How metabolomics evolved and what it might become
A personal story

Stephen Barnes, PhD

Department of Pharmacology & Toxicology

University of Alabama at Birmingham

"First electricity, now telephones. Sometimes I feel as if I were living in an H.G. Wells novel!" Dowager Countess

Iconic UK telephone box

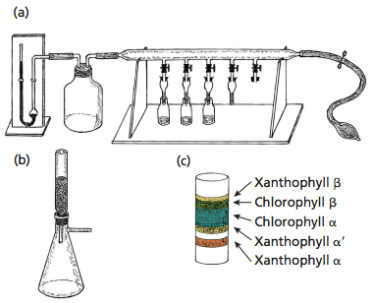
The telephone box evolved into a time machine

1985

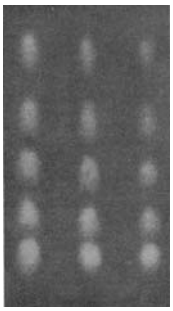
1995

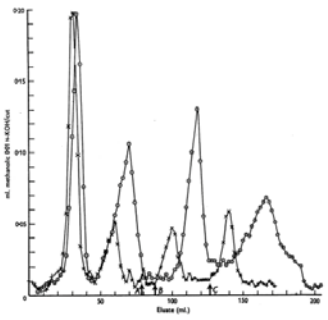
2007 - iPhone

iPhone 7 super computer



Mikhail Tswett, inventor of chromatography, 1906





1950 – fatty acid separations – left, paper chromatography of C_2-C_6 FAs; right, partition chromatography of C_{12}-C_{18} FAs

151. A NEW FORM OF CHROMATOGRAM EMPLOYING TWO LIQUID PHASES

1. A THEORY OF CHROMATOGRAPHY

2. APPLICATION TO THE MICRO-DETERMINATION OF THE HIGHER MONOAMINO-ACIDS IN PROTEINS

By A. J. P. MARTIN and R. L. M. SYNGE

From the Wool Industries Research Association, Torridon, Headingley, Leeds

(*Received 19 November 1941*)

"Consideration led us to try absorbing water in silica gel etc., and - then using the water-saturated solid as one phase of a chromatogram, the other being some fluid immiscible with water, **the silica acting merely as mechanical support"**.

"Separations in a chromatogram of this type thus depend upon **differences in the partition-between two liquid phases of the substances to be separated**, and not, as in all previously described chromatograms, on differences in adsorption between liquid and solid phases."

Gas-liquid Partition Chromatography: the Separation and Micro-estimation of Volatile Fatty Acids from Formic Acid to Dodecanoic Acid

By A. T. JAMES and A. J. P. MARTIN

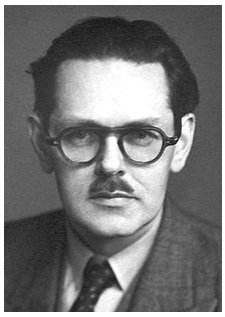
National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received 5 June* 1951)

Martin and Synge's 1941 paper contained the thought "The mobile phase need not be a liquid but may be a vapour. By means of this, refined separations may be carried out."

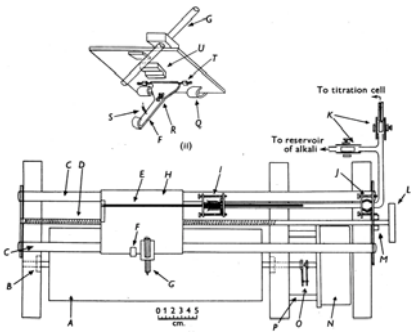
Also, "Very refined separations of volatile substances should be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent..''

First presented at a Biochemical Society Meeting on October 20, 1950

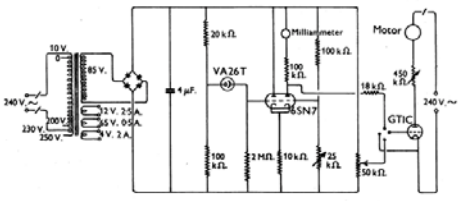


A.T. James reminisces in 1979

- (At the Lister Institute) I .. developed a liquid-liquid column chromatographic system for the separation of the 2:4 dinitrophenyl derivatives of the amino sugars.
- Dr. Martin invited me to join him as co-worker at the National Institute in order to attempt to develop a technique of continuous counter current crystallation. We got very poor results. Seeing my dejection, Martin suggested that we attempt to turn the suggestion in the original paper on the liquid-liquid partition chromatogram (for which they [Martin and Synge] were to receive the Nobel Prize in 1953) into reality.
- We very quickly did after an initial setback and opened up this new field of GLC.
- Our results with **paraffin hydrocarbons quickly got the attention of workers in the petroleum industry** and the subject took off into its present ramifications. Other British and also American workers took it up and most of the potential power of the technology was soon outlined.

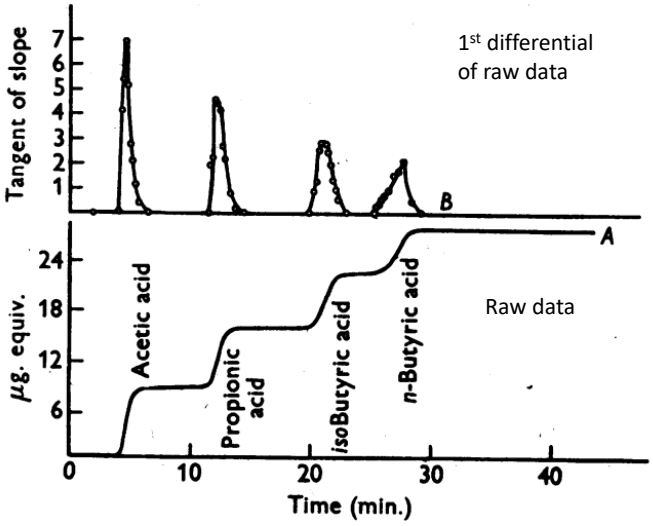


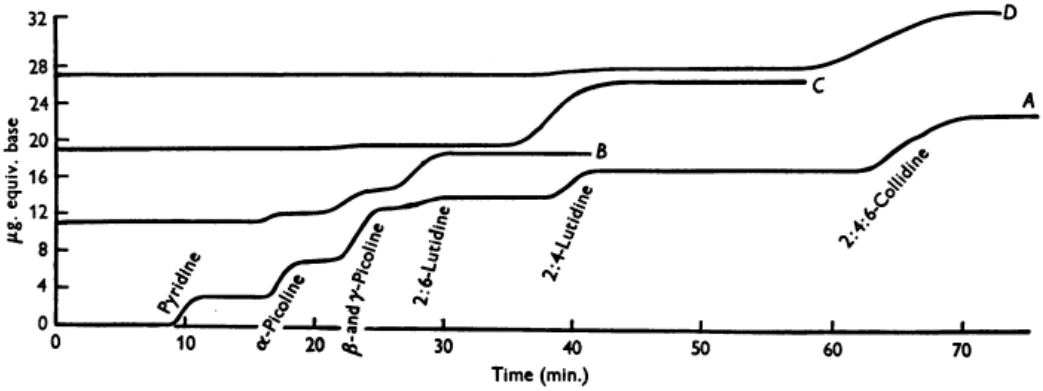
Automated burette



Circuit controlling the burette

The operation of the burette to add NaOH was controlled by changes of the pH indicator color as the fatty acid in the column effluent lowered the pH in the bath.
An example of analytical engineering.





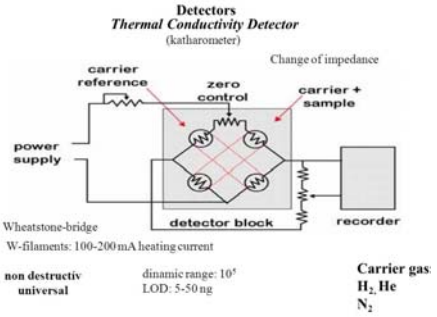
Separation of pyrimidines by gas chromatography on a 4' column at 137°C using a liquid paraffin phase – N_2 flow rate of 15 ml/min. AT James Biochem J (1952) 52:242

More pragmatic advice from A.T. James

- It was very pleasant for Martin and me for a few years to know more about a major development than anyone else in the world, **but it did not last long**.
- Our later development of the technique for the separation, identification, and quantitative analysis of long chain fatty acids, led me directly into fatty acid biochemistry, where I have remained ever since.
- In science and bureaucracy, I have attempted to apply the fundamental precepts I learned from A.J.P. Martin: (1) Nothing is too much trouble **provided someone else does it**; (2) Never answer the first letter; if it's important, they'll write again; and (3) If there are twelve ways of tackling a problem, **they're all wrong**

GLC since then

- **Development of differential detectors**
  - Gas Density Balance (non-destructive)
  - Katharometer (non-destructive or destructive)
  - Flame ionization (destructive, sensitive, linear)
  - Electron capture (non-destructive, v. sensitive, non-linear)



- **Re the Katharometer, there was a problem related to flow**
  - Although Martin solved it, it was characteristic of him that this work was not published; it is likely that James was frustrated by his colleague's **lack of interest in publication**. However, such was his huge regard for Martin as a scientist and as a human being that he was meticulously careful in not referring to such frustrations. (He later learned of Martin's sensitivity over dyslexia.)

The lipid group at Colworth House (Unilever)

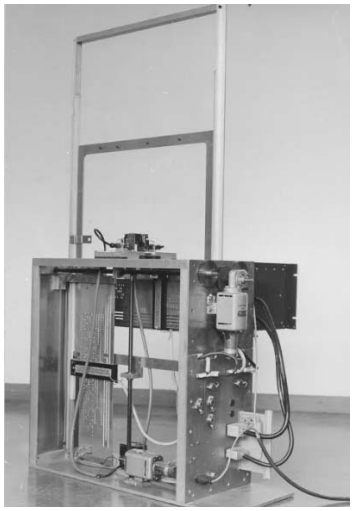
- In 1962, James set up his Lipid Biosynthesis Group at the Unilever Research Laboratory Colworth House, near Bedford.

- For the rest of the decade this laboratory was a world centre for studies of the metabolism of (mainly) plant lipids and their associated acyl groups and in particular trying to understand the mechanism by which double bonds were introduced into saturated fatty acids to form unsaturated ones.
  - Lindsay Morris, Bryan Nichols, Ron Harris and Kit Hitchcock
  - I joined them in the 3rd year of my applied chemistry degree in 1965
  - Investigated the origin of unsaturated C_{16} and C_{18} fatty acids in *E. gracilis*

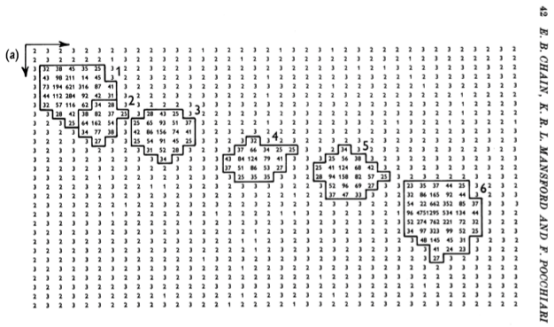
On to graduate school at Imperial College

- **Mentored by Sir Ernst Chain (1945 Nobel Laureate – the biochemist who characterized penicillin)**
  - **Also renown for his work on microanalysis**
- **Used 2D-paper chromatography to resolve glycolytic, Krebs cycle and amino acids derived from ^{14}C-glucose**
  - **Geiger counter mounted on a typewriter frame**
  - **Digitized the collected data and prepared computer-generated figures**
- **METABOLOMICS!!!**

Keith Mansford

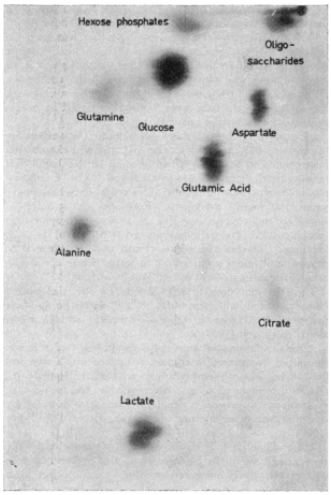


Radiochromatography examples



43 E. B. CHAIN, K. R. L. MANSFORD AND F. POCCHIARI

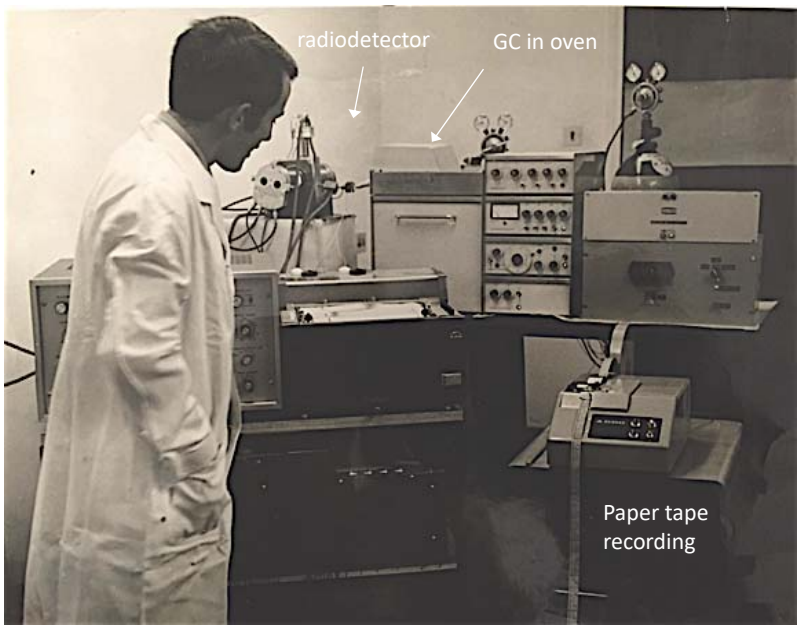
J Physiol (1960) 154:39
E.B. Chain, K.R.L. Mansford and F. Pocchiari



Autoradiogram of ^{14}C-glucose metabolites from an isolated perfused Langendorff rat heart preparation. The metabolites were separated by 2D-paper chromatography.

The conditions were:
1st dimension: butan-l-ol-acetic acid-water (40:11:25, by vol.) for 16hr.;
2nd dimension: (-) phenol-aq. NH_3 (sp.gr. 0.88)-water (80:1:20, by vol.) for 24hr.

Biochem. J. (1969) 115, 537
E.B. Chain, K.R.L. Mansford and L.H. Opie



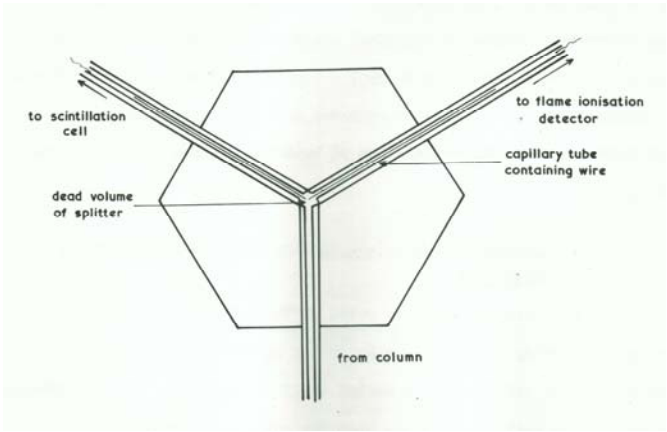
Radio-GC analysis

metabolomics in its infancy

Radio gas-liquid chromatography with digitization of collected data

Developed this for my PhD work (1967-1970) to study glucose metabolism in acellular slime mold, *physarum polycephalum*

Radio-gas chromatography



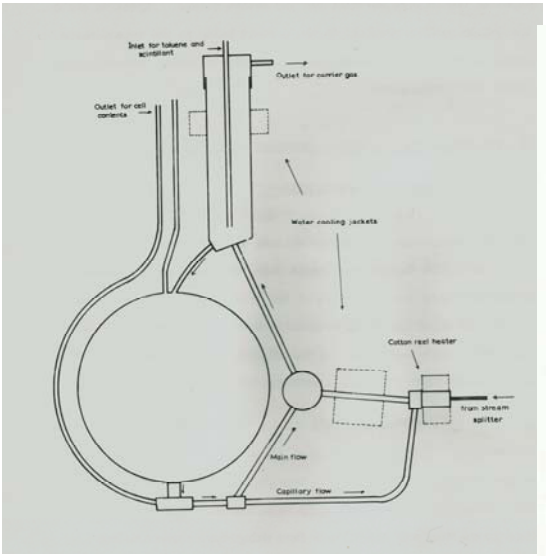
Essential engineering – low dead volume splitter

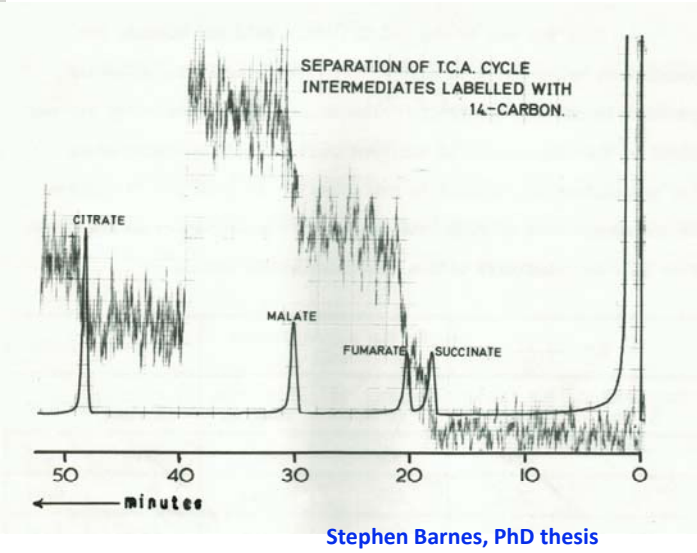
Arms of the splitter were heated to a temperature above that of the column to ensure a constant split ratio during temperature programming

My version of metabolomics in 1969

Stephen Barnes, PhD thesis

Radio-GC of Krebs cycle intermediates

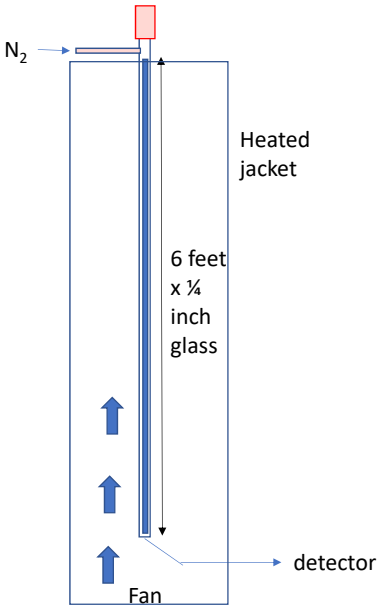




Stephen Barnes, PhD thesis

BS1

Evolution of GC columns

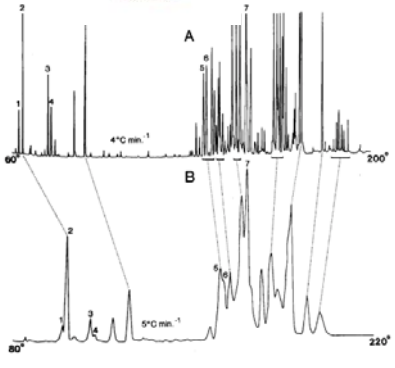


Coiled or U-shaped columns

Back pressure was a problem due to gas compressibility

Open tubular capillary (Golay) was patented in 1956. Not developed until the 1970s.

Quartz capillary GC column

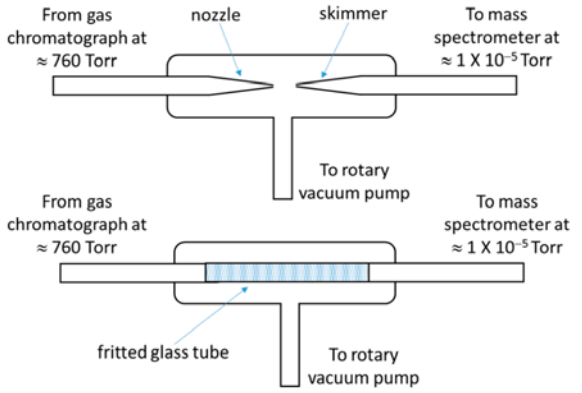


Slide 16

BS1 Barnes, Stephen, 8/21/2017

Combining GC with mass spectrometry
An oxymoron?

The gas from a GC system going into mass spectrometer operating in vacuum (10^{-5} torr)



HPLC

- **Its principle**
  - Martin and Synge (1941) again.. "*the smallest HETP (height equivalent to a theoretical plate) should be obtainable by using very small particles and a high pressure difference across the length of the column*."
- **It has several advantages over GC**
  - Not necessary for the biochemical to go into the gas phase
  - The stationary phase can be modified to many different chemistries
  - The mobile phase (a liquid) is essentially non-compressible
    - Linear flow velocity is the same at the top and bottom of the column
- **One big disadvantage**
  - Smaller particles = smaller HETP & better efficiency, but = greater back pressure

$$\Delta P = \frac{\eta F L}{K^0 \pi r^2 d_p^2}$$

viscosity, flow, length; specific permeability, column radius, particle diameter

UPLC operates at 15,000 psi

The HPLC-MS interface

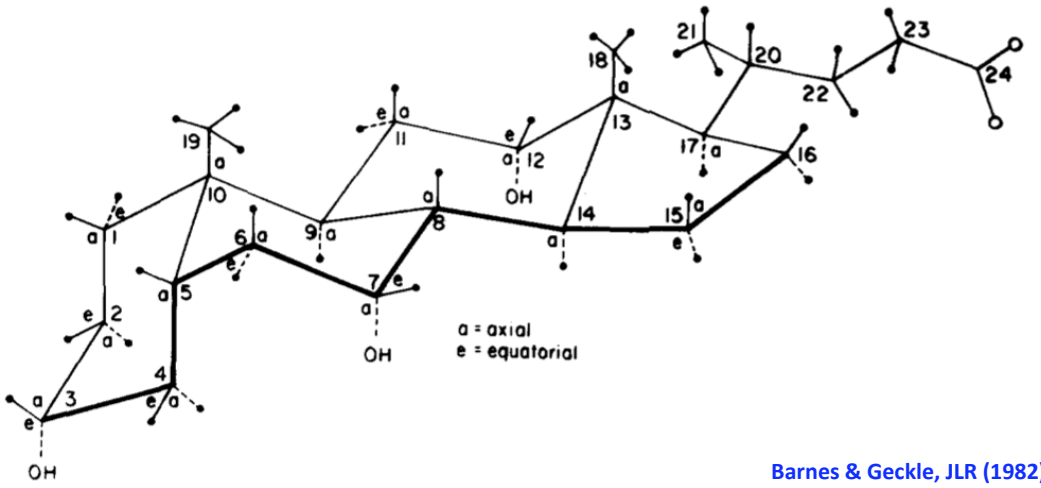
- **The challenge was even bigger than that for GC-MS**
  - 1 ml of water generates >1 liter of water vapor
- **Two technologies provided the solution**
  - Electrospray ionization (works at room temperature and atmospheric pressure)
    - The spray can be heated, but does not change temperature inside the droplet
    - Metabolite ions should be charged or form charged adducts
    - Spray is off-axis and ions move down a potential gradient into the mass spectrometer
  - Atmospheric pressure chemical ionization (works at atmospheric pressure)
    - Discharge needle ionizes the air which in turn ionizes the neutral metabolites

I didn't forget NMR

- **As a graduate student, I remember the implementation of FT-NMR**
  - Before that, spectra were obtained, as for UV/Vis, by continuous wavelength scanning
  - Reduced the amount of sample needed from 1 g to 10 mg
- **At UAB (1979), I made permethylated derivatives of bile acids**
  - Studied them using an iron (90 MHz) magnet
    - Saw protons epimeric to –OH groups and the methyl groups – the rest were a "hump"
  - Offered the chance to repeat the measurement on the newly installed Bruker 400 MHz superconducting magnet NMR
    - Amazing increase in resolution
    - Asked if I could repeat the work with cholic acid – was told "no"
    - Instead "snuck in" during the graveyard shift (Sat 12 midnight to Sun 8 am) with the collusion of the facility manager, Mike Geckle
    - Did this for 8 months – **sometimes you just have to!!**

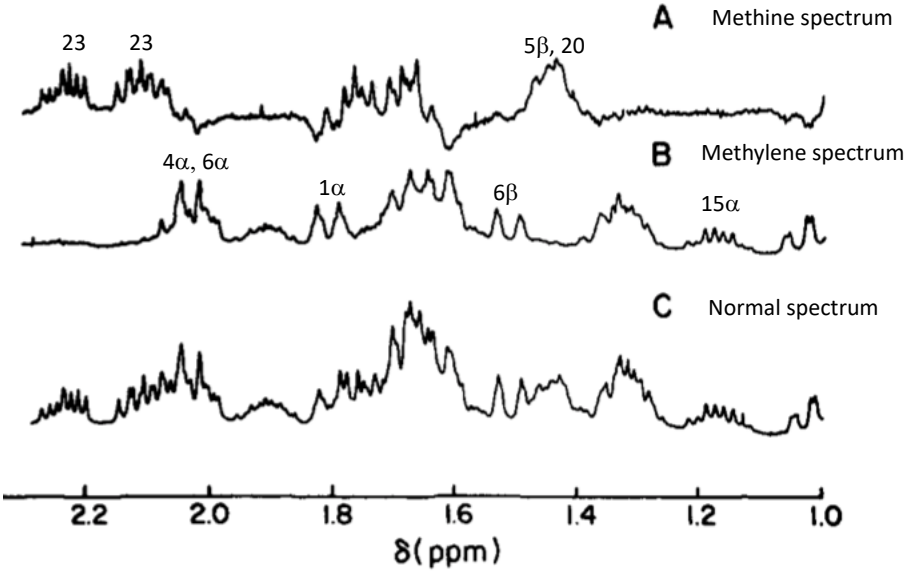
Structure of cholate

3α,7α,12α-trihydroxy-5β-cholanoate

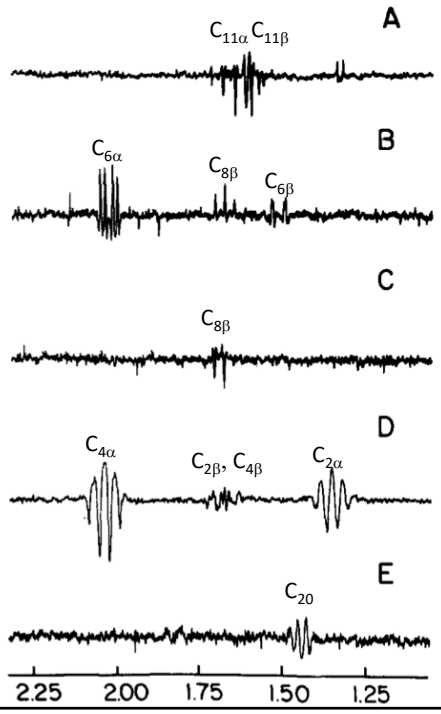


Barnes & Geckle, JLR (1982) 26:1082

^{1}H-NMR of sodium cholate – partial relaxation



Barnes & Geckle, JLR (1982) 26:1082



Decoupled proton

12β non-relaxed

7β non-relaxed

7β methine

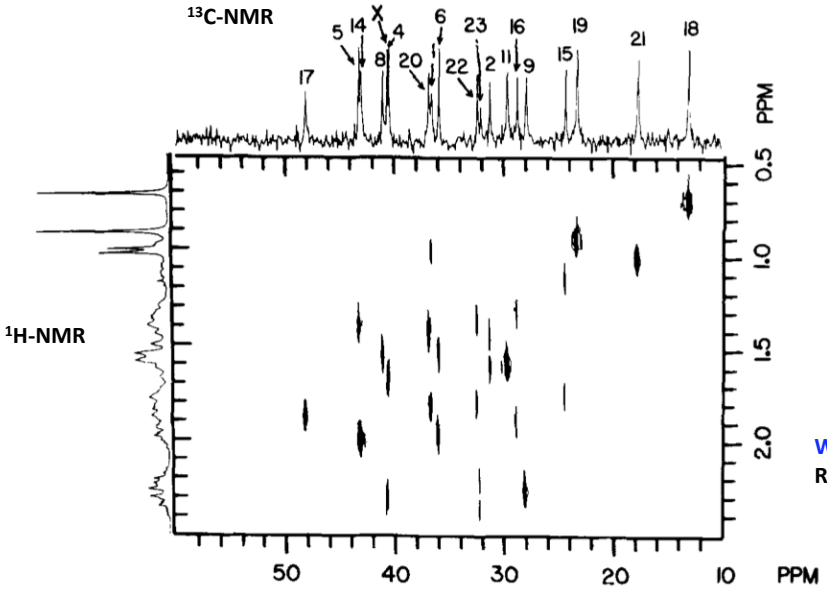
3β non-relaxed

21-CH_3

Selective proton on-resonance decoupling combined with partial relaxation

Barnes & Geckle, JLR (1982) 26:1082

Hetero-correlated 2D $^{1}H/^{13}C$ NMR - cholate

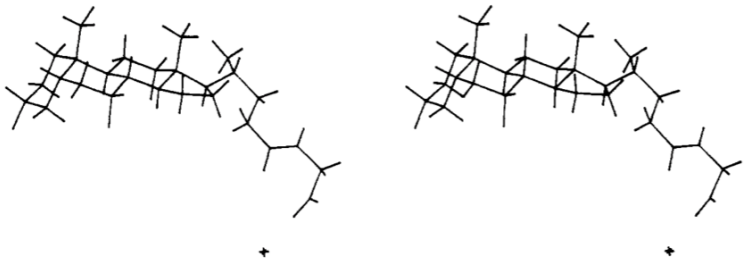


Data acquired in 21 h, processed in 2 h, inspection of data and assignments took 10-15 min

Waterhous et al., JLR (1985) 23:1068

Remembered what A.T. James said

Stereoview of dysprosium glycocholate



Determined by the effect of Dy (a lanthanide that replaces Ca) on the induced relaxation rates ($1/T_1$) of 3β, 7β, 12β, 18-CH_3 and 19-CH_3 proton resonances.

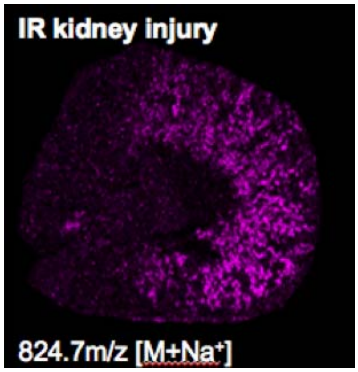
Mukidjam et al., Biochem (1987) 26:6785

Other precursors to metabolomics

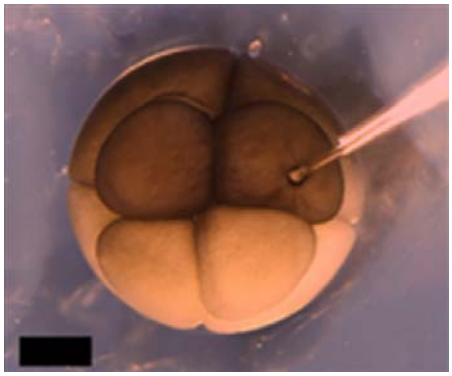
- **Earliest forms of "metabolomics"**
  - Date back to ancient China – the "ant test" to test for sugar (diabetes)
  - In the Middle Ages diagnosis of diseases were based on taste, smell and color
- **Combination of GC and mass spec**
  - Evan C and Margaret G Horning – urine and tissue metabolites
  - Institute of Child Health, Great Ormond Street, London – amino acid and organic acidurias
  - Royal Free Hospital – bile acids and hepatobiliary/GI disease – Kenneth Setchell

KDR Setchell

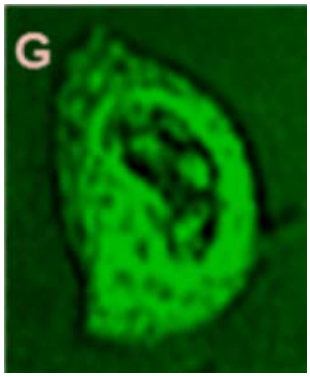
Where to next?



MALDI-Imaging of a phospholipid
Janusz Kabarowski/Kelly Walters



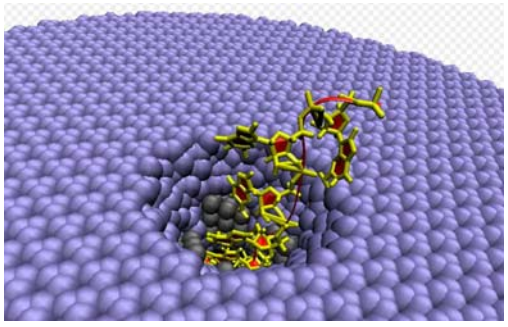
Multiple sampling single cells – Nemes group



CARS imaging of a cancer cell – spectroscopic, real time Raman imaging

OR, two people with disparate abilities and insights will create something we've never heard of (yet)

What might it be?

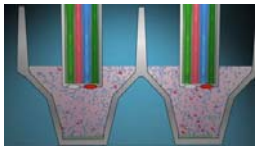


Nanoprobe inserted into the wall of a cell recording changes in metabolism in real time – sub nl sampling/analysis

TOMORROW?

Measuring O_2 uptake using a Warburg apparatus – 10 ml incubations

YESTERDAY



The O_2 and pH probes of a Seahorse™ apparatus – 7 µl incubations

TODAY

Acknowledgements

A.T. James, PhD

Sir Ernst Boris Chain

Dame Sheila Sherlock

Alan F. Hofmann, MD

And many others, and multiple agencies for grant support

Thank you and enjoy science, wherever it may lead you