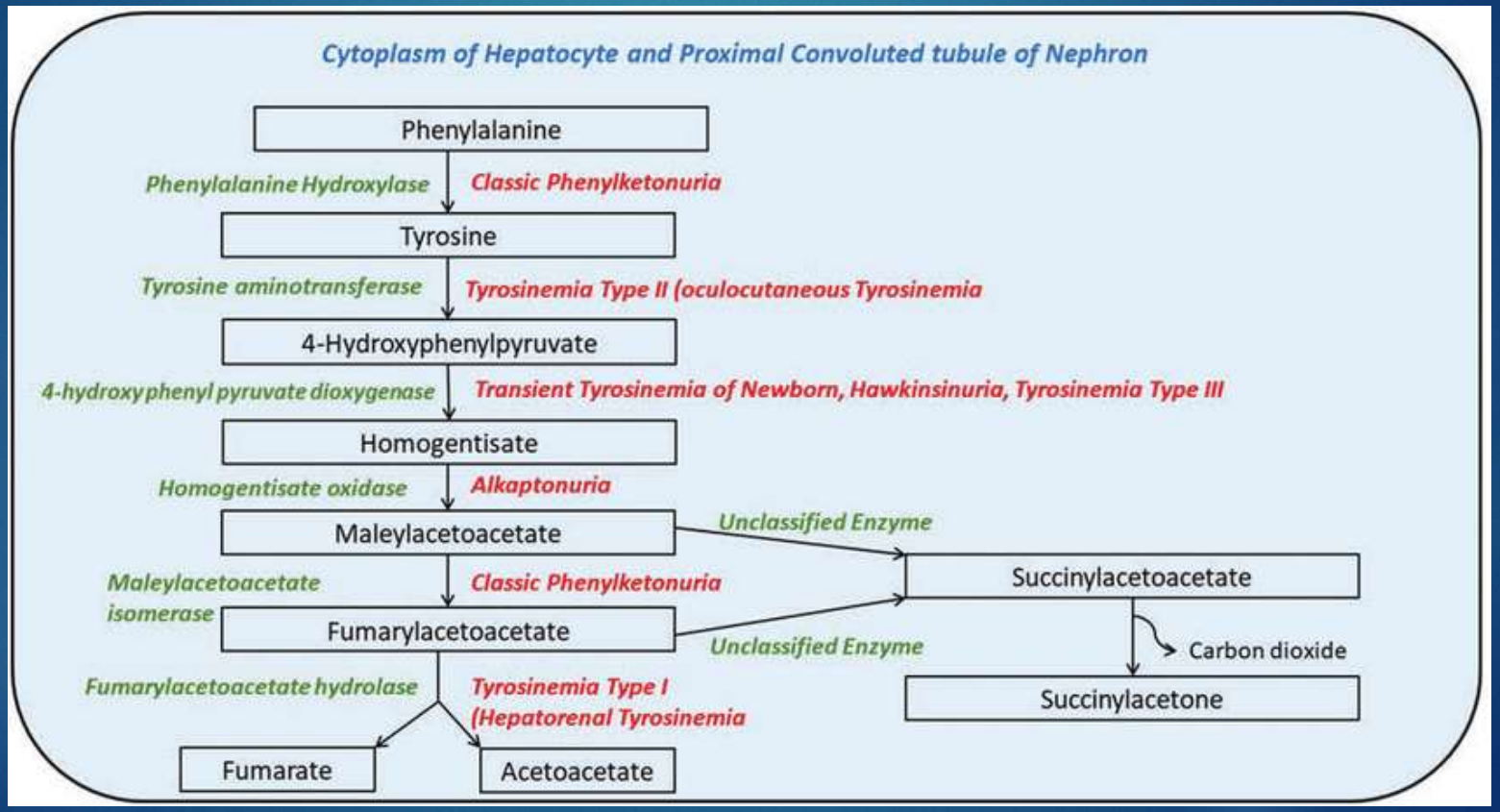
MSUD





pathognomonic disease marker

Tyrosine Metabolism Disorders

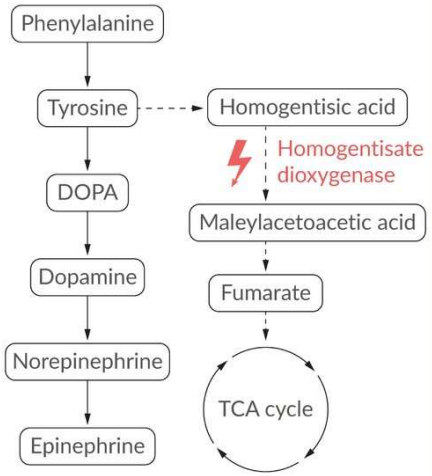


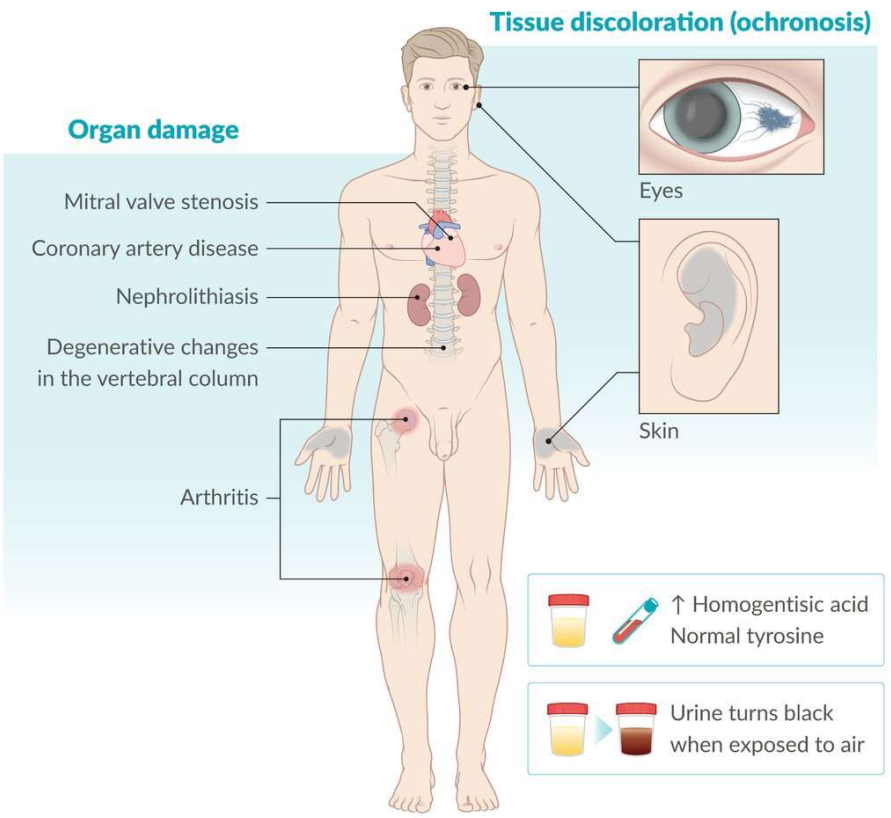
Alkaptonuria

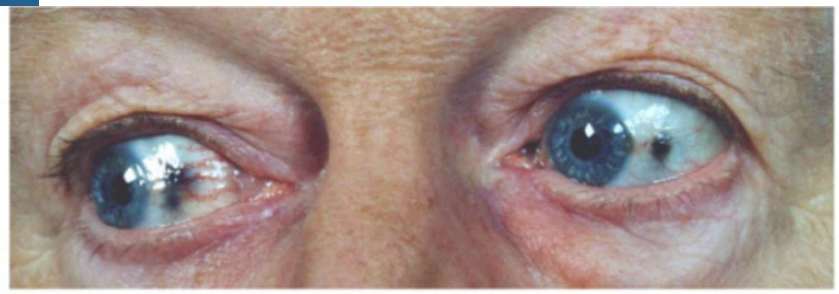
Etiology
Autosomal recessive inheritance
Mutation in the *HGD* gene
Impaired homogentisate dioxygenase

Treatment
Diet low in tyrosine and phenylalanine

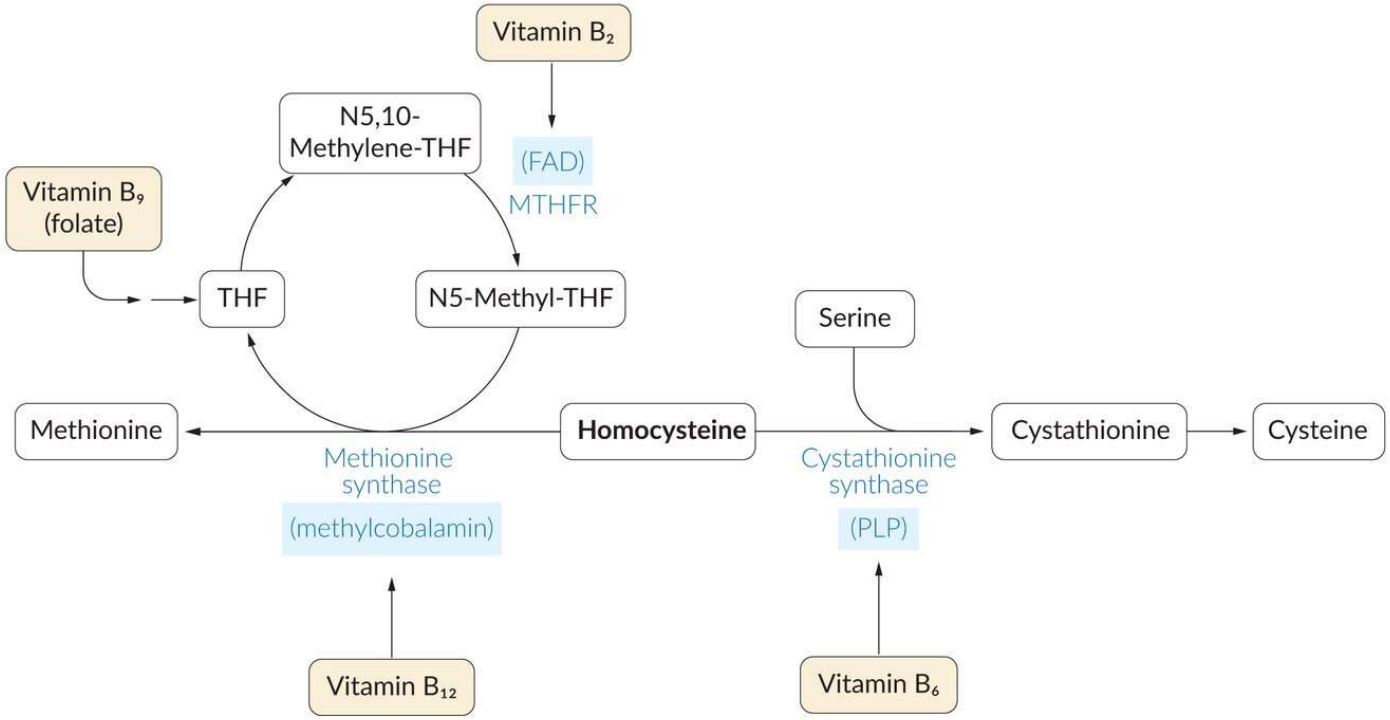
Biochemical pathway







Homocystinuria

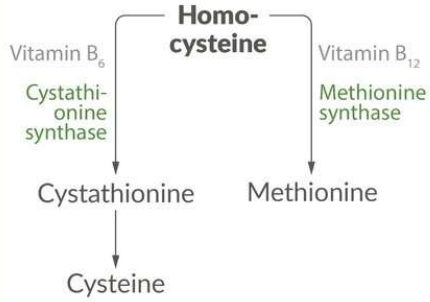


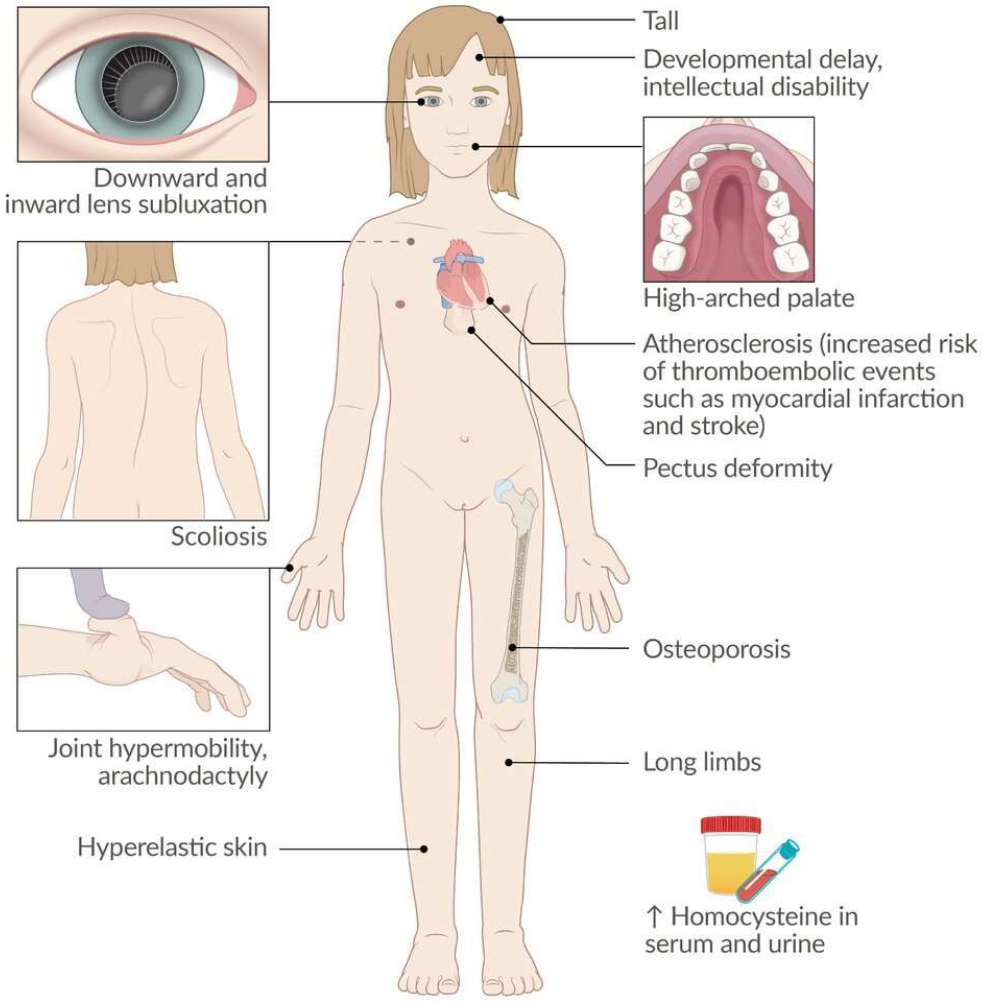
Homocystinuria

Etiology
- Most commonly, deficiency of:
  - Methionine synthase
  - Cystathionine synthase
- Autosomal recessive inheritance

Treatment
- Pyridoxine (vitamin B_6)
- Specific diet (e.g., high-methionine diet)

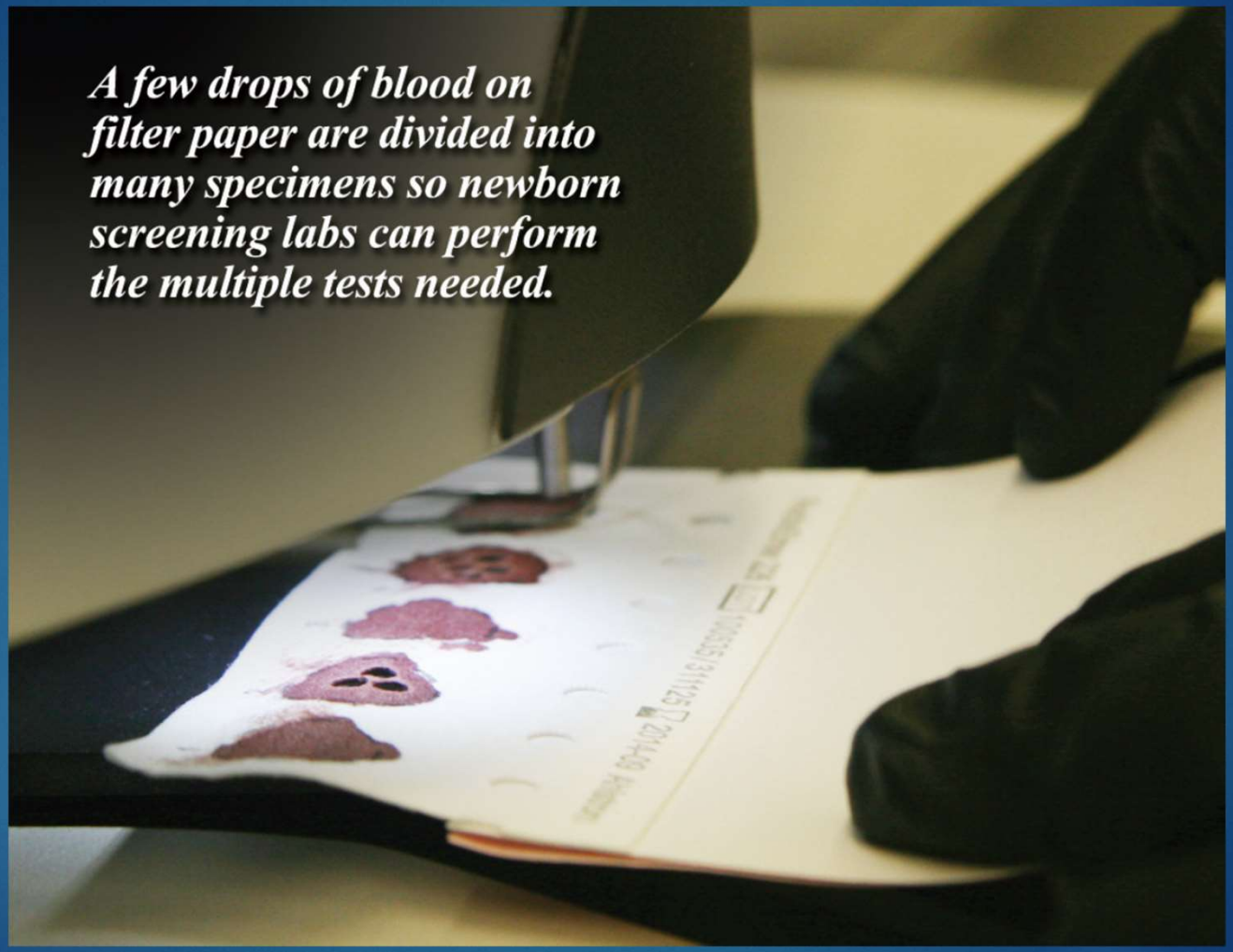
Biochemical pathway





Organic acidemias

Disorders	Primary metabolite in MS/MS	Confirmatory tests / follow-up	Findings in confirmatory tests
Glutaric aciduria 1 (GA I)	↑ Glutarylcarnitine (C5-dicarboxylic)	UOA, PACP	↑ Glutaric acid, 3-hydroxygluratic acid, glutaconic acid on UOA; ↑Glutarylcarnitine (C5-dicarboxylic) on PACP
HMG- CoA lyase deficiency	↑ 3- Hydroxyisovalerylcarnitine (C5-OH)	UOA, PACP	↑ 3-Hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric, 3-hydroxy-3-methylglutaric acids on UOA; ↑ C5- Hydroxyisovalerylcarnitine (C5-OH), 3 methylglutarylcarnitine (C6DC) on PACP
Isovaleric acidemia	↑ Isovalerylcarnitine (C5)	UOA, PACP	↑ Isovalerylglycine, 3-hydroxyisovaleric acid on UOA; ↑ isovalerylcarnitine (C5) on PACP
3-Keto(oxo) thiolase deficiency	↑ Tiglylcarnitine (C5:1), ↑ 3-hydroxy-2-methylbutyrylcarnitine (C5-OH)	UOA, PACP	↑ 2-Methyl-3- hydroxybutyrate, 2 methylacetoacetic, tiglylglycine on UOA; ↑ tiglylcarnitine (C5:1), ↑ 3-hydroxy-2-methylbutyrylcarnitine (C5-OH) on PACP
3-MCC deficiency	↑ 3- Hydroxyisovalerylcarnitine (C5-OH)	UOA, PACP	↑ 3-Hydroxyisovaleric, 3-methylcrotonylglycine on UOA; ↑ 3- hydroxyisovalerylcarnitine (C5-OH) on PACP
2-Methylbutyryl CoA dehydrogenase	↑ 2-Methylbutyrylcarnitine (C5)	UOA	↑ 2-Methylbutyrylglycine on PACP
3-Methylglutanoyl CoA hydratase deficiency	↑ 3 Hydroxyisovalerylcarnitine (C5-OH)	UOA, PACP	↑ 3-Hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric on UOA; ↑ 3 hydroxyisovalerylcarnitine (C5-OH) on PACP
Methylmalonic acidemia	↑ Propionylcarnitine (C3)	UOA, PACP	Methylmalonic, 3-hydroxypropionate, methylcitrate, propionylglycine on UOA; ↑propionylcarnitine (C3) on PACP
Multiple CoA carboxylase deficiency	↑ Propionylcarnitine (C3), ↑ 3-hydroxyisovalerylcarnitine (C5-OH)	UOA, PACP	↑ 3-OH-isovaleric, 3-methylcrotonylglycine, methylcitrate, 3-OH-propionic, lactate, pyruvate, acetoacetate, 3-OH-butyrate on UOA; ↑ propionylcarnitine (C3),↑ 3 hydroxyisovalerylcarnitine (C5-OH) on PACP
Propionic acidemia	↑ Propionylcarnitine (C3)	UOA, PACP	↑ 3-Hydroxypropionate, methylcitrate, propionylglycine; ↑ propionylcarnitine (C3) on PACP


A few drops of blood on
filter paper are divided into
many specimens so newborn
screening labs can perform
the multiple tests needed.


Each year, 12,500 babies with serious but treatable conditions grow up healthy.
Thanks to newborn screening.


Thanks

References

- https://www.chem.pitt.edu/facilities/mass-spectrometry/mass-spectrometry-introduction0
- https://www.intechopen.com/chapters/76164
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7896279/
- https://pubs.acs.org/doi/10.1021/acsomega.9b03764
- https://www.utsc.utoronto.ca/~traceslab/PDFs/MassSpec_QuadsInfo.pdf
- https://www.technologynetworks.com/analysis/articles/how-a-mass-spectrometer-works-types-of-instrumentation-and-interpreting-mass-spectral-data-347878
- Fire Debris Analysis, Book (2008), CHAPTER 8 - Gas Chromatography and Gas Chromatography—Mass Spectrometry, Eric Stauffer, Julia A. Dolan, Reta Newman.