How MS/MS Revolutionized Newborn Screening

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NEWBORN SCREENING IN NORTH CAROLINA (1994)

- 1965 - Phenylketonuria (BIA, then HPLC)
- 1979 - Hypothyroidism (Fluorometric assay for TSH; thenT4 if positive)
- 1987 - Sickle cell disease (limited – Isoelectric Focusing Gel Electrophoresis)
- 1988 - Galactosemia (Fluorometric assay for galactose, then Beutler GALT assay)
- 1989 - Congenital adrenal hyperplasia (Fluorometric assay for 17-OH progesterone)
- 1994 - Sickle cell disease (extended – IEF, then HPLC)

ESTIMATED FREQUENCY OF SOME INBORN ERRORS OF METABOLISM AMONG LIVE-BORN INFANTS IN THE US

• Phenylketonuria	1 in 15,000
• Congenital Hypothyroidism	1 in 4,000
• Galactosemia	1 in 60,000
• Congenital Adrenal Hyperplasia	1 in 16,000
• Biotinidase	1 in 60,000
• Cystic Fibrosis	1 in 3,000

Most inborn errors of metabolism are rare with incidence of less than 1 in 100,000 live births

How MS/MS became involved in Newborn Screening: stage 1

- **Historically, MS/MS was developed as a clinical diagnostic test for disorders of fatty acid oxidation, by detection of abnormal acylcarnitines (Duke Laboratory 1984-89):**

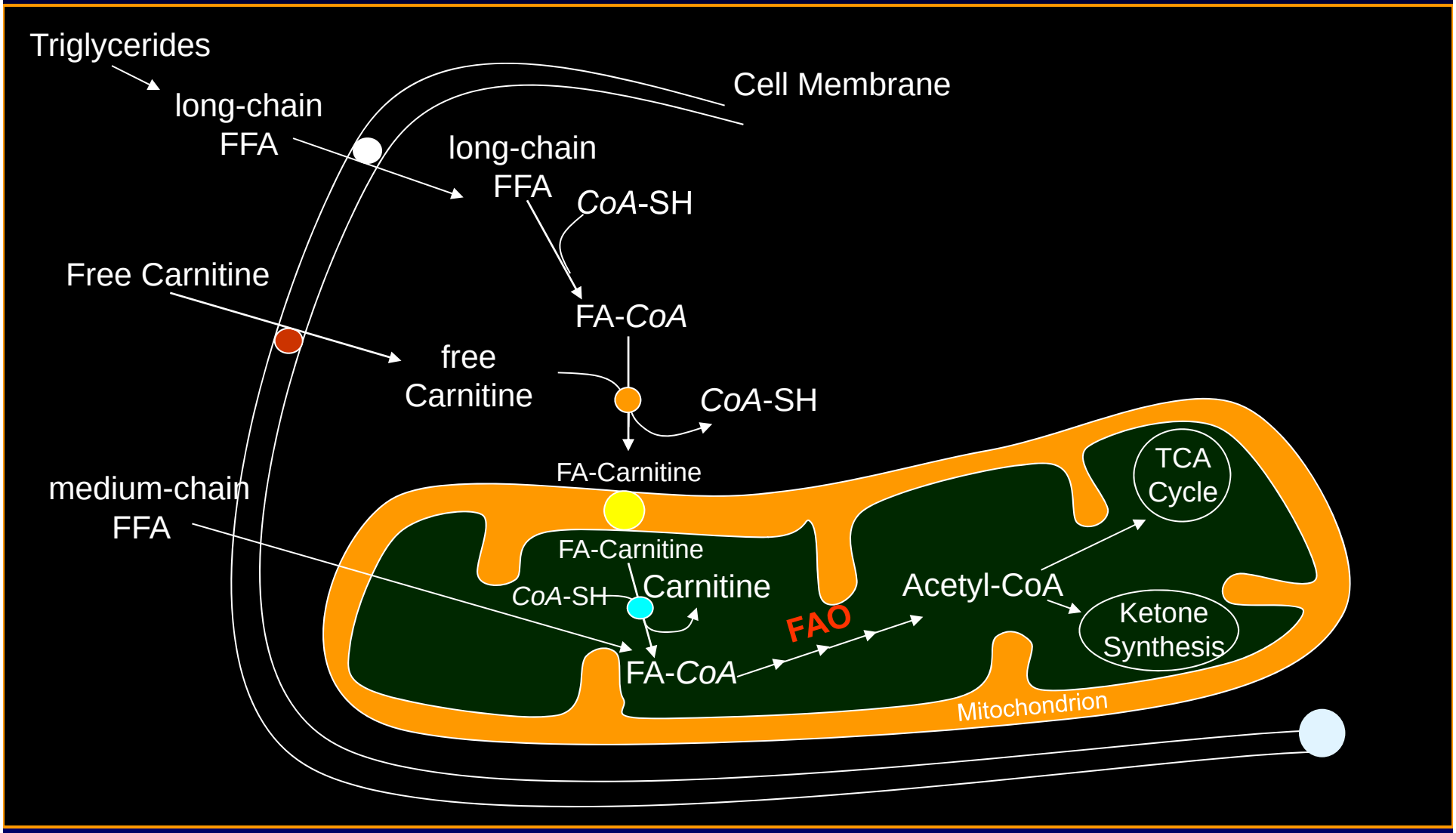
Application of fast atom bombardment and constant B/E ratio liked scanning to the identification and analysis of acylcarnitines in metabolic disease:

Millington DS, Roe CR, Maltby DA. Biomed Mass Spectrom 11:236-241 (1984)

Link between SIDS and defects of fatty acid oxidation

- **"Recognition of Medium-Chain Acyl-CoA Dehydrogenase Deficiency in Asymptomatic Siblings of Children Dying from Sudden Infant Death or Reye-Like Syndromes".** ***Roe, et al. J. Pediatr. 108:1-13, (1986).***
- **"Sudden infant death syndrome and inherited disorders of fatty acid beta-oxidation"** ***Harpey, et al. Biol Neonate. 58 Suppl 1:70-80 (1990).***

Mitochondrial Fatty Acid Metabolism



Carnitine Transporter; Carnitine Palmitoyl Transferase I (CPT I); Carnitine-acylcarnitine translocase;
Long-chain FFA Transporter Carnitine Palmitoyl Transferase II (CPT II); **FAO**: Fatty Acid Oxidation

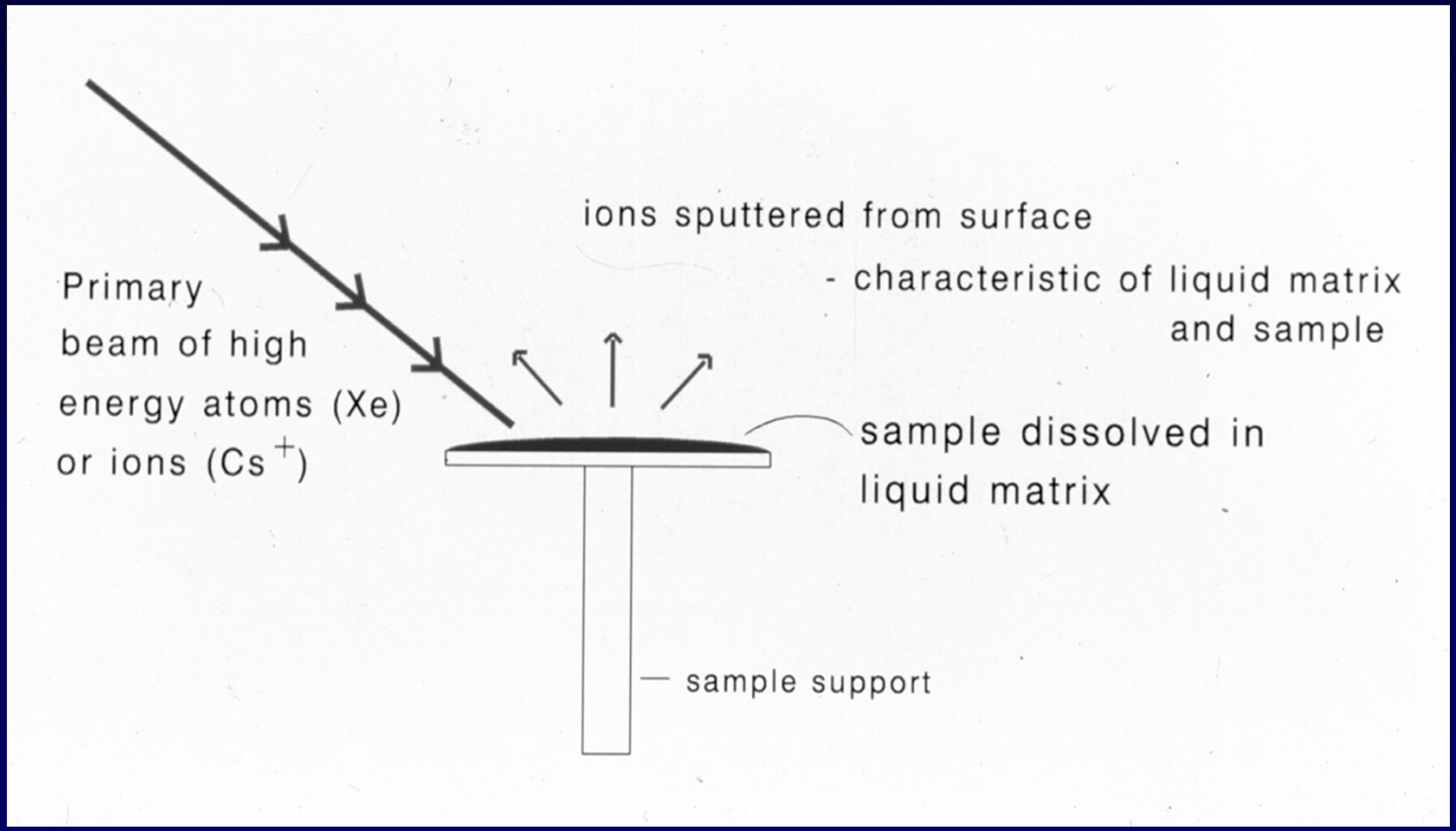
Mitochondrial oxidation/catabolism

- The mitochondrial pathway for fatty acid oxidation involves multiple enzymes and is an essential energy source when reserves of glucose are exhausted (e.g. after a prolonged fast)
- The catabolism of several amino acids (especially LEU, ILE and VAL) also involves mitochondrial enzymes
- Enzymatic defects in these pathways lead to accumulation of abnormal acyl-coA intermediates, many of which are cytotoxic
- Trapped Acyl-coA intermediates can exit the mitochondria and the cell as acylcarnitines, which can be detected and analyzed in urine & blood by MS/MS

Acylcarnitines: The Analytical Challenge

- Acylcarnitines are relatively small, very polar molecules
- Not readily amenable to GC/MS
- Not selectively analyzed by HPLC – share similar structural features to many other biological compounds
- In 1984, the only recourse was to try Fast Atom Bombardment with MS/MS

Principle of Fast Atom/Fast Ion Bombardment (LSIMS)



How the Tandem Mass Spectrometer Works

Specimen mixture introduced directly into system - usually without on-line chromatography

All sample mixture components are ionized at once - e.g. by FAB or electrospray - to produce predominantly molecular or quasi-molecular ions with little fragmentation

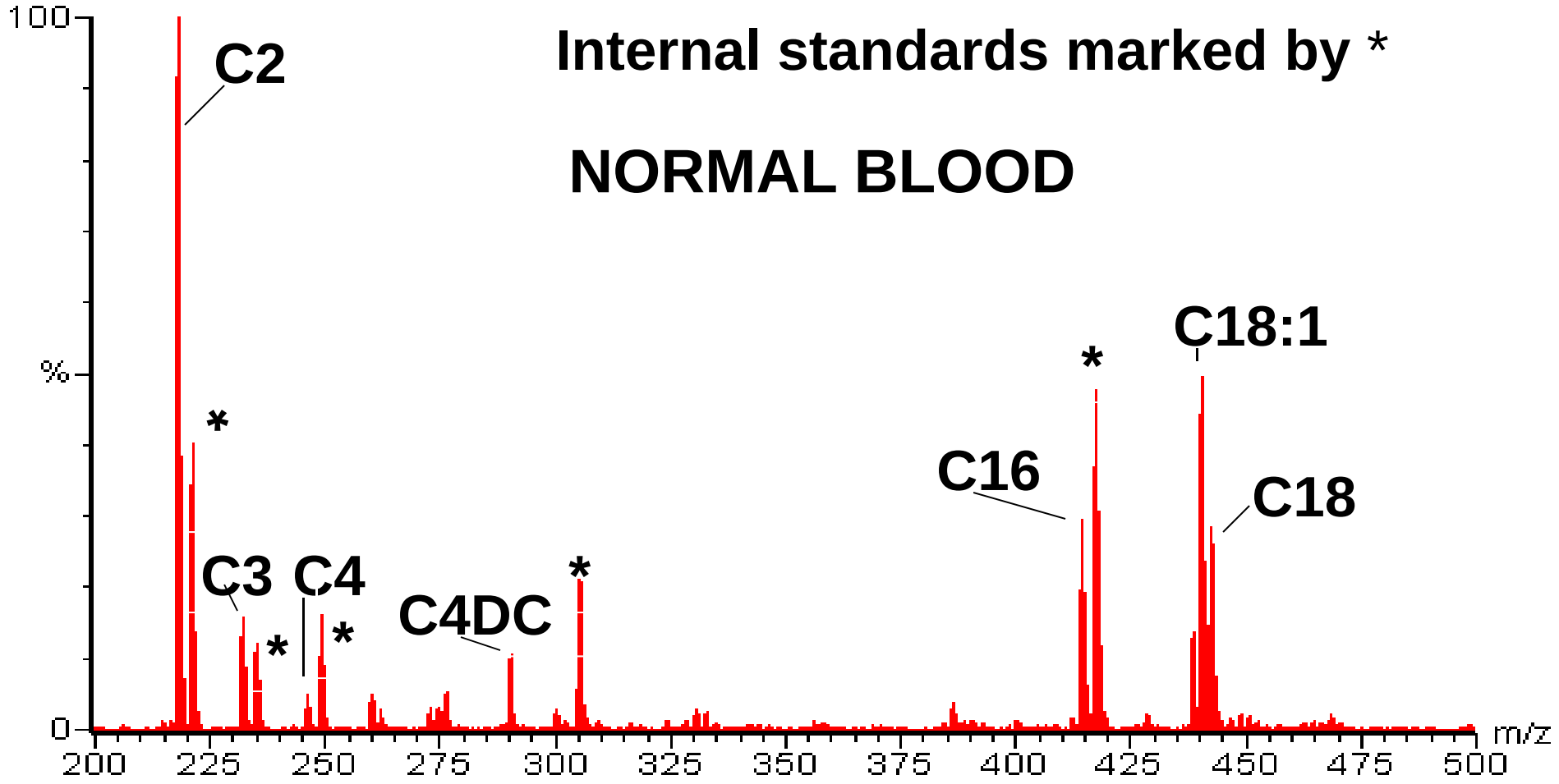
Molecular ions are separated in MS-I according to their mass/charge (m/z) ratio

Mass-selected ions from MS-I are induced to fragment in the collision cell

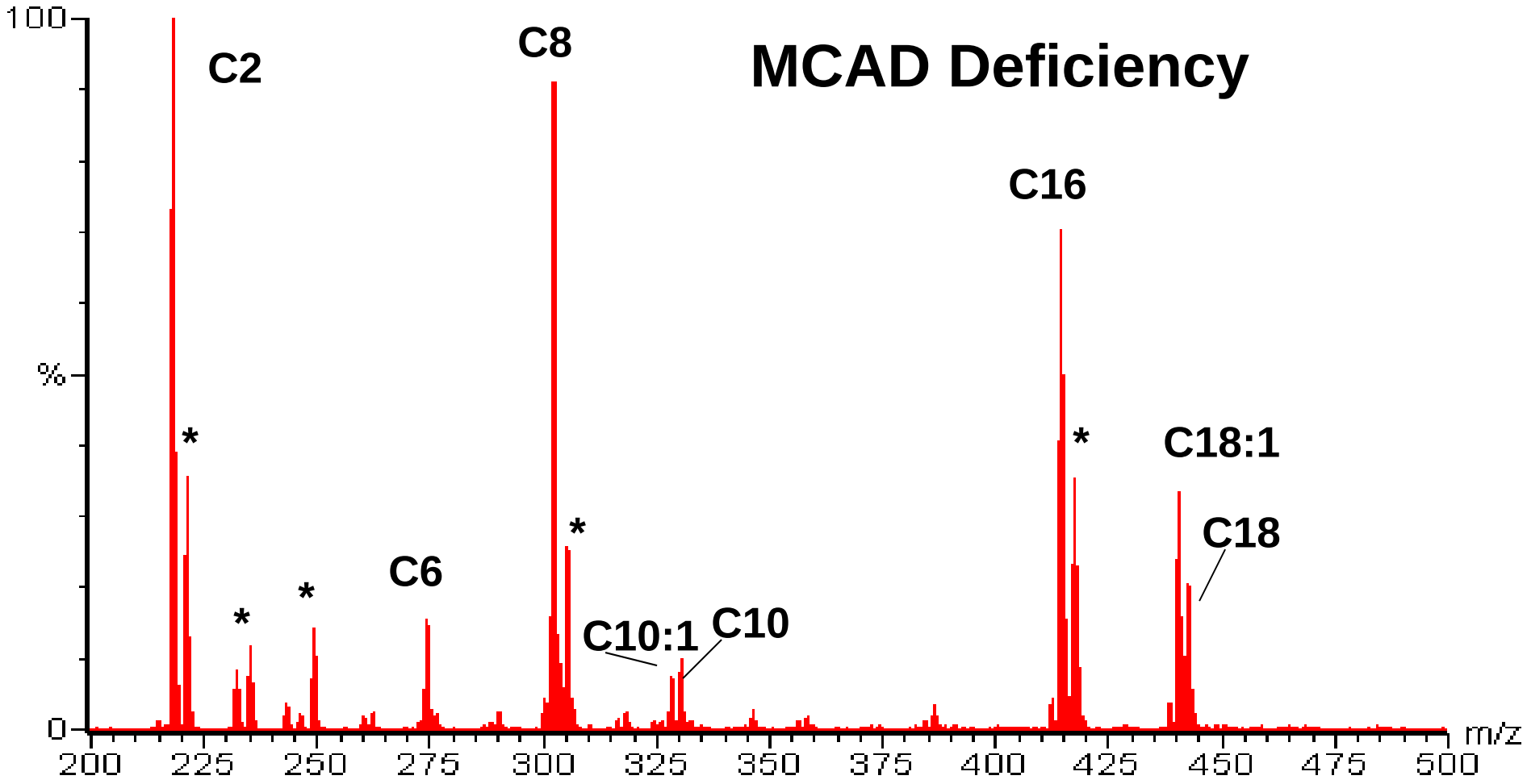
The fragments are separated according to their m/z ratio in MS-II

Mixtures are analyzed in seconds using sophisticated computer programs that target groups of analytes with similar chemical structure

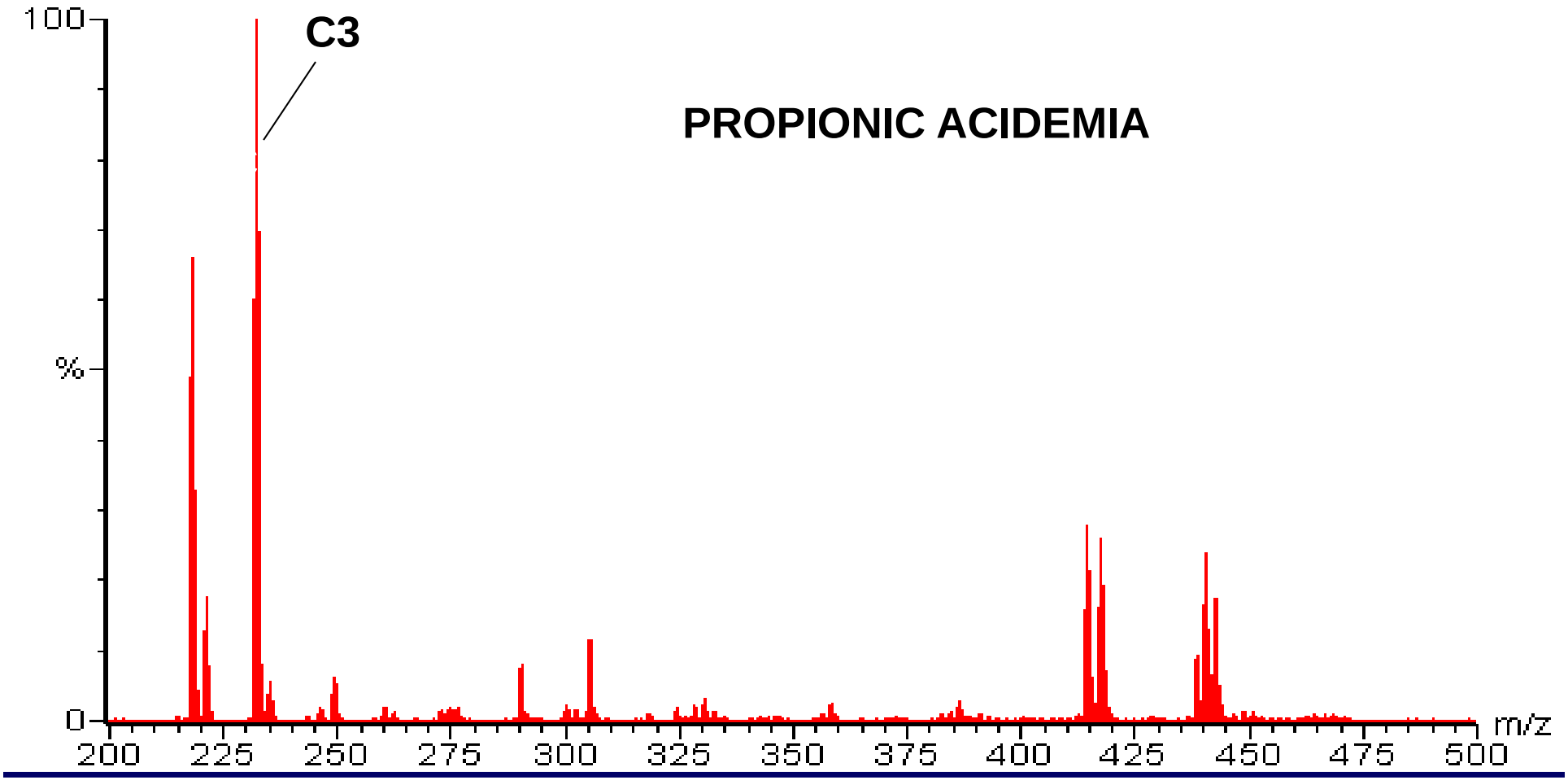
MS/MS targeted to acylcarnitines (butyl esters, precursors of m/z 85 scan)



MS/MS targeted to acylcarnitines: MCAD deficiency



Acylcarnitines in propionic acidemia - note elevated C3



How MS/MS became involved in Newborn Screening: stage 2

- **Historically, MS/MS was developed as a clinical diagnostic test for disorders of fatty acid oxidation, by detection of abnormal acylcarnitines (Duke Laboratory 1984-89)**
- **The method was found to be applicable to dried blood spots**

Tandem Mass Spectrometry: A New Method for Acylcarnitine Profiling with Potential for Neonatal Screening for Inborn Errors of Metabolism:

D.S. Millington, N. Kodo, D.L. Norwood, C.R. Roe.

J. Inher. Metab. Dis. 13:321-324, 1990

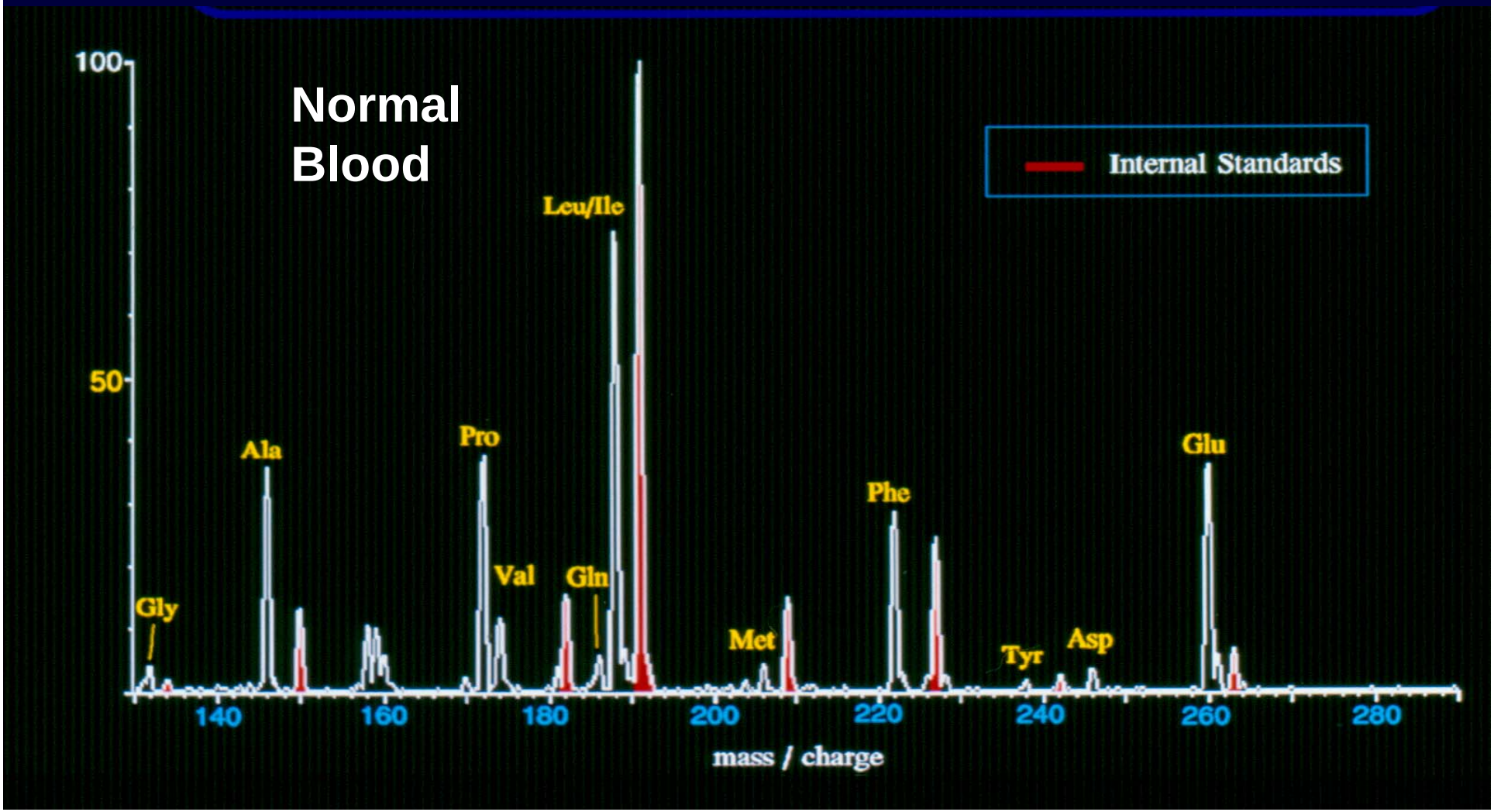
How MS/MS became involved in Newborn Screening: stage 3

- **Historically, MS/MS was developed as a clinical diagnostic test for disorders of fatty acid oxidation, by detection of abnormal acylcarnitines (Duke Laboratory 1984-89)**
- **The method was found to be applicable to dried blood spots (Millington, et al. JIMD 1990 13:321-324)**
- **MS/MS targeted to detect about 8 amino acids at the same time as 20 acylcarnitines, and can recognize over 30 metabolic diseases at once (including PKU):**

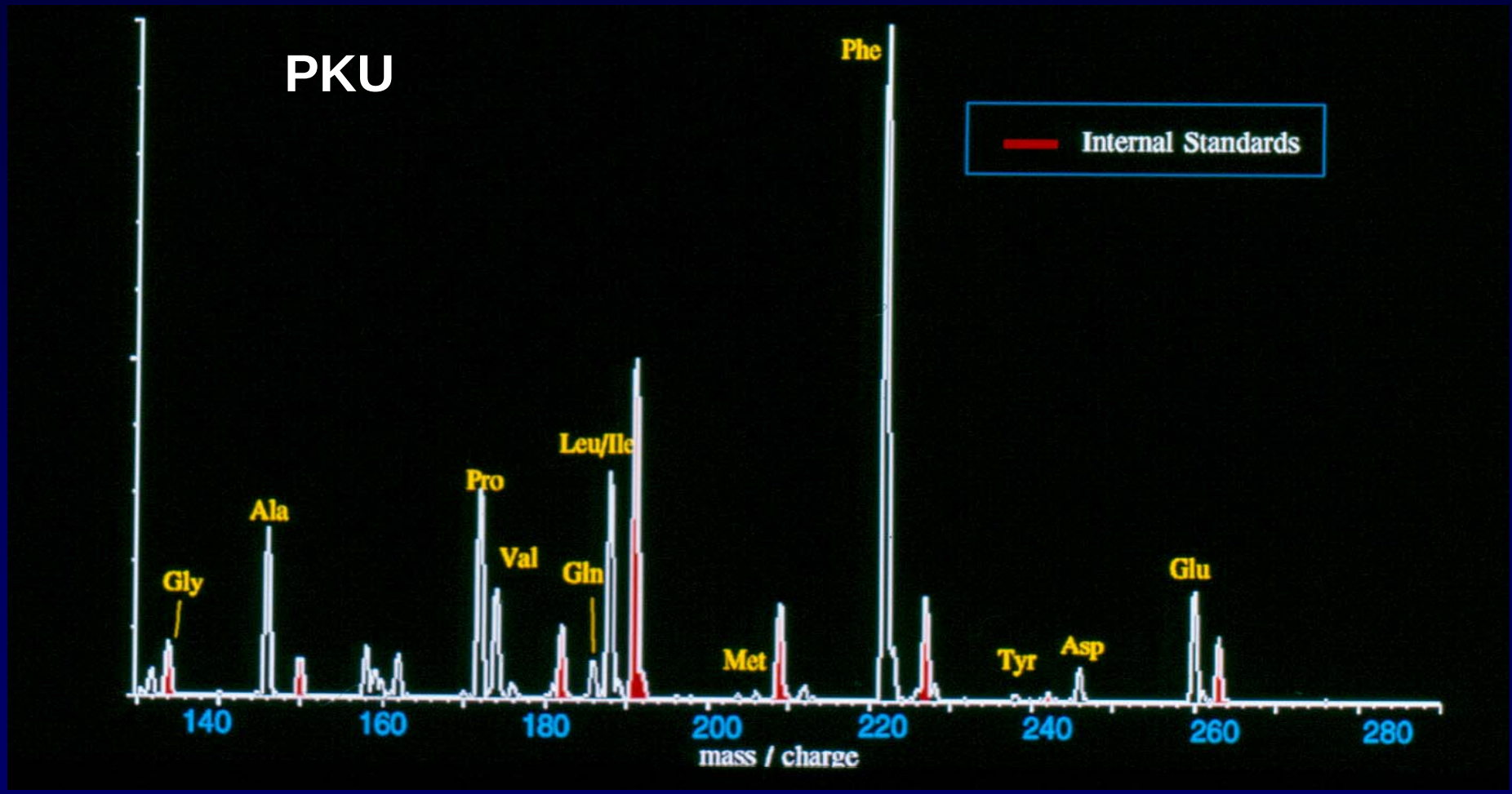
Diagnosis of Phenylketonuria by Quantitative Analysis of Phenylalanine and Tyrosine in Neonatal Blood Spots Using Tandem Mass Spectrometry:

D.H. Chace, D.S. Millington, N. Terada, S.G. Kahler, C.R. Roe, L.F. Hofman. Clin Chem 39:66-71, 1993.

MS/MS targeted to amino acids using neutral loss of 102 Da scan function: acquisition time 30s



Abnormal elevation of phenylalanine in PKU



How MS/MS became involved in Newborn Screening: final stages

- Historically, MS/MS was developed as a clinical diagnostic test for disorders of fatty acid oxidation, by detection of abnormal acylcarnitines (Duke Laboratory 1984-89)
- The method was found to be applicable to dried blood spots (Millington, et al. JIMD 1990 13:321-324)
- MS/MS targeted to detect amino acids at the same time as acylcarnitines, and can recognize over 30 metabolic diseases at once (including PKU)
- Commercial Lab (Neogen) founded in 1994 by Dr. Ed Naylor – motivated interest in expansion of NBS using MS/MS
- Dr M Rashed (Riyadh) developed microplate technology and an automated analytical protocol (1994)
- Collaboration between NC state lab and Duke led to first state-wide expanded screening program in 1997

Disorders Accessible to Newborn Screening by MS/MS

Type of disorder	*Clinical Manifestations*
• Fatty acid oxidation disorders (e.g. MCAD, VLCAD, LCHAD deficiencies)	• Hypoglycemia, coma, sudden death, seizure,cardio-myopathies
• Branched-chain amino acid disorders (e.g. propionic, methylmalonic, isovaleric acidemias)	• Metabolic acidosis, lethargy, coma, respiratory distress, recurrent metabolic crises
• Aminoacidemias (e.g. PKU, MSUD, Urea Cycle disorders)	• Mental Retardation, Hyperammonemia, failure to thrive

INCIDENCE OF METABOLIC DISORDERS DETECTED BY NEWBORN SCREENING USING MS/MS IN NORTH CAROLINA

D. Frazier, et al J Inherit Metab Dis 2006;29:76-85

Total screened = 944,078 (7/28/97 to 7/28/05)
Initial presumptive positive rate: ~0.45%

- **Overall: 219 cases with confirmed diagnoses**
  Incidence 1:4,300 PPV ~ 4% (>60% after repeat NBS)
- **Organic acidurias (58): 1:19,000**
- **Fatty acid oxidation disorders (99): 1:9,000**
- **Amino acid disorders (62): 1:13,000**

Challenges of MS/MS –based expanded newborn screening

- Significant “paradigm shift” – departure from Wilson & Jungner criteria
- MS/MS is expensive, technologically complex and can detect disorders for which no effective treatment is available
- Multiple analytes and possible disorders detected with one test – sudden expansion of presumptive positive cases
- Many detectable disorders are very rare – natural course poorly understood.
- No clear guidelines as to how many disorders/conditions are detectable by MS/MS – confusion of the “numbers game”
- Detection of “mild/variant” forms of diseases (e.g. SCAD, MCAD, 3-MCC) with uncertain clinical significance
- Staff re-training for unfamiliar and difficult roles

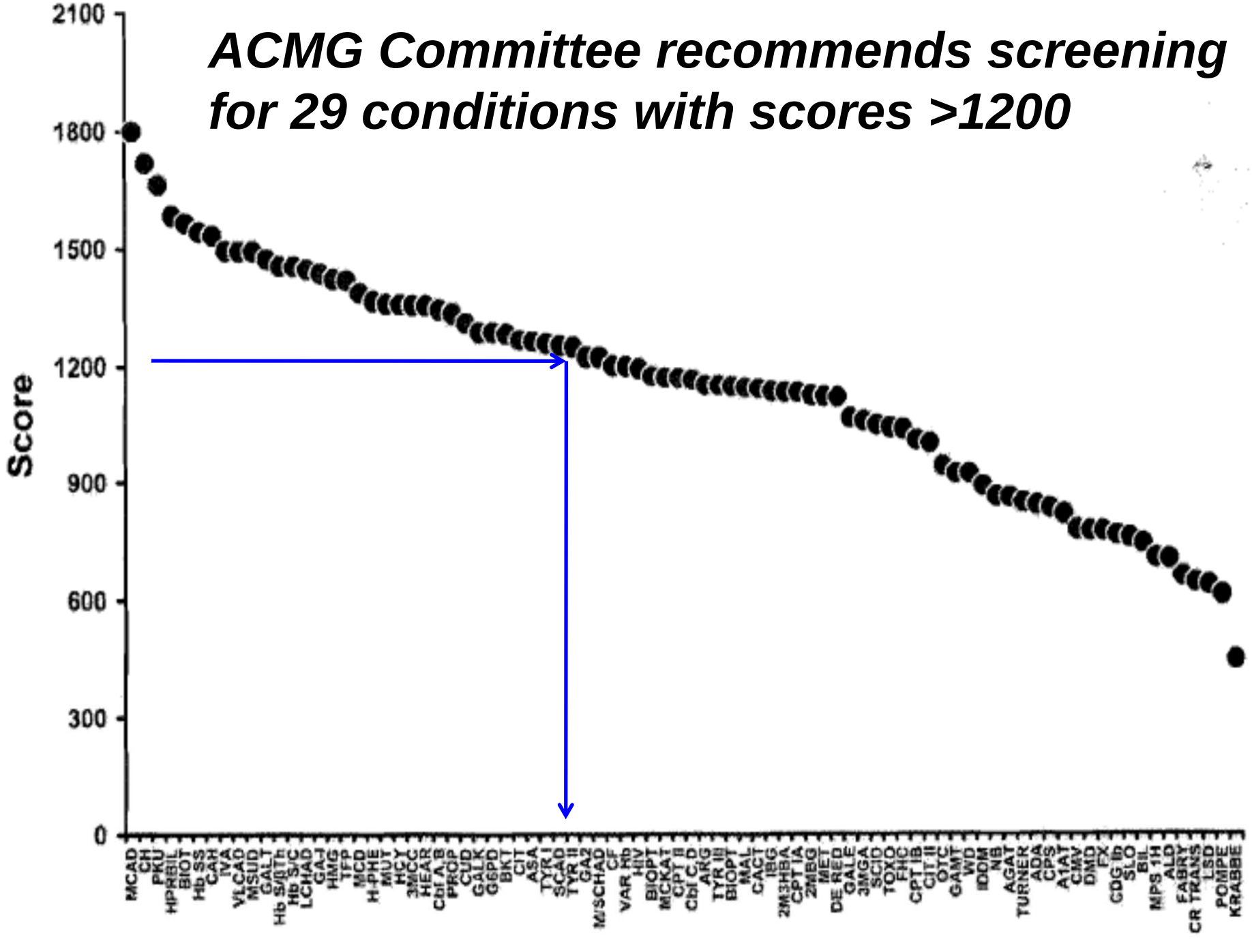
Bringing order to the chaos: Concensus-based recommendations

- **A working group of experts, jointly convened by HRSA and ACMG, generated a ranking system for disorders and issued a recommended panel of 29 primary disorders (plus an additional 26 "secondary conditions") as a guideline for State labs:**

"Newborn Screening: toward a uniform screening panel" (Watson, et al. Pediatrics 2006;117:S296) See also "Counting disorders (conditions) for newborn screening panels" (Sweetman et al. Pediatrics 2006:117;S308)

- **Reviews and recommendations are now made by the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC), a section of HRSA that advises the secretary (USDHHS)**

ACMG Committee recommends screening for 29 conditions with scores >1200



Inherited Metabolic Diseases: the big picture

- See: The Metabolic & Molecular Bases of Inherited Disease (Scriver, et al) www.ommbid.com
- Most are monogenic - affect ~ 1 in 100 live births
- a. Autosomal dominant (~ 7 in 1000)
- b. Autosomal recessive (~ 2.5 in 1000)
- c. X-linked (~ 0.4 in 1000 l.b.)

Summary

- **MS/MS is now integrated routinely into NBS and has markedly enhanced the scope of NBS to include many disorders that were previously inaccessible**
- **The expansion of NBS by MS/MS has stimulated global interest in newborn screening and the possibilities of using dried blood spots for other health applications**
- **New multiplex technologies such as digital microfluidics and DNA tests could significantly expand NBS to include many other treatable conditions**

Further reading: *Newborn Screening for Metabolic Disorders: How are we doing, and Where are We Going?*

P. Rinaldo, et al. Clin Chem 2012:58;324-331

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