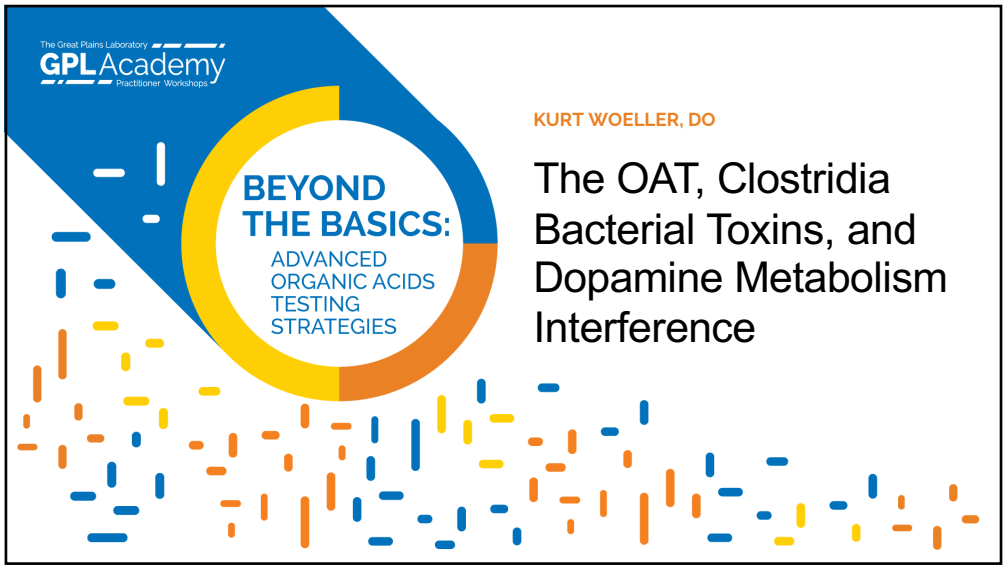
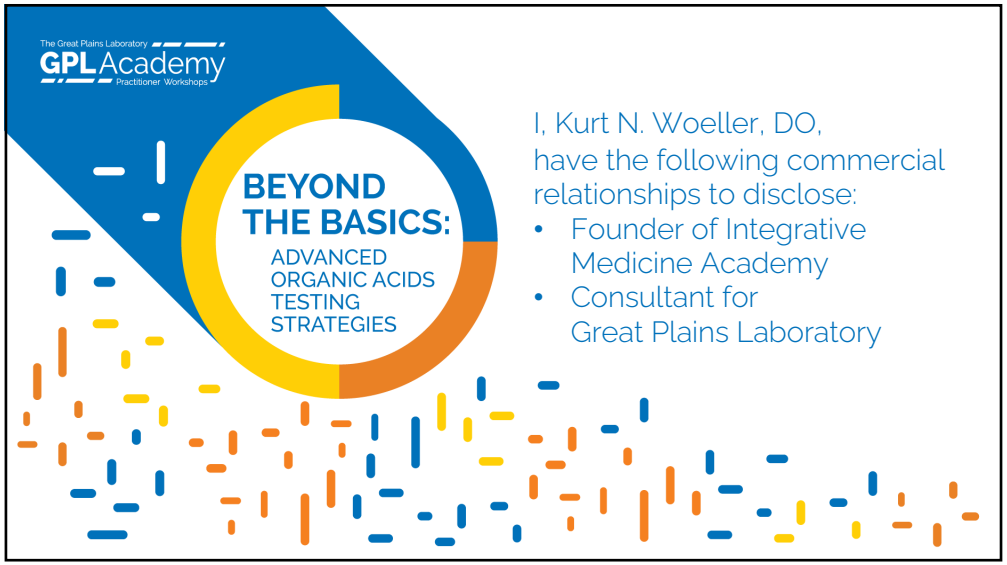

The Great Plains Laboratory
GPLAcademy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
KURT WOELLER, DO
The OAT, Clostridia Bacterial Toxins, and Dopamine Metabolism Interference

1


The Great Plains Laboratory
GPLAcademy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
I, Kurt N. Woeller, DO,
have the following commercial relationships to disclose:
• Founder of Integrative Medicine Academy
• Consultant for Great Plains Laboratory

2

Disclaimer

- The material contained within this presentation is not intended to replace the services and/or medical advice of your personal licensed health care professional.
- This material is for educational purposes only
- This information is not meant to encourage diagnosis and treatment of disease.
- Any application of suggestions set forth in the following portions of this presentation is at the reader's discretion.
- Implementation and/or experimentation with any supplements, herbs, dietary changes, medications, and/or lifestyle changes, etc., is done so at your sole risk and responsibility.

3

3

Lecture Overview

- *Clostridia* bacteria toxicity and various health problems.
- *Clostridia* bacteria and how some of their toxins negatively alter brain and nervous system function.
- HPHPA & 4-cresol as the main culprits for dopamine converting enzyme inhibition.
- Potential dopamine toxicity
- Vagus nerve and gut toxins
- Introduction to treatment options

4

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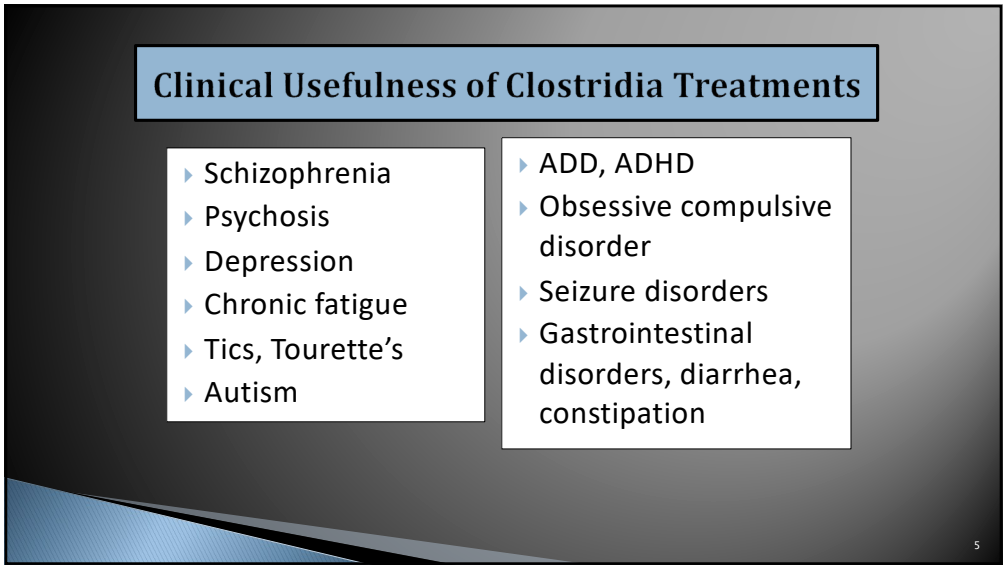
Clinical Usefulness of Clostridia Treatments
Schizophrenia
Psychosis
Depression
Chronic fatigue
Tics, Tourette's
Autism
ADD, ADHD
Obsessive compulsive disorder
Seizure disorders
Gastrointestinal disorders, diarrhea, constipation
5

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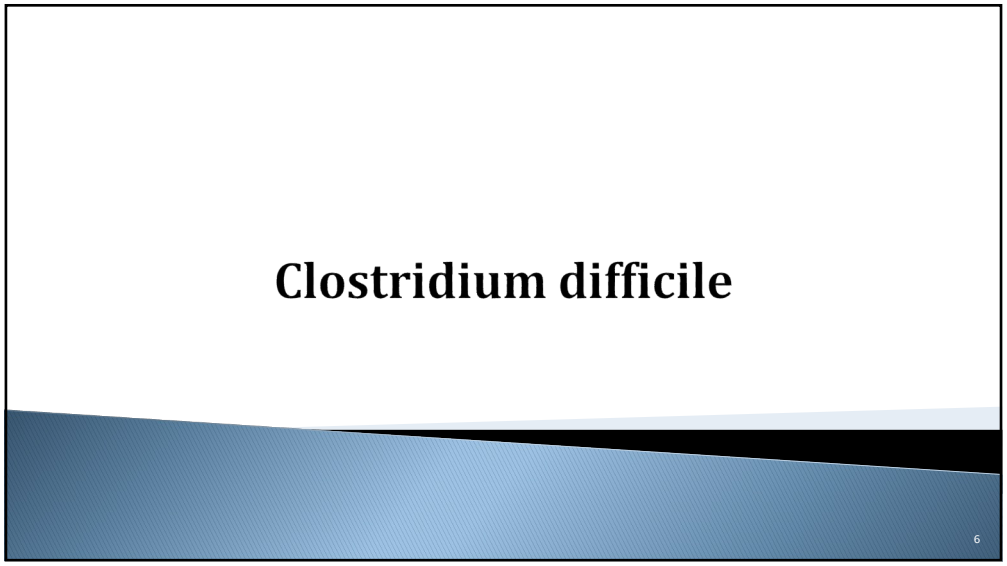

Clostridium difficile
6

6

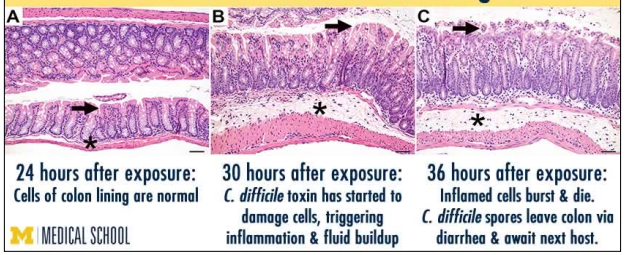
Lessa FC, Mu Y, Bamberg WM, et al. *Burden of Clostridium difficile infection in the United States.* N Engl J Med. 2015;372:825-834

- The Centers for Disease Control estimates that there were 453,000 documented cases of *Clostridium difficile* infection (CDI) in the United States in 2011 leading to 29,300 deaths.
- **Between 10% and 30%** of people who have an initial episode of CDI will develop at least one recurrence.
- The number of incident cases found in 2011, 45,300 - 135,900 people developed recurrent CDI.

7

7

What does *C. difficile* do to the gut?

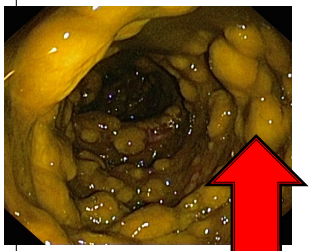


University of Michigan Medical School

8

Toxin A & Toxin B

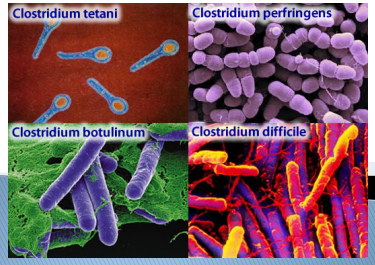
- These two toxins are the main virulence factors related to mucosal damage from *C. difficile*.
- Toxins A & B lead to digestive tract inflammation, e.g., Pseudomembranous colitis or clostridia difficile associated diarrhea (CDAD).
- Toxin A & Toxin B are both capable of causing mucosal damage (Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP; October 2010. "The role of toxin A and toxin B in Clostridium difficile infection". *Nature* 467 (7316): 711–3).



Pseudomembrane

9

Other Toxins of Clostridia Bacteria



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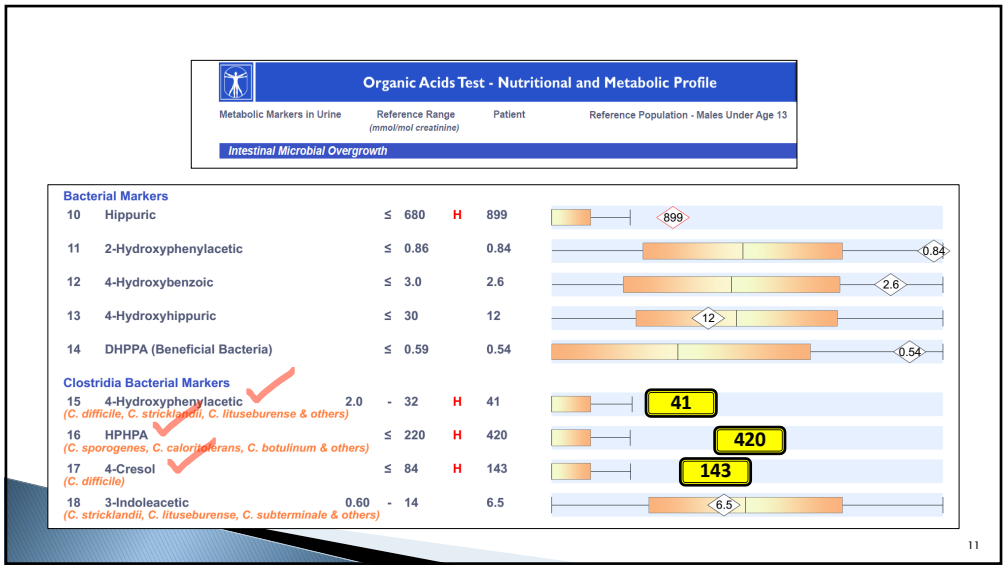
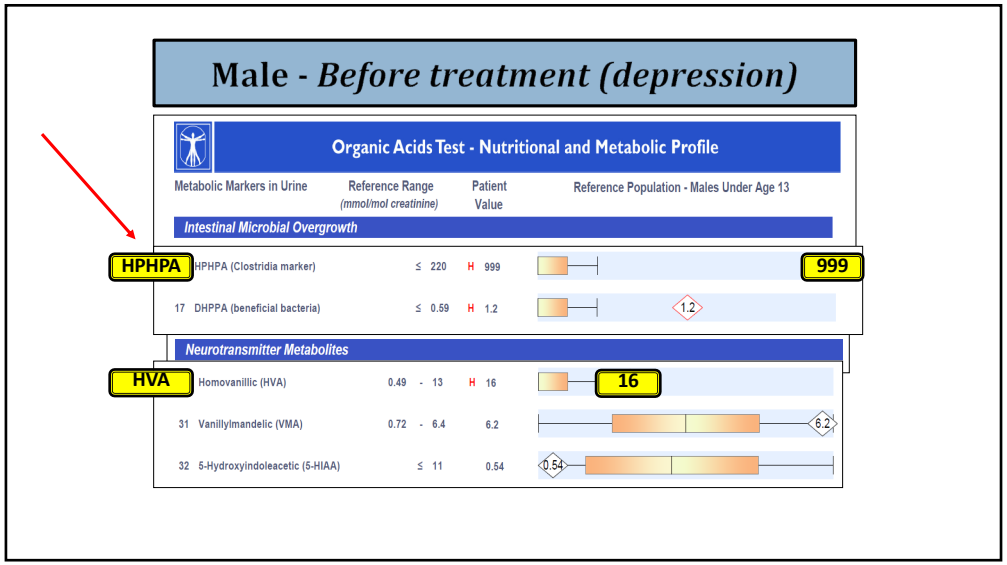
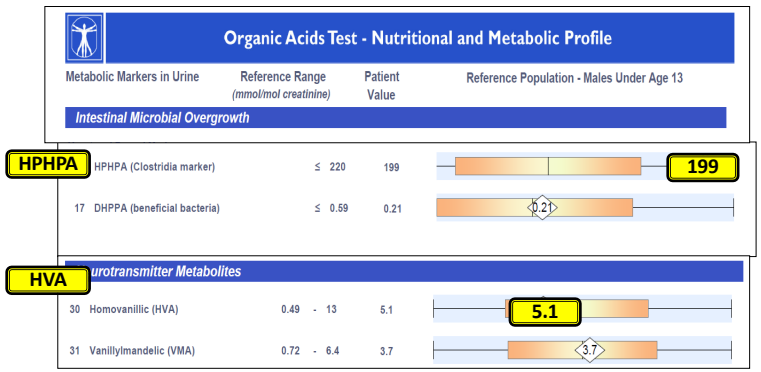

Organic Acids Test - Nutritional and Metabolic Profile
Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient
Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth
Bacterial Markers
10 Hippuric ≤ 680 H 899
11 2-Hydroxyphenylacetic ≤ 0.86 0.84
12 4-Hydroxybenzoic ≤ 3.0 2.6
13 4-Hydroxyhippuric ≤ 30 12
14 DHPPA (Beneficial Bacteria) ≤ 0.59 0.54
Clostridia Bacterial Markers
15 4-Hydroxyphenylacetic 2.0 - 32 H 41
(C. difficile, C. stricklandii, C. lituseburense & others)
16 HPHPA ≤ 220 H 420
(C. sporogenes, C. caloritolerans, C. botulinum & others)
17 4-Cresol ≤ 84 H 143
(C. difficile)
18 3-Indoleacetic 0.60 - 14 6.5
(C. stricklandii, C. lituseburense, C. subterminale & others)
899
0.84
2.6
12
0.54
41
420
143
6.5
11

11


Male - Before treatment (depression)
Organic Acids Test - Nutritional and Metabolic Profile
Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient Value
Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth
HPHPA
HPHPA (Clostridia marker) ≤ 220 H 999
17 DHPPA (beneficial bacteria) ≤ 0.59 H 1.2
999
1.2
Neurotransmitter Metabolites
HVA
Homovanillic (HVA) 0.49 - 13 H 16
31 Vanillylmandelic (VMA) 0.72 - 6.4 6.2
32 5-Hydroxyindoleacetic (5-HIAA) ≤ 11 0.54
16
6.2
0.54

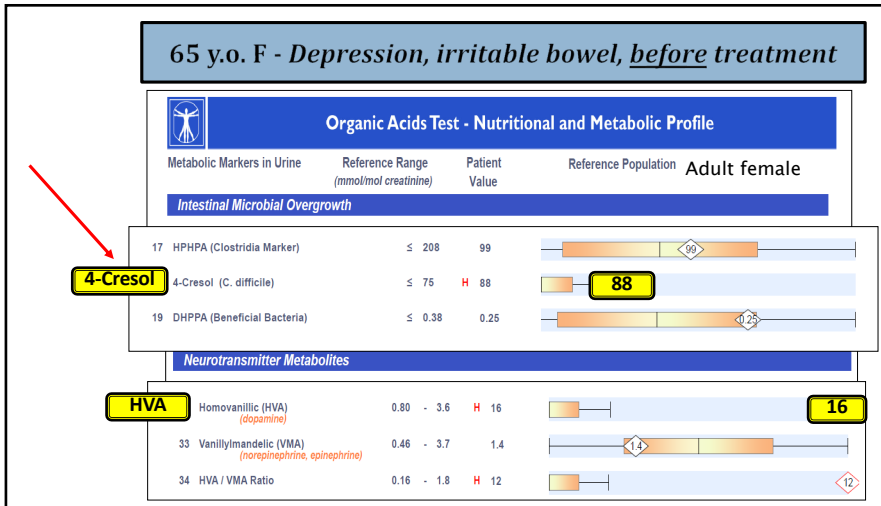
12

Male after treatment *(no depression)*



13

65 y.o. F - *Depression, irritable bowel, before treatment*



14

65 y.o. F - *Depression, irritable bowel, after treatment*

Organic Acids Test - Nutritional and Metabolic Profile

	Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Adult female
Intestinal Microbial Overgrowth					
17	HPHPA (Clostridia Marker)	≤ 208		44	44
4-Cresol	4-Cresol (C. difficile)	≤ 75		61	**61**
19	DHPPA (Beneficial Bacteria)	≤ 0.38	H	0.59	0.59
Neurotransmitter Metabolites					
HVA	Homovanillic (HVA) (dopamine)	0.80 - 3.6		2.9	**2.9**
33	Vanillylmandelic (VMA) (norepinephrine, epinephrine)	0.46 - 3.7		1.6	1.6
34	HVA / VMA Ratio	0.16 - 1.8		1.8	1.8

15

Nature Reviews Neuroscience | AOP, published - September 2012

Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour

John F. Cryan[1,2] and Timothy G. Dinan[1,3]

Abstract | Recent years have witnessed the rise of the gut microbiota as a major topic of research interest in biology. Studies are revealing how variations and changes in the composition of the gut microbiota influence normal physiology and contribute to diseases ranging from inflammation to obesity. Accumulating data now indicate that the gut microbiota also communicates with the CNS — possibly through neural, endocrine and immune pathways — and thereby influences brain function and behaviour. Studies in germ-free animals and in animals exposed to pathogenic bacterial infections, probiotic bacteria or antibiotic drugs suggest a role for the gut microbiota in the regulation of anxiety, mood, cognition and pain. Thus, the emerging concept of **Microbiome-Gut-Brain Axis** suggests that modulation of the gut microbiota may be a tractable strategy for developing novel therapeutics for complex CNS disorders.

16

16

Cell

December 5, 2013

"Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders" - Elaine Y. Hsiao, et. al.

- Demonstrate GI barrier defects and microbiota alterations in the immune activation (MIA) mouse model that is known to display features of ASD.
- Oral treatment of MIA with the **human commensal *Bacteroides fragilis*** corrects gut permeability, alters microbial composition, and ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors. MIA display an altered serum metabolomic profile **(4-ethylphenylsulphate),** and *B. fragilis* modulates levels of several metabolites.
- Treating naive mice with a metabolite that is increased by MIA and restored by *B. fragilis* causes certain behavioral abnormalities, suggesting that gut bacterial effects on the host metabolome impact behavior.

"Taken together, these findings support a gut-microbiome-brain connection in a mouse model of ASD and identify a potential probiotic therapy for GI and particular behavioral symptoms in human neurodevelopmental disorders."

17

17

THE OCCURRENCE OF (−)-β-*m*-HYDROXYPHENYL-HYDRACRYLIC ACID IN HUMAN URINE*

By MARVIN D. ARMSTRONG and KENNETH N. F. SHAW

(From the Laboratory for the Study of Hereditary and Metabolic Disorders, and the Departments of Biological Chemistry and Medicine, University of Utah College of Medicine, Salt Lake City, Utah)

(Received for publication, June 26, 1956)

Journal of Biological Chemistry 225:269–278,1957

- "It was observed that mentally ill patients, in general, seem to excrete much larger amounts of **HPHPA** than do most normal people."
- "Most patients with mental retardation excrete very low amounts of HPHPA."

18

18

Nutritional Neuroscience 2010 Vol. 13 No 3: 1-10

Research article

Increased urinary excretion of a 3-(3-hydroxy-phenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of *Clostridia* spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia

William Shaw

The Great Plains Laboratory, Inc., Lenexa, Kansas, USA

A compound identified as 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) was found in higher concentrations in urine samples of children with autism compared to age and sex appropriate controls and in an adult with recurrent diarrhea due to *Clostridium difficile* infections. The highest value measured in urine samples was 7500 mmol/mol creatinine, a value 300 times the median normal adult value, in a patient with acute schizophrenia during an acute psychotic episode. The psychosis remitted after treatment with oral vancomycin with a concomitant marked decrease in HPHPA. The source of this compound appears to be multiple species of anaerobic bacteria of the *Clostridium* genus. The significance of this compound is that it is a probable metabolite of *m*-tyrosine (3-hydroxyphenylalanine), a tyrosine analog which depletes brain catecholamines and causes symptoms of autism (stereotypical behavior, hyperactivity, and hyper-reactivity) in experimental animals.

19

19

Effect of anti-Clostridia therapy on urine excretion of HPHPA* in young woman with acute psychosis-auditory hallucinations

	patient	normals
During acute psychosis	7489	0-150
After treatment (depressed but no psychosis)	673	0-150

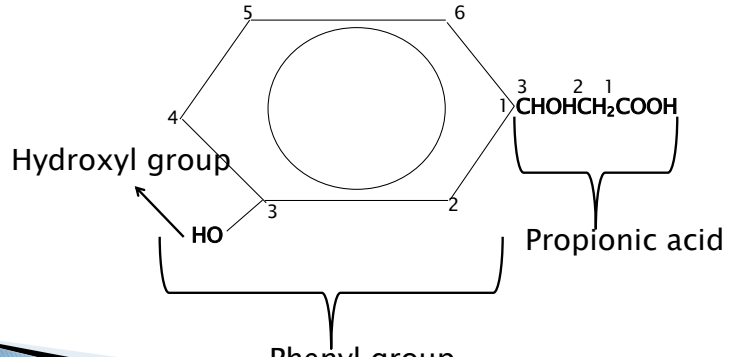
Still very high even after treatment

*mmol/mol creatin

20

20

Structure of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid *(HPHPA)*



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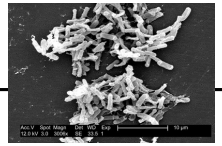
HPHPA

- Known producers of HPHPA: *C. botulinum*, *C. sporogenes* (similar to C. botulinum, but doesn't contain the neurotoxin), *C. caloritolerans* (and a few others) - Shaw, Nutritional Neuroscience 2010 Vol 13 No 3: 1-10
- **Severe botulinum infection:**
  - *Sudden onset weakness (arms, legs, chest), trouble seeing and speaking, and eventual flaccid paralysis.*
  - *Some cases of food borne C. botulinum can have mild symptoms with people seeking no medical attention.*
- **Autism-Spectrum Disorders (often seen with high HPHPA):**
  - *little to no speech*
  - *low muscle tone*



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Clostridia species that do not produce HPHPA precursors

Nutritional Neuroscience - 2010 Vol 13 No 3: 1-10

- C. tetani
- C. sticklandii
- C. lituseburense
- C. subterminale
- C. putifaciens
- C. propionicum
- C. malenomenatum
- C. coclearium
- C. histolyticum
- C. aminovalericum
- C. sporospheroides
- C. limosum
- C. lentoputrescens
- C. tetanomorphum

Approximately 100 species of Clostridia can exist in the GI tract, but not all are pathogenic.

23

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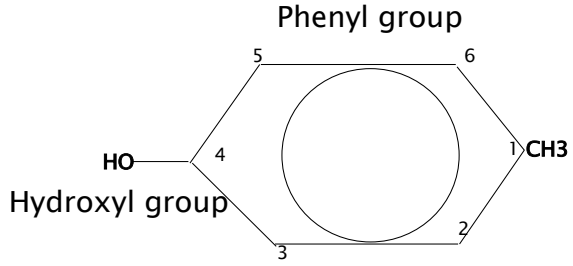
Structure of p-cresol *(methylphenol)*



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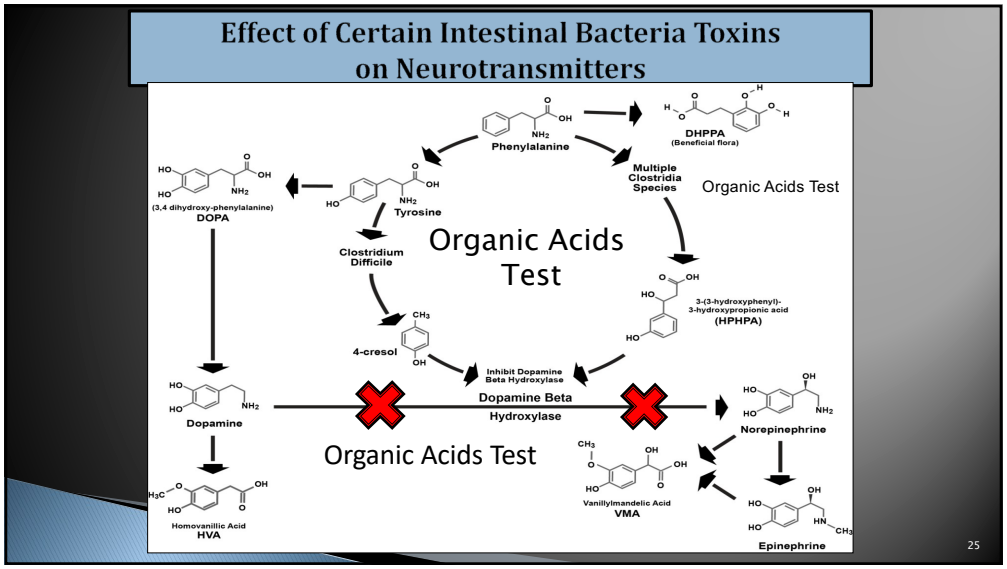
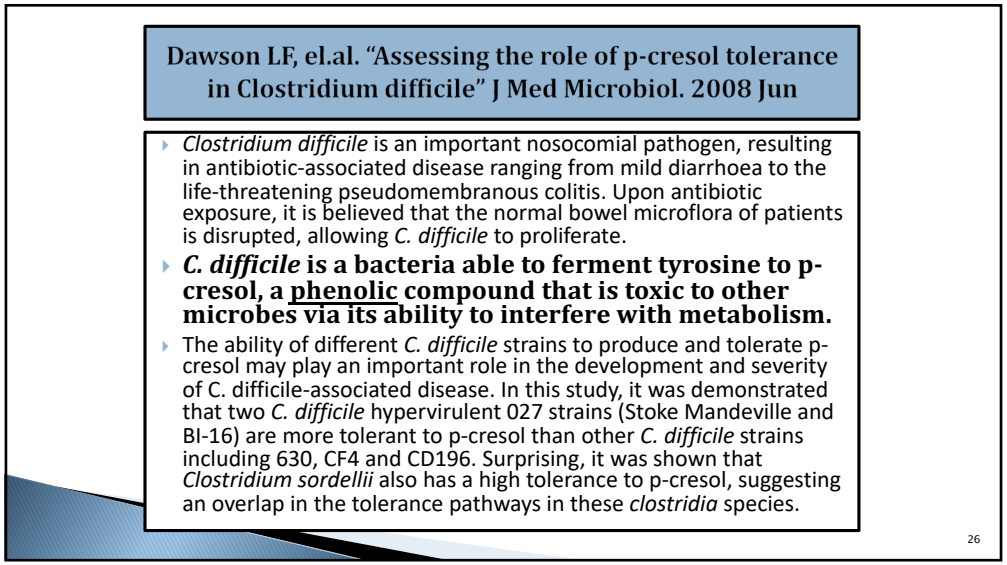

Effect of Certain Intestinal Bacteria Toxins on Neurotransmitters
Phenylalanine
DHPPA
(Beneficial flora)
Multiple Clostridia Species
Organic Acids Test
Tyrosine
(3,4 dihydroxy-phenylalanine)
DOPA
Clostridium Difficile
Organic Acids Test
3-(3-hydroxyphenyl)-3-hydroxypropionic acid
(HPHPA)
4-cresol
Inhibit Dopamine Beta Hydroxylase
Dopamine Beta Hydroxylase
Dopamine
Norepinephrine
Organic Acids Test
Vanillylmandelic Acid
VMA
Homovanillic Acid
HVA
Epinephrine
25

25

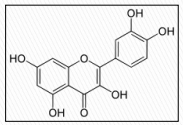

Dawson LF, el.al. "Assessing the role of p-cresol tolerance in Clostridium difficile" J Med Microbiol. 2008 Jun
Clostridium difficile is an important nosocomial pathogen, resulting in antibiotic-associated disease ranging from mild diarrhoea to the life-threatening pseudomembranous colitis. Upon antibiotic exposure, it is believed that the normal bowel microflora of patients is disrupted, allowing C. difficile to proliferate.
C. difficile is a bacteria able to ferment tyrosine to p-cresol, a phenolic compound that is toxic to other microbes via its ability to interfere with metabolism.
The ability of different C. difficile strains to produce and tolerate p-cresol may play an important role in the development and severity of C. difficile-associated disease. In this study, it was demonstrated that two C. difficile hypervirulent 027 strains (Stoke Mandeville and BI-16) are more tolerant to p-cresol than other C. difficile strains including 630, CF4 and CD196. Surprising, it was shown that Clostridium sordellii also has a high tolerance to p-cresol, suggesting an overlap in the tolerance pathways in these clostridia species.
26

26

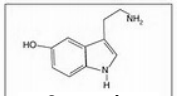
What Are Phenols?

- Class of chemical compounds where a hydroxyl group (-OH) is bonded directly to an aromatic hydrocarbon.
- Can be produced industrially.
- Can be produced naturally from plants and microorganisms.
- Estrogen, Serotonin, Dopamine are phenols.
- L-Tyrosine is a phenol

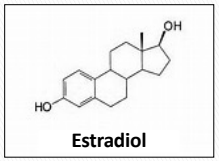
Benzene



Quercetin



Serotonin



Estradiol

27

27

Other Potential Causes of High HVA

If HVA is elevated and VMA is normal and the patient has elevated Clostridia markers, avoid supplementation with L-DOPA, phenylalanine or tyrosine until *Clostridia* is treated. Homovanillic acid (HVA), a dopamine metabolite, is often elevated due to stress-induced catecholamine output from the adrenal gland which depletes vitamin C. Supplementation with vitamin C (ascorbate) may be helpful in such cases.* Elevated HVA can result from the intake of L-DOPA, dopamine, phenylalanine, or tyrosine. Elevated HVA may also result from ingestion of aspartame (Nutrasweet®), salicylates (aspirin), and dietary salicylates. For more information about salicylates in foods go to <http://www.feingold.org/salicylate.php>. Elevated HVA may also result from toxic metal exposure (including lead, aluminum, manganese,arsenic, and mercury), presumably due to DBH inhibition. Heavy metal testing (blood or hair) might be useful to determine if such exposure is significant.

- Aspartame
- Salicylates, e.g. aspirin, diet
- Heavy metals, e.g. aluminum
- Toxoplasmosis or tumors

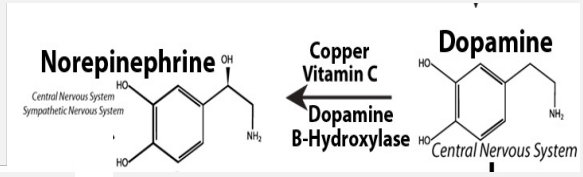
If values are more than double the upper limit of normal, toxoplasmosis and tumors such as neuroblastoma, or other catecholamine-secreting tumors should be ruled out. Catecholamine-secreting tumors can be ruled out by 24- hour VMA and/or HVA testing in urine. Even in this subgroup, the incidence of tumors is extremely rare.

28

28

Goodhart PJ, DeWolf WE Jr, Kruse LI. Mechanism-based inactivation of dopamine beta-hydroxylase by p-cresol and related alkylphenols. *Biochemistry.* 1983 Jun 21; 22(13):3091-6.

- A wide range of phenols, including p-cresol, a major *Clostridia* metabolite, are strong inhibitors of *dopamine-beta-hydroxylase* which converts dopamine to norepinephrine.



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Int J Biomed Sci. 2009 Dec; 5(4): 395–401.
PMCID: PMC3614808
PMID: 23675164

Dopamine-β-Hydroxylase (DBH), Its Cofactors and Other Biochemical Parameters in the Serum of Neurological Patients in Bangladesh

Md. Khalilur Rahman,[1] Farhana Rahman,[1] Tania Rahman,[1] and Takeshi Kato[2]

Abstract Go to:

Dopamine-β-hydroxylase (DBH) is a neurotransmitter synthesizing enzyme which catalyzes the formation of norepinephrine from dopamine. In this study, we measured the level of DBH activity in the serum of patients of three different age groups (8–14 yrs, 20–40 yrs and 45–60 yrs) suffering from neurological diseases. Serum DBH activity was measured in 38 neurological patients and 38 normal individuals in order to determine significant variables for its potential use to diagnose the neurological patients. It was found that the DBH activity decreased in the patients of all age groups. A considerable decrease in activity was observed in the patients of 8–14 yrs age group (14.2 nmoles/min/ml in patients compared to the normal value of 22.6). A significant decrease in activity was found in the 20–40 yrs age group (23.4 nmoles/min/ml in patients compared to the normal value of 33.0). The decrease in DBH activity was also found in the patients of 45–60 yrs age group but to a lesser extent (26.4 nmoles/min/ml in the patients compared to the normal value of 30.2). The kinetic studies of DBH exhibited an increase of K_m value and a decrease in V_{max} in the neurological patients. Serum copper and ascorbic acid levels (cofactors of DBH) were found to be decreased in neurological patients and hence are in agreement with the decrease in DBH activity in these patients. Other parameters such as glucose and cholesterol levels increased, protein and zinc levels decreased and ALT, AST, creatinine and urea content remained nearly unchanged in the patients' serum.

Keywords: dopa

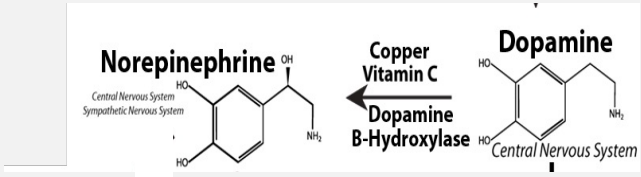
"Serum copper and ascorbic acid levels (cofactors of DBH) were found to be decreased in neurological patients…"

30

30

Goodhart PJ, DeWolf WE Jr, Kruse LI. Mechanism-based inactivation of dopamine beta-hydroxylase by p-cresol (4-cresol) and related alkylphenols. *Biochem.* 1983 Jun 21; 22(13):3091-6.

- Most importantly, the reaction is irreversible, and the p-cresol is incorporated into the enzyme active site so that further enzyme activity is destroyed.



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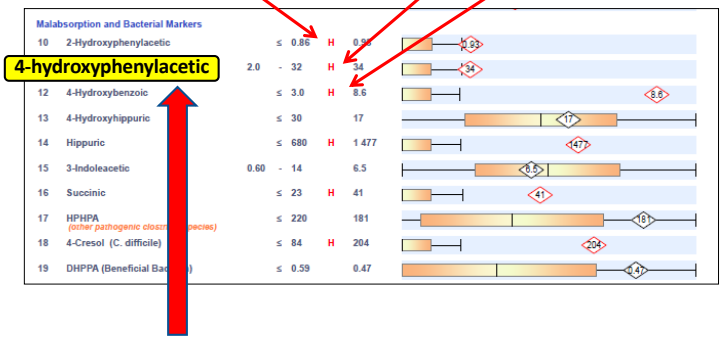
Suspicion about other markers negatively influencing Dopamine-Beta Hydroxylase



4-hydroxyphenylacetic acid – linked to *C. difficile* and others

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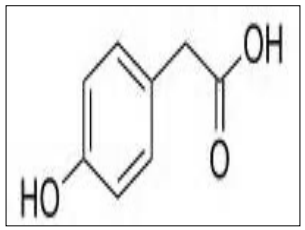
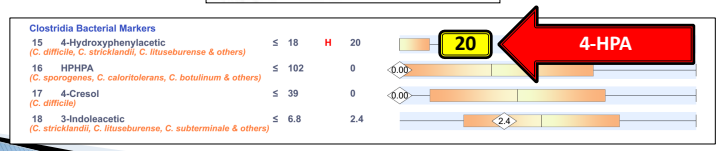
4-Hydroxyphenylacetic acid *(4-HPA)*





33

Cell **December 5, 2013**

"Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders" - Elaine Y. Hsiao, et. al.

- Demonstrate GI barrier defects and microbiota alterations in the immune activation (MIA) mouse model that is known to display features of ASD.
- Oral treatment of MIA with the **human commensal *Bacteroides fragilis*** corrects gut permeability, alters microbial composition, and ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors. MIA display an altered serum metabolomic profile **(4-ethylphenylsulphate),** and *B. fragilis* modulates levels of several metabolites.
- Treating naive mice with a metabolite that is increased by MIA and restored by *B. fragilis* causes certain behavioral abnormalities, suggesting that gut bacterial effects on the host metabolome impact behavior.

"Taken together, these findings support a gut-microbiome-brain connection in a mouse model of ASD and identify a potential probiotic therapy for GI and particular behavioral symptoms in human neurodevelopmental disorders."

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4-Ethylphenylsulphate

The researchers further dissected what was going on by screening for chemicals in the blood that differed between autistic and wild-type mice. One compound, 4-ethylphenylsulphate (4EPS), stood out:

1. **It was found at levels 46 times higher in autistic mice.**
2. **Injecting 4EPS into wild-type mice resulted in autism-like behaviors.**

4EPS – Is similar in chemical structure to 4-Cresol which is known to inhibit *Dopamine Beta-Hydroxylase*.

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Potential Toxicity of Elevated Dopamine

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Problems with Elevated Dopamine

- Overstimulation of dopamine tracts
- Potential infiltration of dopamine into norepinephrine tracts and sympathetic nervous system.
- Damage to neurons producing excess dopamine due to oxidative damage of abnormal dopamine metabolites.
- Depletion of glutathione in brain making it susceptible to other toxic chemicals.

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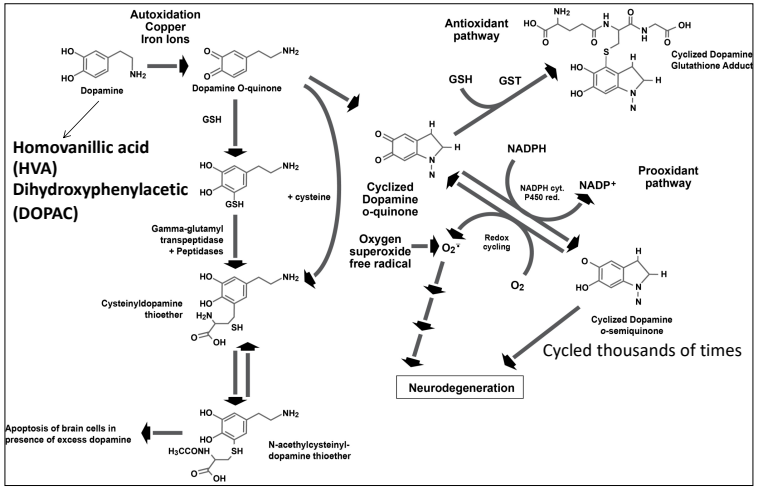
Dopamine Related Toxicity



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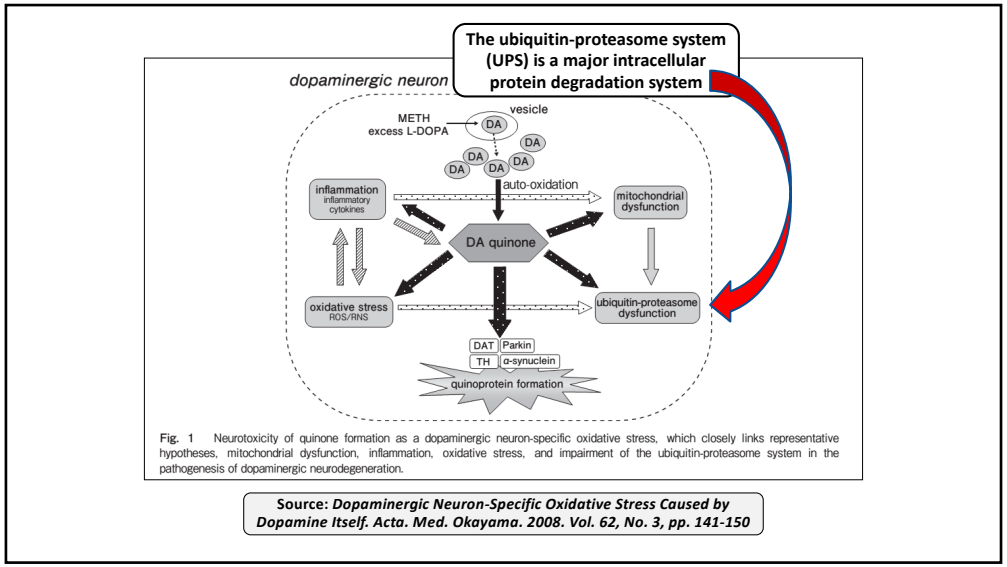

Fig. 1 Neurotoxicity of quinone formation as a dopaminergic neuron-specific oxidative stress, which closely links representative hypotheses, mitochondrial dysfunction, inflammation, oxidative stress, and impairment of the ubiquitin-proteasome system in the pathogenesis of dopaminergic neurodegeneration.

Source: ***Dopaminergic Neuron-Specific Oxidative Stress Caused by Dopamine Itself. Acta. Med. Okayama. 2008. Vol. 62, No. 3, pp. 141-150***

39

Linan Chen, et al. Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. J. Neurosci. 2008. pg. 425–433

- Dopamine is a very reactive molecule (particularly the cytosolic dopamine) compared with other neurotransmitters, and dopamine degradation naturally produces oxidative species.
- More than 90% of dopamine in dopamine neurons is stored in abundant terminal vesicles and is protected from degradation.
- However, a small fraction of dopamine is cytosolic, and it is the **major source of dopamine metabolism and presumed toxicity leading to <u>glutathione depletion</u>**.

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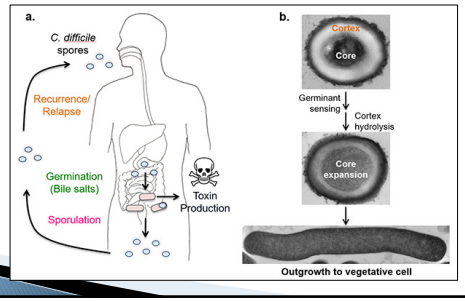
Properties of Clostridia Bacteria

- Anaerobic
- Causes tetanus, diarrhea and botulism (food poisoning)
- Forms spores that are highly resistant to heat and antibiotics
- May be controlled by vancomycin, metronidazole and certain probiotics, e.g., Acidophilus GG, soil-based organisms.



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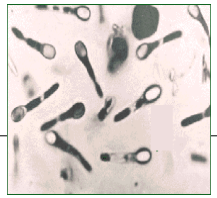
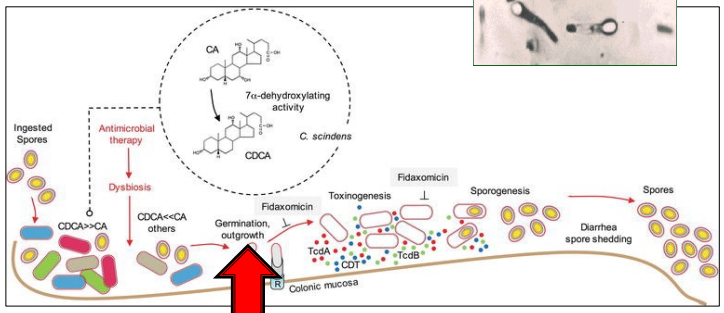
Spores





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Spore formation increases potential for recurrence of infection

- Recurrences occur after use of Metronidazole and/or Vancomycin.
- Spores not destroyed by common disinfectants like alcohol hand wipes.
- Bleach can destroy spores
- Carriers without symptoms may spread spores

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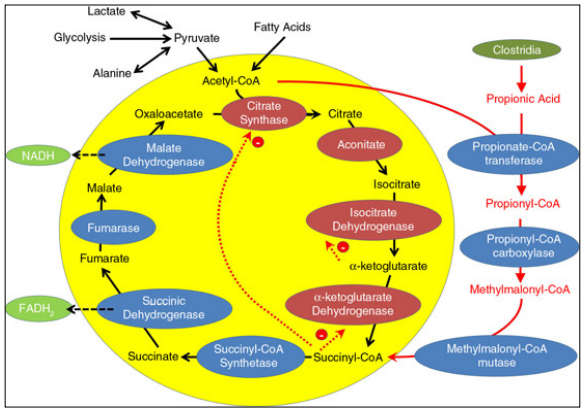

From: "Gastrointestinal dysfunction in autism spectrum disorders - the role of the mitochondria and the enteric microbiome (2015)."

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HPHPA versus DHPPA

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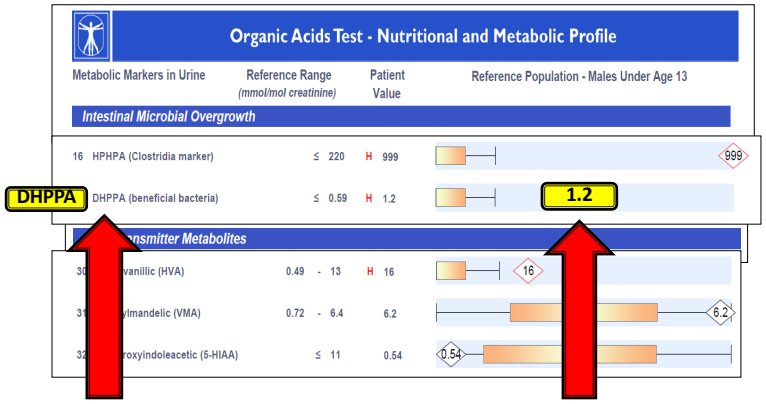

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DHPPA

- **3,4-dihydroxyphenylpropionic acid (DHPPA)** is a marker for beneficial bacteria in the gastrointestinal tract such as Lactobacilli, Bifidobacteria, and E. coli. The exception is *Clostridia orbiscindens* which converts certain flavonoids (e.g., parsley, thyme, celery) *into DHPPA.*
- The quantity of *C. orbiscindens* in the gastrointestinal tract is negligible (approximately 0.1% of the total bacteria) compared to the predominant flora.
- DHPPA is an antioxidant that lowers cholesterol, reduces pro-inflammatory cytokines, and protects against pathogenic bacteria.

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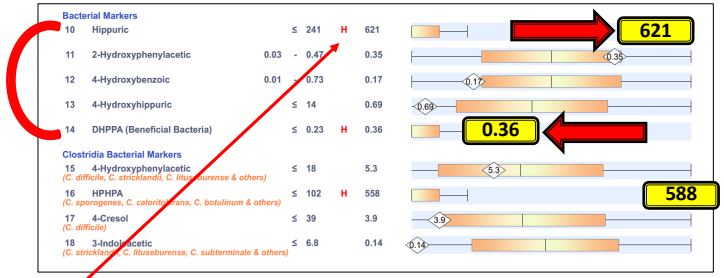

High hippuric acid (Marker 10) may derive from food, GI bacterial activity, or exposure to the solvent toluene. Hippuric acid is a conjugate of glycine and benzoic acid formed in the liver. Most hippuric acid in urine is derived from microbial breakdown of chlorogenic acid to benzoic acid. Chlorogenic acid is a common substance in beverages and in many fruits and vegetables, including apples, pears, tea, coffee, sunflower seeds, carrots, blueberries, cherries, potatoes, tomatoes, eggplant, sweet potatoes, and peaches. Benzoic acid is present in high amounts in cranberry juice and is a food preservative. The workplace is the most common source of toluene exposure, but toluene may be absorbed from outgassing of new carpets and other building materials, or absorbed during recreational abuse of solvents such as glue-sniffing. Because most hippuric acid in urine is from GI sources, this marker is a poor indicator of toluene exposure and is being replaced by other markers in occupational safety testing. Bacterial overgrowth can be treated with natural anti-bacterial agents and/or probiotics (30-50 billion cfu's) that include *Lactobacillus rhamnosus*.

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16050–16055 | PNAS | September 20, 2011 | vol. 108 | no. 38

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo[a,1], Paul Forsythe[b,c,1], Marianne V. Chew[b], Emily Escaravage[b], Hélène M. Savignac[a,d], Timothy G. Dinan[a,e], John Bienenstock[b,f,2], and John F. Cryan[a,d,g,2]

[a]Laboratory of NeuroGastroenterology, Alimentary Pharmabiotic Centre, [d]School of Pharmacy, and Departments of [e]Psychiatry and [g]Anatomy, University College Cork, Cork, Ireland; [b]The McMaster Brain-Body Institute, St. Joseph's Healthcare, Hamilton, ON, Canada L8N 4A6; and Departments of [c]Medicine and [f]Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada L8S 4L8

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved July 27, 2011 (received for review February 27, 2011)

There is increasing, but largely indirect, evidence pointing to an effect of commensal gut microbiota on the central nervous system (CNS). However, it is unknown whether lactic acid bacteria such as *Lactobacillus rhamnosus* could have a direct effect on neurotransmitter receptors in the CNS in normal, healthy animals. GABA is the main CNS inhibitory neurotransmitter and is significantly involved in regulating many physiological and psychological processes. Alterations

tant pharmacological targets for clinically relevant antianxiety agents (e.g., benzodiazepines acting on $GABA_A$ receptors), and alterations in the GABAergic system have important roles in the development of stress-related psychiatric conditions.

Probiotic bacteria are living organisms that can inhabit the gut and contribute to the health of the host (14). Accumulating clinical evidence suggests that probiotics can modulate the stress response

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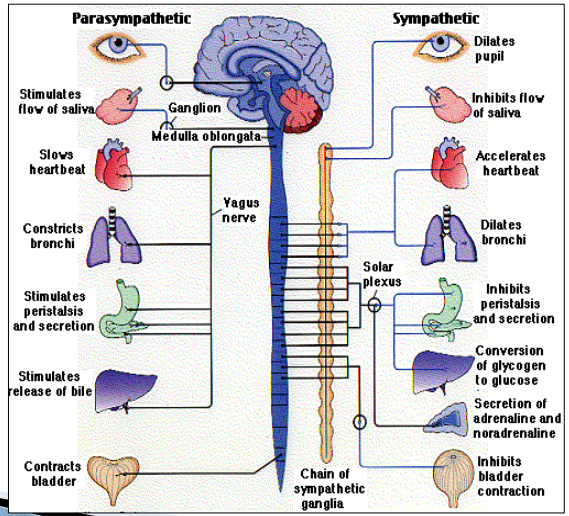

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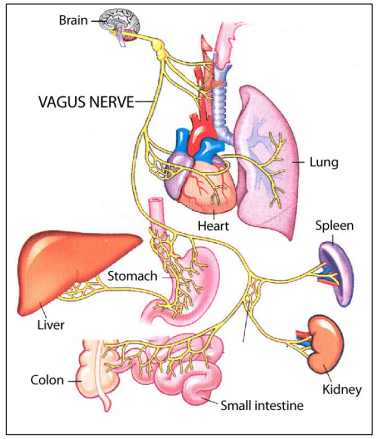
"Gut Microbiome and Brain-Gut Axis in Autism — Aberrant Development of Gut-Brain Communication and Reward Circuitry" Intech, 2013

Elizabeth M. Sajdel-Sulkowska[1] and Romuald Zabielski[2]
[1] Dept. Psychiatry Harvard Medical School and BWH, USA
[2] DDept. Physiological Sciences, Warsaw University of Life Sciences, Poland



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Stress

- "Animal studies have also shown that stress can change the composition of the microbiome, where the changes are associated with **increased vulnerability to inflammatory stimuli** in the gut."
- "Stress is known to inhibit gut contraction, one of the crucial defense strategies against bacterial colonization of gut mucosa.

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Vagus Nerve and Immune Function

- "Evidence indicates that the **vagus nerve** is involved in immunomodulation attenuating the production of pro-inflammatory cytokines in experimental models of inflammation."
- "The microbiome also plays an important role in anxiety-like and depressive behaviors. Effects are diminished in vagatomized animals."
- "Suggests either the direct communication between the bacteria and the brain or through the brain-gut axis."

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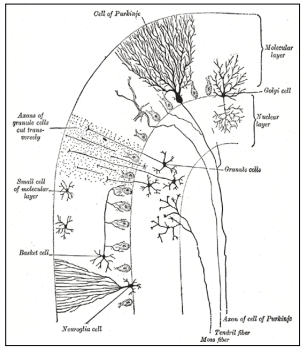
Vagus Nerve Transport of Neurotoxins

- *Clostridium tetani* produces a potent neurotoxin, tetanus neurotoxin (TeNT), that is transported by the Vagus Nerve from the GI to the CNS.
- Neurons in the cerebellum called Purkinje Cells that release the inhibitory neurotransmitter **GABA** are a preferred target for tetanus neurotoxins.



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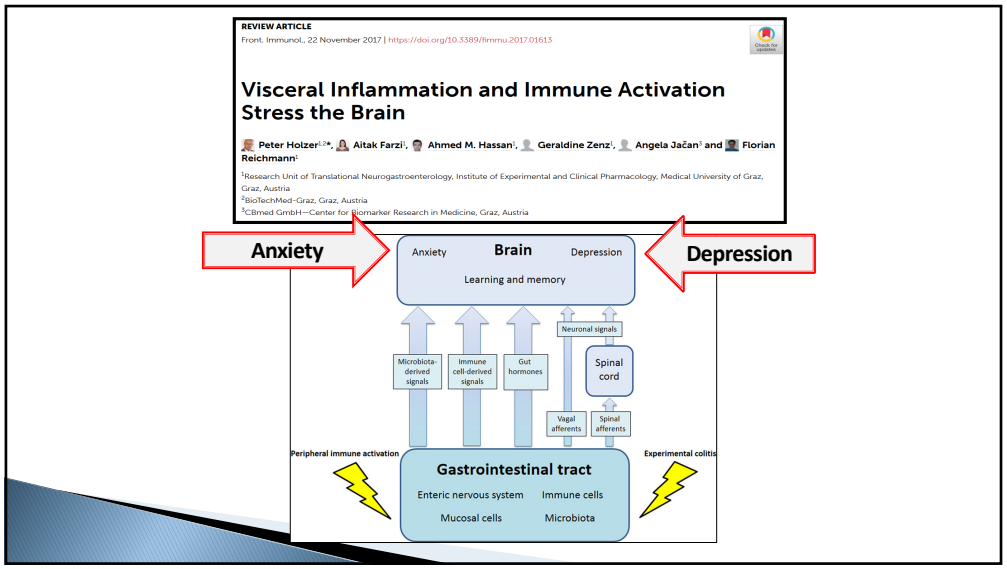
REVIEW ARTICLE
Front. Immunol., 22 November 2017 | https://doi.org/10.3389/fimmu.2017.01613
Visceral Inflammation and Immune Activation Stress the Brain
Peter Holzer[1,2]*, Aitak Farzi[1], Ahmed M. Hassan[1], Geraldine Zenz[1], Angela Jačan[3] and Florian Reichmann[1]
[1]Research Unit of Translational Neurogastroenterology, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria
[2]BioTechMed-Graz, Graz, Austria
[3]CBmed GmbH—Center for Biomarker Research in Medicine, Graz, Austria
Anxiety
Depression
Anxiety
Brain
Depression
Learning and memory
Neuronal signals
Microbiota-derived signals
Immune cell-derived signals
Gut hormones
Spinal cord
Vagal afferents
Spinal afferents
Peripheral immune activation
Experimental colitis
Gastrointestinal tract
Enteric nervous system
Immune cells
Mucosal cells
Microbiota

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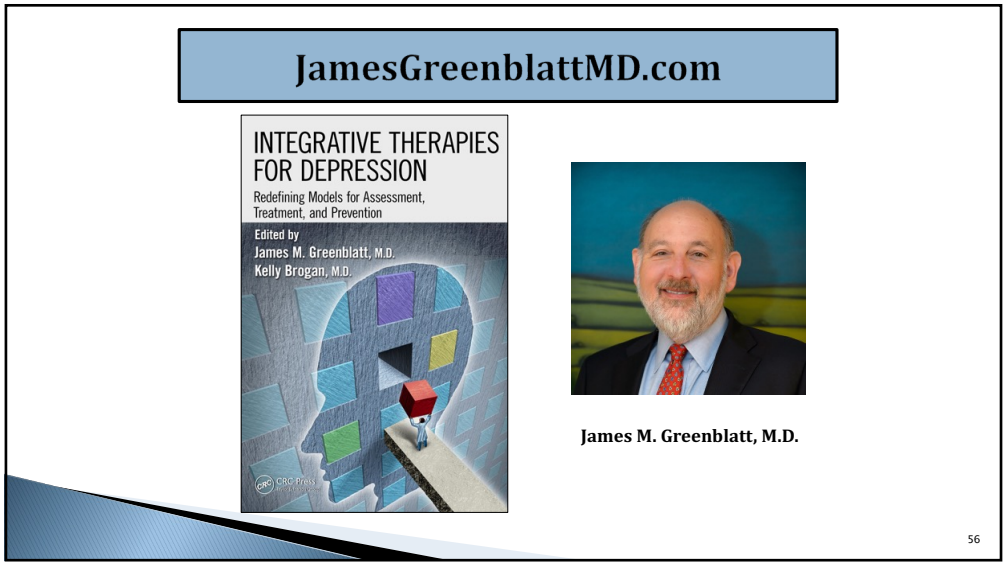
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INTEGRATIVE THERAPIES FOR DEPRESSION
Redefining Models for Assessment, Treatment, and Prevention
Edited by
James M. Greenblatt, M.D.
Kelly Brogan, M.D.
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James M. Greenblatt, M.D.
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4 Clostridia Bacteria in the GI Tract Affecting Dopamine and Norepinephrine Metabolism

William Shaw, PhD

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Parkinson's Disease

Organic acids test—Nutritional and metabolic profile

Metabolic markers in urine		Reference range (mmol/mol creatinine)		Patient	Reference population—Males age 13 and over
Intestinal microbial overgrowth					
17	HPHPA (*other pathogenic clostridia species*)	≤ 102	H	507	507
18	4-Cresol (*C. difficile*)	≤ 39	H	64	64
Neurotransmitter metabolites					
32	Homovanillic (HVA) (*dopamine*)	0.39 - 2.2	H	14	14
33	Vanillylmandelic (VMA) (*norepinephrine, epinephrine*)	0.53 - 2.2	H	2.3	2.3
34	HVA/VMA ratio	0.32 - 1.4	H	6.1	6.1
Nutritional markers					
	Vitamin C				
53	Ascorbic	10 - 200	H	301	301

FIGURE 4.10 Urine organic acid profile of elderly man with beginning symptoms of Parkinson's disease. Both HPHPA and 4-cresol are significantly elevated. It is interesting that both HVA and VMA are elevated, but the dopamine metabolite HVA is markedly elevated as is the HVA/VMA ratio. Vitamin C is also elevated which is a cofactor in the inactivation of dopamine-beta-hydroxylase. Parkinson's disease is associated with decreased amounts of dopamine in the brain, but in the early stages of the disease clostridia metabolites HPHPA and 4-cresol may paradoxically cause elevated dopamine which at high concentrations leaks from the synaptic vesicles and is converted to quinones and amino acid adducts which damage dopamine containing neurons. If a metabolite is elevated, only the upper part of the normal range is graphed. The letter H indicates high, meaning the value exceeds the upper limit of normal. The patient's value is indicated in a diamond on each graph.

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E. Svensson, et.al. *Vagotomy and subsequent risk of Parkinson's disease*; Ann Neurol 2015;78:522–52

Parkinson's disease (PD) may be caused by an enteric neurotropic pathogen entering the brain through the vagal nerve, a process that may take over 20 years.

We investigated the risk of PD in patients who underwent vagotomy and hypothesized that truncal vagotomy is associated with a protective effect, whereas superselective vagotomy has a minor effect. Cohorts were constructed of all patients in Denmark who underwent vagotomy during 1977–1995 and a matched general population cohort by linking Danish registries. We used Cox regression to compute hazard ratios (HRs) for PD and corresponding 95% confidence intervals (CIs), adjusting for potential confounders.

Results:

Risk of PD was decreased in patients who underwent truncal (HR = 0.85; 95% CI = 0.56–1.27; follow-up of >20 years: HR = 0.58; 95% CI: 0.28–1.20) compared to superselective vagotomy. Risk of PD was also decreased after truncal vagotomy when compared to the general population cohort (overall adjusted HR = 0.85; 95% CI: 0.63–1.14; follow-up >20 years, adjusted HR = 0.53; 95% CI: 0.28–0.99). In patients who underwent superselective vagotomy, risk of PD was similar to the general population (HR = 1.09; 95% CI: 0.84–1.43; follow-up of >20 years: HR = 1.16; 95% CI: 0.80–1.70). Statistical precision of risk estimates was limited.

Full truncal vagotomy is associated with a decreased risk for subsequent PD, suggesting that the vagal nerve may be critically involved in the pathogenesis of PD.

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Some Treatment Options

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Vancomycin (Vancocin)

- Mostly used as an intravenous antibiotic against gram positive bacteria that have resistance to other antibiotics:
  - *Methicillin-resistance Staph aureus*
- Early reports of high rate of kidney and hearing toxicity secondary to IV use seemed to be related to impurities.
- Vancocin is the oral antibiotic for Pseudomembranous colitis and C. difficile associated diarrhea.

<u>Pediatric Dosing (less than 18 years of age)</u>: 30mg to 35mg mg/kg in 3 to 4 divided dosages for 7 to 10 day – *not to exceed 2 grams (2000mg) in 24-hours.*

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Flagyl (Metronidazole)

- Effective against a variety of intestinal pathogens such as:
  - *Fusabacteria, Bacteroides, Clostridia, as well as Giardia lamblia and Entamoeba histolytica.*
- Available in both intravenous and oral route (capsules or oral suspension).

<u>Pediatric Dosing</u>: 5mg/kg every 8 hours (TID) either as capsule or oral suspension.

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Dificid (Fidaxomicin)

- For the use against *Clostridia difficile* or suspected *C. difficile* infections.
- 200mg BID x 10 days with or without food

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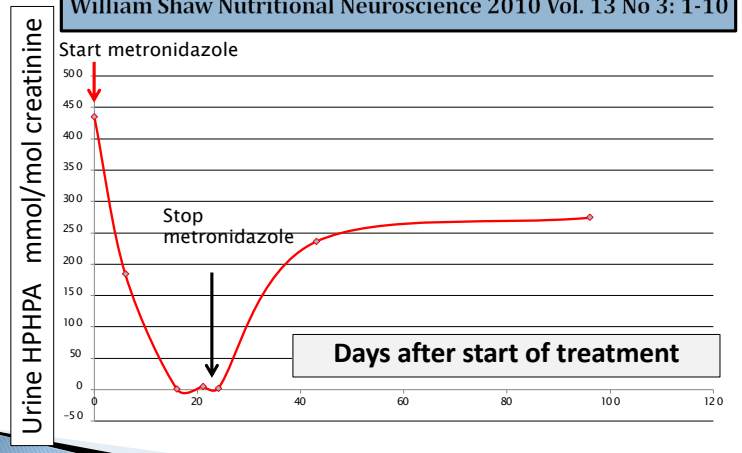
Effect of metronidazole on urine HPHPA levels in autism
William Shaw Nutritional Neuroscience 2010 Vol. 13 No 3: 1-10



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Clinical Infectious Diseases

BRIEF REPORT

Vancomycin Taper and Pulse Regimen With Careful Follow-up for Patients With Recurrent *Clostridium difficile* Infection

Benjamin D. Sirbu,[1,2] Melinda M. Soriano,[3] Carl Manzo,[1,a] Jessica Lum,[1,b] Dale N. Gerding,[1,2] and Stuart Johnson[1,2]

[1]Loyola University Medical Center, Maywood and [2]Edward Hines Jr Veterans Affairs Hospital, Hines, Illinois; and [3]Merck & Co, Inc, Kenilworth, New Jersey

We retrospectively studied vancomycin taper and pulse treatment on 100 consecutive, evaluable patients with recurrent *Clostridium difficile* infection. Following taper to once-daily vancomycin dosing, 22 of 36 patients (61%) who received every-other-day dosing (QOD) and 50 of 64 (81%) who received QOD followed by every-third-day dosing were cured ($P = .03$).

Keywords. *C. difficile*; recurrence; vancomycin; taper; pulse.

IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Received 3 April 2017; editorial decision 24 May 2017; accepted 5 June 2017; published online June 7, 2017.

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Clinical Infectious Diseases® 2017;65(8):1396–9

DOI: 10.1093/cid/cix529

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METHODS

A records review was conducted for all patients seen at our clinic between 1 January 2009 and 31 December 2014. These patients were seen monthly with more frequent contacts as needed. All patients had at least 1 of their prior CDI episodes confirmed with a *C. difficile* stool assay (prior to July 2011 by toxin A/B immunoassay and afterward by polymerase chain reaction). Recurrent CDI was defined as ≥2 CDI episodes at the time of referral. Following referral, these patients were treated with a VAN-TP regimen defined as a taper of vancomycin to once daily, followed by every-other-day (QOD) dosing, or once daily followed by QOD followed by every-third-day (Q3D) dosing for at least 2 weeks. All patients had follow-up documented for at least 90 days after completion of the VAN-TP regimen. Recurrent CDI was defined as recurrence of diarrheal symptoms requiring re-treatment with a CDI-specific agent. Some

- 100 people with recurrent CDI (*clostridia difficile* infection) was defined as ≥ 2 CDI.
- VAN-TP (vancomycin taper):
  - *Taper of Vancomycin to once daily followed by QOD (every other day) X 2 weeks.*
  - *Taper of Vancomycin to once daily followed by QOD and then Q3D (every 3rd day) X 2 weeks.*
- All patients had follow-up for at least 90 days after completion of VAN-TP.
- Recurrent CDI = return of diarrheal symptoms requiring re-treatment.

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Results:

- 22 of 36 patients (61%) doing QOD dosing were cured.
- 50 of 64 patients (78%) doing QOD + Q3D were cured.

Hypothesis:

- Pulse dosing promotes a cyclical decrease in spore burden.
- Pulse dosing permits for reestablishment of normal flora.
- Extension of pulse regimen (QOD to Q3D) might promote thorough clearance of spores…eradication of resultant vegetative organisms.

Our finding of improved cure rates with QOD + Q3D dosing over QOD dosing is consistent with the hypothesis that pulse dosing promotes a cyclical decrease in spore burden while also permitting the reestablishment of normal microbiota [2, 12]. Extension of the pulse regimen from QOD to Q3D might promote more thorough clearance of spores by allowing more time between dosing for spore germination and eradication of the resultant vegetative organisms.

Conclusions in our study are limited because this was a retrospective, nonrandomized observational study, and included patients treated at a single referral clinic. Despite these limitations, VAN-TP with careful follow-up can be a very effective treatment strategy for patients with rCDI, and this strategy warrants further study. A randomized, double-blinded, controlled trial that investigates VAN-TP for the treatment of rCDI is currently being conducted through the Veterans Administration Cooperative Studies Program (CSP 596; ClinicalTrials.gov identifier NCT02667418). This trial compares a standard 10-day course of vancomycin to a 10-day course of fidaxomicin and a 10-day course of vancomycin followed by a taper and pulse vancomycin regimen.

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The Lancet Infectious Diseases
Volume 18, Issue 3, March 2018, Pages 296-307

Articles

Extended-pulsed fidaxomicin versus vancomycin for *Clostridium difficile* infection in patients 60 years and older (EXTEND): a randomised, controlled, open-label, phase 3b/4 trial

Prof Benoit Guery MD [a], Prof Francesco Menichetti MD [b], Veli-Jukka Anttila MD [c], Nicholas Adomakoh MBBS [d], Prof Jose Maria Aguado MD [e], Karen Bisnauthsing BSc [f], Areti Georgopali MD [d], Simon D Goldenberg MD [f], Andreas Karas MD [d], Gbenga Kazeem PhD [d], Chris Longshaw PhD [d], Jose Alejandro Palacios-Fabrega PhD [d], Prof Oliver A Cornely MD [g], Maria J G T Vehreschild MD [h], EXTEND Clinical Study Group [†]

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https://doi.org/10.1016/S1473-3099(17)30751-X

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Fidaxomicin (Dificid) 200mg BID (days 1 - 5), then once daily QOD (days 7 – 25) versus Vancomycin 125mg QID (days 1 - 10)

Methods

In this randomised, controlled, open-label, superiority study, we recruited hospitalised adults aged 60 years and older with confirmed *C difficile* infection at 86 European hospitals. Patients were randomly assigned (1:1) using an interactive web response system to receive extended-pulsed fidaxomicin (200 mg oral tablets, twice daily on days 1–5, then once daily on alternate days on days 7–25) or vancomycin (125 mg oral capsules, four times daily on days 1–10), stratified by baseline *C difficile* infection severity, cancer presence, age (≥75 years *vs* <75 years), and number of previous *C difficile* infection occurrences. The primary endpoint was sustained clinical cure 30 days after end of treatment (day 55 for extended-pulsed fidaxomicin and day 40 for vancomycin), assessed in all randomised patients who met the inclusion criteria and received at least one dose of study medication (modified full analysis set). Adverse events were assessed in all patients who received at least one dose of study drug. The study is registered with ClinicalTrials.gov, number NCT02254967.

Primary endpoint was sustained clinical cure 30 days after end of treatment.

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Findings

Between Nov 6, 2014, and May 5, 2016, 364 patients were enrolled and randomly assigned to receive extended-pulsed fidaxomicin or vancomycin. 362 patients received at least one dose of study medication (181 in each group). 124 (70%) of 177 patients in the modified full analysis set receiving extended-pulsed fidaxomicin achieved sustained clinical cure 30 days after end of treatment, compared with 106 (59%) of 179 patients receiving vancomycin (difference 11% [95% CI 1·0–20·7], p=0·030; odds ratio 1·62 [95% CI 1·04–2·54]). Incidence of treatment-emergent adverse events did not differ between extended-pulsed fidaxomicin (121 [67%] of 181) and vancomycin (128 [71%] of 181) treatment arms.

"70% of patients receiving Dificid extended-pulse achieved cure 30 days after end of treatment, compared with 59% receiving Vancomycin."

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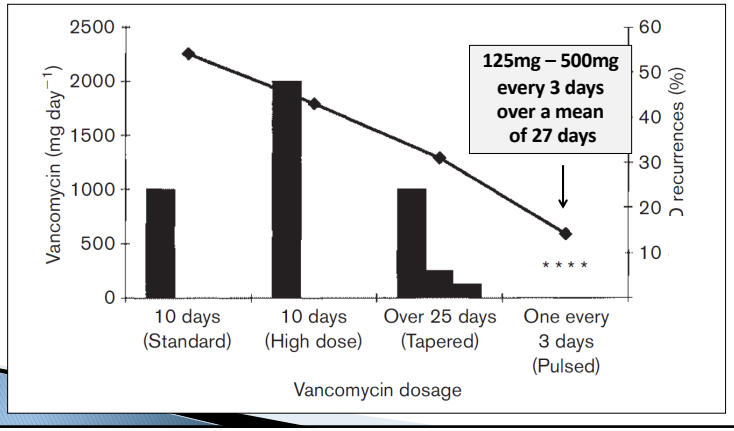
Alternative treatments for Clostridium difficile disease: what really works? Lynne V. McFarland Journal of Medical Microbiology (2005), 54, 101–111



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Clostridia – *Antibiotic Treatment*

- **Flagyl** (Metronidazole) – 250mg to 500 mg TID X 10 days
- **Vancomycin** (Vancocin) – 125mg to 500 mg QID X 10 days
- **Dificid** (Fidaxomicin) - 200mg BID X 10 days

Cyclical Dosing Options (examples):

1. Every 3rd day for 27 days *(McFarland, 2005)*
2. Dificid 200mg BID x 5 days, then once daily QOD days 7 to 25 (Lancet 2018).
3. Vancomycin QOD + Q3D *(IDSA, 2017)*
4. One dose TID for 10 days, then:
   - *One treatment day (one dose three times/day) every 72 hours for 3 weeks*

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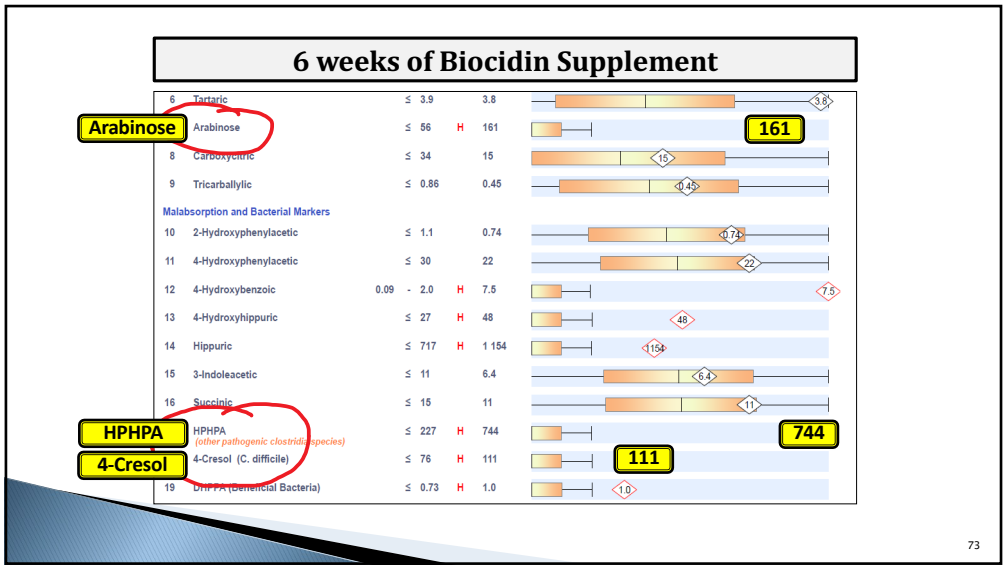
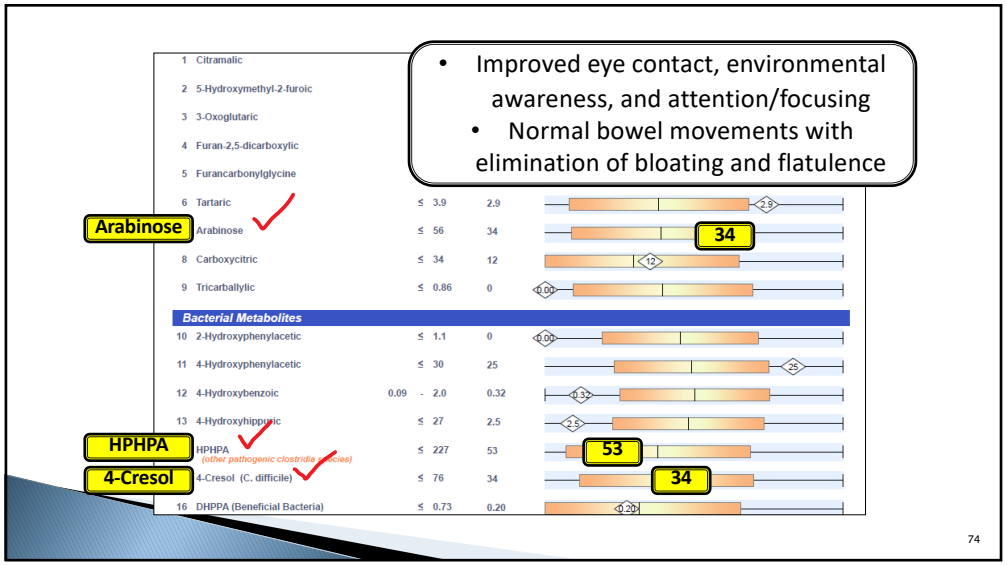

6 weeks of Biocidin Supplement
Arabinose
HPHPA
4-Cresol
161
744
111
73

73


• Improved eye contact, environmental awareness, and attention/focusing
• Normal bowel movements with elimination of bloating and flatulence
Arabinose
HPHPA
4-Cresol
34
53
34
74

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Dr. Woeller OAT Lecture Support Document

Integrative Medicine Academy

Recurrent Clostridia Antibiotic or Natural Remedy Intervention Protocols (examples)

By Kurt N. Woeller, D.O.

These intervention options are typically used for individuals with recurrent *clostridia* problems seen on the Organic Acids Test and without severe illness and/or digestive disease secondary to *Clostridia difficile* infection. People suffering with severe health problems such as fever, weight loss, abdominal cramping, loose and/or bloody stools should be evaluated and treated medically.

Antibiotic Option:

The goal with this intervention approach for recurrent *clostridia* is to hit the *clostridia* bacterial colonies with a 10-day course of an antibiotic, then stop for a few days before hitting the bacterial colonies again with a series of cyclical treatment days. This cycle then repeats itself over a 3 week period of time. The total program is approximately 3-1/2 weeks. The typical dose for Flagyl or Vancocin is 30-40mg/kg split dose three times daily. Vancocin can be given four times per day, but compliance is an issue and three times daily has worked well, particularly when the dosing schedule is spread out over time:

- One dose of Vancocin or Flagyl three times daily for ten days straight, then
- Every 3rd day thereafter administer another treatment day (at three doses for that one day) for an additional 3 weeks.

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The Great Plains Laboratory GPL Academy Practitioner Workshops

BEYOND THE BASICS: ADVANCED ORGANIC ACIDS TESTING STRATEGIES

THANK YOU

Next Lecture

Clinical Considerations of Elevated Oxalic Acid, Its Causes, and Intervention Strategies

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