The Berry's Enigma

Mary Ann Lila
North Carolina State University

-Traditional Knowledge

-Antioxidant

-Anti-inflammatory

-Bioassays:

- *-chemical*
- *-In vitro*
- *-In vivo*
- *-clinical*

Multiplicity of bioactivities

http://www.thespoof.com/news/spoof.cfm?

(The issues with)
Berry bioactives

Stability

-Hollands et al. 2008, Blackcurrant juice

-Lee et al. 2002, Blueberry juice

Bioavailability

-apparently very low

-plasma v blood

Absorption

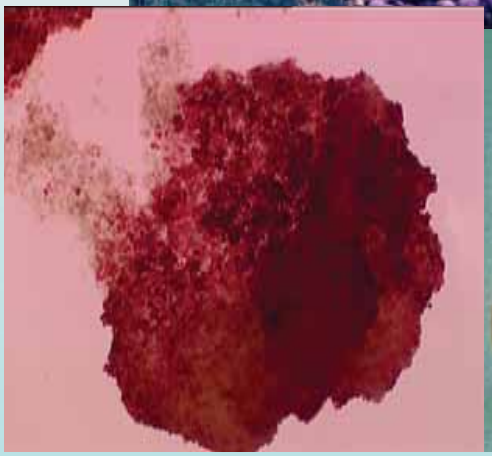
-codelivery

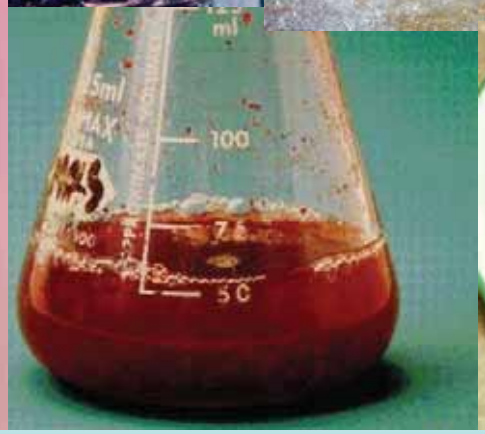
Low [ANC]

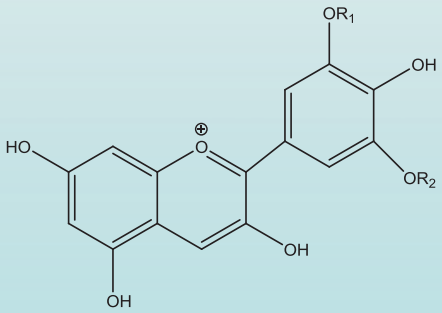
-Nicholson et al. 2008, 0.1 micromole

Colonic microflora

ANTHOCYANINS



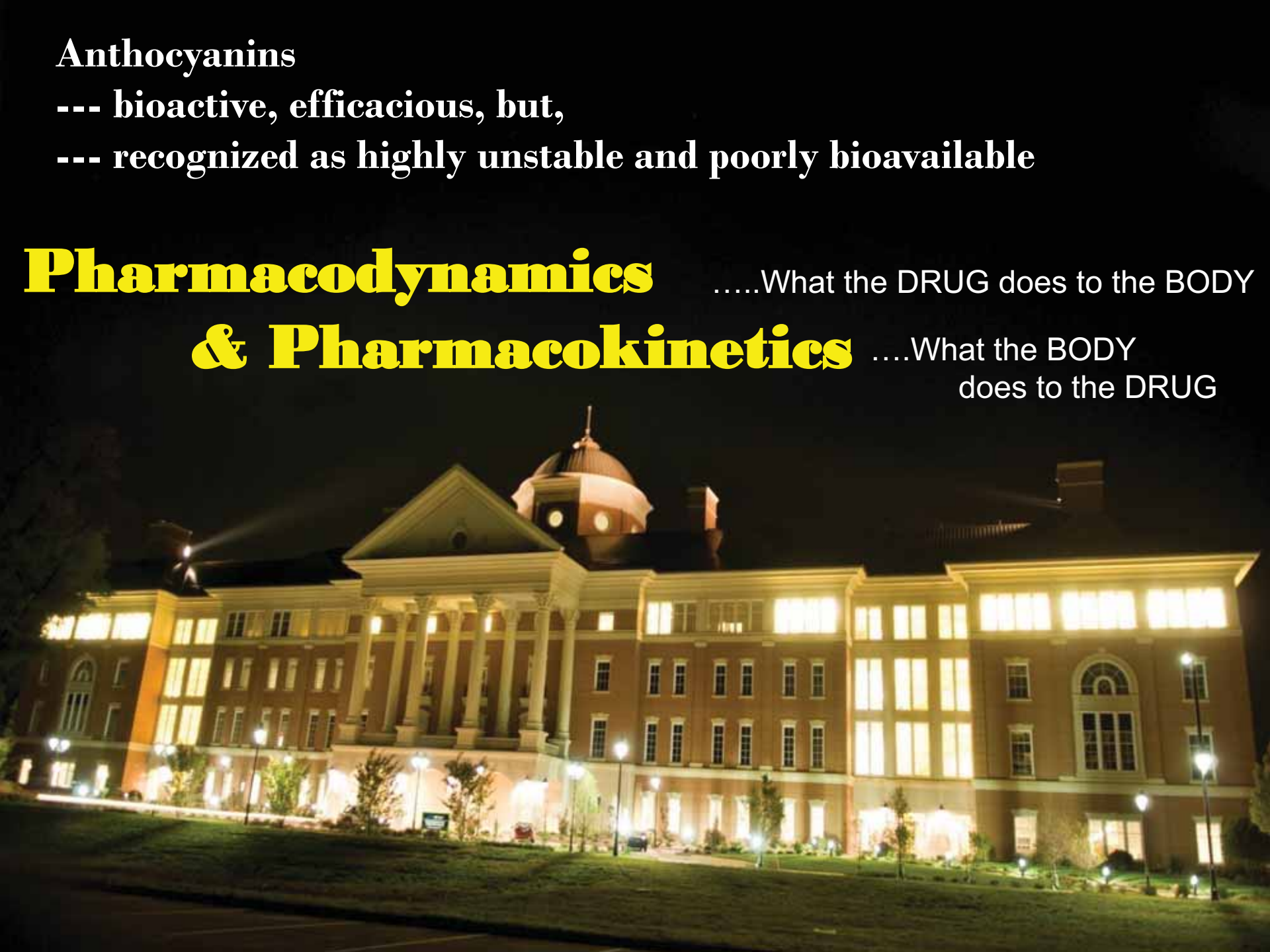




Anthocyanins
--- bioactive, efficacious, but,
--- recognized as highly unstable and poorly bioavailable

Pharmacodynamics .....What the DRUG does to the BODY

& Pharmacokinetics ....What the BODY does to the DRUG



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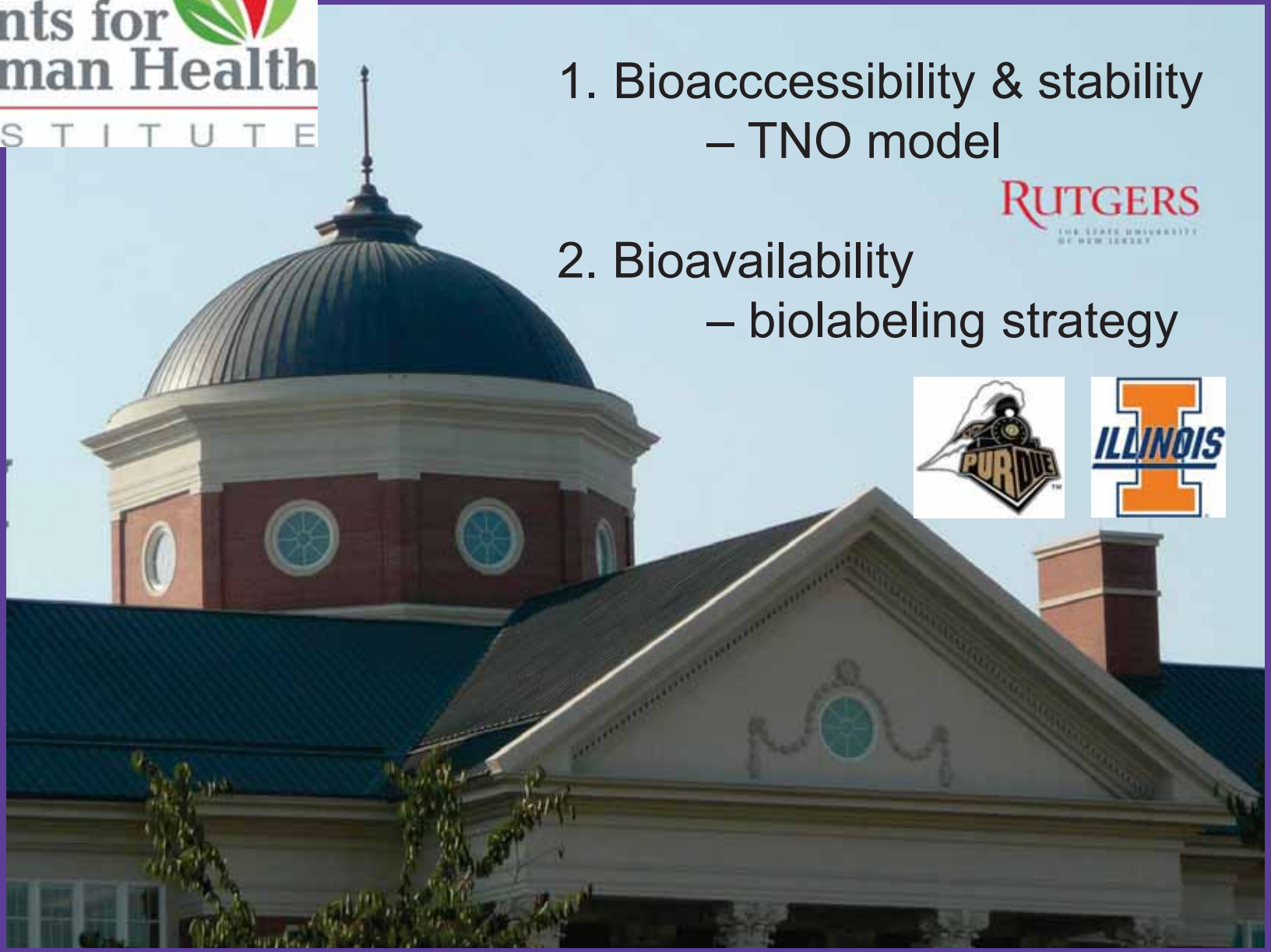


1. Bioacccessibility & stability
   – TNO model

RUTGERS
THE STATE UNIVERSITY OF NEW JERSEY

2. Bioavailability
   – biolabeling strategy

PURDUE

ILLINOIS

TNO gastro Intestinal Model (TIM)

Evaluation of gastrointestinal stability and bioaccessibility of botanical compounds or drugs;

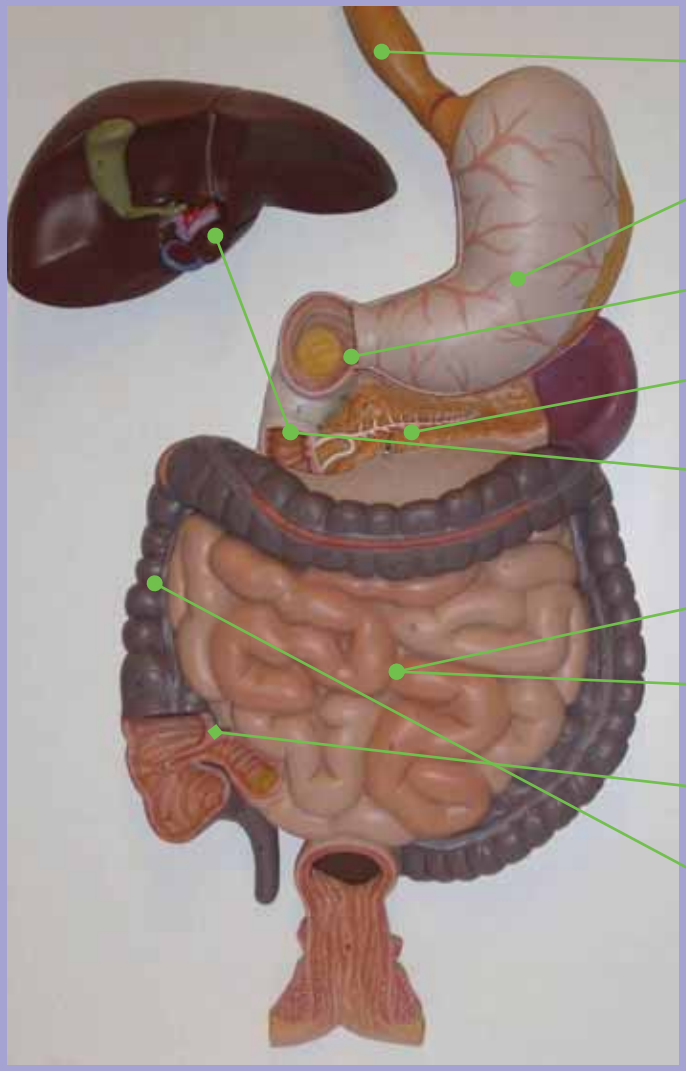
Evaluation of the fate of anthocyanins from dietary sources to in vivo sites of biological activity

Stability: how much of the anthocyanin remains intact as it travels through the GIT?

Bioaccessibility: how much of the intact anthocyanin is released from the food matrix and is able to pass through membranes with a cutoff of 5 kDa during transit through the stomach and the small intestine, reflecting the availability for absorption in vivo?

[Stomach, duodenum, jejunum, and ileum]

GI Tract Features Successive Dynamic Conditions



Swallowing of food & saliva

Gastric enzymes, gastric acid, peristalsis

Gastric emptying

Secretion of digestive enzymes

Bile secretion

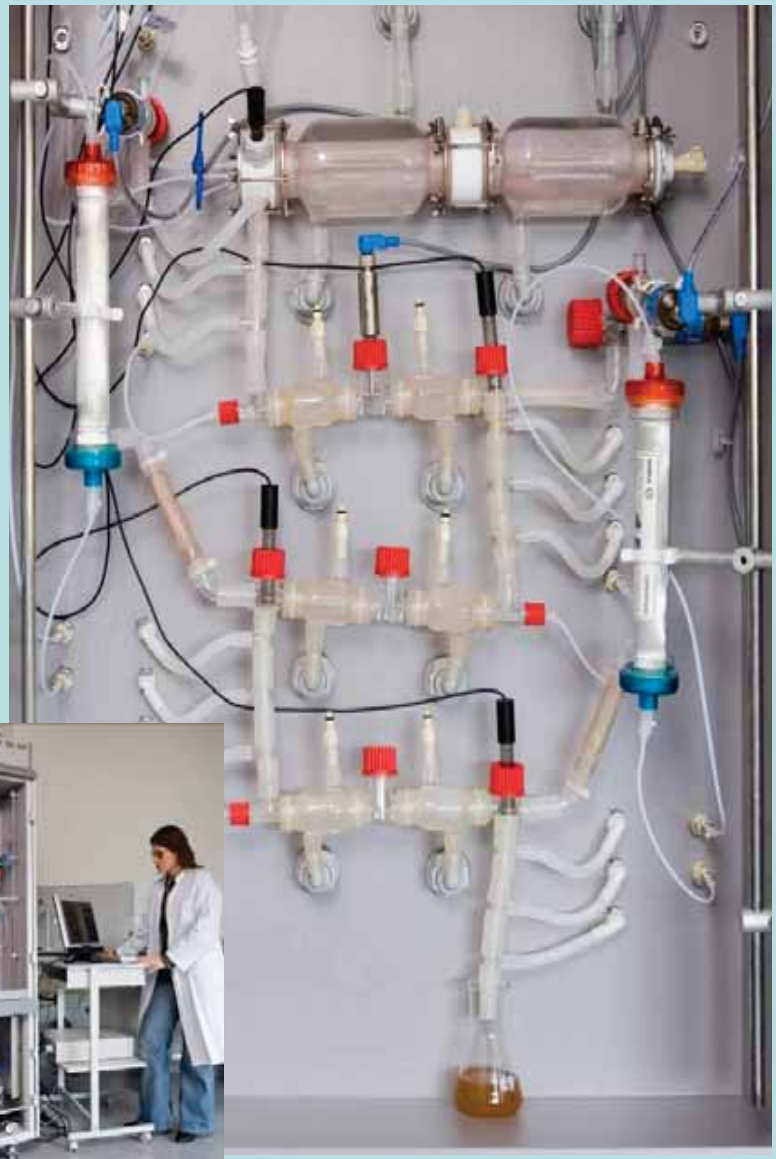
Peristalsis, intestinal transit

Absorption of dissolved products and water

Ileum effluent to colon

TIM-1 System

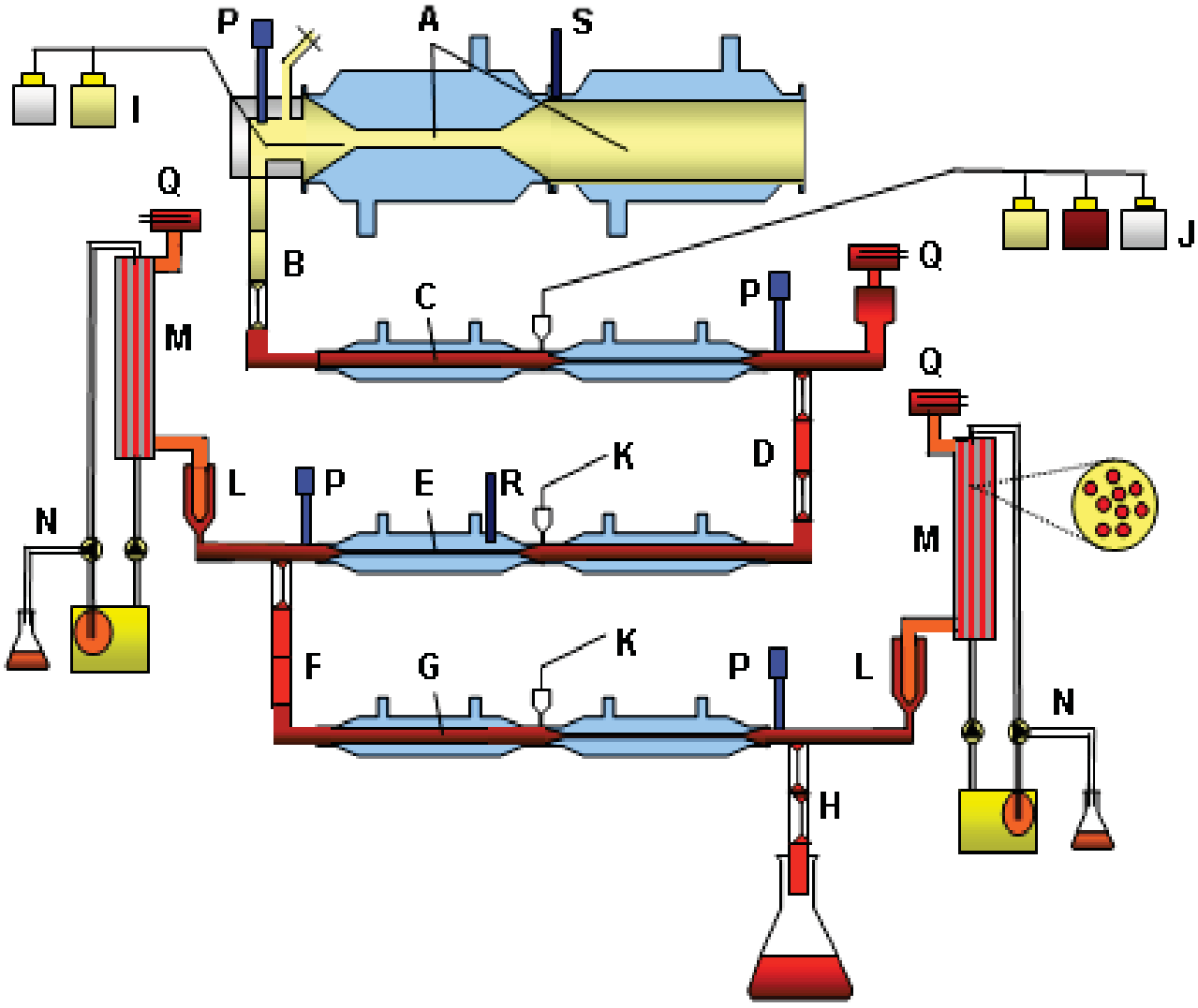
Upper GI Model



- body temperature
- peristaltic movements
- gastrointestinal pH curves
- gastrointestinal transit times
- secretion of gastric acid and enzymes (pepsin, lipase)
- secretion of bile, pancreatic juice
- absorption of digested products and water
- continuous process control and data collection
- modular set-up, high flexibility

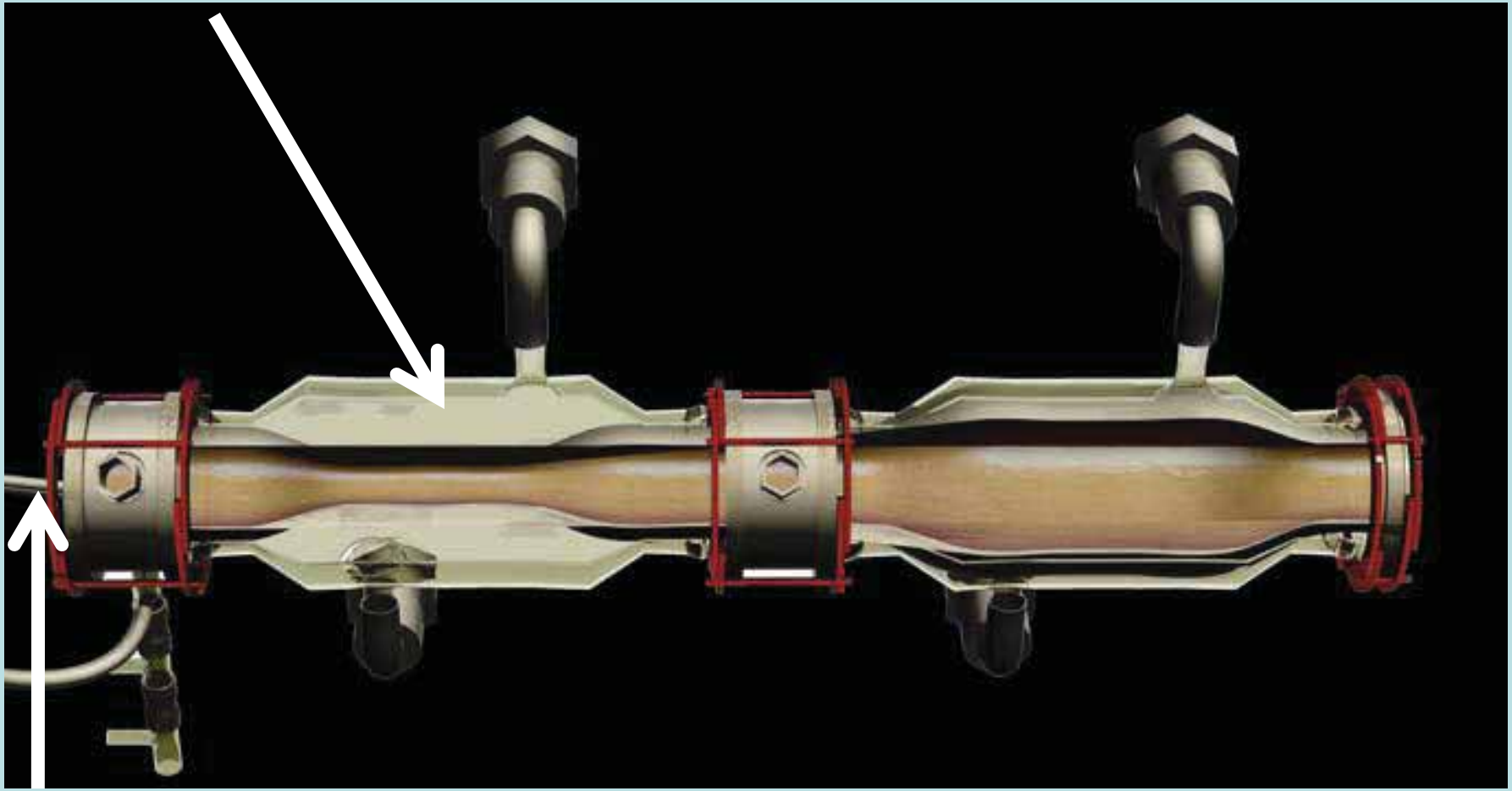
TIM Schematic

Front panel of the TIM system and schematic diagram showing the stomach compartment (A) and three small- intestinal compartments: duodenum (C), jejunum (E), and ileum (G) connected by vertical peristaltic valves, including the pyloric sphincter (B) and the ileo-cecal valve (H). Each compartment with secretion tubes, such as gastric acid and pepsin in the stomach (I), and pancreatin and bile in the duodenum (J), pH electrodes (P), pressure (S) or level sensors (Q) and temperature sensors (R). Connected to the jejunum (left) and ileum (right) are semi-permeable hollow-fiber membrane units (M) for continuous absorption of digested products and water absorption (N)



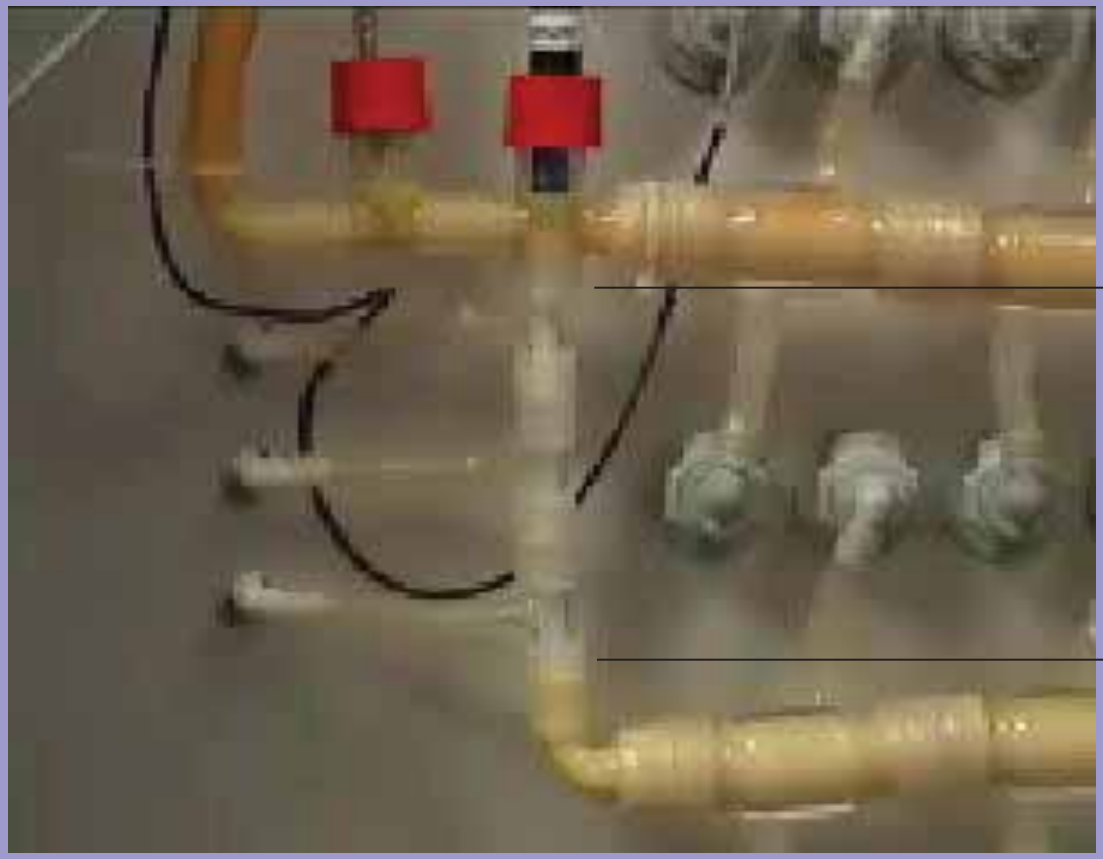
TIM Stomach Cross Section

Jacket water controls temperature, mixing and gastric emptying



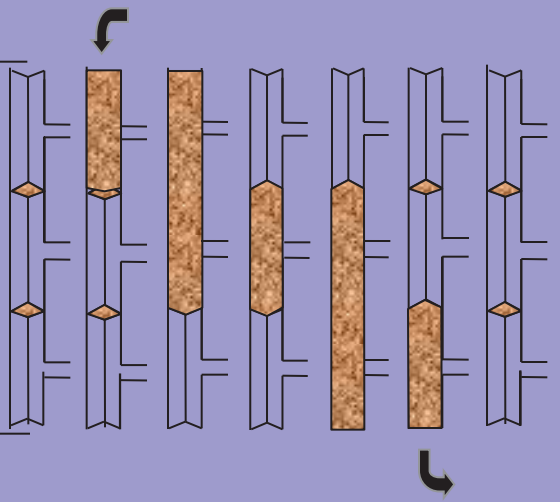
Secretion of water, enzymes and acid into lumen controlled by program

Gastrointestinal Transit



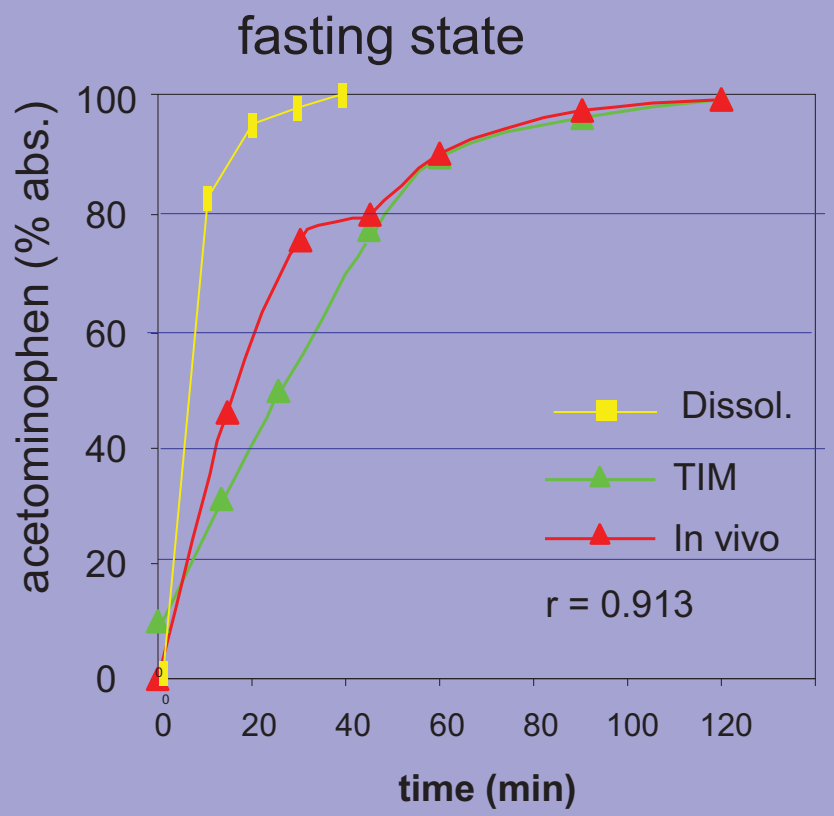
Controlled by a series

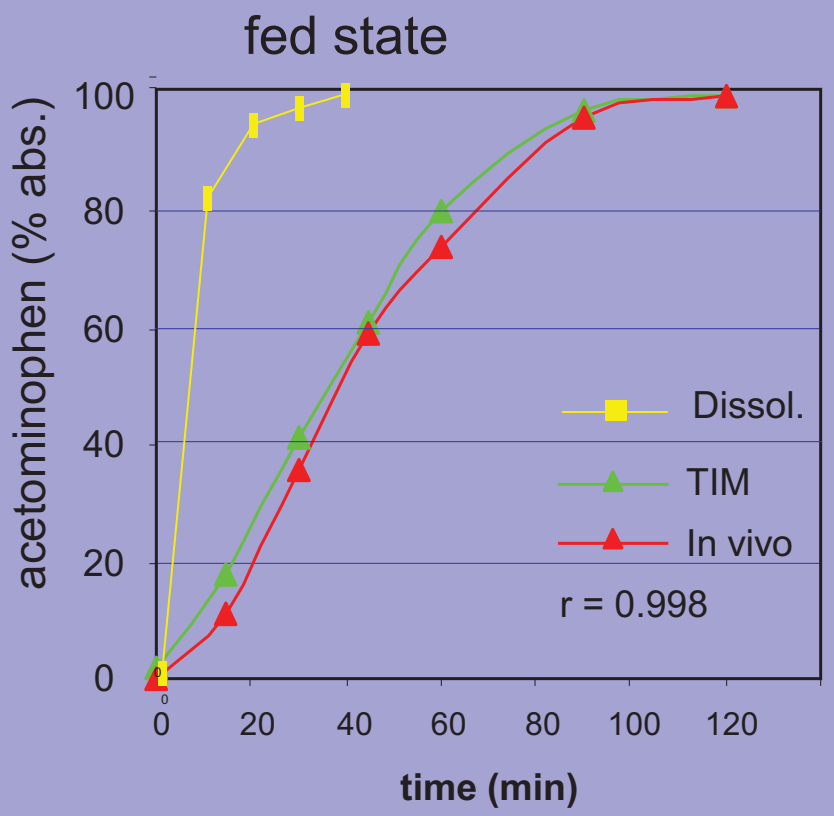
of 3 peristaltic valves



Pharmaceutical Validation of TIM

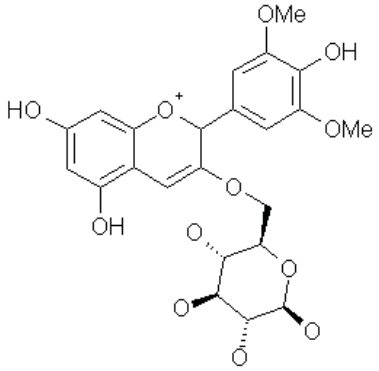
Bioaccessibility / Bioavailability of acetominophen TIM-1 versus humans



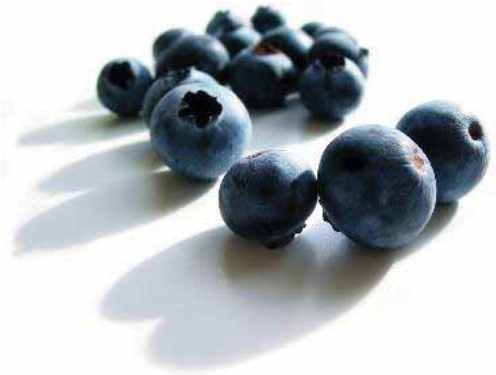


Souliman et al. Eur.J.Pharm.Sci. 27, 2006

TIM experiments



Malvidin-3-glucoside

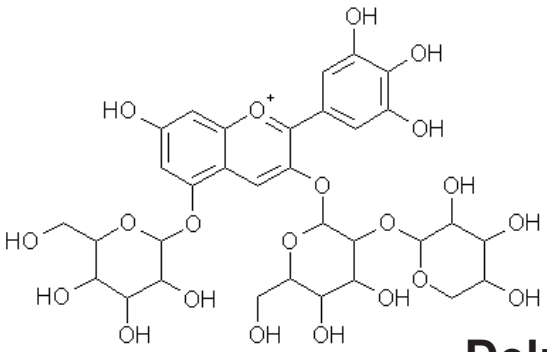


Anthocyanin sources:

IQF wild blueberries (*V. angustifolium* Aiton)

&

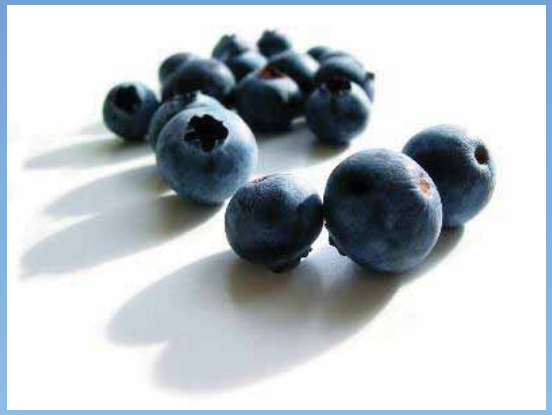
FD maquiberries (*Aristotelia chilensis)*



Delphinidin-3-sambubioside-5-glucoside

Malvidin-containing ANC – 17.1% bioaccessibility (highest)

Delphinidin--containing ANC – 6.5% bioaccessibility (lowest)



Total bioaccessibility of individual ANC ranged from 4.3% to 36.9%, and DID NOT CORRELATE to the amount of ANC in the extract;
ANC with acetyl groups and sugar were more bioaccessible;
Total bioaccessibility only slightly higher in the fed state

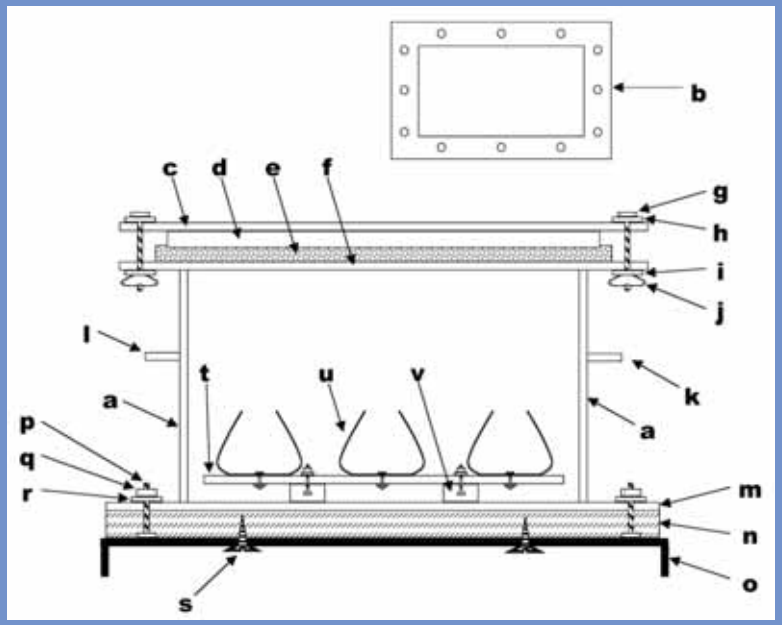
Grace, M., David M. Ribnicky, Peter Kuhn, Alex Poulev, Sithes Logendra, Gad G. Yousef, Ilya Raskin, and Mary Ann Lila. 2009. Hypoglycemic activity of a novel anthocyanin-rich formulation from lowbush blueberry, *Vaccinium angustifolium* Aiton. Phytomedicine 16:406-415.

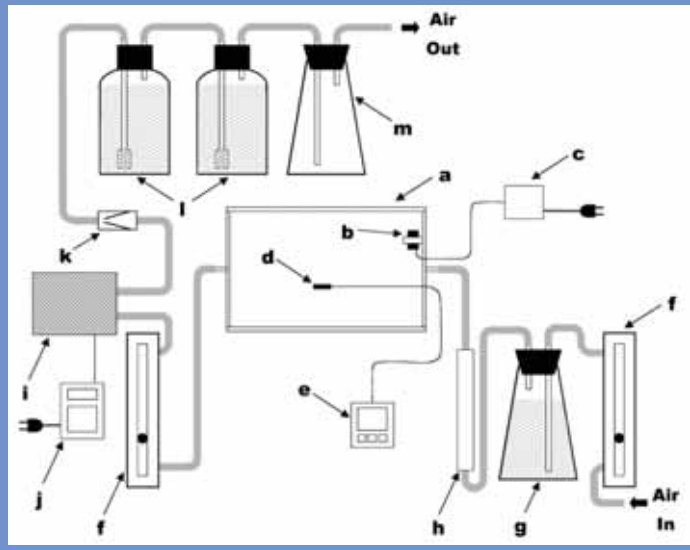
Bioaccessibility of Maquiberry Anthocyanins in the Fasted State

Peak #	ID Chemical Structure	PAE BB extract (mg)	Total Anthocyanin Amount (mg) Averaged	Total Bioaccessibility as a % of intake (averaged)	Bioaccessibility as a % of intake for Efflux alone (averaged)
1	Delphinidin-3-sambubioside-5-glucoside	**38.28**	**1.64**	**4.28**	**0**
2	Delphinidin-3,5-diglucoside	44.47	1.35	**3.05**	0
3	Cyanidin-3-sambubioside5-glucoside + cyanidin3,5-diglucoside	27.99	4.94	**17.64**	1.4
4	Delphinidin-3 sambubioside	**0.02**	**0.89**	**4186.60**	**86.55**
5	Delphinidin-3- glucoside	34.64	2.18	**6.30**	0.3
6	Cyanidin-3-sambubioside	84.10	0.04	**0.00**	0
7	Cyanidin-3-glucoside	36.39	2.61	**7.16**	0.25
	Total	265.89	13.31	**5.00**	0.25

Stability & bioaccessibility via TIM

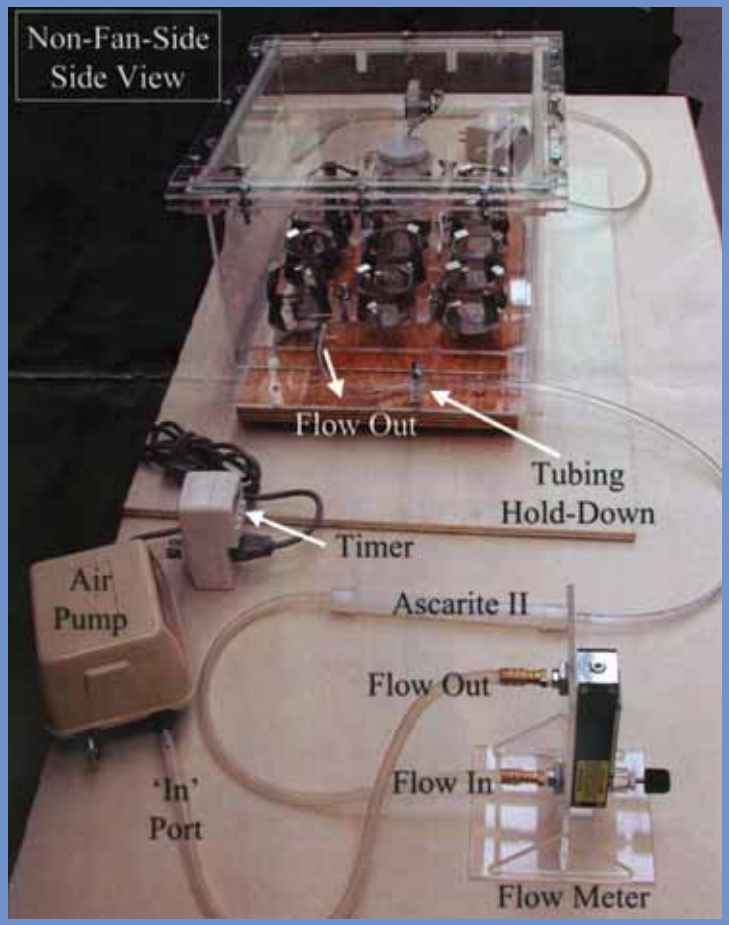
- A unique opportunity to explore the fate of phytochemicals between ingestion and bioconversion in the colon or modification during absorption in vivo
- Predictive for bioavailability
- Determine stability and digestibility
- Limits the need for test animals



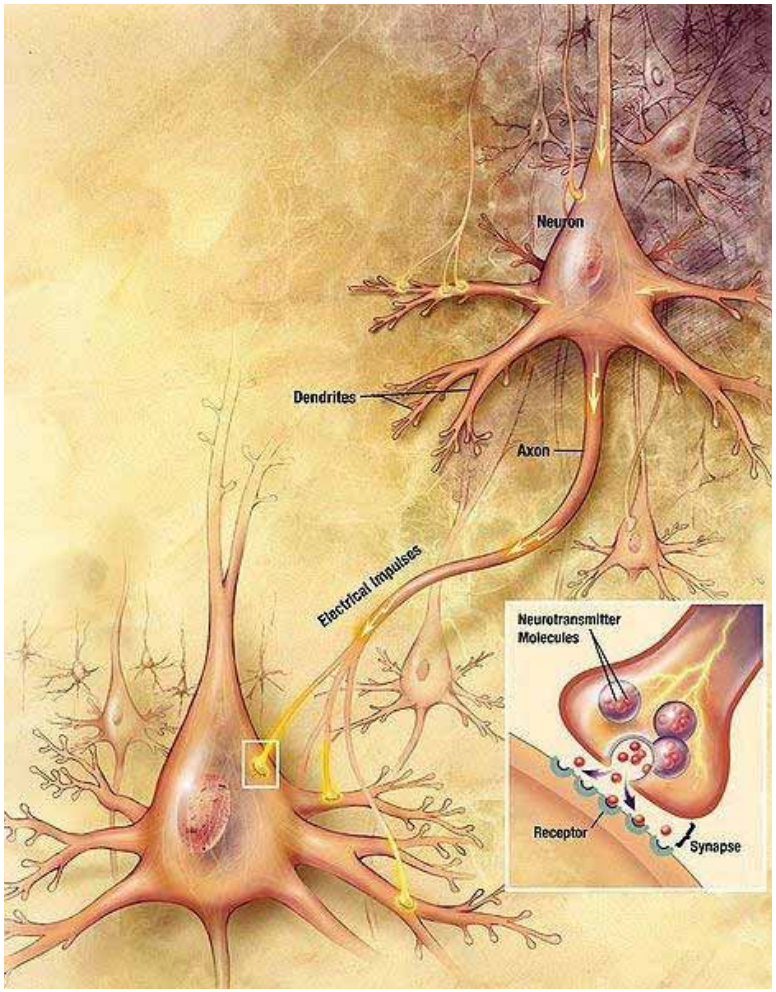


Grusak et al. 2004.
In Vitro Cell Devel. Biol. Plant 40:80-85.

Yousef et al. 2004.
J. Ag. Food Chem. 52:1138-1145.


Non-Fan-Side
Side View
Flow Out
Tubing
Hold-Down
Timer
Air
Pump
Ascarite II
Flow Out
'In'
Port
Flow In
Flow Meter

DDL



↓ oxidative stress

↓ inflammation

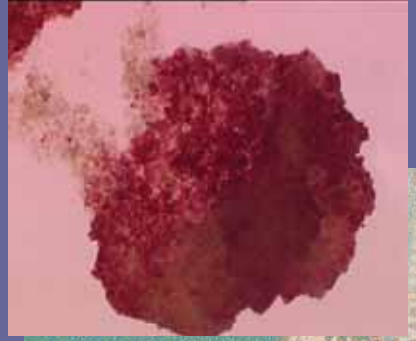
DIRECT evidence still n/a

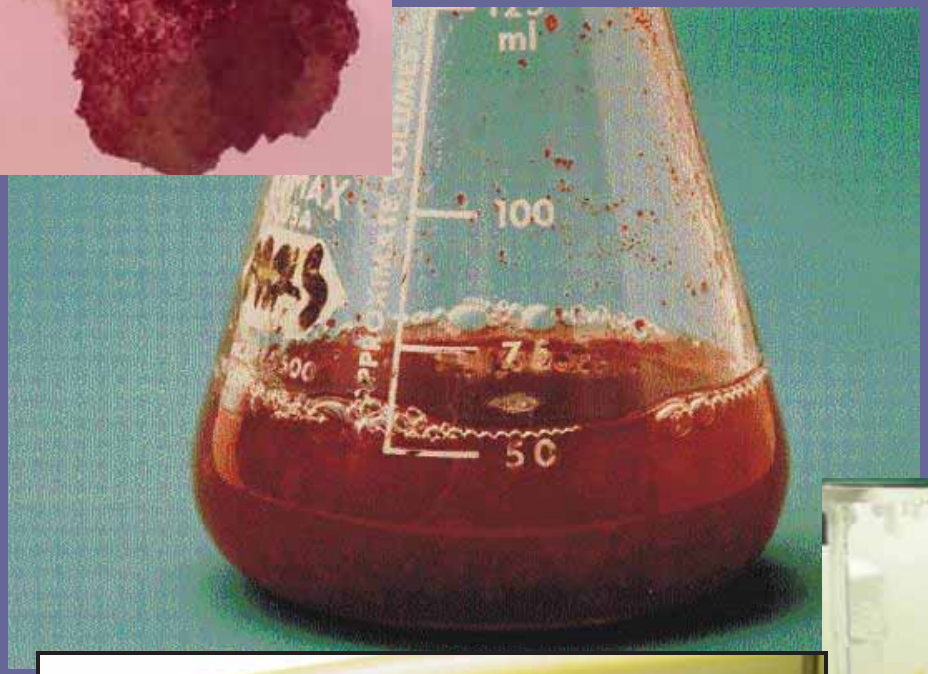
PTC : dietary relevant conc. (fentamolar); complex mixtures; biodistribution

Grape polyphenols inhibition of Alzheimer's Disease. Wang et al. 2008. J. Neuroscience 28:6388-6392

Flavonoids

Feeding resveratrol ↓ plaque pathology in brain. Karuppagounder et al. 2009. Neurochem Int, 54:111-118





Janle et al. 2010.
J Med. Food 13:1-8.

Janle et al. 2010.
Nuclear Instr. & Methods Physics Res. 268: 1313-1316.

^{14}C labeling of flavonoids from ohelo and grape cell cultures

Cell extracts --- 70% acetone

↓

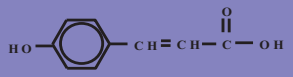
Toyopearl

↓

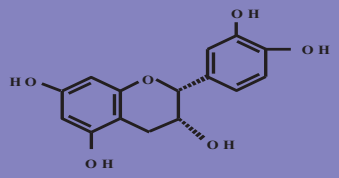
Vacuum chromatography on silica gel

1-3
Triglycerides and fatty acids

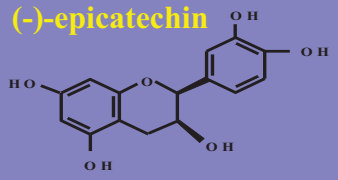
4
E- and Z-*P*-coumaric Acid



(-)-epicatechin and (+)-catechin



(-)-epicatechin

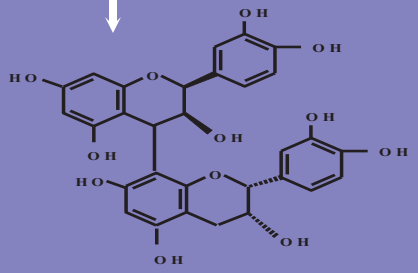


(+)-catechin

5
(-)-Epicatechin

6-9
***p*-hydroxybenzoic and *p*-coumaric acid**

10

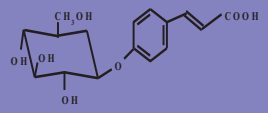


B-2

11
Dimer B-type
Trimer A-type
Tetramer A-type

12
Trimers A type
Tetramers and Pentamers A type
Pentamers and hexamers A type
Insoluble tannins

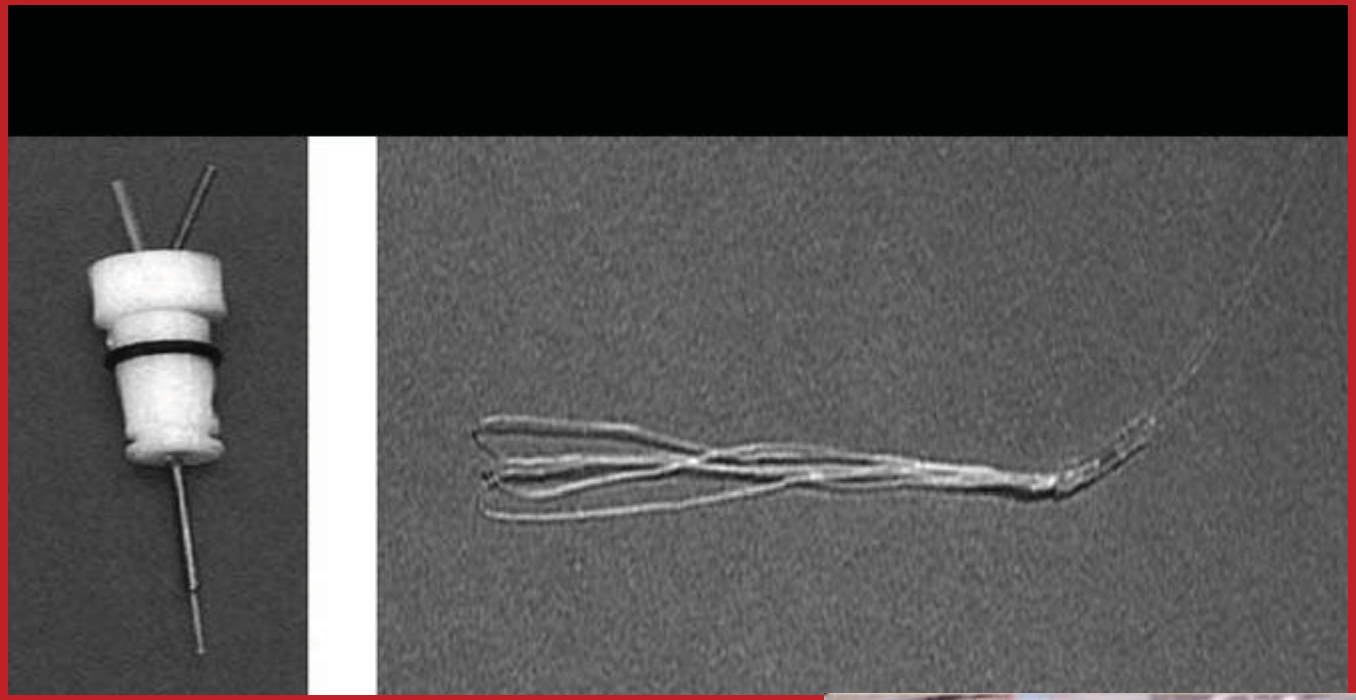
13
4-O-glucoside of E- and Z-*p*-coumaric acid



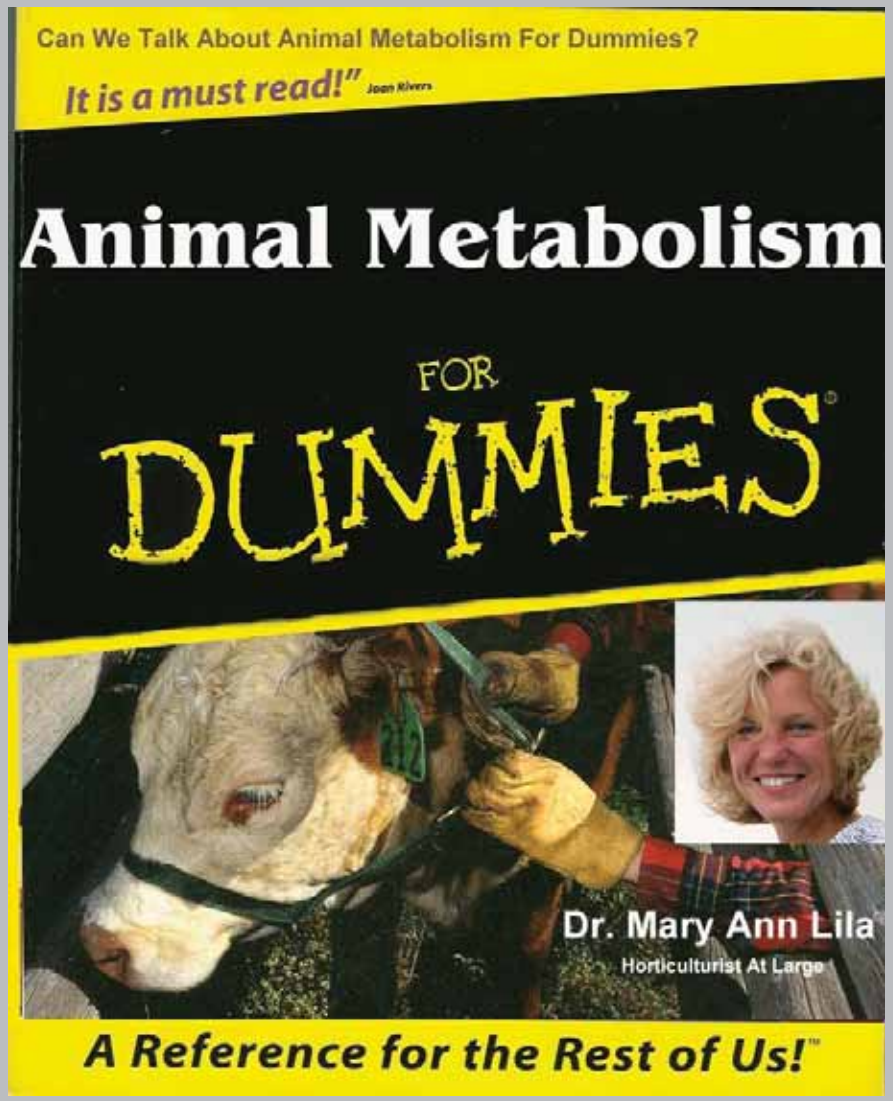
14-17
Anthocyanidins + Phenolic glycosides + Proanthocyanidins

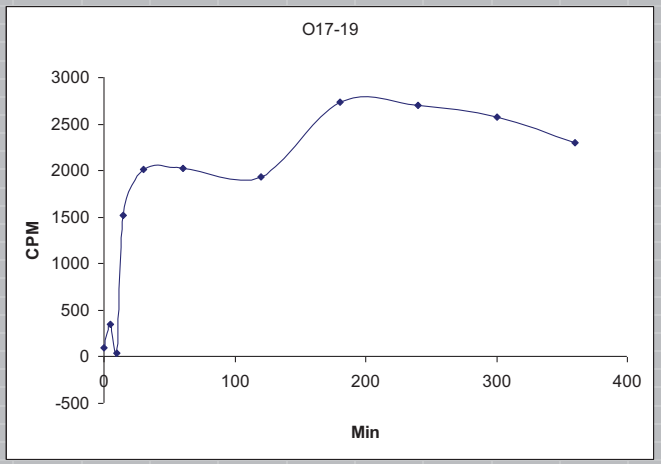
18-23
Free sugars

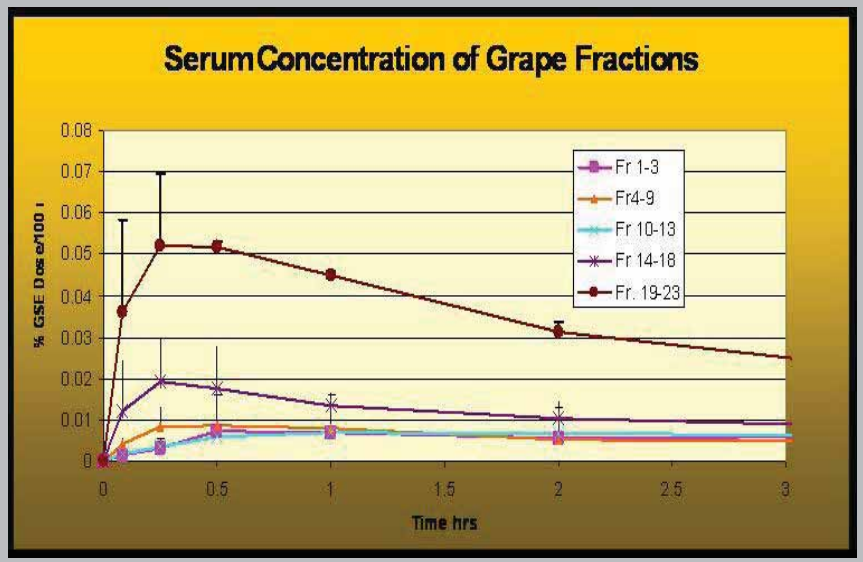
I, II, III = PAC monomers, dimers, oligomers (less polar)
IV, V = ANC 3-*O*- glycosides (more polar)



gut ⇒ blood ⇒ interstitial fluids ⇒ cells & tissues







Metabolic Tracking of Crude Extracts in Rats

- Rats were fasted 8 h
- Baseline samples were collected
- 100 mg/kg bw
- Blood automatically sampled 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600, 720, and 1440 minutes after gavage

Outcomes:

① ANC glycosides (IV, V) better absorbed than PACS
Blood peak @ 15 min-4 hours

② ISF kinetics paralleled blood, with lag time

③ Serum concentrations not indicative of concentrations that reach target tissues (ISF conc. did not correlate with serum conc,; fraction IV low serum conc., but higher ISF conc. than fraction V)

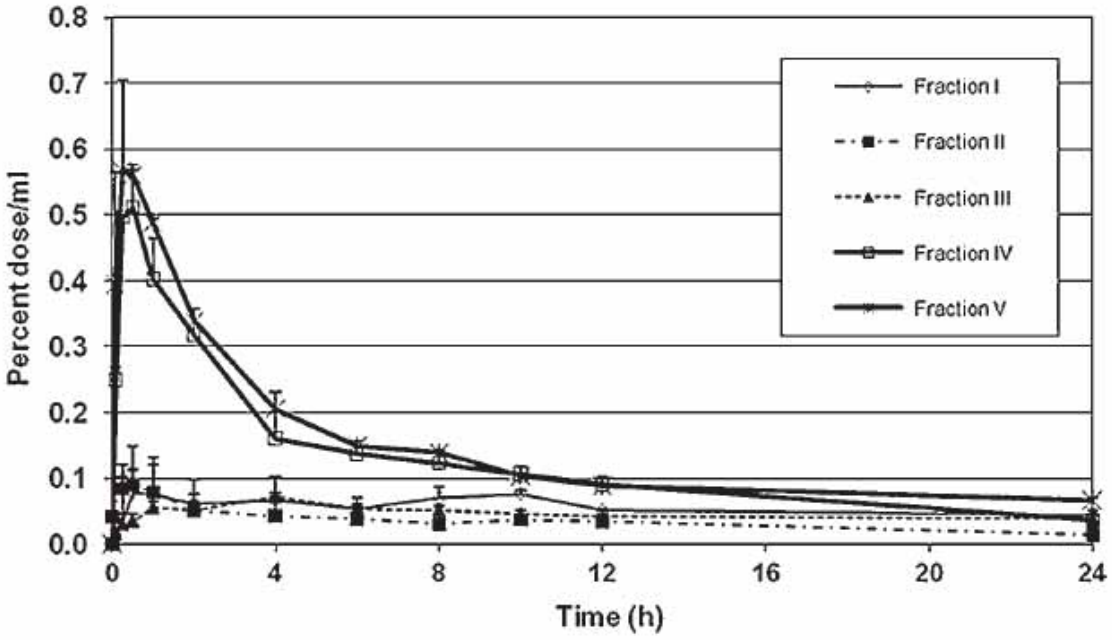


FIG. 2. Serum concentrations (+SEM) of labeled fractions after oral dosing expressed as percentage of dose/mL. There was a 7.8-fold difference in the C_{max} between the best and most poorly absorbed fractions. Fractions containing anthocyanin glycosides were better absorbed than proanthocyanidin fractions.

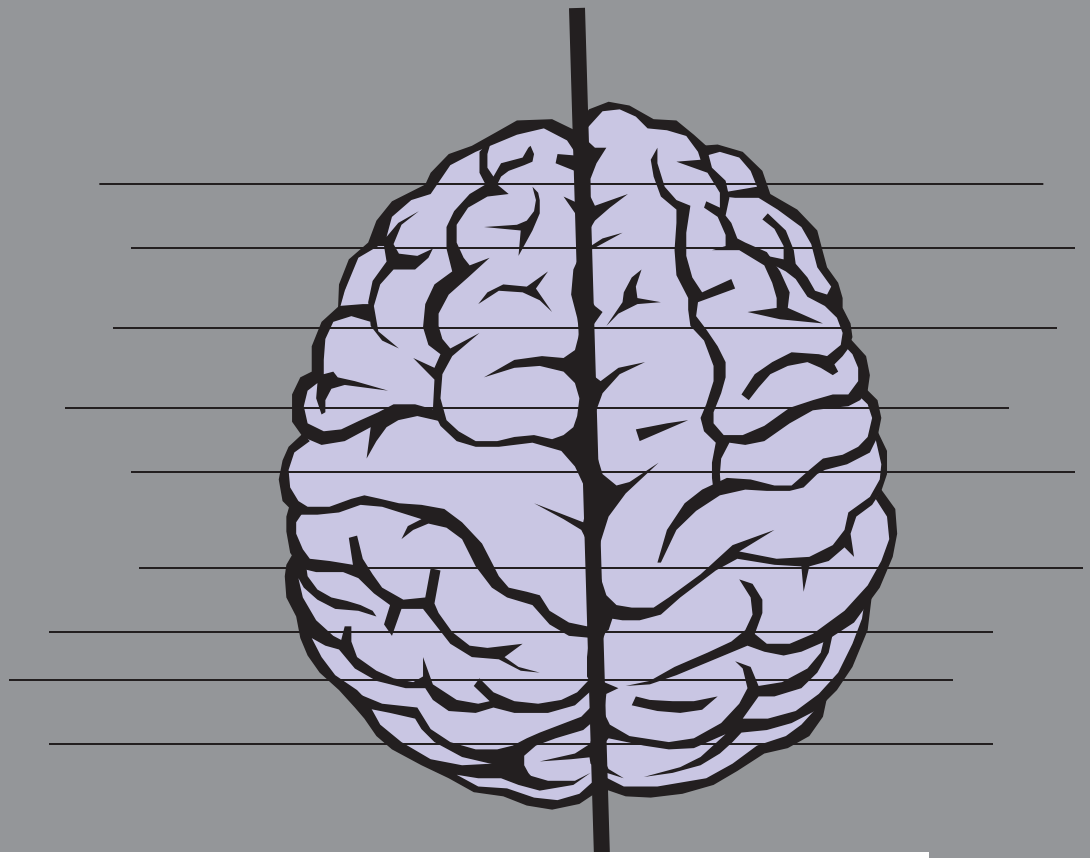
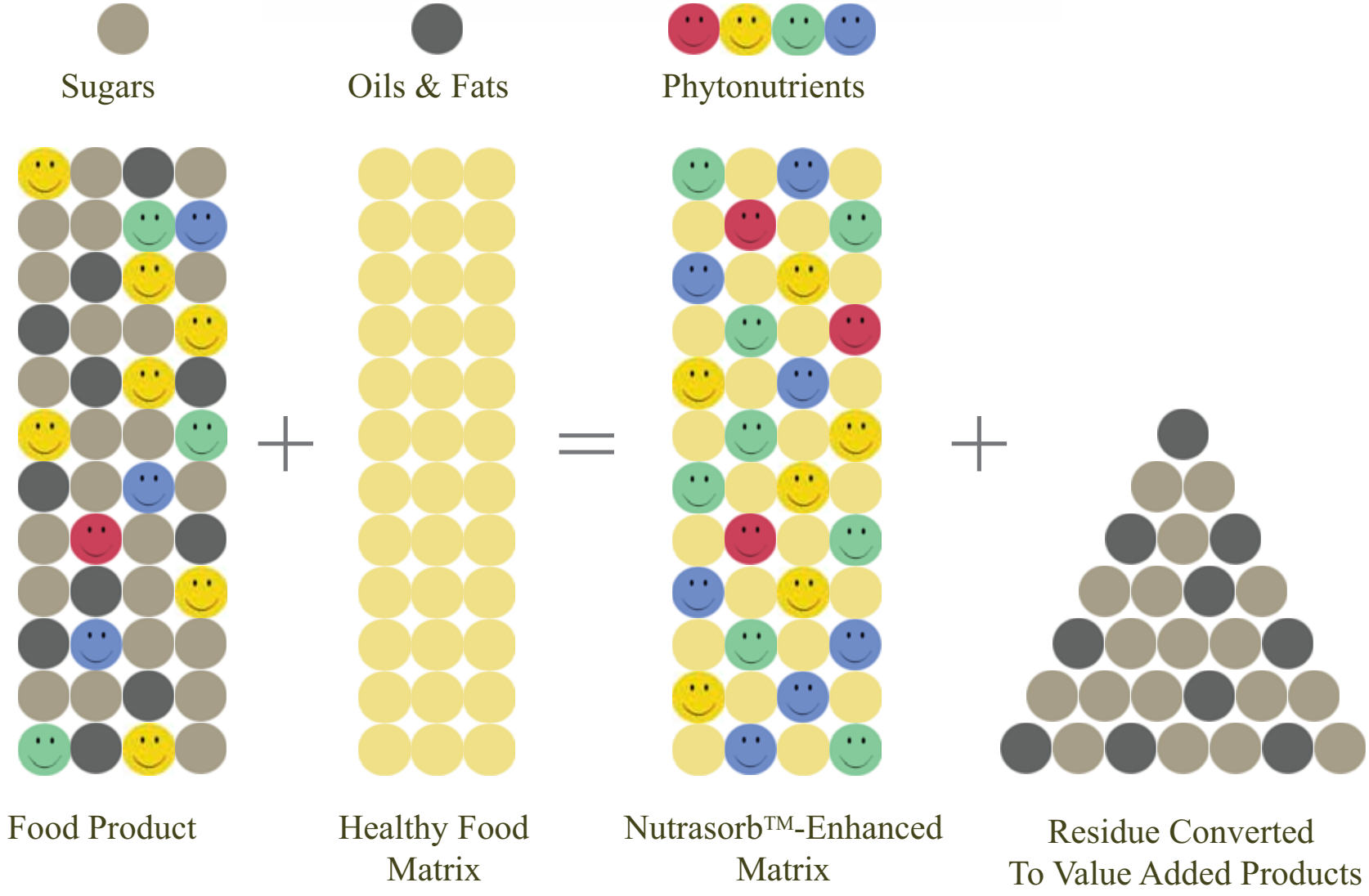


Table 1
^{14}C label in plasma and brain.

Fraction	Serum AUC (% dose × h)	% Dose in brain	Brain ^{14}C/serum AUC
I	1.34	.007%	0.0050
II	0.82	.016%	0.0200
III	1.11	.011%	0.0101
IV	2.07	.125%	0.0605
V	3.46	.119%	0.0343

Nutrasorb™ Process



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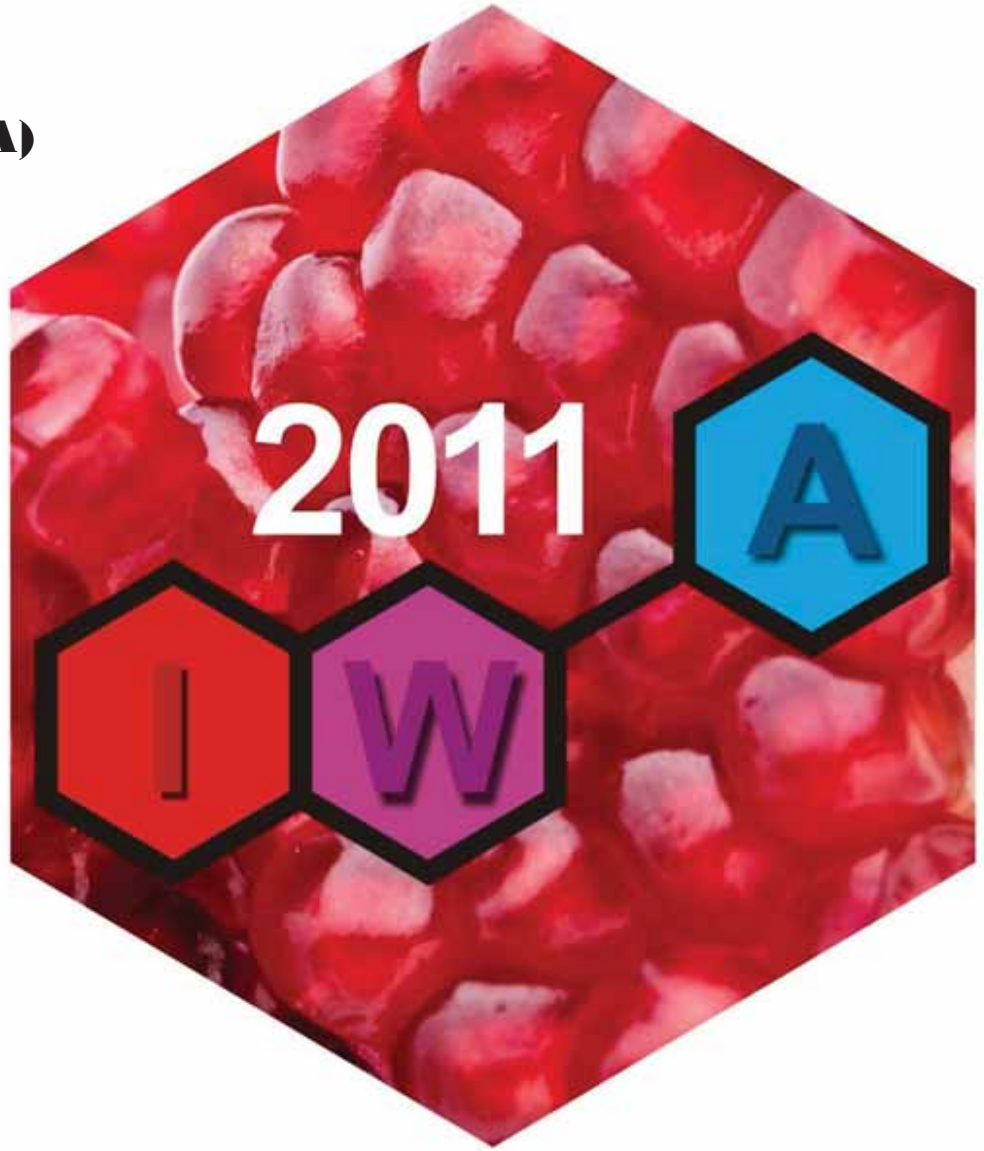
International Workshop on Anthocyanins

Hosted by: NCRC & the PHHI
(8 universities, industries, & USDA)



11-14 September 2011

The Great Wolf Lodge
& Waterpark

Charlotte/Concord
North Carolina

Bluegrass, Barbeque, NASCAR

www.iwa2011.org