Knowledge that will change your world

Ist UAB Metabolomics Workshop
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How metabolism became metabolomics

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Really early history of (bio)chemistry

- Lavoisier – when iron rusted, it gained, not lost, weight
- Isolation of the elements
  - Mendeleev's atomic table
- Late 1800s – isolation of organic materials as chemical substances
- Realization that organic materials in nature could be made from non-organic precursors
- Early 20th century – isolation and identification of biologically active compounds
  - Vitamins, steroids, insulin, pituitary hormones

Nutritional needs revealed by new environments

Captain James Cook

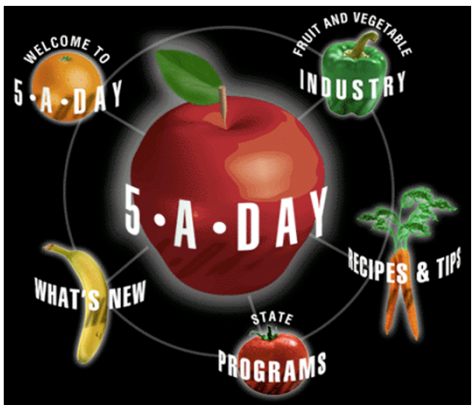
- Scurvy (found in the 20th century to be due to a deficiency in vitamin C) was a major problem for seafaring explorers in the 17th and 18th centuries
- Captain Cook in his voyages to the South Seas forced his men to eat fresh food
- Only one man on the second voyage of the *Endeavour* had scurvy
- Early example of preventive medicine and of a food metabolite that enters the body

Enthusiasm for apples

The value of apples as part of the diet has been appreciated for 200 years

"An apple-a-day keeps the doctor away"

The NCI adopted a 5-a-day strategy for fruits and veggies that in 2003 evolved to a 9-a-day for men and 7-a-day for women - the centerpiece is an apple

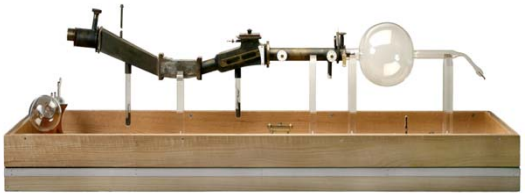


Apples contain proanthocyanidins

Nuclear physics moves to biology

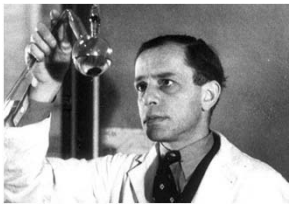
- 1897 JJ Thomson discovers the electron (cathode rays)

- 1919 Aston using a mass spectrograph shows that Neon with a non-integer MW (20.2 Da) is composed of two isotopes, ^{20}Ne and ^{22}Ne



http://www.asms.org/Publications/Historical/HistoryofMassSpectrometry/tabid/94/Default.aspx

Transition to biology

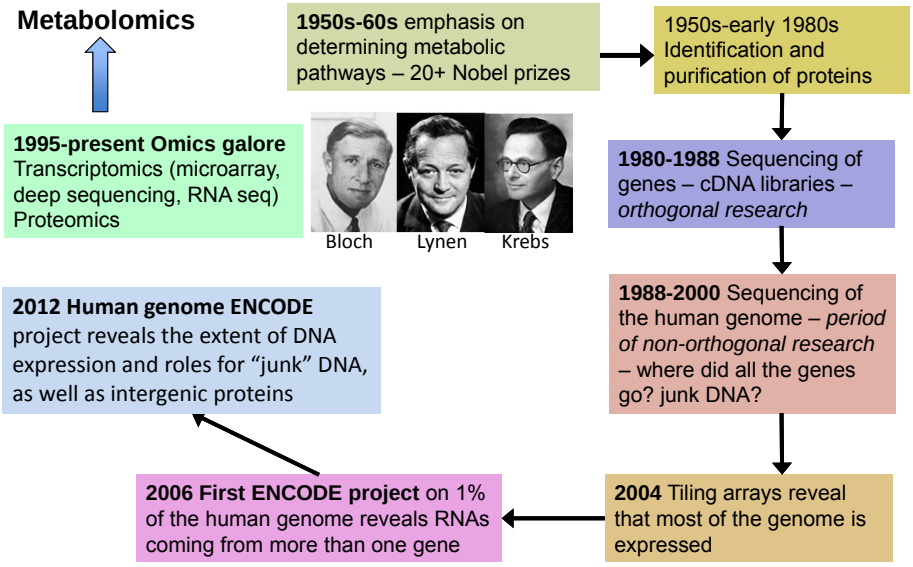


Ralf Schoenheimer

David Rittenberg

- While the politicians, tyrants, dictators and despots were salivating at the thought of developing nuclear weapons from unstable isotopes in the early part of the 20th Century, two scientists began the pursuit of the peaceful use of stable isotopes, initially deuterium (^{2}H), and later carbon (^{13}C) and nitrogen (^{15}N), to study biochemical pathways
- Understanding the pathways of metabolism was born

Direction of NIH Research 1950-2012

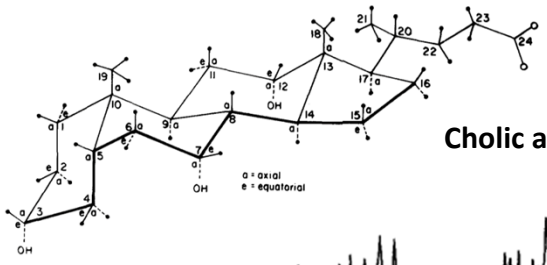


Metabolism to metabolomics

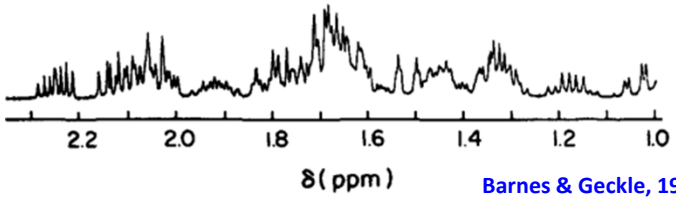
- **Measured with enzymes – NAD(P)H absorbance/fluorescence**
  - **Studies of glycolytic and the TCA cycle intermediates one at a time**
- **Organic acids, fatty acids and amino acids by GC**
  - **Volatile derivatives, Flame Ionization Detection**
  - **GC-MS started in mid-70s**
  - **Capillary GC gave far higher chromatographic resolution than the packed ¼" ID columns (1975/6)**

How NMR became a player

- Mid 60s – introduction of Fourier transform analysis
- Late 70s – introduction of superconducting magnets

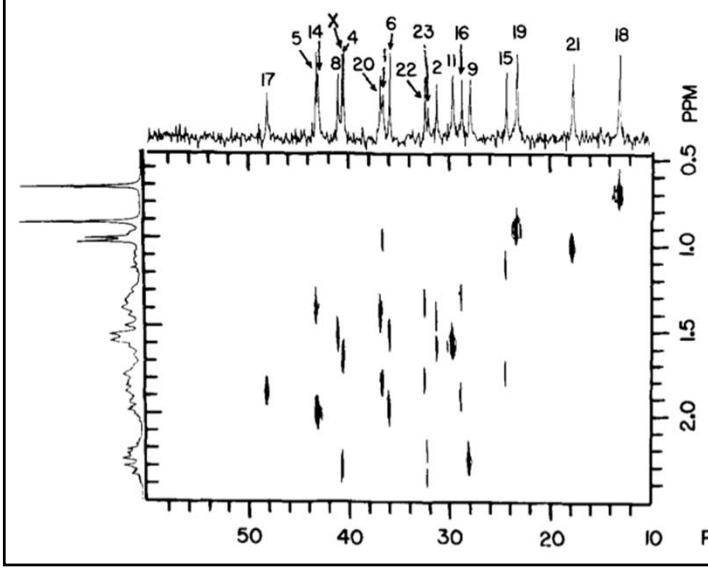


Cholic acid



Barnes & Geckle, 1982

Pulse sequences in NMR (HetCor)



Waterhous et al. (1985)

Progress in LC-MS

- Commercial HPLC appeared in the early 1970s to separate thermally stable and unstable molecules
- The challenge remained to find a way to get the unstable compounds into the gas phase
  - Applied to macromolecules (peptides, proteins) as well as metabolites
- Thermospray had some initial success
- Electrospray ionization and chemical ionization radically changed analysis, allowing compounds to go into the gas phase at atmospheric pressure and room temperature

LC-MS

- Suddenly, there were what appeared to be no limits (or very few) to what could be analyzed
- Unheard of, robust mass spectrometers came into play
  - "A reliable mass spectrometer" was considered in 1990 to be an oxymoron

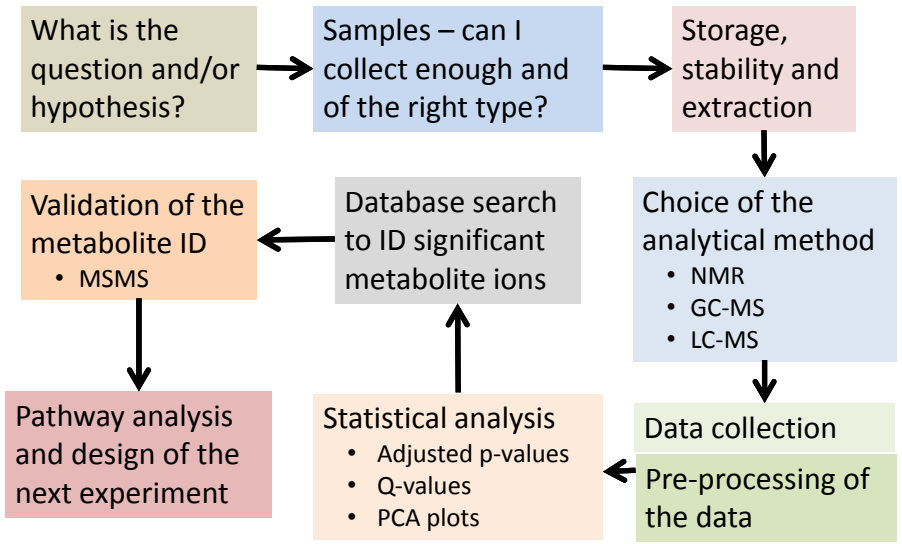
Types of LC-MS analysis

Single quadrupole LC-MS analysis	Triple quadrupole LC-MS analysis
LC-time-of-flight (TOF)-MS	Multiple reaction monitoring (MRM)
FT-ICR MS	Q-TOF
Orbi-trap	TripleTOF

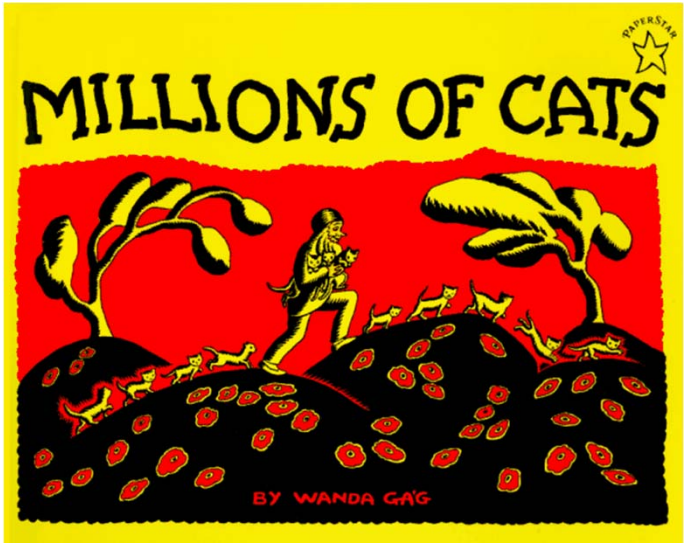
World without gas!

PHOTO: BRENDAN SMIALOWSKI/AFP/GETTY IMAGES

Metabolomics workflow

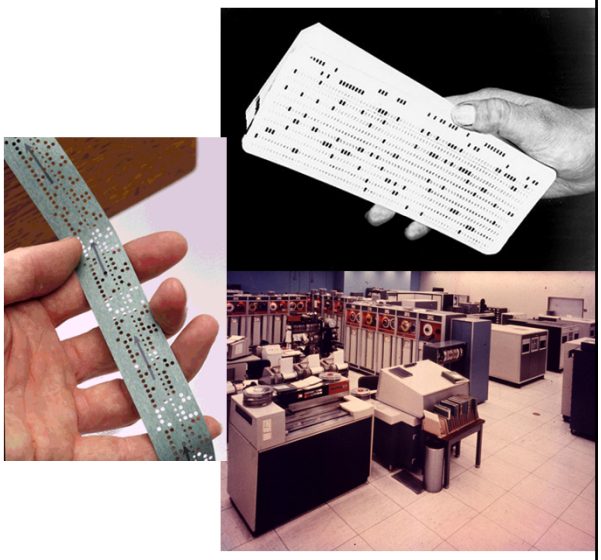


Data explosion



Changing times in Computing

- 1950 The Cambridge colleagues of Watson and Crick calculated the structure of DNA by putting data onto punched cards and taking them by train to London for analysis – and to the fog – the "cloud" in 1950s
- 1964 Seymour Cray develops the CDC 6600 (1 Mflops)
- 1967 I used paper tape to collect data from a radio gas chromatograph and then submitted them via a terminal reader to the CDC 6600 at the University of London



Today in Computing

On my desk in 2013

- The Apple MacBook Air with 2 quad core Intel i7 processors
  - Operates at 2.0 GHz
  - Memory of 8 GB
    - Access 1.333 GHz
  - 512 GB Flash memory storage
  - 10 Gbs Thunderbolt I/O
- Also costs ~$2,000

IBM Blue-Gene

- Parallel processing with 2,048 700 MHz computers operating at 4.733 Tflops
- Replaced by Cheaha, in its current configuration it has 48 compute nodes with two 2.66GHz 6-core Intel CPUs per node (576 cores total)
- It operates at 6.125 Tflops

Great challenges in metabolomics

- The extent of the metabolome
  - From gaseous hydrogen to earwax
- Having complete databases
  - METLIN has 60,000+ metabolite records, but your problem always creates a need to have more
  - Current lack of a MSMS database
- Storing and processing TBs of data
- Standards and standard operating procedures
- Being able to do the analyses in real time