ICDRC

International Child Development Resource Center

*Opening windows of hope for children with developmental disorders.*

# Advanced Lab Interpretation with Summary: Connecting Lab Data to Treatment and Outcome

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# Grounds for Intervention and Defensible Practice

- Objective Biomarker indicating serious condition – diabetic model with glucose and insulin.
- Apply this to oxidative stress, autoimmunity etc.
- Serious, dangerous and life-changing medical conditions justify treatment based on reasoned approach even if proof of cure is not available.
- Risk benefit estimates of any intervention become more critical where objective safety and efficacy are lacking.
- Cost analysis of intervention should include time stress and money.

# Biomarkers

- We must define individual needs, issues and pathologies.
- Assists the selection of proper treatment instead of trial and error and shotgun approaches.
- Monitors efficacy of interventions
  - When are we done treating
  - Did we give enough or too much treatment
  - Evaluation of possible side-effects

# OXIDATIVE STESS

- ***Urine***
  - **8-OHG** (prepub work Bradstreet et al**)**
  - **Isoprostane** (Ming, et al. Prostaglandins Leukot Essent Fatty Acids. 2005 Nov;73(5):379-84.)
- ***Blood***
  - Transferrin, Ceruloplasmin, (Chauhan, et al. Life Sci. 2004 Oct 8;75(21):2539-49.)
  - **Ammonia and Lactate** (Filipek et al. J Autism Dev Disord. 2004 Dec;34(6):615-23.)
  - Reduced Glutathione or GSSG - if available.

# IMMUNOLOGICAL (ref later)

- Urinary: Neopterin and Biopterin
- Blood:
  - **Anti-endothelial Antibodies at WUSTL**,
  - ASO and Anti-DNase B,
  - IgG subclasses, IgM, IgA and IgE,
  - CRP, Cytokines, TNF alpha,
  - Complete Blood Count,
  - Immune cell counts (CD and NK cell specifics)
- Special: intestinal permeability to lactulose and mannitol.
- Fecal
  - IgA Gluten, Casein, Egg, Soy etc.
  - Pathogens: Yeast, Bacteria (both Probiotic and Pathogens)
  - **Calprotectin**
  - **Eosinophil Protein X**
  - Blood and Leukocytes
- Antibody and PCR testing for HHV6, EBV, CMV, Chlamydia and Mycoplasma

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ISSN 0002-9270
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# Noninvasive Markers in the Assessment of Intestinal Inflammation in Inflammatory Bowel Diseases: Performance of Fecal Lactoferrin, Calprotectin, and PMN-Elastase, CRP, and Clinical Indices

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(Am J Gastroenterol 2007;102:1–8)

**Table 1.** Descriptive Statistics for PMN-e, Cal, and Lf for Endoscopy-Based Classification of Inflammation for UC, CD, and IBS

| | UC No Inflammation (N = 15) | UC Inflammation (N = 27) | CD No Inflammation (N = 10) | CD Inflammation (N = 33) | IBS No Inflammation (N = 54) |
|---|---|---|---|---|---|
| Lf | | | | | |
| Median | 4.34[b] | 51.1[b] | 6.4[a,†] | 55.1[a] | 1.82[*,†] |
| Range | (0–104) | (1–1,669) | (0.01–103) | (1.3–1,795) | (0–90) |
| Cal | | | | | |
| Median | 34.5[b,†] | 108.6[b] | 11.2[b] | 105.0[b] | 7.1[*,†] |
| Range | (5.0–134) | (8.7–311) | (0 –168) | (12.4–347) | (0–77) |
| PMN-e | | | | | |
| Median | 0.035[a,†] | 0.18[a] | 0.03[a] | 0.12[a] | 0.02[*,†] |
| Range | (0–1.3) | (0.01–0.81) | (0–0.48) | (0.01–1.04) | (0–0.3) |
| CRP | | | | | |
| Median | 0.20[a] | 0.70[a] | 0.45 | 0.9 | 0.20* |
| Range | (0–2.9) | (0–3.9) | (0–3.2) | (0–13.7) | (0–1.2) |
| CAI/CDAI | | | | | |
| Median | 2.0[b] | 7.0[b] | 76.0 | 98.0 | — |
| Range | (0–5) | (3–13) | (7–132) | (5–237) | |

Inflammation was defined as endoscopic score of $\geq 1$; no inflammation was defined as endoscopic score = 0.
Data are shown as $\mu$g/mL. PMN-e = polymorphonuclear-elastase; Cal = calprotectin; Lf = lactoferrin. (Published cutoffs: $\geq 0.062$ $\mu$g/mL for PMN-e, $>6$ $\mu$g/mL for Cal, $>7.25$ $\mu$g/mL for Lf).
[aa,bb] Equal letters indicate significant differences between inflamed and noninflamed patients (Mann-Whitney tests: [aa] $P < .05$; [bb] $P < 0.01$).
*For all indices, IBS patients had significantly lower scores than UC or CD patients with endoscopic inflammation (all $P < 0.001$, Mann-Whitney tests).
†IBS patients had significantly lower scores than UC without endoscopic inflammation (for Cal $P < 0.01$, for PMN-e $P < 0.05$) and CD without inflammation (for Lf $P < 0.05$) (all Mann-Whitney tests).

(Am J Gastroenterol 2007;102:1–8)

**Table 3.** Sensitivity and Specificity for Lf, Cal, PMN-e, CRP, and Clinical Disease Scores Based on Endoscopy With Adjusted Cutoffs

| | All (N = 139) | | UC (N = 42) | | CD (N = 43) | | IBS (N = 54) |
|---|---|---|---|---|---|---|---|
| | Sensitivity | Specificity | Sensitivity | Specificity | Sensitivity | Specificity | Specificity |
| Lf | 86.7% | 77.2% | 92.6% | 66.7% | 81.8% | 60% | 83.3% |
| Cal | 81.7% | 83.5% | 81.5% | 72.3% | 81.8% | 80% | 87% |
| PMN-e | 76.7% | 77.2% | 70.4% | 66.7% | 81.8% | 70% | 81.5% |
| CRP | 57.4% | 87.3% | 46.4% | 93.3% | 66.7% | 60% | 90.7% |
| Clinical disease score (CAI/CDAI) | 77% | 84% | 89.3% | 93.3% | 66.7% | 70% | — |
| Comprehensive activity index* | 85.2% | 88% | 92.9% | 100% | 78.8% | 70% | — |

PMN-E = polymorphonuclear-elastase; Cal = calprotectin; Lf = lactoferrin.
Adjusted cutoffs: for PMN-E >0.062 μg/mL, for Cal >48 μg/mL, for Lf >0.705 μg/mL, for CRP >0.7 mg/dL, for CAI >4, for CDAI >80.
*Comprehensive activity index: rated positive if at least two out of the following three conditions were fulfilled: (a) at least two stool parameters elevated, (b) elevated CRP, (c) clinical disease score elevated; rated negative if two or more of these three conditions were not fulfilled.

**Table 4.** Overall Diagnostic Accuracy for Lf, Cal, PMN-e, CRP, and Clinical Disease Scores Based on Endoscopy With Adjusted Cutoffs

| | All N = 139 | IBD (N = 85) | UC (N = 42) | CD (N = 43) | IBS (N = 54) |
|---|---|---|---|---|---|
| Lf | 80.7% | 80.0% | 83.3% | 76.7% | 83.3% |
| Cal | 82.1% | 80.0% | 78.6% | 81.4% | 87.0% |
| PMN-e | 76.4% | 74.1% | 69.0% | 79.1% | 81.5% |
| CRP | 74.3% | 64.0% | 62.8% | 65.1% | 90.7% |
| CAI/CDAI | | 79.0% | 90.7% | 67.4% | — |
| Comprehensive activity index* | | 86.0% | 95.3% | 76.7% | — |

Lf = lactoferrin; Cal = calprotectin; PMN-e = polymorphonuclear-elastase.
Adjusted cutoffs: for PMN-E >0.062 μg/mL, for Cal >48 μg/mL, for Lf >0.705 μg/mL, for CRP >0.7 mg/dL, for CAI >4, for CDAI >80.
*Comprehensive activity index: rated positive if at least two out of the following three conditions were fulfilled: (a) at least two stool parameters elevated, (b) elevated CRP, (c) clinical disease score elevated; rated negative if two or more of these three conditions were not fulfilled.

- Lactoferrin, calprotectin, and polymorphonuclear neutrophil-elastase differentiate active IBD from inactive IBD, and irritable bowel syndrome (IBS).
- None of the three investigated stool markers is superior in the ability to reflect endoscopic inflammation, but all three are superior to CRP in diagnostic accuracy.
- A combination of the stool markers with CRP and a disease-specific activity index can increase the diagnostic accuracy with reference to the endoscopic inflammation in UC.

# Rectal nitric oxide and fecal calprotectin in inflammatory bowel disease

*Scandinavian Journal of Gastroenterology*, 2007; 42: 1151–1157

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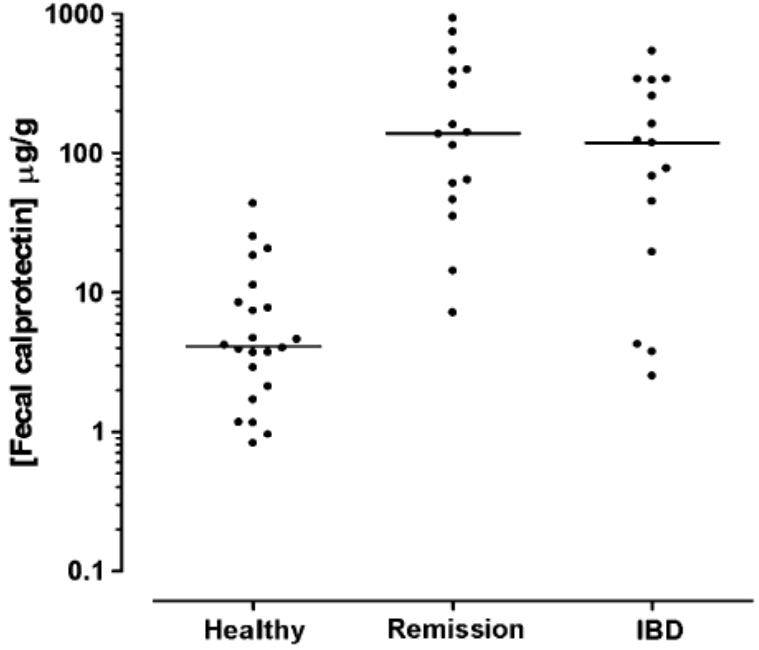


Figure 2. Individual and median fecal calprotectin levels in healthy volunteers, patients with IBD in clinical remission (Remission) and patients with active inflammatory bowel disease (IBD).

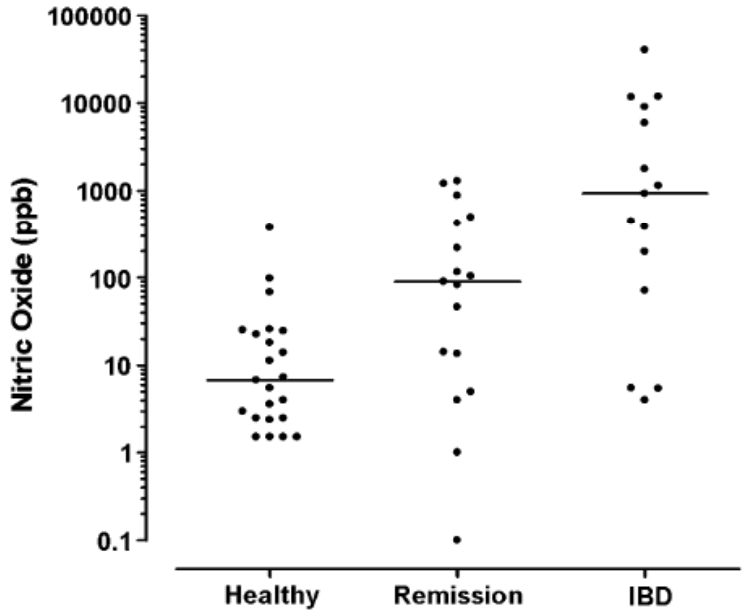


Figure 1. Individual and median rectal nitric oxide (NO) levels in healthy volunteers, patients with inflammatory bowel disease (IBD) in clinical remission (Remission) and patients with active IBD (IBD).

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REGULAR ARTICLE

# No evidence for a clear link between active intestinal inflammation and autism based on analyses of faecal calprotectin and rectal nitric oxide

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**Keywords**
Autism, Intestinal inflammation, Nitric oxide, Calprotectin

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DOI:10.1111/j.1651-2227.2007.00298.x

**Abstract**

Aim: Due to parental concern regarding the child's bowel habits and the ongoing discussion whether there might be an association between autism and intestinal inflammation, two inflammatory markers were analysed in a group of children with autism.

Methods: Twenty-four consecutive children with autism (3–13 years) of unknown aetiology were investigated with respect to faecal calprotectin and rectal nitric oxide (NO).

Results: One child who previously had a severe *Clostridium difficile* infection displayed raised levels of both these inflammatory markers and one child with extreme constipation for whom only calprotectin was possible to measure had raised levels. The remaining children displayed results that did not indicate an active inflammatory status in the intestine when the two inflammatory markers were combined.

Conclusion: By the use of two independent markers of inflammatory reactions in the gut, i.e. rectal NO and faecal calprotectin we were not able to disclose evidence of a link between the autistic disorder and active intestinal inflammation.

# Measurements of eosinophil activation before and after food challenges in adults with food hypersensitivity.

**van Odijk J et al**

Int Arch Allergy Immunol. 2006;140(4):334-41.

Department of Clinical Nutrition, Faculty of Medicine, Sahlgrenska Academy at Goteborg University, Gothenburg, Sweden.

RESULTS: **Patients with IgE-mediated food allergy had increased levels of F-EPX compared to controls** and tended to have lower serum levels of eotaxin compared to non-allergic patients and controls. Reported symptoms, abdominal pain, distension, flatulence and nausea were similar in the allergic and non-allergic patients. CONCLUSION: **The results strongly indicate that eosinophils are activated in the gastrointestinal tract of food-allergic patients but not in patients with non-allergic food hypersensitivity.**

# Clostridia, Aerobes and Yeast

- Urine: Organic Acid test for markers of anerobic bacterial metabolism: Proprionic acid derivatives (HPHPA and others) and yeast metabolites. Arabinose by itself is not reliable.
- Feces
  - Culture is difficult and not reliable
  - PCR also challenging but will over time gain enough reliability to be gold standard – currently a research tool in my opinion.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2004, p. 6459–6465 Vol. 70, No. 1
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.11.6459–6465.2004


# Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children

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*Research Service[1] and Infectious Diseases Section,[2] VA Medical Center West Los Angeles, and Department of Medicine[3] and Department of Microbiology, Immunology, and Molecular Genetics,[4] UCLA School of Medicine, Los Angeles, California*

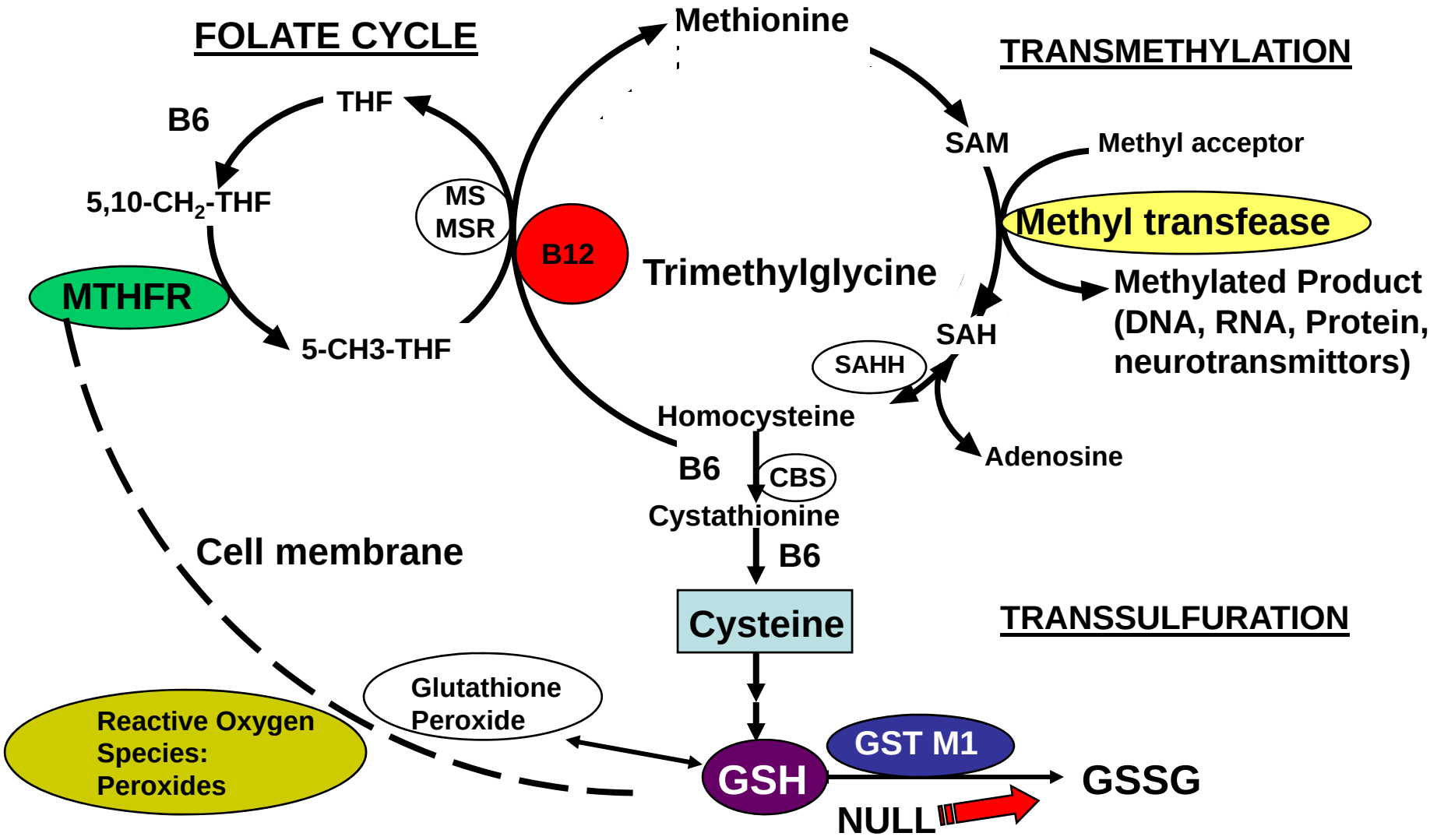
Received 11 February 2004/Accepted 27 June 2004

**Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three *Clostridium* clusters and one *Clostridium* species, *C. bolteae*, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle ($C_T$) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of *C. bolteae* could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for *C. bolteae* and the following *Clostridium* groups were statistically significant: mean counts of *C. bolteae* and clusters I and XI in autistic children were 46-fold ($P = 0.01$), 9.0-fold ($P = 0.014$), and 3.5-fold ($P = 0.004$) greater than those in control children, respectively, but not for cluster XIVab ($2.6 \times 10^8$ CFU/g in autistic children and $4.8 \times 10^8$ CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.**

# METHYLATION AND TRANSSULFATION

- ***Urine***
  - ***Methylmalonic acid – B12 indicator*** (James et al. Clin Chim Acta. 2009 Feb;400(1-2):77-81. )
- ***Plasma*** (Am J Clin Nutr. 2009 Jan;89(1):425-30.) (J Cell Mol Med. 2008 Aug 9.)
  - Fasting Cysteine, Methionine, Taurine & Sulfate
- ***B12 and Folate levels***

# The Methylation and Transsulfuration Pathways Provide the Reduced Glutathione (GSH) to Repair Oxidative Damage.




Courtesy of Jill James, PhD, University of Arkansas

# Cerebral Spinal Fluid (CSF)

- If Blood is positive for pathogens by PCR (DNA or RNA) then comparative CSF PCR data is important if treatment is going to be prescribed. Examples: HHV6, Mycoplasma and Chlamydia (As with HHV6 and limbic sclerosis: Neurology. 2007 Jul 10;69(2):156-65.)
- Measles Virus: No commercially reliable testing available at this time.
- Immune Markers: TNF alpha, cytokines, neopterin, autoantibodies. (Pediatr Neurol. 2007 Jun;36(6):361-5.)
- CSF Methylfolate in some rare cases: (J Autism Dev Disord. 2008 Jul;38(6):1170-7.)

Chez et al. *Pediatric Neurology:* 2007;36:361-365.

Original Articles

# Elevation of Tumor Necrosis Factor-Alpha in Cerebrospinal Fluid of Autistic Children

Michael G. Chez, MD*†, Tim Dowling, BS‡, Pikul B. Patel, MS*, Pavan Khanna, MS*, and Matt Kominsky*

**Table 2. Tumor necrosis factor-alpha levels in serum and cerebrospinal fluid**

| Patient | Autoimmune Treatment | CSF (pg/mL) | Serum (pg/mL) | CSF/Serum Ratio |
|---|---|---|---|---|
| 1 | No | 124 | 1.6 | 77.5 |
| 2 | No | 140 | 2.0 | 70.0 |
| 3 | No | 181 | 8.2 | 22.1 |
| 4 | No | 16 | 2.1 | 7.6 |
| 5 | No | 155 | 2.7 | 57.4 |
| 6 | No | 385 | 1.4 | 275 |
| 7 | Yes | 12 | 1.7 | 7.1 |
| 8 | Yes | 13 | 1.3 | 10.0 |
| 9 | Yes | 11 | 1.3 | 8.5 |
| 10 | Yes | 4 | 2.3 | 1.7 |
| | Mean | 104.1 | 2.5 | 53.7 |
| | S.D. | 121.3 | 2.3 | |

# HEAVY METALS

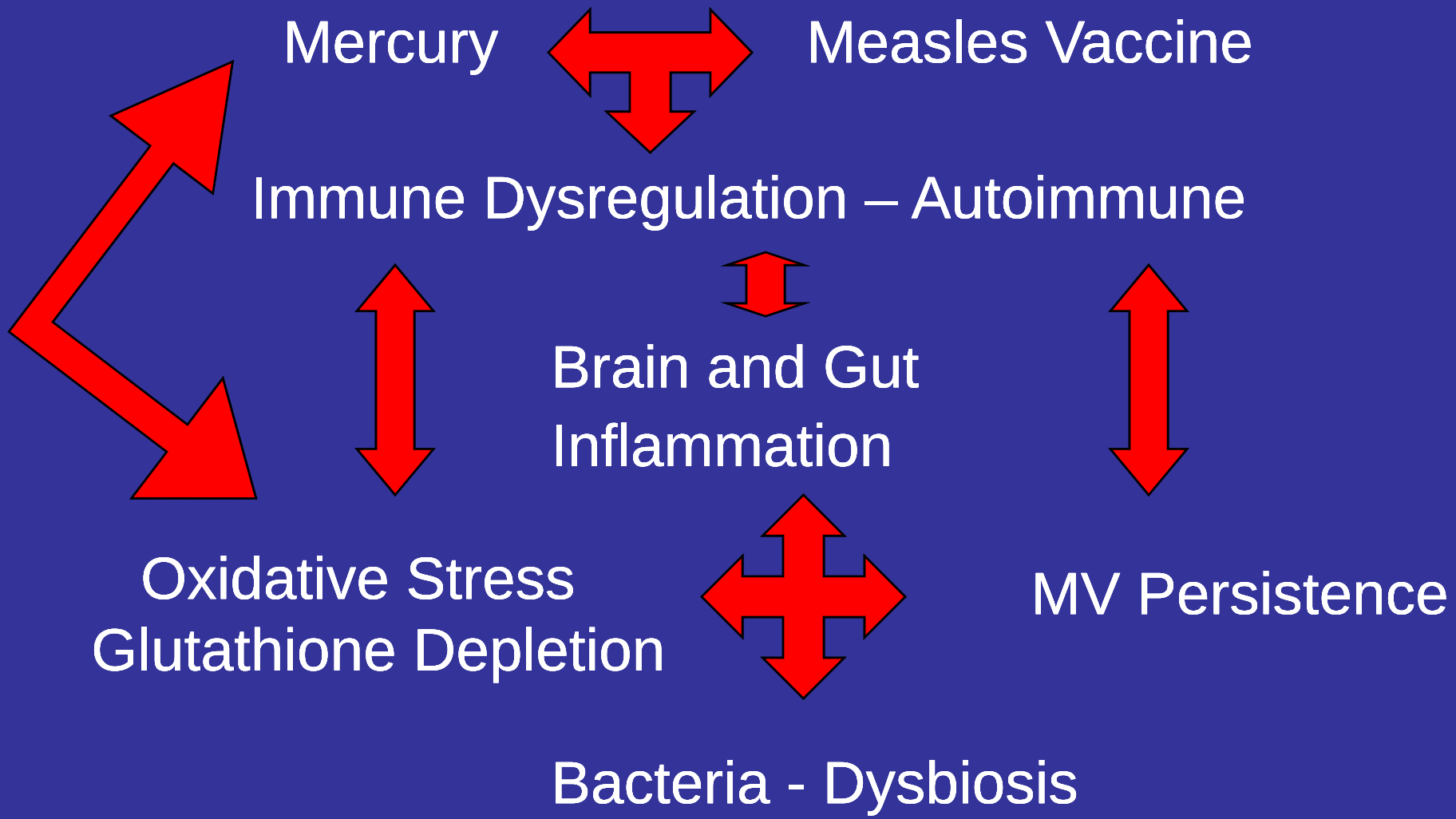
- Blood:
  - Packed Erythrocyte Minerals and Toxic Metals (Biol Trace Elem Res. 1990 Jul-Dec;26-27:683-9.)
  - Lymphocyte Metallthionein pre/post induction (Scand J Immunol. 1982 Feb;15(2):217-20.)
- Urinary:
  - Fractionated Porphyrins (ref later)
  - If porphyrins elevated or history strongly suggestive get post-chelation challenge = 6 or 24 hour urine toxic metal assay

# Evaluating Cases Studies

# Regressive Autism Variant

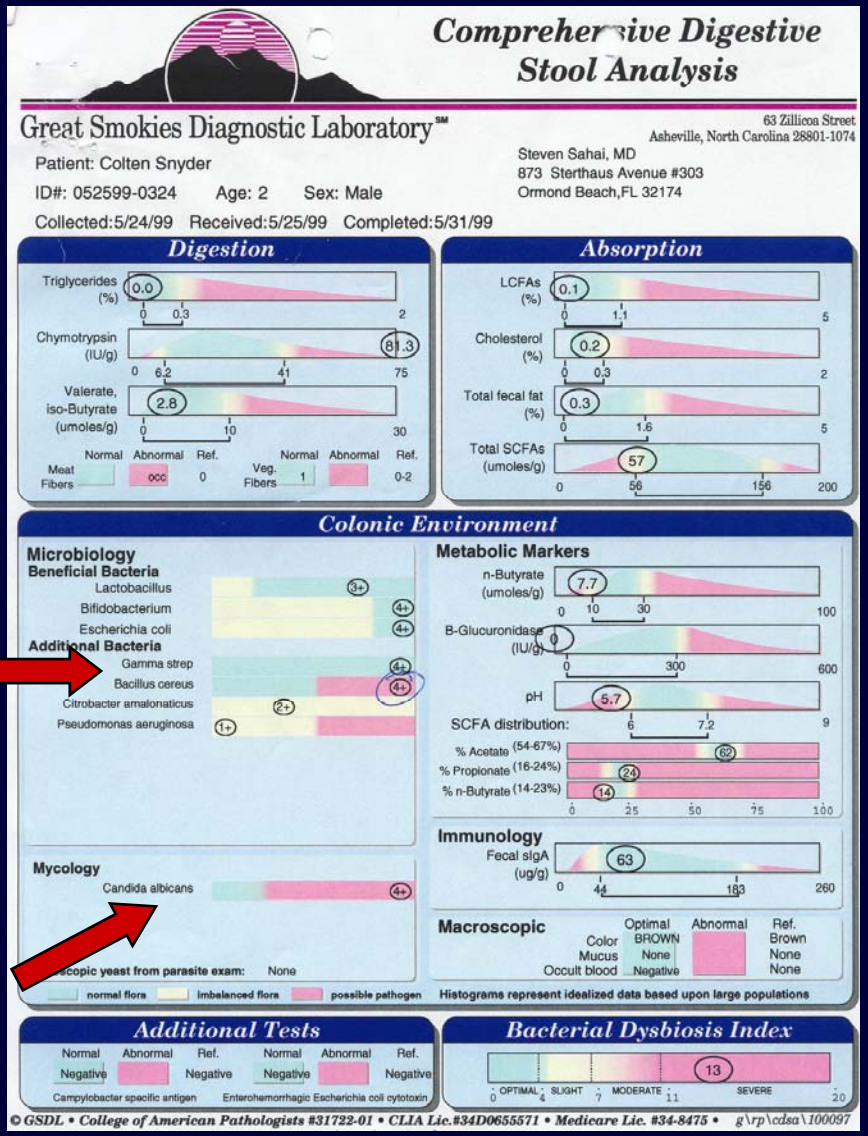
- Colton Snyder
- Medical Records are in the Public Domain secondary to being a test case in the Omnibus Proceedings regarding Vaccine Related injuries and autism
- Full discussion is available from my NAA talk in November of 2007

# My Clinical Concept of Colton's Medical Problems



# Colton Snyder's Case Review

- Prenatal Maternal use of Prozac (ex 2: 0002), also positive ANA in 1996 (ex 2: 0056)
- Pregnancy: Mild Hypertension final weeks (ex 2: 0009)
- Born Jan 9, 1997 (ex 4: 0001) Term without apparent distress.
- APGARS 9/9 with wt of 7Lb 3 oz (ex 3: 0003)
- Several episodes of febrile illnesses in first 15 months of life with multiple courses of antibiotics.
- MMR-II administered on 4/23/1998
- Pharyngitis and Regressive Symptoms begin 5/6/1998
- ER visit high fever 5/24/98 - Antibiotics fail to help
- Hospitalized 5/26/98
- See my report Jan 29, 2001 (P Ex 1)
- Strongly Favorable Response to IVIG
- Intestinal Bx (+) MV RNA, CSF (+) MV RNA, Blood negative.
- Detection of Measles Virus Genomic RNA in Cerebrospinal Fluid of Children with Regressive Autism: a Report of Three Cases. Bradstreet et al, ***Journal of American Physicians and Surgeons.*** **Volume 9 Number 2 Summer 2004**




# THE GREAT PLAINS LABORATORY

9335 W 75 St
Overland Park,KS 66204
Phone 913 341-8949
Fax 913 341-6207
CLIA ID#17D0919496
William Shaw Ph.D.,Laboratory Director

| | | | |
|---|---|---|---|
| Patient Name | Colten Joseph Snyder | Date of collection | 5/12/99 |
| Patient Age | 28 mo. | Time of collection | 10:24 AM |
| Physician Name | Dr. Steven Sahai | Patient Sex | Male |

| Name | mmol/mol creatinine | | Reference range mmol/mol creatinine |
|---|---|---|---|
| **Glycolysis** | | | |
| lactic | 89.43 | | 0-100 |
| pyruvic | 8.32 | | 0-50 |
| 2-hydroxybutyric | 1.87 | | 0-2 |
| glyceric | 50.93 | H | 0-10 |
| **Amino Acid Metabolites** | | | |
| 2-hydroxyisovaleric | 0.49 | | 0-2 |
| 2-oxoisovaleric | 0.28 | | 0-2 |
| 3-methyl-2-oxovaleric | 0.65 | | 0-2 |
| hydroxyisocaproic | 0.36 | | 0-2 |
| 2-oxoisocaproic | 0.15 | | 0-2 |
| 2-oxo-4-methiolbutyric | 0.27 | | 0-2 |
| mandelic | 0.1 | | |
| phenyllactic | 1.0 | | |
| phenylpyruvic | 2.87 | | 0-5 |
| homogentisic | 1 | | 0-2 |
| 4-hydroxyphenyllactic | 1 | | 0-50 |
| pyroglutamic | 34 | | 20-115 |
| 3-indoleacetic | 4.27 | | 0-10 |
| kynurenic | 1.44 | | 0-2 |
| **Fatty Acid Metabolites** | | | |
| 3-hydroxybutyric | 3.25 | | 0-10 |
| acetoacetic | 5.65 | | 0-10 |
| ethylmalonic | 2.56 | | 0-10 |
| methylsuccinic | 1.44 | | 0-5 |
| adipic | 3.43 | | 0-12 |
| suberic | 1.03 | | 0-2 |
| sebacic | 0.50 | | 0-2 |
| **Miscellaneous** | | | |
| glutaric | 0.80 | | 0-2 |
| methylmalonic | 2.07 | | 0-5 |
| N-acetyl aspartic | 10.00 | | 0-100 |
| ascorbic | 9.69 | L | 10-200 |
| orotic | 0.85 | | 0-3.5 |
| 3-hydroxy-3-methylglutaric | 7.44 | | 0-36 |
| hydroxyhippuric | 2.96 | | 0-20 |

| Name | mmol/mol creatinine | | Reference range mmol/mol creatinine |
|---|---|---|---|
| **Yeast/Fungal** | | | |
| citramalic | 0.40 | | 0-2 |
| 5-hydroxymethyl-2-furoic | 27 | | 0-80 |
| 3-oxoglutaric | 0.48 | | 0-0.5 |
| furan-2,5-dicarboxylic | 12.23 | | 0-50 |
| furancarbonylglycine | 1 | | 0-60 |
| tartaric | 4 | | 0-16 |
| arabinose | 40 | | 0-115 |
| carboxycitric | 0.63 | | 0-46 |
| **Bacterial** | | | |
| 2-hydroxyphenylacetic | 0.33 | | 0-10 |
| 4-hydroxyphenylacetic | 17 | | 0-50 |
| **Anaerobic Bacterial** | | | |
| DHPPA analog | 334 | H | 0-150 |
| VMA analog | 0.24 | | 0-31 |
| **Krebs Cycle** | | | |
| succinic | 8 | | 0-20 |
| fumaric | 1.54 | | 0-10 |
| 2-oxo-glutaric | 137.47 | | 15-200 |
| aconitic | 21 | | 0-25 |
| citric | 615 | H | 20-200 |
| **Neurotransmitters** | | | |
| HVA | 6.09 | | 0-25 |
| VMA | 9.97 | | 0-18 |
| 5-hydroxyindoleacetic | 2.53 | | 0-20 |
| **Pyrimidines** | | | |
| uracil | 6.38 | | 0-22 |
| thymine | 0.48 | | 0-2 |
| **Miscellaneous** | | | |
| glycolic | 109 | H | 0-100 |
| oxalic | 148.06 | H | 0-100 |
| malonic | 0.59 | | 0-10 |
| methylglutaric | 0.59 | | 0-10 |
| hippuric | 148 | | 10-400 |
| 4-hydroxybutyric | 2.49 | | 0-5 |
| phenylcarboxylic | 1.30 | | 0-15 |
| indole-like compound | 6.36 | | 0-60 |

# Yeast Metabolism

http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/03%20Yeast%20Metabolism.pdf

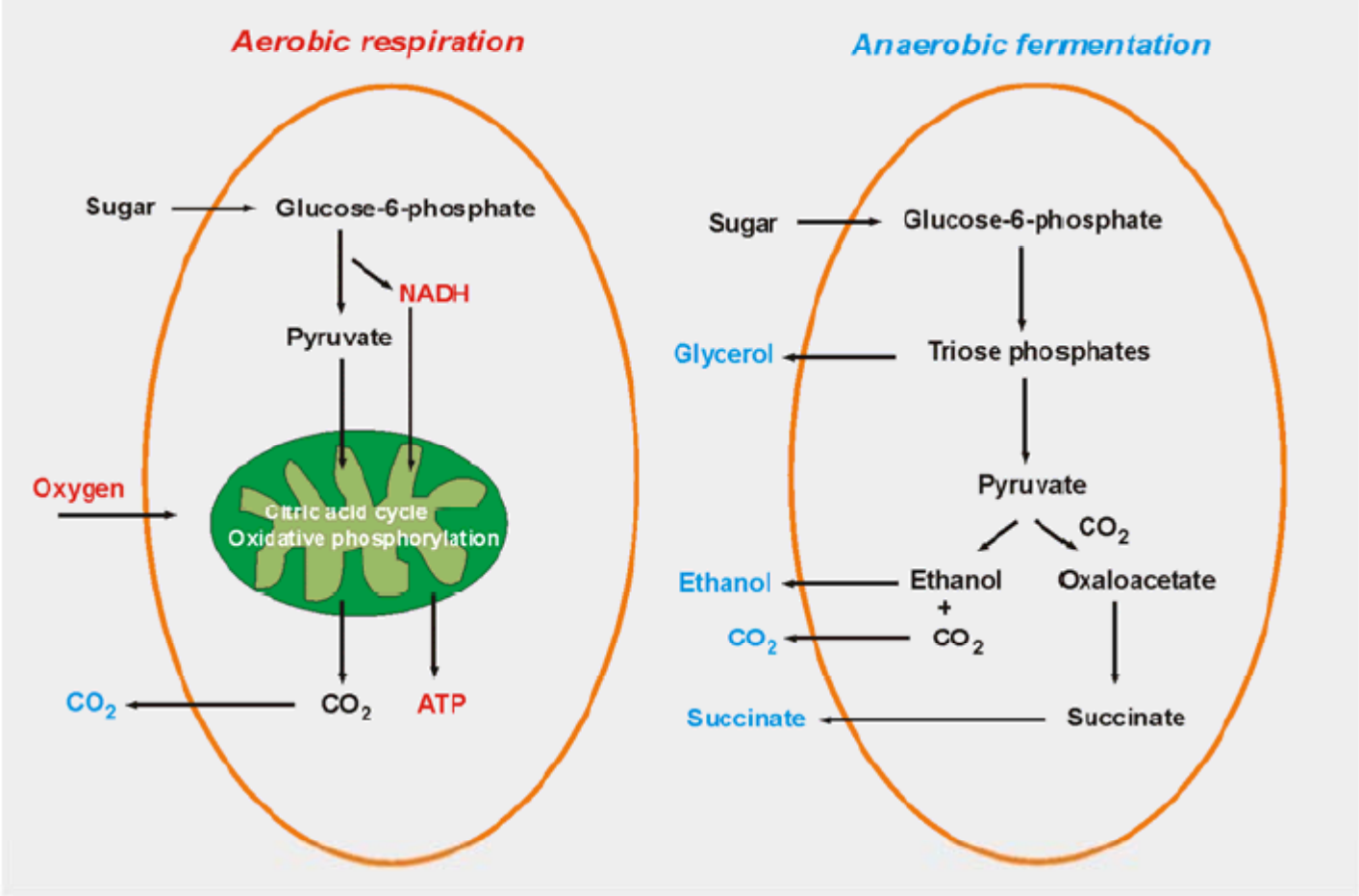



*Figure 3-1: Metabolism in yeast under aerobic and anaerobic conditions.*

# Potential Relationship of Anerobes and Aerobes to Various Yeast

http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/03%20Yeast%20Metabolism.pdf

***Table 3-2: Principal modes of respiration in yeasts.***

| Types | Examples | Respiration | Fermentation | Anaerobic growth |
|---|---|---|---|---|
| Obligate respirers | Rhodotorula spp.<br>Cryptococcus spp. | YES | NO | NO |
| Anaerobic respirers | Candida spp.<br>Kluyveromyces spp.<br>Pichia spp. | YES | Anaerobic in pregrown cells | NO |
| Aerobic fermenters | S. pombe | Limited | Aerobic and anaerobic | NO |
| Facultative aerobic fermenters | S. cerevisiae | Limited | Aerobic and anaerobic | Facultative |
| Obligate fermenters | Torulopsis | NO | Anaerobic | YES |

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2004, p. 6459–6465 Vol. 70, No. 11
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.11.6459–6465.2004


# Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children

Yuli Song,[1]* Chengxu Liu,[1] and Sydney M. Finegold[2,3,4]

*Research Service[1] and Infectious Diseases Section,[2] VA Medical Center West Los Angeles, and Department of Medicine[3] and Department of Microbiology, Immunology, and Molecular Genetics,[4] UCLA School of Medicine, Los Angeles, California*

Received 11 February 2004/Accepted 27 June 2004

**Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three *Clostridium* clusters and one *Clostridium* species, *C. bolteae*, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle ($C_T$) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of *C. bolteae* could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for *C. bolteae* and the following *Clostridium* groups were statistically significant: mean counts of *C. bolteae* and clusters I and XI in autistic children were 46-fold ($P = 0.01$), 9.0-fold ($P = 0.014$), and 3.5-fold ($P = 0.004$) greater than those in control children, respectively, but not for cluster XIVab ($2.6 \times 10^8$ CFU/g in autistic children and $4.8 \times 10^8$ CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.**

# One Year Check-Up

Steven H. Sahai, M.D.
Memorial Office Centre
873 Sterthaus Avenue, Suite 303
Ormond Beach, FL 32174

Phone: 904/677-0780 Fax: 904/677-0855

**Snyder, Colton** **1/15/98**

**Subjective**: 12 m/o male presents today for his 12 month check up. Hgb. 11.0. TB tine placed on his right forearm. Handouts on prevention of tooth decay, helping your child get enough iron, normal weaning, normal weaning resistance, prevention of lead poisoning and prevention of accidents were given to and discussed with mom. Height of 79.5 cm. at the 80th percentile. Weight of 23 lbs. 9 ounces at the 55th percentile. Head circumference at 41.5 cm. at the 25th percentile. Passes all his developmental milestones. Seems to be doing well. Slowly transitioning into milk without much of a problem.

**Objective** The child is normocephalic, atraumatic. Anterior fontanel is soft and flat. His neck is supple. Skin is clear. No acute lesions. His lungs are clear. Abdomen is benign. Normal neuro. RRR without murmurs. TM's are both normal. His throat is clear. Eyes have a good red reflex. No hip clicks. Normal genitalia. Descended testes bilaterally.

**Assessment**: Well child.

**Plan**: I asked mom to increase his iron intake with green leafy vegetables. Transition him into milk a little slower and we will see him again at 15 months of age. Call with any changes.

P. Ex. 8 - 0122

Steven H. Sahai, M.D.
Memorial Office Centre
873 Sterthaus Avenue, Suite 303
Ormond Beach, FL 32174

Phone: 904/677-0780 Fax: 904/677-0855

**Colten Snyder** **5/26/98**

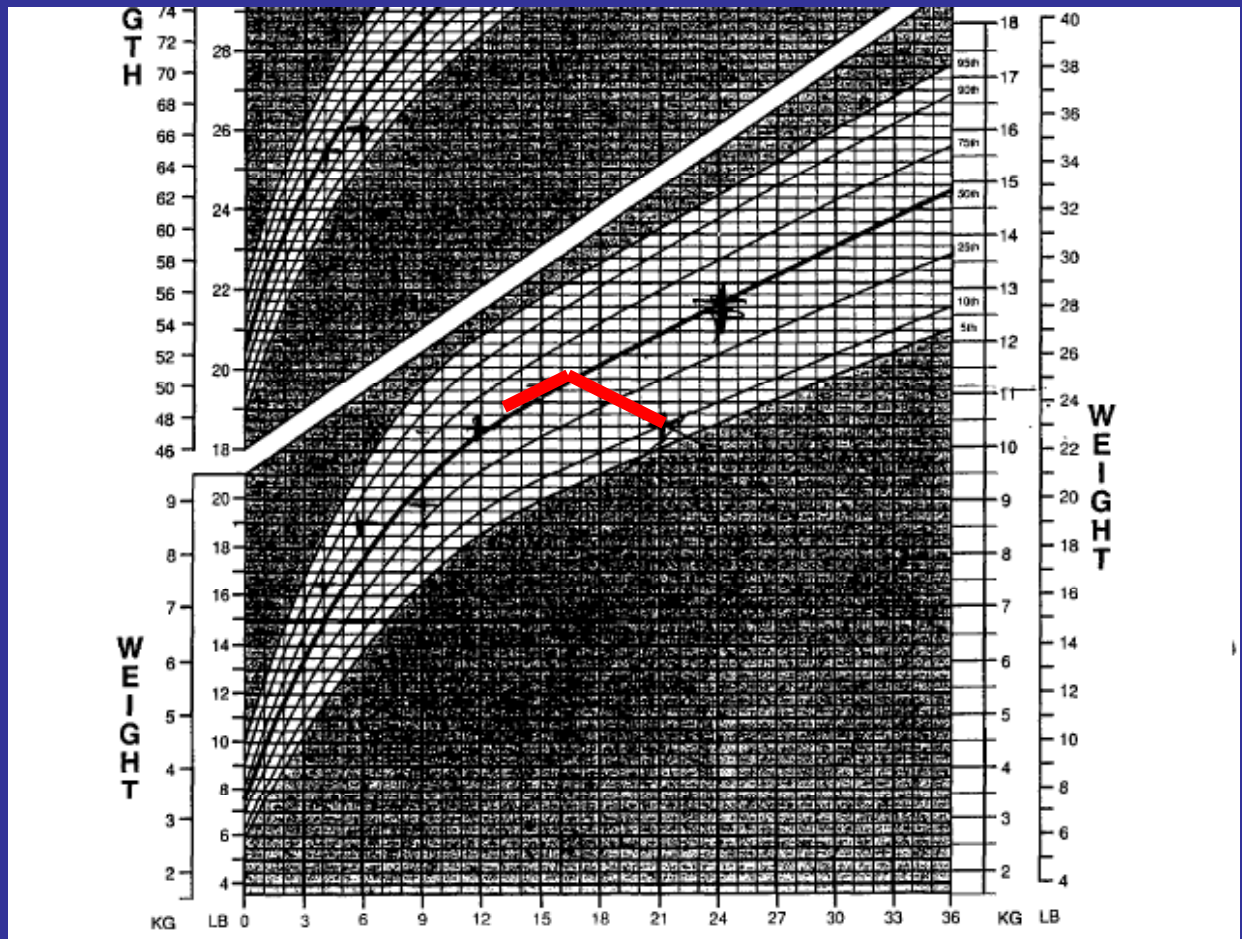
**Subjective**: 17 m/o male presents today with temp. of 102.6 He has been febrile up to 104.8 for the past 4 days. Went to the ER on Saturday, 3 days ago, received a shot of antibiotics. Was given Penicillin. Was at Halifax ER last night for shaking and poor appetite, taking fluids relatively well, however has been having severe diarrhea as well as some vomiting. He is now on Penicillin, Advil, Tylenol supp. with very poor control of any of his symptoms. He does appear quite limp, dry, easily accessible which for Colten is an abnormal finding.

**Objective** He is normocephalic, atraumatic. His anterior fontanel is almost closed, it is sunken. His neck is supple. Skin appears clear. No acute lesions, his lungs are clear. No wheeze, no rales or ronchi. Abdomen is benign. Mildly hyperactive bowel sounds, no hepatosplenomegaly, no masses, no guarding, no rebound. RRR without any murmur. His TM's both appear normal. His throat is quite inflamed, erythematous with enlarged tonsils, white patchy exudates covering the surface of both tonsils. The rest of the exam appears normal.

**Assessment**: Pharyngitis, r/o bacteremia.

**Plan**: We will admit him for IV hydration, IV Rocephin q 24 hours, force fluids. Maintain good hydration and I will follow him in the hospital this afternoon. Arrangements have been made.

# Lost Weight After MMR




| Date | Age in Months | Recumbent Length | Weight | Head Circumference |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

| Date | Age in Months | Recumbent Length | Weight | Head Circumference |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |

P. Ex. 8 - 0058

Department of Health, Education, and Welfare, Public Health Service
Health Resources Administration, National Center for Health Statistics, and Center for Disease Control

# Diagnostic Evaluation

**DATE OF EVALUATION:** **March 25, 1999**

| | | | |
|---|---|---|---|
| **CLIENT'S NAME:** | **SNYDER, Colten** | **CA:** | **~~UNKNOWN~~** 26m, 16dy |
| **UNIQUE #:** | **593657639** | **AA:** | **N/A** |
| **DOB:** | **01-09-1997** | **BW:** | **7 POUNDS 3 OUNCES** |
| **GEST:** | **40 WEEKS** | | |

**MULTIDISCIPLINARY TEAM PARTICIPANTS:**

Richard Hartmann, MD
Nancy M. Wenk, Psy.D.
Patty Daubin, M.S., RN
Suzy Wilson, OTR/L
Kathy Timlin, CCC/SLP

P. Ex. 8 - 0039

# Language Deficit

In speech/language skills, Colten appears to be functioning at a total language age equivalent of nine months, according to the PLS-3. Standard scores place his communication skills greater than 2 ½ standard deviations below the mean. Specific subtests, standard scores, age equivalents and age ranges follow utilizing a 90% confidence level:

| Subtests | SS | Age Equivalent | Age Ranges |
|---|---|---|---|
| Auditory Comprehension | 61 | 7 Months | 3 to 14 Months |
| Expressive Communication | 63 | 10 Months | 5 to 17 Months |
| Total Language Score | 58 | 9 Months | 5 to 14 Months |

Colten appears to understand a specific word or phrase although he does not attend to specific directions with or without cues; he uses a variety of vowel combinations with a few consonants |m, b, h |; he uses gestures to request objects and recurrence. He does not use single words or sound combinations approximating words, engage in an activity with another person; or display typical play patterns with familiar objects.

Oral motor/articulation skills: Perioral and intraoral structures appeared to be typical in function and configuration and adequate for the production of speech sounds and feeding skills.

**Kathy Timlin, CCC/SLP**

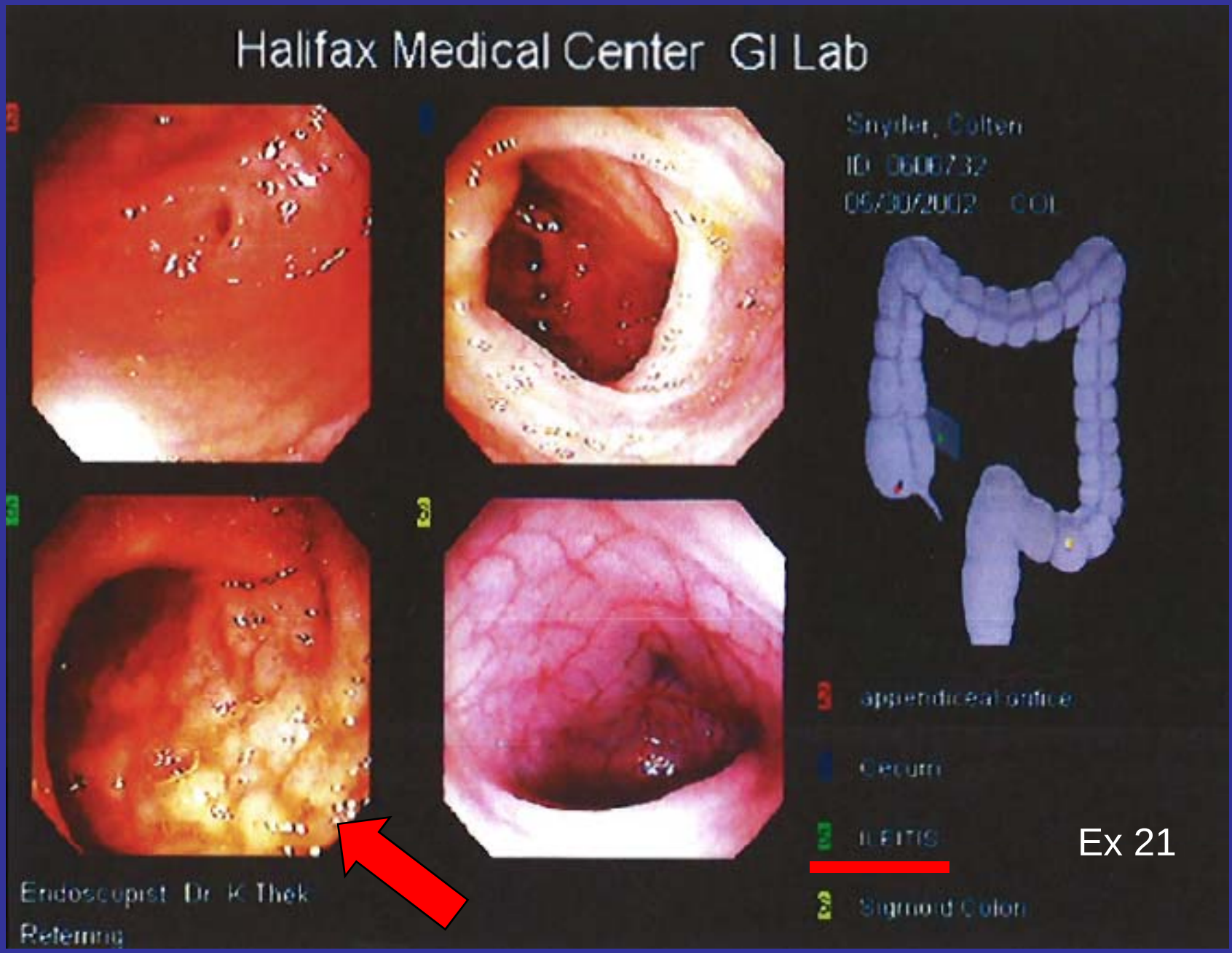
# Diagnostic Elements of ASD

Due to Colten's disinterest in the test items and assessment process, The Bayley Scales of Infant Development Second Edition (BSID-II) could not be administered at this time. He primarily walked about the room restlessly. He demonstrated limited social interaction and a lack of responsiveness to this novel situation. His mother reported the presence of characteristics consistent with a pervasive developmental disorder, (e.g. loss of communication skills, lining up of toys, intense emotional reactions) to change. In order to obtain a measure of Colten's adaptive behavior, the Vineland Adaptive Behavior Scales were completed. The following scores were obtained;

| | **Standard Score** | **Age Equivalent** |
|---|---|---|
| Communication Domain | 63 | 11 Months |
| Daily Living Skills Domain | 73 | 18 Months |
| Socialization Domain | 63 | 9 Months |
| Motor Skills Domain | 79 | 20 Months |
| Adaptive Behavior Composite | 64 | 14-15 Months |

**Nancy M. Wenk, Psy.D. Licensed Psychologist #PY0004765**

P. Ex. 8 - 0041


Halifax Medical Center GI Lab
Snyder, Colten
Endoscopist Dr K Thek
Referring
Ex 21

# Detection of Measles Virus Genomic RNA in Cerebrospinal Fluid of Children with Regressive Autism: a Report of Three Cases

**J.J. Bradstreet, M.D.; J. El Dahr, M.D.; A. Anthony, M.B., Ph.D.; J.J. Kartzinel, M.D.; A.J. Wakefield, M.B.**


## ABSTRACT

In light of encephalopathy presenting as autistic regression (autistic encephalopathy, AE) closely following measles-mumps-rubella (MMR) vaccination, three children underwent cerebrospinal fluid (CSF) assessments including studies for measles virus (MV). All three children had concomitant onset of gastrointestinal (GI) symptoms and had already had MV genomic RNA detected in biopsies of ileal lymphoid nodular hyperplasia (LNH).

Presence of MV Fusion (F) gene was examined by TaqMan real-time quantitative polymerase chain reaction (RT-PCR) in cases and control CSF samples. The latter were obtained from three non-autistic MMR-vaccinated children with indwelling shunts for hydrocephalus. None of the cases or controls had a history of measles exposure other than MMR vaccination. Serum and CSF samples were also evaluated for antibodies to MV and myelin basic protein (MBP).

MV F gene was present in CSF from all three cases, but not in controls. Genome copy number ranged from $3.7 \times 10^4$ to $2.42 \times 10^7$ per ng of RNA total. Serum anti-MBP autoantibodies were detected in all children with AE. Anti-MBP and MV antibodies were detected in the CSF of two cases, while the third child had neither anti-MBP nor MV antibodies detected in his CSF.

Findings are consistent with both an MV etiology for the AE and active viral replication in these children. They further indicate the possibility of a virally driven cerebral immunopathology in some cases of regressive autism.


### Background

A possible association between MMR vaccination and autistic encephalopathic regression (AE) in previously developmentally normal children has been reported.[1-5] Evidence for this link is controversial, reflecting the widely differing conclusions of basic and clinical science[1-5] vs. epidemiology,[6-10] although the association is supported by a recent report based upon the CDC's Vaccine Adverse Events Reporting System (VAERS).[11]

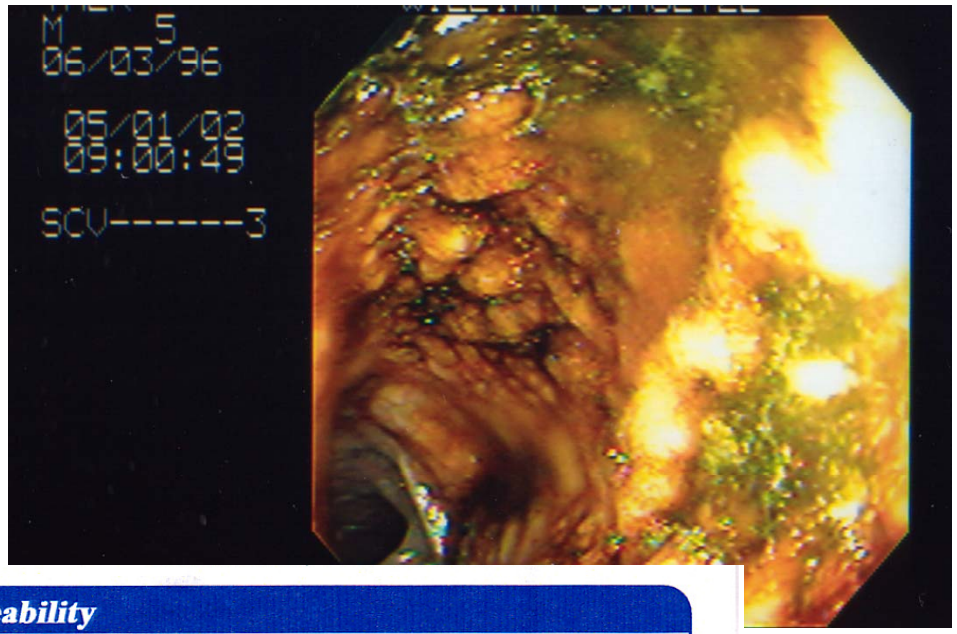
Measles, mumps, and rubella viruses, in their natural form, have been linked to childhood developmental disorders including autistic spectrum disorder (ASD),[12-14] disintegrative disorder,[15] and developmental regression.[16] Deykin and MacMahon compared exposure patterns of 183 children with autism and 355 sibling controls to the encephalitogenic viruses, measles, mumps, rubella, and chickenpox.[12] They found that autistic manifestations were associated with prenatal experience with measles and mumps. Ring et al., using statistical modelling of the number of autism births compared with epidemics of measles, rubella, poliomyelitis, viral meningitis, and viral encephalitis in Israel, found that children born during epidemics of measles were at greater risk of developing autism.[13]

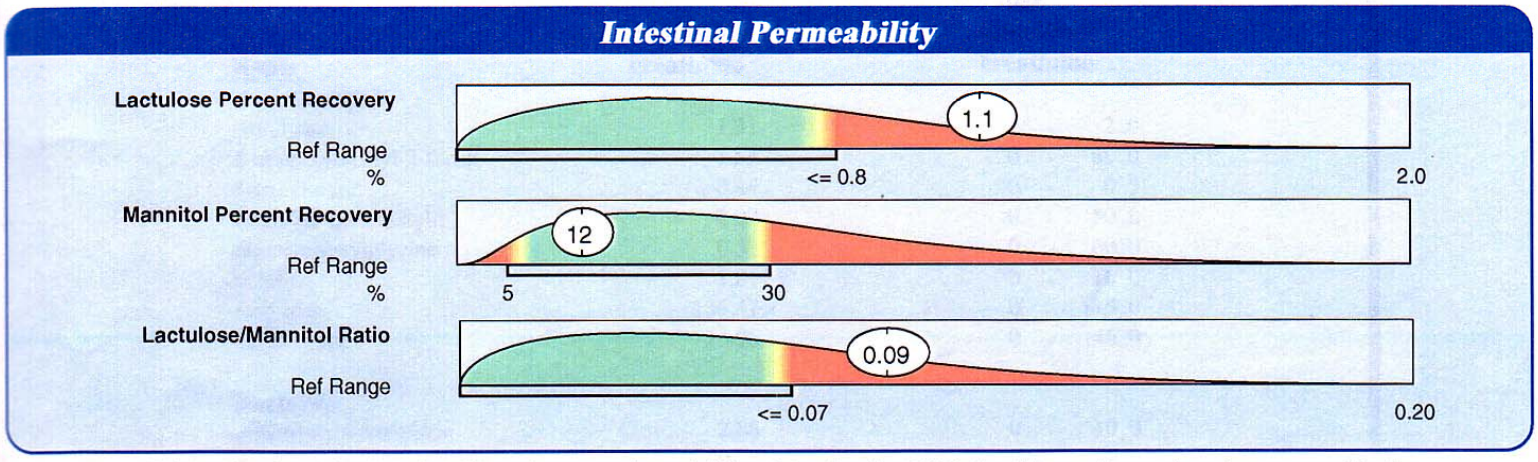
Pathogenetic studies of children with regressive autism and gastrointestinal (GI) symptoms have identified an intestinal mucosal lesion that is consistent with a viral etiology.[17-20] The salient features include ileocolonic lymphodular hyperplasia (LNH) and a patchy, panenteric mucosal immunopathology characterized by an increased lymphocyte density, predominantly of $CD3^+$, $CD8^+$, and $CD19^+$ phenoytpes. Flow cytometric analysis of mucosal lymphocyte intracellular cytokine profiles has identified extensive immunodysregulation in affected children, characterized by a significant excess of tumor necrosis factor alpha (TNF-$\alpha$), raised interferon gamma (INF-$\gamma$), and a reduced counter-regulatory interleukin-10 (IL-10), in biopsies from duodenum, ileum, and colon.[21] The data are consistent with evidence of systemic up-regulation of proinflammatory cytokines in similarly affected children.[22] The findings are reminiscent of human immunodeficiency virus (HIV) enteropathy, which has been reviewed by Schneider et al.[23] and Zeitz.[24]

Uhlmann et al. have reported the presence of measles virus (MV) genomic RNA in the hyperplastic ileal lymphoid tissues of affected children at significantly higher prevalence than in developmentally normal controls.[3] MV nucleocapsid (N) protein has been identified in the same location.[25] Singh et al. have reported atypical humoral immune responses to MV in children with a similar autistic presentation.[24] Serum anti-MV, but not rubella and mumps virus, immunoglobulin G (IgG) antibody levels were significantly higher in children with AE than in developmentally normal children of the same age, including siblings of autistic children. MV IgG titers exhibited a significant positive correlation with serum antibody titers to MBP. In addition, specific IgG antibody to a 74kD protein extracted from MMR vaccine, and consistent with MV Hemagglutinin (H) protein, was identified in 80% of affected children, but not in control sera.

# Intestinal immune changes lead to *increased intestinal permeability*

Abnormal lactulose absorption can be measured in the urine after a standard oral dose in many children with autism.
***Acta Paediatr.*** **1996 Sep;85(9):1076-9.**



# Terminal Ileum RNA

Type of Sample(s) Received: Fresh Frozen Biopsy (t-ileum)

Condition of Sample(s): Satisfactory

---

**TEST - MEASLES VIRUS DETECTION**

RNA was extracted from the biopsy and measles virus was detected using a reverse transcriptase PCR procedure [TaqMan RT-PCR (5'nuclease assay)].

The detected measles virus per ng of RNA: F Gene: > 7 copies/ng total RNA.

Result: **Positive for Measles virus.**

Approved by: _____
Prof. John O'Leary

Date: 31/1/03.

P. Ex. 12 - 0390

# PERSISTENT ILEAL MEASLES VIRUS in a Large Cohort of Regressive Autistic Children WITH ILEOCOLITIS AND LYMPHONODULAR HYPERPLASIA: ReVisitation of an Earlier Study

**June 1st-3rd , 2006 (IMFAR), Montreal,**

Walker, S.J., Hepner K., Segal, J., & Krigsman A., Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27101 USA

**Methods:** Patients who had been diagnosed with autism and who were referred to a pediatric gastroenterologist for evaluation of chronic GI symptoms were eligible to participate…Terminal ileum (TI) biopsy tissue was assayed by RT-PCR for the presence of measles virus RNA and PCR-positive samples were sequenced.

**Results:** Medical and clinical data have been collected for >275 patients who fit the study inclusion criteria. PCR analysis on TI biopsy tissue from an initial 82 patients showed that 70 **(85%) were positive for the F gene amplicon**.

**Conclusions:** Preliminary results from this large cohort of pediatric autistic patients with chronic GI symptoms confirm earlier findings of measles virus RNA in the terminal ileum and support an association between measles virus and ileocolitis /LNH. (personal communcation with the lead author indicates all strains are vaccine related except one which is both WT and vaccine type.

SPECIALTY LABORATORIES
2211 Michigan Avenue 310·828·6543
Santa Monica, CA 90404-3900 800·421·4449

ACCT NUMBER: 42500
S.L.I. ACCESSION NUMBER: 098-3362950
PATIENT NAME: SNYDER, COLTON
REFERRING PHYSICIAN: BRADSTREET, J.J.
NOTES:

DR. J. BRADSTREET
1663 GEORGIA ST #700

PALM BAY FL 32907

PATIENT I.D. NUMBER:
DRAWN: 01/13/00 02:00
RECEIVED: 01/15/00 04:09
REPORTED: 01/25/00 16:09
FINAL
AGE: 3 Y SEX: Male

| | RESULT | | REFERENCE RANGE |
|---|---|---|---|
| **MYELIN BASIC PROTEIN (MBP) AUTOANTIBODIES** | | | |
| MBP Autoabs | 46 | H | EIA Units (< 10) |

This test result or one or more of its components was developed and its performance characteristics determined by Specialty Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

| | | | |
|---|---|---|---|
| **NEUROFILAMENT-HEAVY (NF-H) SUBUNIT AUTOABS** | | | |
| NF-H Autoabs | < 10 | | EIA Units (< 10) |

This test result or one or more of its components was developed and its performance characteristics determined by Specialty Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

REPORT COMPLETED PLEASE FILE
Tests Requested:
MYELIN BASIC PROTEIN (MBP) AUTOANTIBODIES,
NEUROFILAMENT-HEAVY (NF-H) SUBUNIT AUTOABS

MYELIN BASIC PROTEIN Autoabs, EIA: MBP autoantibody titers are highly correlated to exacerbation and relapse in MS, and are found in up to 77% of MS patients and up to 89% of ON patients. MBP

M42500 5800 1
Page 1

P. Ex. 12 - 0593

James B. Peter, M.D., Ph.D.

R10271 (12/99)

# CSF Measles RNA

Type of Sample(s) Received: CSF

Condition of Sample(s): Satisfactory

## TEST - MEASLES VIRUS DETECTION

RNA was extracted from spinal fluid and measles virus was detected using a reverse transcriptase PCR procedure [TaqMan RT-PCR (5'nuclease assay)].

The detected measles virus per ng of RNA: F Gene: 3.7 x 10^4 copies/ng total RNA.

Result: **Positive for Measles virus.**

Approved by: Prof. John O'Leary

Date: 05/06/2007

P. Ex. 12 - 0419

# An Adult Case of Acute Disseminated Encephalomyelitis Accompanied with Measles Infection

*Key words:* encephalitis, measles, acute disseminated encephalomyelitis, adult

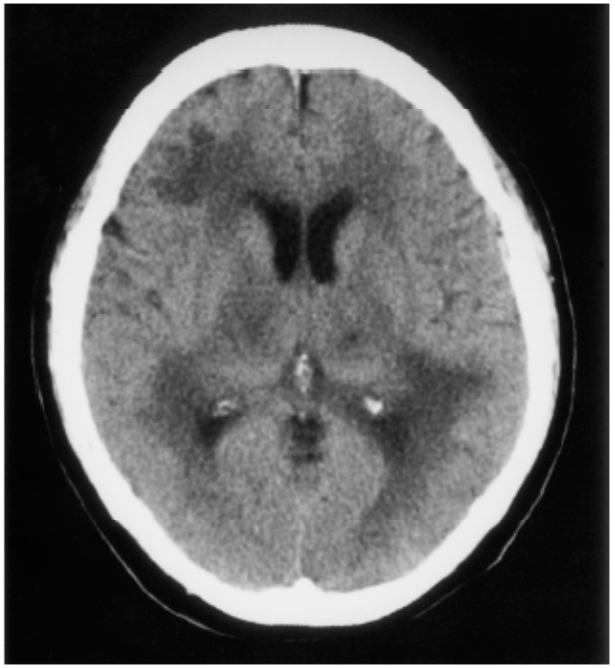


Figure 1. Brain CT on 2nd hospital day. The white matter density is relatively low and there are low density areas in the right frontal lobe and left parietal lobe. The low density area reaches the cortex. The low density areas in the bilateral thalami are more prominent compared to the previous study.

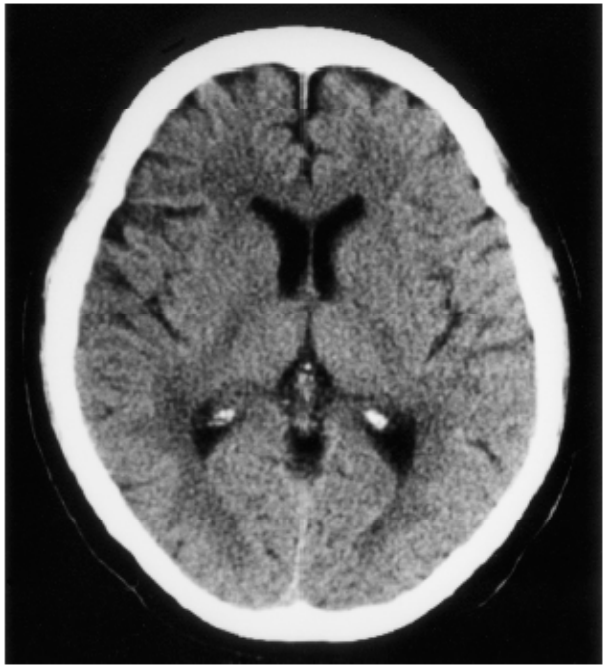


Figure 2. Brain CT on 33rd hospital day. Brain CT on the 33rd hospital day after treatment showed no abnormal image.

**IVIG Responder**

**Intern Med. 2005 Nov;44(11):1204-5**

Yokoyama T, et al First Department of Internal Medicine, Tokyo Medical University, Tokyo.

*Journal of Autism and Developmental Disorders, Vol. 26, No. 4, 1996*

*Brief Reports*

# Brief Report: Dysregulated Immune System in Children with Autism: Beneficial Effects of Intravenous Immune Globulin on Autistic Characteristics[1]

**Sudhir Gupta,[2] Sudeepta Aggarwal, and Cathy Heads**
*Division of Basic and Clinical Immunology, University of California, Irvine*

**Table III.** Effect of Intravenous Immune Globulin on Autistic Characteristics

| Patient Age (years) | Sex | Degree of improvement[a] | Characteristic changes following 6 months of infusion |
|---|---|---|---|
| 3 | M | +++ | Very calm, better eye contact, counting numbers, painting X-mas cards, responding "normal for age" |
| 4 | M | +++ | Speech dramatically improved (400+ nouns, 300+ verbs), plays appropriately, good eye contact, stims only when under stress, regular school with speech therapy |
| 4 | M | + | No hyperactivity, speaking full sentences, more articulate speech, more aware of surrounding, good eye contact, more social |
| 3 | M | +++ | Increased eye contact, improved attention span, speech improved, however not always coherent speech, calmer behavior, much more independent and expressive |
| 5 | M | + | Calmer, sleeps well, increased verbal expression, improved and appropriate behavior, better eye contact |
| 4 | M | +++ | Verbalizing, much calmer, no stimming, good eye contact. Curently in kindergarten with tutor |
| 5 | M | + | Better eye contact, more aware of surroundings, much calmer, no echolalia, still no spontaneous speech, however speech is articulate |
| 3 | M | ++++ | Speech almost normal, behavior normal, good eye contact, more social, attending regular school |
| 3 | M | + | Improved focus, better eye contact, more verbal, much calmer, improved awareness of surroundings |
| 6 | F | + | More initiative and appropriate physical play, calmer affect, better eye contact |

[a] + = minimal, ++ = modest, +++ = marked, ++++ = striking.

*Journal of Nutritional & Environmental Medicine*, December 2006; 15(4): 1–8

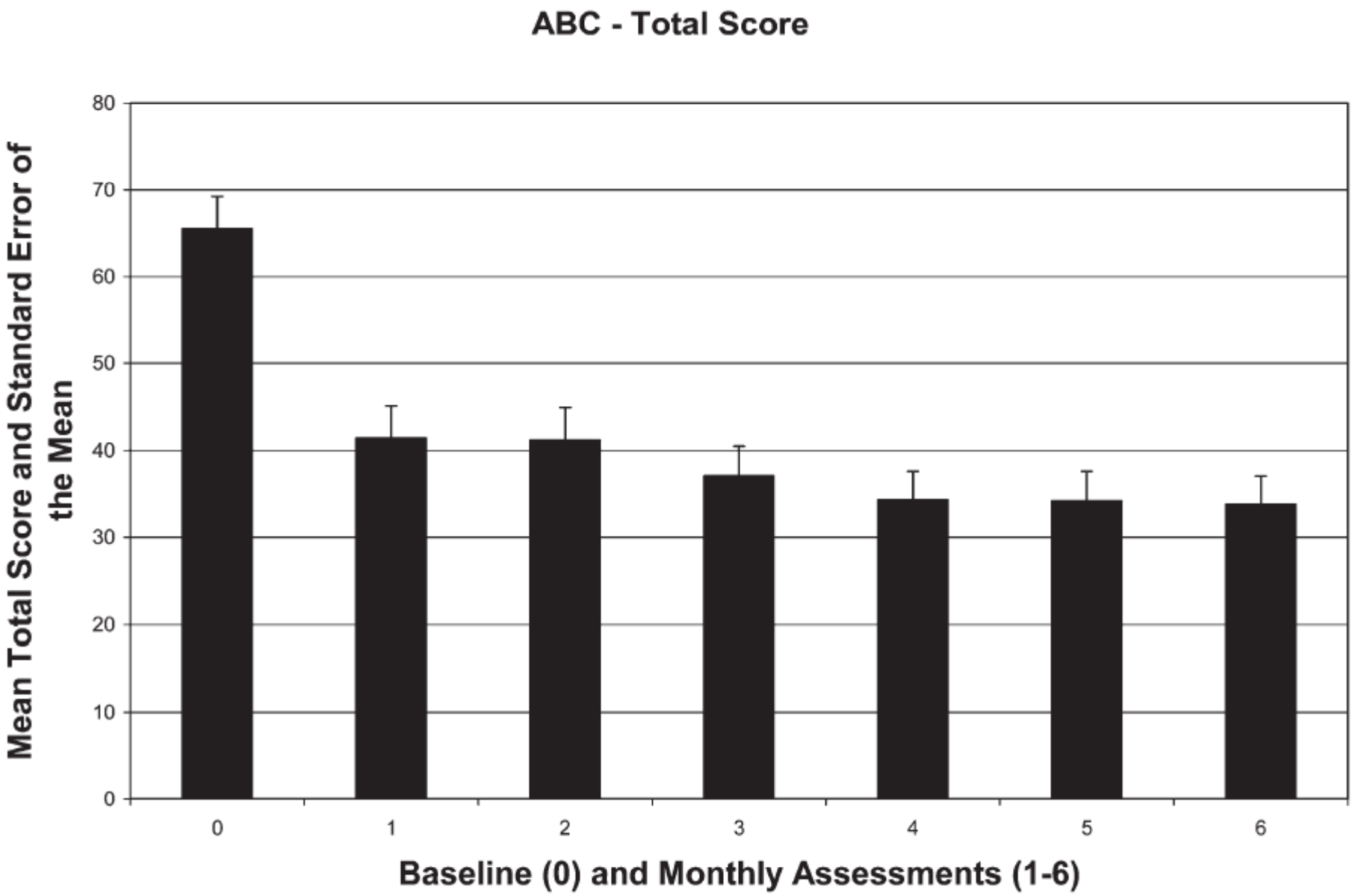
Taylor & Francis
Taylor & Francis Group

**ORIGINAL ARTICLE**

# Improvement in children with autism treated with intravenous gamma globulin

MARVIN BORIS, MD[1], ALLAN GOLDBLATT, PA-C[2] &
STEPHEN M. EDELSON, PHD[3]

[1]*New York University School of Medicine, New York, New York, USA,* [2]*Truro College, New York, New York, USA, and* [3]*Autism Research Institute, 4182 Adams Ave, San Diego, CA 92116, USA*


ABC - Total Score
Mean Total Score and Standard Error of the Mean
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Baseline (0) and Monthly Assessments (1-6)

# Another view of autism

by James Oleske

Despite the lack of epidemiological studies confirming an association between autism and the MMR (measles/mumps/rubella) vaccine, some parents report anecdotal data suggesting such a link. Research indicates that some children with autism have immune abnormalities on laboratory testing. Operating on the premise that their immunodysregulation may be associated with the pathogenesis of autism, I tested for the possible beneficial effects of intravenous gamma globulin therapy on these children.

UMDNJ RESEARCH
Volume 5, Number 1, Winter 2004

JAMES OLESKE, MD, MPH, FRANCOIS-XAVIER BAGNOUD PROFESSOR OF PEDIATRICS; DIRECTOR OF THE DIVISION OF PULMONARY, ALLERGY, IMMUNOLOGY AND INFECTIOUS DISEASES, UMDNJ-NEW JERSEY MEDICAL SCHOOL; MEDICAL DIRECTOR OF THE FXB CENTER AT UMDNJ

"Parents and physicians also reported an improvement in autistic behaviors in 21/27 children, and one child had reported improvement in autistic features without improvement in recurrent infections."

*James M. Oleske is one of the country's foremost pediatric HIV/AIDS specialists. He is the Francois-Xavier Bagnoud Professor of Pediatrics and director of the Division of Pulmonary, Allergy, Immunology and Infectious Diseases at UMDNJ-New Jersey Medical School (NJMS), and medical director of the FXB Center at UMDNJ, one of the nation's largest treatment centers for childhood HIV/AIDS. He earned his MD from NJMS in 1971 and an MPH from Columbia University. After*

# Medical therapy = mostly IVIG Resulted in Remarkable Recovery

First Step Therapeutics
Physical Therapy Occupational Therapy Speech Therapy
1104 Beville Road, Suite J Daytona Beach, Fl 32114
Phone: (904) 252-7837 Fax: (904) 252-0021

NAME: SNYDER, Colten
D.O.B.: 1-9-97
C/A: 4 YEARS, 10 MONTHS
DIAGNOSIS: receptive language delay
DATE: 11-21-01

P. Ex. 14 - 0007

SPEECH/LANGUAGE RE-EVALUATION

BACKGROUND INFORMATION: Colten has been receiving ongoing speech/language therapy services since April, 1999. He has been attending a structured preschool program during the past year and currently is enrolled in preschool at Temple Beth El. Colten met and surpassed the annual goal and short term objectives established.

TESTS ADMINISTERED:
Preschool Language Scale-3 (PLS-3)

| | Standard Score | Deviation from Mean | Previous Standard Score (4/01) |
|---|---|---|---|
| Auditory Comprehension | 79 | >-1 | 80 |
| Expressive Communication | 104 | n/a | 73 |
| Total Language Score | 91 | n/a | 74 |

# Medical therapy = IVIG, Dysbiosis Therapy and Special Diet


**First Step Therapeutics**

Pediatric - Physical Therapy - Occupational Therapy - Speech Therapy

1104 Beville Road, Suite J - Daytona Beach, Florida 32114

Phone: (386) 252-7837 Fax: (386) 252-0021


NAME: SNYDER, Colten
D.O.B.: 1-9-97
C/A: 6 YEARS, 3 MONTHS
DATE: 4-14-03

P. Ex. 14 - 0002

SPEECH/LANGUAGE EVALUATION

BACKGROUND INFORMATION: Colten had received language therapy at this center from April, 1999 until discharge in May, 2002. He continues to attend school at Temple Beth-el and is followed for medical management by Dr. Jeff Bradstreet.

TESTS ADMINISTERED:
Preschool Language Scale-4 (PLS-4)

| | Standard Score | Deviation from Mean (Mean=100) |
|---|---|---|
| Auditory Comprehension | 103 | n/a |
| Expressive Communication | 107 | n/a |
| Total Language Score | 106 | n/a |

# Medical Therapy
# Remarkable Recovery

## First Step Therapeutics

Pediatric - Physical Therapy - Occupational Therapy - Speech Therapy

1104 Beville Road, Suite J - Daytona Beach, Florida 32114
Phone: (386) 252-7837 Fax: (386) 252-0021

P. Ex. 14 - 0001

NAME: SNYDER, Colten
D.O.B.: 1/9/97
C/A: 7 years 4 months
DATE: 5/27/04

SPEECH/LANGUAGE RE-EVALUATION

BACKGROUND INFORMATION: Colten returned to this center for an annual reevaluation of speech and language skills. He is currently enrolled at Sugar Mill Elementary School in a regular education curriculum with resource speech/language therapy.

TESTS ADMINISTERED:
Test for Auditory Comprehension of Language-3 (TACL-3)

| | Age Equivalent | Standard Score | Range of Performance |
|---|---|---|---|
| I. Vocabulary | 9 years 0 months | 13 | Above Average |
| II. Grammatical Morphemes | 7 years 6 months | 10 | Average |
| III. Elaborated Phrases and Sentences | 9 years 0 months | 12 | Average |

# Additional Cases

# Immune/Gut Case male age 3

**Immune system** Methode : HPLC

| | patient µmole / mole Cr | reference | result |
|---|---|---|---|
| ***urinary Neopterine (NEO)*** | 1147 | 110-180 | increased cellular immunity activation |
| ***Urinary Biopterin*** | 434 | | |
| ***Biopeterin / Neopterin*** | 0,4 | >1,5 | very low ratio |

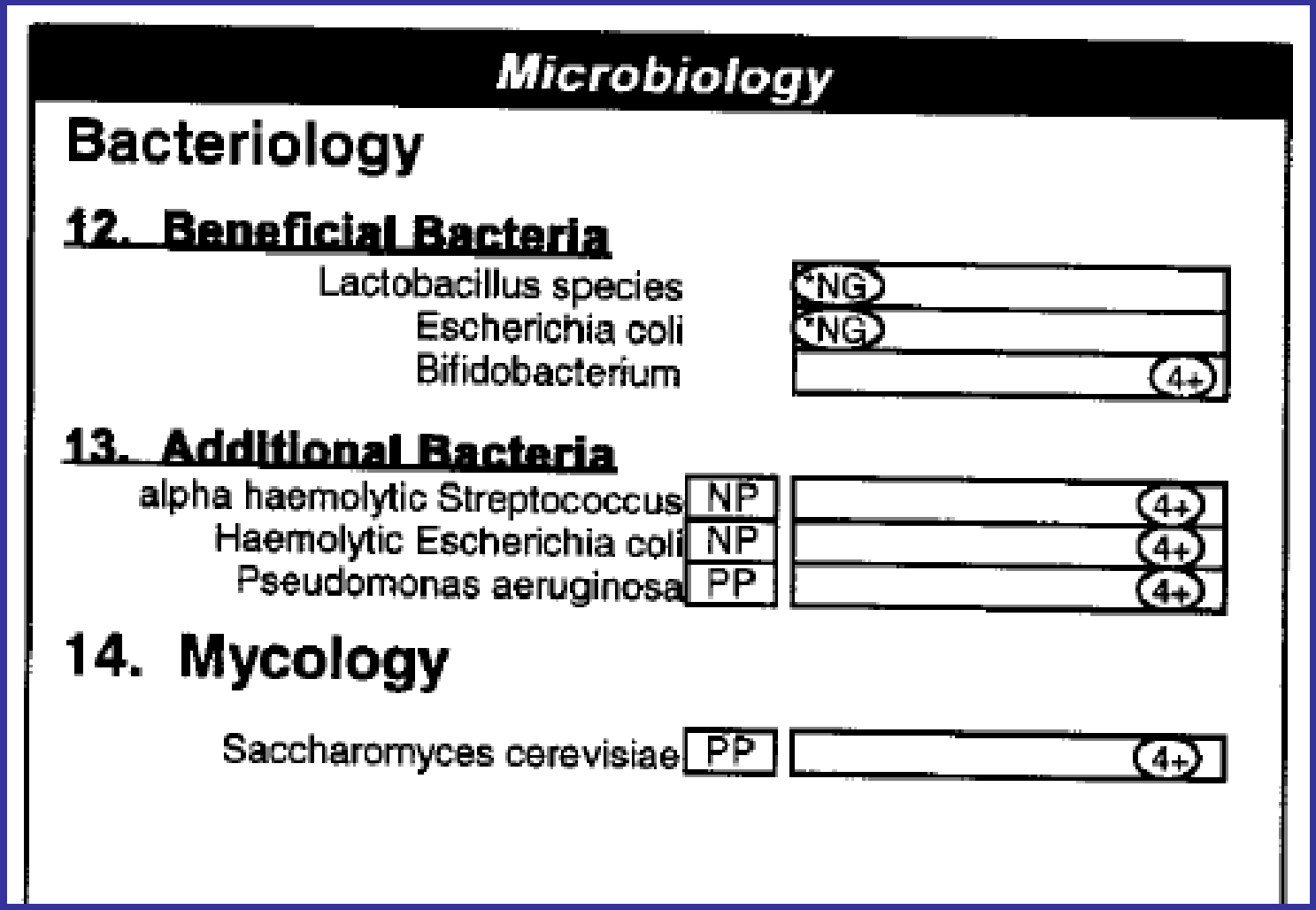
## *Gut Immunology*

| Analyte | Result | Reference Range |
|---|---|---|
| 3. Eosinophil Protein X | 4.0 | <= 7.0 mcg/g |
| 4. Calprotectin | 122 | <= 50 mcg/g |

# Immune/Gut Case male age 3

| Name | mmol/mol creatinine | | Reference range mmol/mol creatinine |
|---|---|---|---|
| **Yeast/Fungal** | | | |
| citramalic | 1.33 | | 0 - 2.0 |
| 5-hydroxymethyl-2-furoic | 2.65 | | 0 - 80.0 |
| 3-oxoglutaric | 3.01 | H | 0 - 0.5 |
| furan-2,5-dicarboxylic | 1.17 | | 0 - 50.0 |
| furancarbonylglycine | 0.71 | | 0 - 60.0 |
| tartaric | 3.13 | | 0 - 16.0 |
| arabinose | 257.88 | H | 0 - 47.0 |
| carboxycitric | 5.65 | | 0 - 46.0 |
| **Bacterial** | | | |
| 2-hydroxyphenylacetic | 0.69 | | 0 - 10.0 |
| 4-hydroxyphenylacetic | 52.64 | H | 0 - 50.0 |
| **Anaerobic Bacterial** | | | |
| HPHPA | 2278.84 | H | 0 - 150.0 |
| VMA analog | 1.96 | | 0 - 31.0 |

# Absent Lactobacillus ~ Anerobic and Likely Clostridia



**Microbiology**

## Bacteriology

### 12. Beneficial Bacteria

| | | |
|---|---|---|
| Lactobacillus species | | *NG |
| Escherichia coli | | *NG |
| Bifidobacterium | | 4+ |

### 13. Additional Bacteria

| | | |
|---|---|---|
| alpha haemolytic Streptococcus | NP | 4+ |
| Haemolytic Escherichia coli | NP | 4+ |
| Pseudomonas aeruginosa | PP | 4+ |

## 14. Mycology

| | | |
|---|---|---|
| Saccharomyces cerevisiae | PP | 4+ |

# Short-Term Benefit From Oral Vancomycin Treatment of Regressive-Onset Autism

Richard H. Sandler, MD; Sydney M. Finegold, MD; Ellen R. Bolte; Cathleen P. Buchanan, MS; Anne P. Maxwell, PhD; Marja-Liisa Väisänen, BS; Michael N. Nelson, PhD; Hannah M. Wexler, PhD

## ABSTRACT

In most cases symptoms of autism begin in early infancy. However, a subset of children appears to develop normally until a clear deterioration is observed. Many parents of children with "regressive"-onset autism have noted antecedent antibiotic exposure followed by chronic diarrhea. We speculated that, in a subgroup of children, disruption of indigenous gut flora might promote colonization by one or more neurotoxin-producing bacteria, contributing, at least in part, to their autistic symptomatology. To help test this hypothesis, 11 children with regressive-onset autism were recruited for an intervention trial using a minimally absorbed oral antibiotic. Entry criteria included antecedent broad-spectrum antimicrobial exposure followed by chronic persistent diarrhea, deterioration of previously acquired skills, and then autistic features. Short-term improvement was noted using multiple pre- and post-therapy evaluations. These included coded, paired videotapes scored by a clinical psychologist blinded to treatment status; these noted improvement in 8 of 10 children studied. Unfortunately, these gains had largely waned at follow-up. Although the protocol used is not suggested as useful therapy, these results indicate that a possible gut flora–brain connection warrants further investigation, as it might lead to greater pathophysiologic insight and meaningful prevention or treatment in a subset of children with autism. (*J Child Neurol* 2000;15:429–435).

# MICs of Selected Antibiotics for *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus mycoides* from a Range of Clinical and Environmental Sources as Determined by the Etest

Peter C. B. Turnbull,* Nicky M. Sirianni,† Carlos I. LeBron, Marian N. Samaan, Felicia N. Sutton, Anatalio E. Reyes, and Leonard F. Peruski, Jr.‡

*Biological Defense Research Directorate, Naval Medical Research Center, Silver Spring, Maryland 20910-7500*

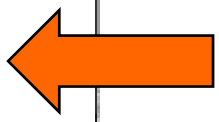
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gentamicin | *B. anthracis* | 75 | 0.064–0.5 | 0.25 | 0.38 | 4[a] | 16[a] | 100 | | |
| | *B. cereus* | 67 | 0.094–0.75 | 0.38 | 0.75 | | | 100 | | |
| | *B. thuringiensis* | 12 | 0.047–0.5 | 0.19 | 0.5 | | | 100 | | |
| | *B. mycoides* | 5 | 0.19–0.38 | 0.25 | 0.25 | | | 100 | | |
| | *S. aureus* 29213 | | 0.8 (0.5–1.5)[d] | | | | | | | |
| Penicillin | *B. anthracis* | 74[c] | <0.016–≥32 | <0.016 | 0.023 | 0.12[a,d] | 0.25[a,d] | 97 | | 3 |
| | *B. cereus* | 67 | 0.012–>32 | >32 | >32 | | | 1 | | 99 |
| | *B. thuringiensis* | 12 | >32 | >32 | >32 | | | | | 100 |
| | *B. mycoides* | 4 | >32 | >32 | >32 | | | | | 100 |
| | *S. aureus* 29213 | | 0.4 (0.25–0.38)[d] | | | | | | | |
| Tetracycline | *B. anthracis* | 71 | 0.016–0.094 | 0.023 | 0.032 | 1 | | 100 | | |
| | *B. cereus* | 67 | 0.05–32 | 1 | 6 | 4[a] | 16[a] | 84 | 9 | 6 |
| | *B. thuringiensis* | 12 | 0.5–24 | 2 | 6 | | | 84 | 8 | 8 |
| | *B. mycoides* | 4 | 0.125–2 | 0.5 | 2 | | | 100 | | |
| | *S. aureus* 29213 | | 0.17 (0.094–0.38)[d] | | | | | | | |
| Vancomycin | *B. anthracis* | 74 | 0.75–5 | 2 | 3 | 4[a] | 32[a] | 99 | 1 | |
| | *B. cereus* | 67 | 1–16 | 3 | 6 | | | 85 | 15 | |
| | *B. thuringiensis* | 12 | 0.75–4 | 2 | 4 | | | 100 | | |
| | *B. mycoides* | 4 | 1.5–4 | 2 | | | | 100 | | |
| | *S. aureus* 29213 | | 1.8 (1.5–2)[d] | | | | | | | |

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3626–3634

# Metronidazole (Flagyl) Kills All Clostrida at Reasonable Doses

TABLE 1. *Inhibitory and bactericidal effect of metronidazole, ornidazole, and tinidazole on anaerobic bacteria*

| Organisms | No. of strains tested | Antimicrobial agent | Cumulative percent of isolates inhibited/killed at various concn (μg/ml)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ≤0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 3.1 | 6.3 | >100 |
| *Bacteroides fragilis* | 39 | Metronidazole | | 15/15 | 38/31 | 62/67 | 97/90 | 100/100 | | |
| | | Ornidazole | 3/5 | 18/15 | 44/44 | 72/79 | 97/95 | 100/97 | 100/100 | |
| | | Tinidazole | 3/3 | 28/23 | 56/56 | 74/82 | 92/95 | 100/97 | 100/100 | |
| *Bacteroides melaninogenicus* | 2 | Metronidazole | 100/100 | | | | | | | |
| | | Ornidazole | 100/100 | | | | | | | |
| | | Tinidazole | 100/100 | | | | | | | |
| *Fusobacterium* sp. | 13 | Metronidazole | 62/62 | 85/85 | 92/100 | | 100/100 | | | |
| | | Ornidazole | 62/54 | 85/85 | 92/92 | 100/100 | | | | |
| | | Trinidazole | 62/54 | 85/77 | 92/92 | | 100/100 | | | |
| *Clostridium perfringens* | 9 | Metronidazole | | 33/33 | 44/44 | 89/89 | 100/100 | | | |
| | | Ornidazole | | 11/22 | 56/44 | 100/89 | 100/100 | | | |
| | | Tinidazole | | 11/11 | 56/33 | 89/78 | 100/100 | | | |
| *Clostridium* sp. (other) | 14/5[b] | Metronidazole | 14/0 | 64/0 | | 86/60 | 100/80 | 100/100 | | |
| | | Ornidazole | 14/0 | 57/0 | 71/0 | 93/80 | 100/100 | | | |
| | | Tinidazole | 14/0 | 57/0 | 64/0 | | 93/80 | 100/100 | | |



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1977, p. 631-637

Vol. 11, No. 4
*Printed in U.S.A.*

# Alinia® (Nitazoxanide)

**DOSAGE AND ADMINISTRATION**

| Indication | Age | Dosage | Duration |
|---|---|---|---|
| Treatment of diarrhea caused by *Giardia lamblia* or *Cryptosporidium parvum, and Clostridia* | 1-3 years | 5 mL of Alinia for Oral Suspension (100 mg nitazoxanide) every 12 hours with food | |
| | 4-11 years | 10 mL of Alinia for Oral Suspension (200 mg nitazoxanide) every 12 hours with food | 3 days |
| | ≥12 years | 1 Alinia Tablet (500 mg nitazoxanide) every 12 hours with food or 25 mL of Alinia for Oral Suspension (500 mg nitazoxanide) every 12 hours with food | |

# The Search for a Better Treatment for Recurrent *Clostridium difficile* Disease: Use of High-Dose Vancomycin Combined with *Saccharomyces boulardii*

Christina M. Surawicz,[1] Lynne V. McFarland,[2,3] Richard N. Greenberg,[4] Moshe Rubin,[5] Robert Fekety,[6] Maury E. Mulligan,[7] Reuben J. Garcia,[5] Sally Brandmarker,[1] Karen Bowen,[4] Delia Borjal,[5] and Gary W. Elmer[2]

[1]*Division of Gastroenterology, Department of Medicine, School of Medicine,* [2]*Department of Medicinal Chemistry, School of Pharmacy, University of Washington, and* [3]*Biocodex, Seattle, Washington;* [4]*Division of Infectious Diseases, Department of Medicine, University of Kentucky, and Lexington Veterans Affairs Medical Center, Lexington, Kentucky;* [5]*Department of Medicine, Columbia University College of Physicians and Surgeons, New York;* [6]*Division of Infectious Diseases, Department of Internal Disease, University of Michigan, Ann Arbor; and* [7]*Department of Infectious Diseases, Veterans Affairs Medical Center, Long Beach, California*

**Clinical Infectious Diseases 2000; 31:1012–7**

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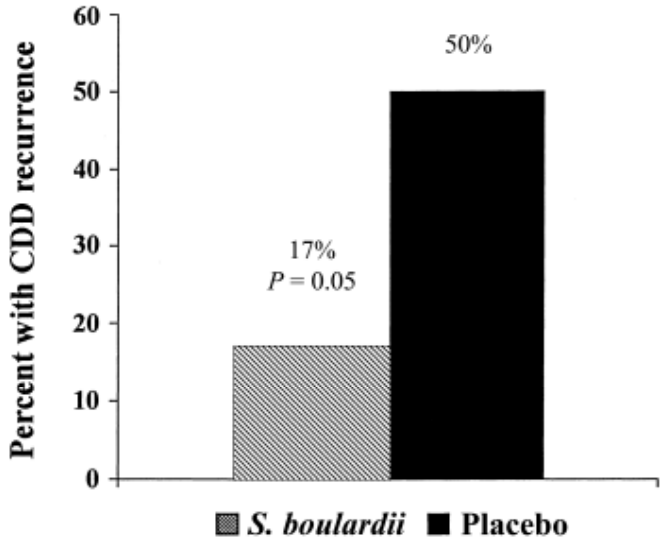



Figure 1. Frequency of a subsequent recurrence of *Clostridium difficile* disease (CDD) after treatment with high-dose vancomycin (2 g/day for 10 days) and either *Saccharomyces boulardii* or placebo (1 g/day for 28 days) in adult patients with active CDD.

# Immune/Gut Case male age 3

**02/01/2008**

## *DNA Oxidative Stress*

| | | reference range |
|---|---|---|
| **8OHdG** | **26** nmole/gCr | 12-25 |

urinary 8-hydroxy deoxyguanosine

***Moderate elevated urinary 8OHdG, reflecting mildly increased DNA oxidative damage***

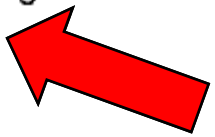
In Children 3-9 years old, urinary 8OHdG is 25% higher than in adults (Bogdanov et al, Free Radical Biol. Med 27, 647-686, 1999)

## *RNA Oxidative Stress*

| | | |
|---|---|---|
| **8 OHG** | **123** nmole/gCr | 20-40 |

urinary 8-hydroxy guanosine

***strongly increased urinary 8OG, reflecting high RNA oxidative damage***



## *RNA/DNA*

| | | |
|---|---|---|
| **8OHG/8OHdG** | **4,7** | <2 |

| | | |
|---|---|---|
| urinary creatinin | **955** | mg/l |

**As noted earlier with immune activation comes oxidative stress: Isoprostane level was 3x greater than normal**

# Immune/Gut Case male age 3

## Membrane Oxidative Damage

### Urinary IsoprostaneF2-alpha

*LC/MS-MS-immunoaffinity cartrige extraction -deuterated standard*

| | | |
|---|---|---|
| **8-iso-PGF2 alpa** | **618** ng/gCr | 80-160 |

*Increased oxidative stress damage*

# Increased excretion of a lipid peroxidation biomarker in autism.

Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC.
**Department of Neurosciences, UMDNJ-New Jersey Medical School**, Newark, 07103, USA.

Prostaglandins Leukot Essent Fatty Acids. 2005 Nov;73(5):379-84.

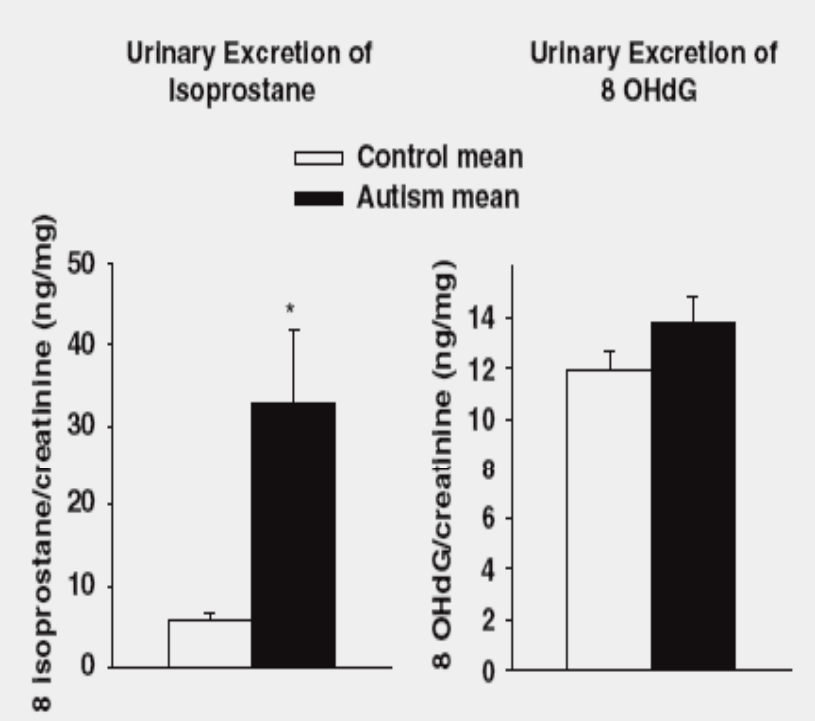



Fig. 1. Urinary excretion of isoprostane and 8-OHdG in children with autism and controls. $*P < 0.05$, Student's *t*-test.

# Antioxidant ORAC/ 1 gram of whole food
# Freeze dried ACAI is > 420

| | |
|---|---|
| Muscadine Grape Seed** | 559 |
| Acai* | 387 |
| Goji Berry* | 253 |
| Noni* | 151 |
| Pomegranates* | 105 |
| Raspberries* | 82 |
| Blueberries* | 77 |
| Red Grapes* | 74 |
| Prunes* | 57 |
| Cherries* | 67 |
| Strawberries* | 36 |

*Antioxidant Capacities of Foods. J. Agric. Food Chem., Vol. 52, No. 12, 2004 4027

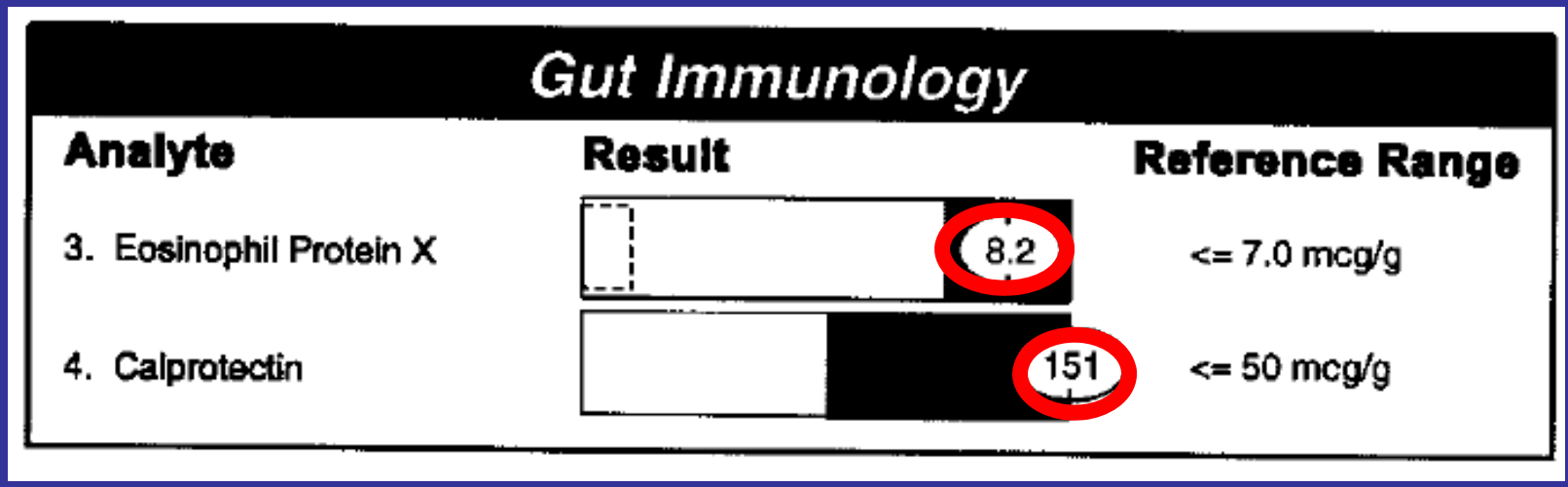
*Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL, J Agric Food Chem. "Lipophilic and hydrophilic antioxidant capacities of common foods in the United States." 2004 Jun 16;52(12):4026-37

# Immune/Gut Case male age 3

- Treatment options (in addition to GFCF)
- Tappering dose of prednisolone (steroids) over 5 weeks
- Probiotics = good bacteria
- Antifungal = Fluconazole, and others in class or Nystatin or Amphotericin
- Vancomycin = antibiotic (gut specific not absorbed orally) or Metronidazole or Nitazoxanide (Alinia™)
- ACAI 1 scoop twice daily as antioxidant.
- Remarkable turnaround within the first 2 weeks

# Allergic Component to Inflammation in Gut

# Gut Immune Issues with Significant Allergic Component: Age 21mo



**Gut Immunology**

| Analyte | Result | Reference Range |
|---|---|---|
| 3. Eosinophil Protein X | 8.2 | <= 7.0 mcg/g |
| 4. Calprotectin | 151 | <= 50 mcg/g |

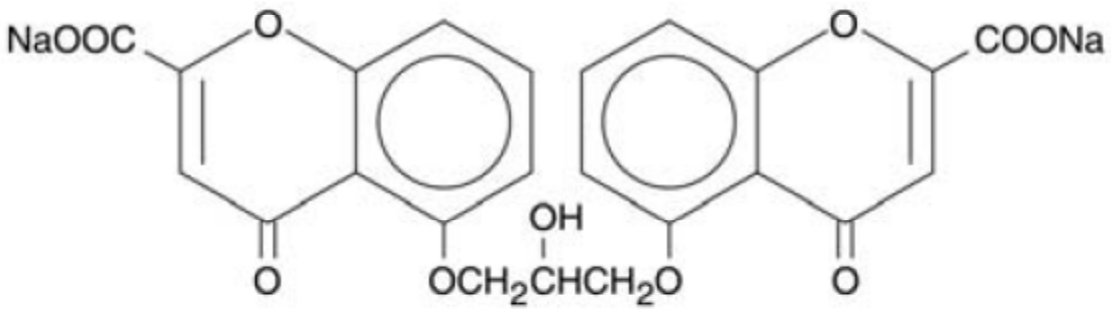
# Gastrocrom®
## (cromolyn sodium, USP)
## Oral Concentrate

Pharmacologic Category: Mast cell stabilizer

Therapeutic Category: Antiallergic

**CLINICAL PHARMACOLOGY:** *In vitro* and *in vivo* animal studies have shown that cromolyn sodium inhibits the release of mediators from sensitized mast cells. Cromolyn sodium acts by inhibiting the release of histamine and leukotrienes (SRS-A) from the mast cell.

Cromolyn sodium has no intrinsic vasoconstrictor, antihistamine, or glucocorticoid activity.



# Do Enzymes Work for Food Allergy? Maybe Not

## Role of pepsin in modifying the allergenicity of bhetki (Lates calcarifer) and mackerel (Rastrelliger kanagurta) fish.

**Indian J Biochem Biophys. 2007 Apr;44(2):94-100.**

**Mondal G**, **Chatterjee U**, **Samanta S**, **Chatterjee BP**

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India.

The effect of pepsin digestion on the allergenicity of raw and thermally processed (boiled and fried) fish muscle extracts of two widely consumed fishes bhetki (Lates calcarifer) and mackerel (Rastrelliger kanagurta) was studied. Sere were collected from 110 patients who were hypersensitive to fish, as evidenced by their clinical history, symptoms and positive skin-prick test results. The various extracts after digestion with pepsin at different times of incubation were tested for specific IgE-binding activity by ELISA and immunoblotting with patients' sera. All the extracts of both the fishes retained their allergenicity as evidenced by ELISA and immunoblotting. Results showed that raw as well as thermally processed allergens of both the fishes maintained strong allergenicity, even after digestion with pepsin for different time periods. The study revealed that the fish proteins played an important role in manifestation of allergy, due to their stable structure, which was retained even after pepsin and heat treatment.

# Very Difficult but Not Impossible to Remove Allergy from Food with Predigestion

## Evaluation of the residual antigenicity of dairy whey hydrolysates obtained by combination of enzymatic hydrolysis and high-pressure treatment.

**J Food Prot. 2006 Jul;69(7):1707-12.**

**Peñas E**, **Restani P**, **Ballabio C**, **Préstamo G**, **Fiocchi A**, **Gomez R**.

Department of Science and Technology of Plant Food, Instituto del Frío, c/José Antonio Nováis 10, 28040-Madrid, España. elenape@if.csic.es

Dairy whey was hydrolyzed for 15 min with five food-grade enzymes (Alcalase, Neutrase, Corolase 7089, Corolase PN-L, and Papain) at atmospheric pressure (0.1 MPa) and in combination with high pressure (HP) at 100, 200, and 300 MPa, applied prior to or during enzymatic digestion. The peptide profile of the hydrolysates obtained was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and their residual antigenicity was assessed by immuno-blotting with anti-beta-lactoglobulin monoclonal antibodies and the sera from pediatric patients allergic to cow's milk proteins. Moreover, to evaluate the presence of residual trace amounts of casein in bovine whey hydrolysates, immunoblotting with anti-cow's milk protein polyclonal antibodies was performed. On the contrary, no residual immunochemical reactivity associated with beta-lactoglobulin was observed in the hydrolysates obtained by Alcalase and Neutrase under HP, and none was observed in any of the hydrolysis products obtained by Papain. The presence of traces of casein was not significant. These results suggest that HP combined with selected food-grade proteases is a treatment that can remove the antigenicity of whey protein hydrolysates for their use as ingredients of hypoallergenic infant formulae.

# Do Probiotics Reduce Atopic Symptoms? Maybe Not

## Allergy in marathon runners and effect of Lactobacillus GG supplementation on allergic inflammatory markers.

**Respir Med. 2007 Jun;101(6):1123-31. Epub 2006 Dec 29.**

**Moreira A**, **Kekkonen R**, **Korpela R**, **Delgado L**, **Haahtela T**.

Laboratory of Immunology, Faculty of Medicine, University of Porto, Porto, Portugal. andremoreira@med.up.pt

OBJECTIVE: We studied the prevalence of asthma and allergy in non-elite marathon runners and investigated the effects of probiotic supplementation on allergic inflammatory markers. METHODS: Asthma and allergies were surveyed by questionnaire, and blood eosinophils, serum eosinophil cationic protein (ECP), total IgE, and Phadiatop were measured in 141 Finnish marathon runners who took part in the Helsinki City Marathon. They were also randomized to receive either Lactobacillus GG (LGG) or placebo during the 3 months of the pollen season prior to the marathon. RESULTS: No differences in changes were seen between groups receiving LGG or placebo. CONCLUSION: LGG supplementation did not prevent the increase of allergic markers during the pollen season, or the eosinopenia induced by the marathon.

# Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants.

**Pediatr Allergy Immunol. 2005 Feb;16(1):65-71.**

**Viljanen M**, **Kuitunen M**, **Haahtela T**, **Juntunen-Backman K**, **Korpela R**, **Savilahti E**.

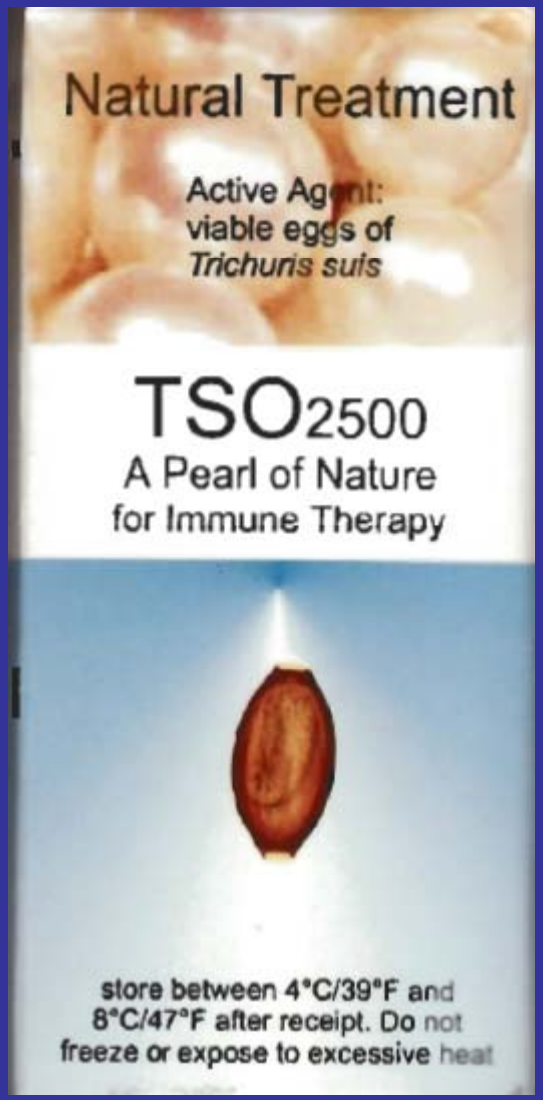
The Skin and Allergy Hospital, University of Helsinki, Helsinki, Finland. mirva.viljanen@hus.fi

Probiotic bacteria are proposed to alleviate intestinal inflammation in infants with atopic eczema/dermatitis syndrome (AEDS) and food allergy. In such infants we investigated effects of probiotic bacteria on faecal IgA, and on the intestinal inflammation markers tumour necrosis factor-alpha (TNF-alpha), alpha1-antitrypsin (AT), and eosinophil cationic protein (ECP). A total of 230 infants with AEDS and suspected cow's milk allergy (CMA) received in a randomized double-blinded manner, concomitant with elimination diet, Lactobacillus GG (LGG), a mixture of four probiotic strains (MIX), or placebo for 4 wk. Four weeks after treatment, CMA was diagnosed with a double-blind placebo-controlled milk challenge. Faecal samples of 102 infants, randomly chosen for analysis, were collected before treatment, after 4-wk treatment, and on the first day of milk challenge. After treatment, IgA levels tended to be higher in probiotic groups than in the placebo group (LGG vs. placebo, p=0.064; MIX vs. placebo, p=0.064), and AT decreased in the LGG group, but not in other treatment groups. After challenge in IgE-associated CMA infants, faecal IgA was higher for LGG than for placebo (p=0.014), and **TNF-alpha was lower for LGG than for placebo, but non-significantly (p=0.111)**. In conclusion, 4-wk treatment with LGG may alleviate intestinal inflammation in infants with AEDS and CMA.

# What can reset the immune system?

**Helminthic therapy** is the treatment of autoimmune diseases and immunological disorders by means of deliberate infection with a helminth or with the ova of a helminth. May represent our best option

# Helminthic Therapy TSO



International Immunology, Vol. 15, No. 1, pp. 59–69 

# Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization

**Diane Sewell[1], Zhu Qing[1], Emily Reinke[1], David Elliot[2], Joel Weinstock[2], Matyas Sandor[1]** and **Zsuzsa Fabry[1]**

[1]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI 53706, USA
[2]Department of Internal Medicine, University of Iowa, Iowa City, IA 52242, USA

*Keywords*: experimental autoimmune encephalomyelitis, *Schistosoma mansoni*, STAT6, $T_h1$, $T_h2$

## Abstract

**Experimental autoimmune encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS) characterized by chronic inflammatory demyelination of the central nervous system (CNS). The pathology of EAE involves autoimmune $CD4^+$ $T_h1$ cells. There is a striking inverse correlation between the occurrence of parasitic and autoimmune diseases. We demonstrate that in mice with *Schistosoma mansoni* ova immunization, the severity of EAE is reduced as measured by decreased clinical scores and CNS cellular infiltrates. Disease suppression is associated with immune deviation in the periphery and the CNS, demonstrated by decreased IFN-γ and increased IL-4, transforming growth factor-β and IL-10 levels in the periphery, and increased frequency of IL-4 producing neuroantigen-specific T cells in the brain. *S. mansoni* helminth ova treatment influenced the course of EAE in wild-type mice, but not in STAT6-deficient animals. This indicates that STAT6 plays a critical role in regulating the ameliorating effect of *S. mansoni* ova treatment on the autoimmune response, and provides the direct link between helminth treatment, $T_h2$ environment and improved EAE. As some intestinal helminthic infections induce minimal pathology, they might offer a safe and inexpensive therapy to prevent and/or ameliorate MS.**

INFLAMMATORY BOWEL DISEASE

# *Trichuris suis* therapy in Crohn's disease

**R W Summers, D E Elliott, J F Urban Jr, R Thompson, J V Weinstock**

*Gut* 2005;**54**:87–90. doi: 10.1136/gut.2004.041749

See end of article for authors' affiliations

Correspondence to: Dr R W Summers, James A Clifton Center for Digestive Diseases, Department of Internal Medicine, University of Iowa Roy J and Lucille A Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA; robert-summers@uiowa.edu

Revised version received 28 March 2004
Accepted for publication 9 April 2004

**Background:** Crohn's disease is common in highly industrialised Western countries where helminths are rare and uncommon in less developed areas of the world where most people carry worms. Helminths diminish immune responsiveness in naturally colonised humans and reduce inflammation in experimental colitis. Thus exposure to helminths may help prevent or even ameliorate Crohn's disease.
**Aims:** The aim of the study was to determine the safety and possible efficacy of the intestinal helminth *Trichuris suis* in the treatment of patients with active Crohn's disease.
**Patients:** Twenty nine patients with active Crohn's disease, defined by a Crohn's disease activity index (CDAI) ⩾220 were enrolled in this open label study.
**Methods:** All patients ingested 2500 live *T suis* ova every three weeks for 24 weeks, and disease activity was monitored by CDAI. Remission was defined as a decrease in CDAI to less than 150 while a response was defined as a decrease in CDAI of greater than 100.
**Results:** At week 24, 23 patients (79.3%) responded (decrease in CDAI >100 points or CDAI <150) and 21/29 (72.4%) remitted (CDAI <150). Mean CDAI of responders decreased 177.1 points below baseline. Analysis at week 12 yielded similar results. There were no adverse events.
**Conclusions:** This new therapy may offer a unique, safe, and efficacious alternative for Crohn's disease management. These findings also support the premise that natural exposure to helminths such as *T suis* affords protection from immunological diseases like Crohn's disease.

# Does the failure to acquire helminthic parasites predispose to Crohn's disease?

DAVID E. ELLIOTT,*,[1] JOE F. URBAN, JR.,[†] CURTIS K. ARGO,* AND JOEL V. WEINSTOCK*,[1]

*Department of Internal Medicine, Division of Gastroenterology/Hepatology, University of Iowa, Iowa City, Iowa 52242, USA; and [†]The Immunology and Disease Resistance Laboratory, U.S. Department of Agriculture, Beltsville, Maryland 20705, USA

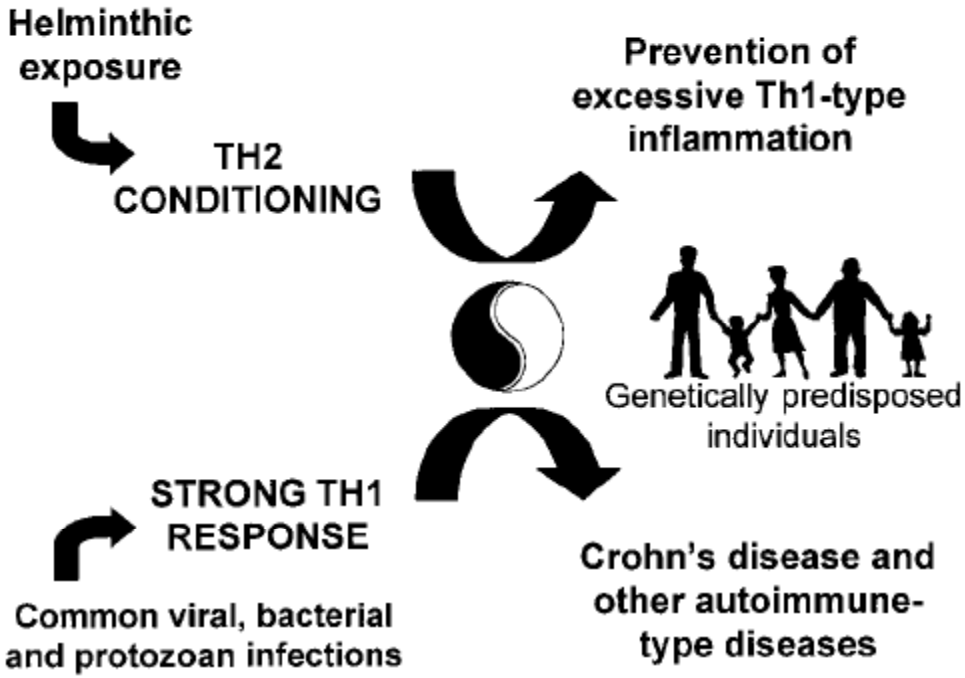
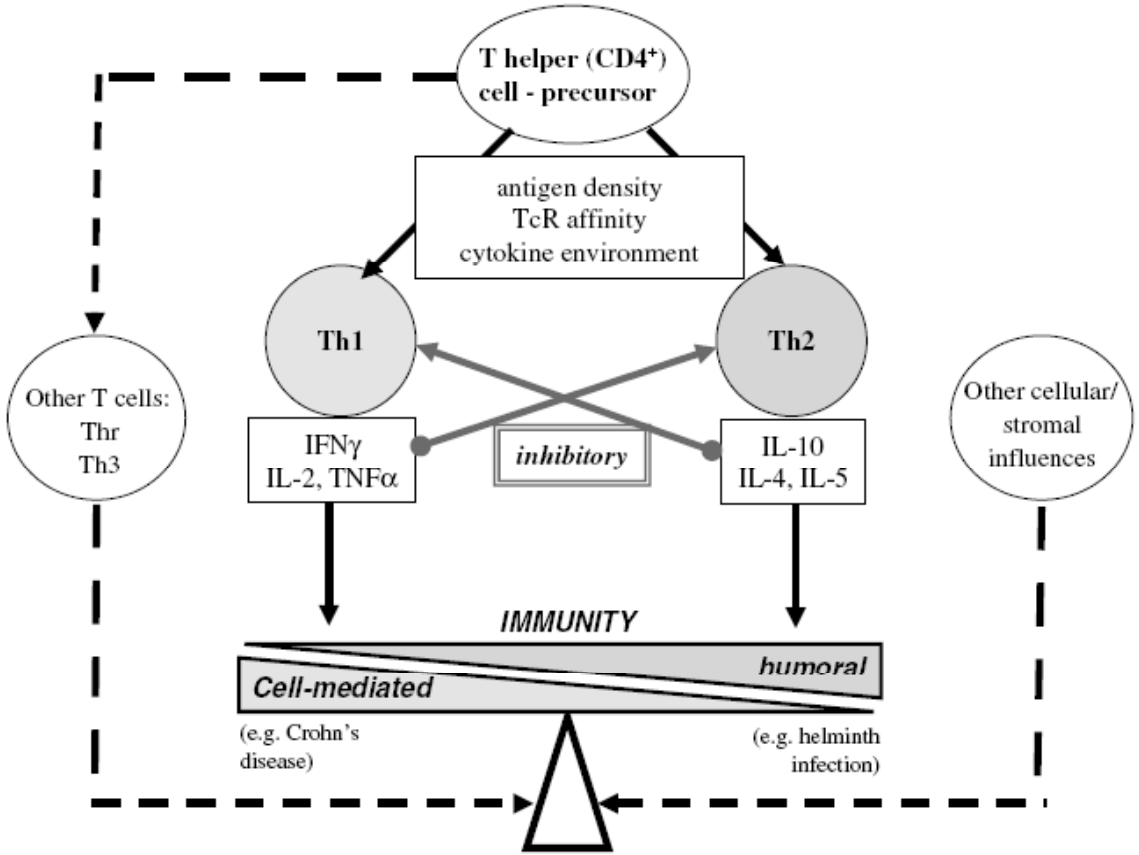


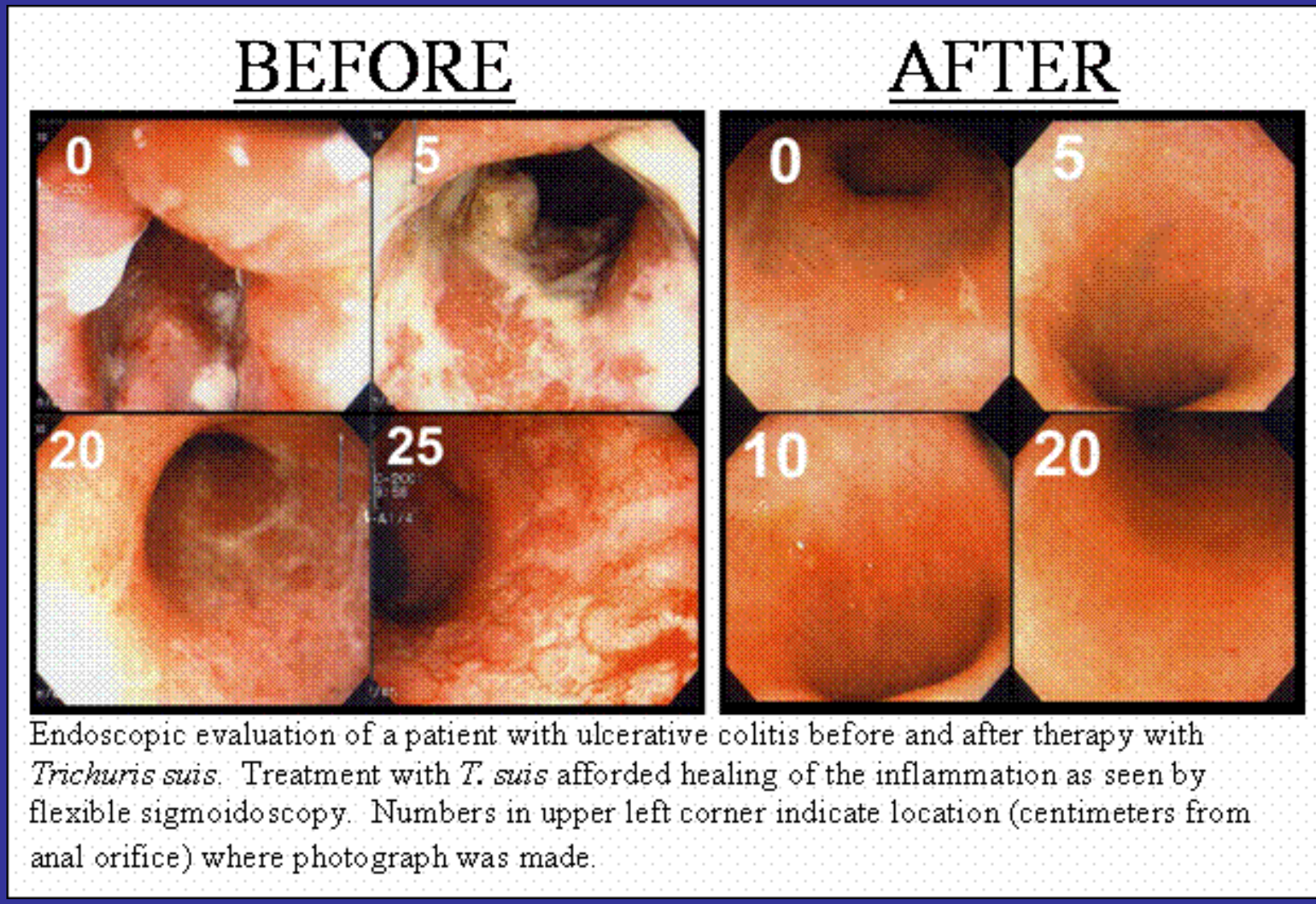

**Figure 2.** Exposure to helminths promotes Th2-type immune responses that may prevent development of the excessive Th1-type inflammatory reactions that cause autoimmune disease in genetically predisposed people.

Aliment Pharmacol Ther 2004; 19: 167–177. doi: 10.1111/j.0269-2813.2004.01803.x

# *Review article: helminths as therapeutic agents for inflammatory bowel disease*

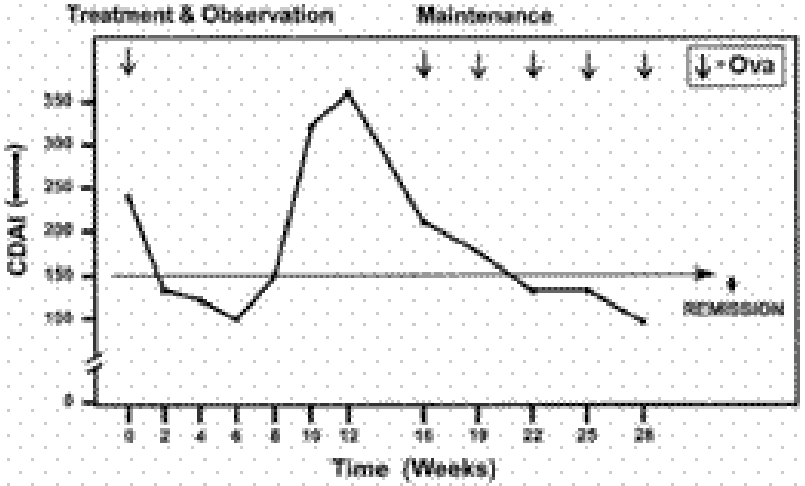
M. M. HUNTER & D. M. McKAY
*Intestinal Disease Research Programme, McMaster University, Hamilton, Ontario, Canada*






Endoscopic evaluation of a patient with ulcerative colitis before and after therapy with *Trichuris suis*. Treatment with *T. suis* afforded healing of the inflammation as seen by flexible sigmoidoscopy. Numbers in upper left corner indicate location (centimeters from anal orifice) where photograph was made.

http://www.ovamed.org/english/home/home.html



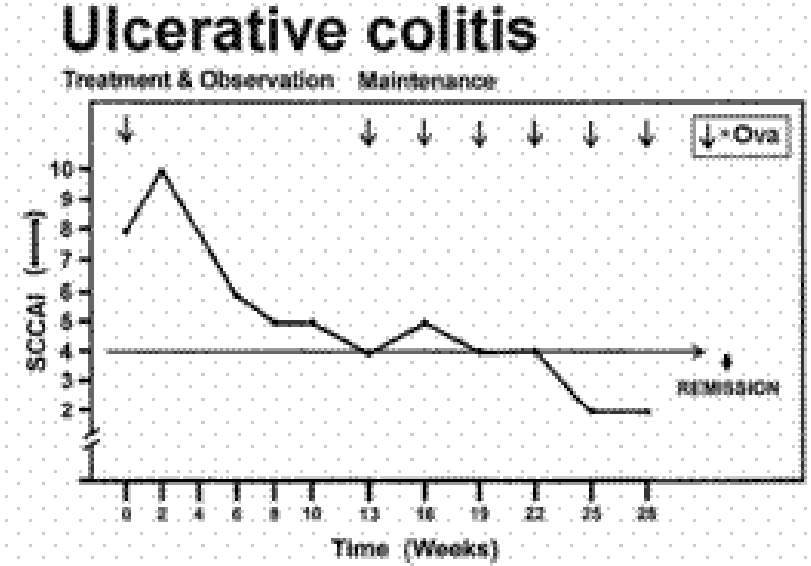




Figure 4. Response profiles of a Crohn's disease patient and an ulcerative colitis patient treated with *T. suis* ova. Adapted from Summers, et. al., Amer. J. Gastro. 98:2034, 2003

# A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors

J Croese, J O'Neil, J Masson, S Cooke, W Melrose, D Pritchard and R Speare

*Gut* 2006;55;136-137
doi:10.1136/gut.2005.079129

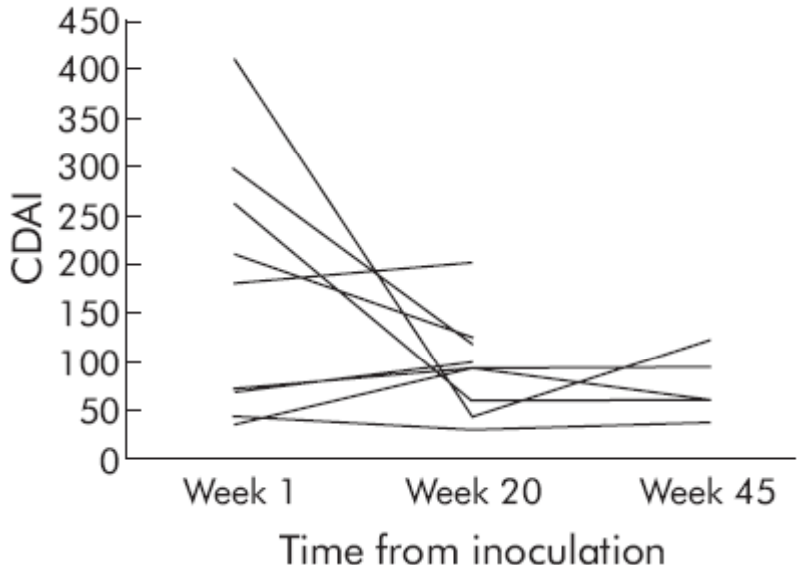
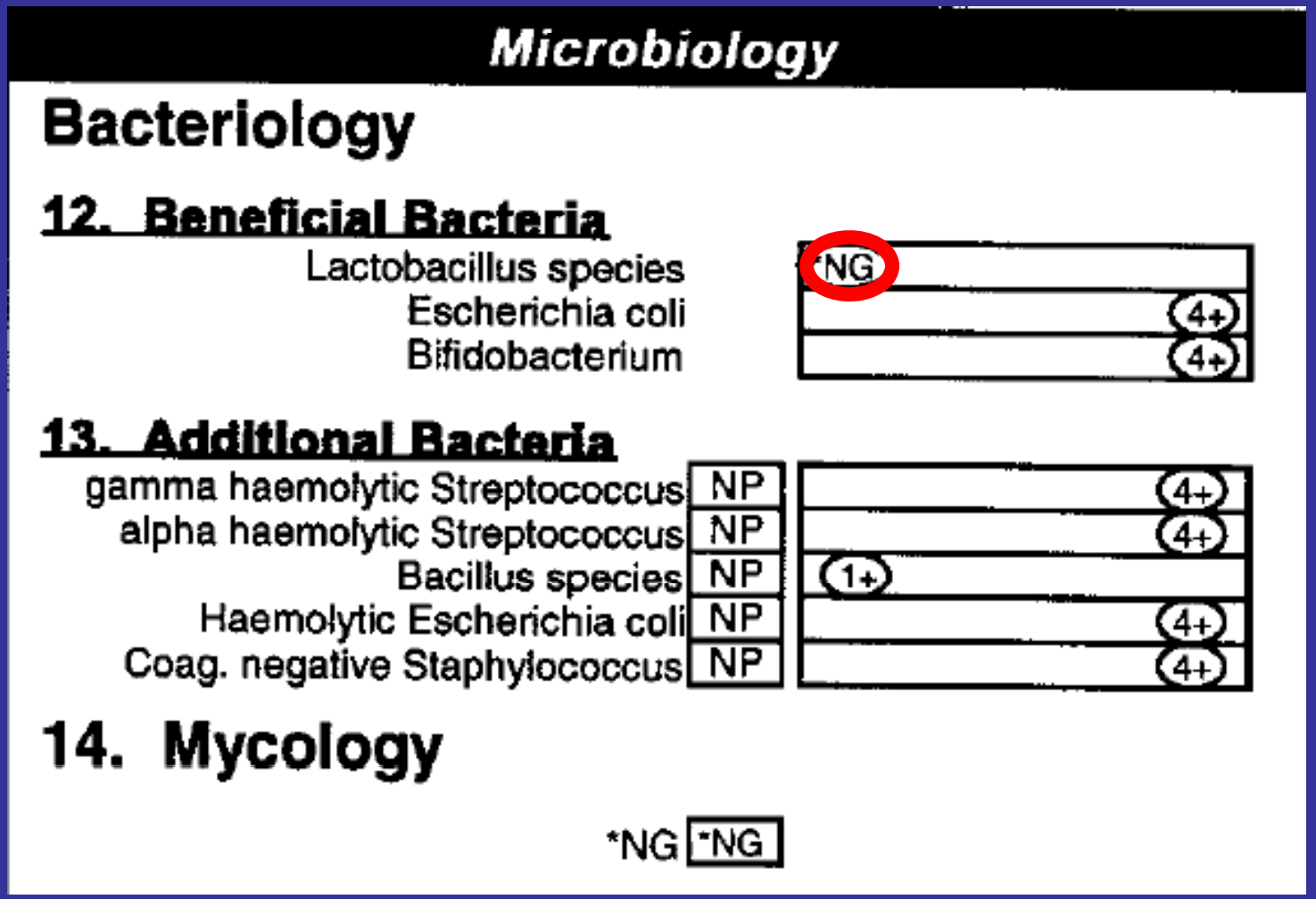



**Figure 1** Initial Crohn's disease activity index (CDAI) score for each CD patient versus score at week 20 and at week 45 for the first five inoculated cases (mean 165 (95% confidence interval 145) *v* 64 (25), p = 0.132; mean 165 *v* 75 (29), p = 0.246).

# Gut Immune Issues with Significant Allergic Component: Age 21mo



**Microbiology**

## Bacteriology

### 12. Beneficial Bacteria

| | | |
|---|---|---|
| Lactobacillus species | | *NG |
| Escherichia coli | | 4+ |
| Bifidobacterium | | 4+ |

### 13. Additional Bacteria

| | | |
|---|---|---|
| gamma haemolytic Streptococcus | NP | 4+ |
| alpha haemolytic Streptococcus | NP | 4+ |
| Bacillus species | NP | 1+ |
| Haemolytic Escherichia coli | NP | 4+ |
| Coag. negative Staphylococcus | NP | 4+ |

## 14. Mycology

| | | |
|---|---|---|
| *NG | *NG | |

# Gut Immune Issues with Significant Allergic Component: Age 21mo

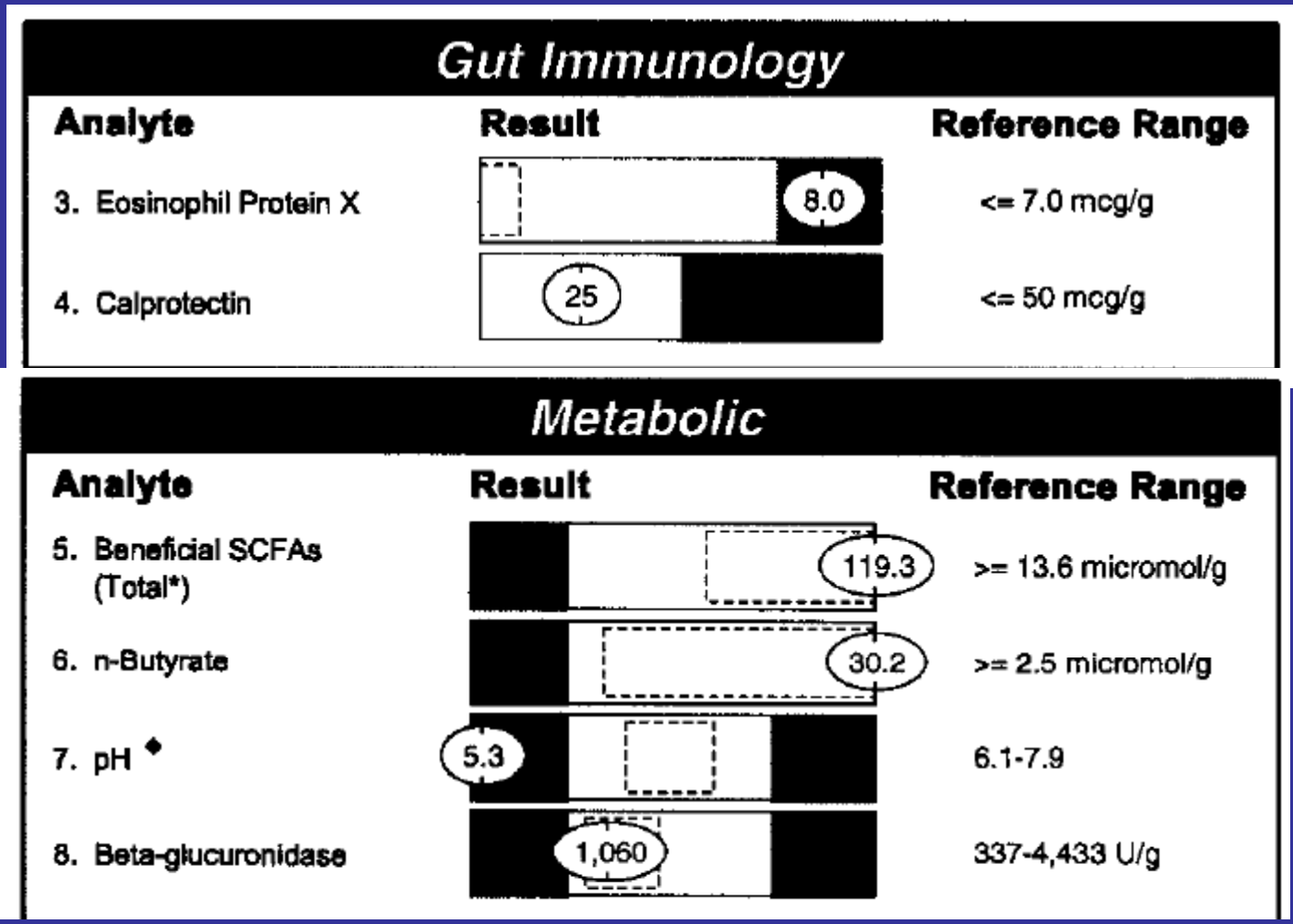
| Name | mmol/mol creatinine | | Reference range mmol/mol creatinine |
|---|---|---|---|
| **Yeast/Fungal** | | | |
| citramalic | 9.35 | H | 0 - 2.0 |
| 5-hydroxymethyl-2-furoic | 33.63 | | 0 - 80.0 |
| 3-oxoglutaric | 7.31 | H | 0 - 0.5 |
| furan-2,5-dicarboxylic | 11.33 | | 0 - 50.0 |
| furancarbonylglycine | 30.94 | | 0 - 60.0 |
| tartaric | 10.46 | | 0 - 16.0 |
| arabinose | 2746.80 | H | 0 - 47.0 |
| carboxycitric | 10.18 | | 0 - 46.0 |
| **Bacterial** | | | |
| 2-hydroxyphenylacetic | 3.81 | | 0 - 10.0 |
| 4-hydroxyphenylacetic | 108.70 | H | 0 - 50.0 |
| **Anaerobic Bacterial** | | | |
| HPHPA | 331.67 | H | 0 - 150.0 |
| VMA analog | 2.78 | | 0 - 31.0 |

# Gut Immune Issues with Significant Allergic Component: Age 21mo

- Treatment here requires more consideration for food allergy.
- Gastrocrom blocks histamine release from allergy cells.
- Enzymes break food down more quickly so it is less antigenic – maybe?
- Rotation of foods (challenging in ASD) varies the allergens.
- Antifungals and Antibiotics remove pathogens.
- Probiotics rebuild immune response.
- Anti-inflammatories may be required.

Androgen (Male Hormone Issues)
Not that common in our practice.
Case of Immune, Gut and
Androgen Problems all Combined

# Immune, Gut and Androgen Dysregulation: Age 3



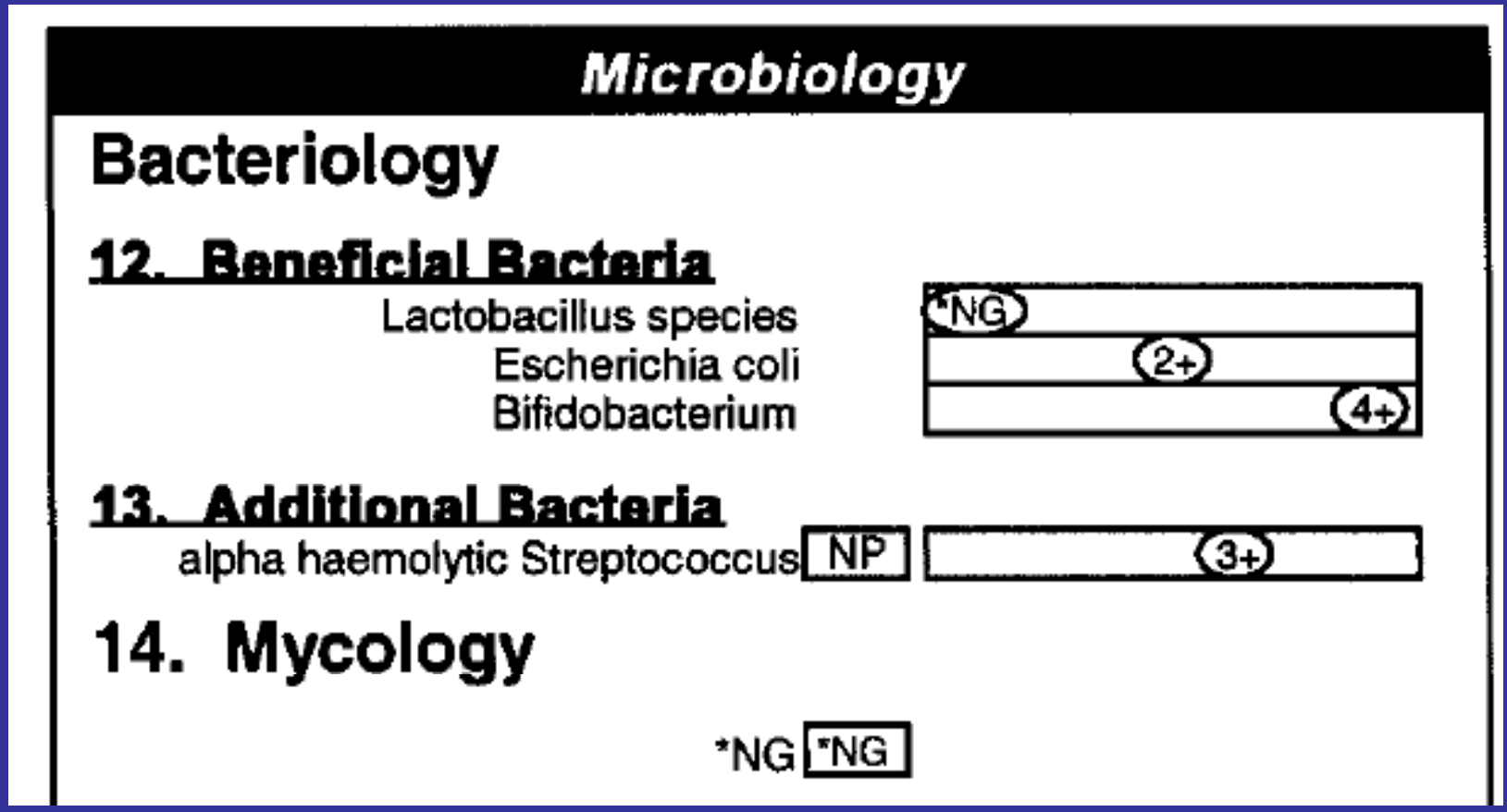
## Gut Immunology

| Analyte | Result | Reference Range |
|---|---|---|
| 3. Eosinophil Protein X | 8.0 | <= 7.0 mcg/g |
| 4. Calprotectin | 25 | <= 50 mcg/g |

## Metabolic

| Analyte | Result | Reference Range |
|---|---|---|
| 5. Beneficial SCFAs (Total*) | 119.3 | >= 13.6 micromol/g |
| 6. n-Butyrate | 30.2 | >= 2.5 micromol/g |
| 7. pH ◆ | 5.3 | 6.1-7.9 |
| 8. Beta-glucuronidase | 1,060 | 337-4,433 U/g |

# Immune and Androgen Dysregulation: Age 3



## Microbiology

### Bacteriology

**12. Beneficial Bacteria**

| | | |
|---|---|---|
| Lactobacillus species | | *NG |
| Escherichia coli | | 2+ |
| Bifidobacterium | | 4+ |

**13. Additional Bacteria**

| | | |
|---|---|---|
| alpha haemolytic Streptococcus | NP | 3+ |

### 14. Mycology

| | |
|---|---|
| *NG | *NG |

# Immune and Androgen Dysregulation: Age 3

## *Organic Acid Profile*

| Compound | Reference Range mmol/mol creatinine | Patient Value | |
|---|---|---|---|
| **Yeast/Fungal** | | | |
| citramalic | 0.0 - 2.0 | 1.00 | |
| 5-hydroxymethyl-2-furoic | 0.0 - 80.0 | 0.57 | |
| 3-oxoglutaric | 0.0 - 0.5 | 0.40 | |
| furan-2,5-dicarboxylic | 0.0 - 50.0 | 0.42 | |
| furancarbonylglycine | 0.0 - 60.0 | 0.42 | |
| tartaric | 0.0 - 16.0 | 0.29 | |
| arabinose | 0.0 - 47.0 | 47.80 | H |
| carboxycitric | 0.0 - 46.0 | 2.27 | |
| **Bacterial** | | | |
| 2-hydroxyphenylacetic | 0.0 - 10.0 | 0.55 | |
| 4-hydroxyphenylacetic | 0.0 - 50.0 | 77.63 | H |
| HPHPA | 0.0 - 150.0 | 788.99 | H |
| VMA analog | 0.0 - 31.0 | 1.15 | |

# Immune and Androgen Dysregulation: Age 3

| | patient µmole / mole Cr | reference | result |
|---|---|---|---|
| ***urinary Neopterine (NEO)*** | 444 | 110-180 | increased cellular immunity activation |
| ***Urinary Biopterin*** | 796 | | |
| ***Biopeterin / Neopterin*** | 1,6 | >1,5 | favorable ratio |

INTERPRETATION: This serum showed IgG immunostaining to brain endothelial cells. This pattern of staining has been described in some children with Landau Kleffer variant and Autism.

INTERPRETATION: This serum showed IgM immunostaining to brain endothelial cells. This pattern of staining has been described in some children with Landau Kleffnar variant and Autism.

Anne Connolly

"The observed increase in urinary native neopterin in autism agrees with our previous observations and indicates activation of cellular immunity in these children thus supporting the possible involvement of **autoimmunity** in the pathogenesis of autism."

**Messahel et al, Neuroscience Letters 241 (1998) 17–20**

| | | | | | |
|---|---|---|---|---|---|
| **Immunoglobulins A/E/G/M, Serum** | | | | | |
| Immunoglobulin A, Qn, Serum | 83 | | mg/dL | 20 - 100 | 01 |
| Immunoglobulin M, Qn, Serum | 142 | | mg/dL | 19 - 146 | 01 |
| Immunoglobulin E, Total | 27 | | IU/mL | 0 - 352 | 01 |
| **Testosterone,Free and Total** | | | | | |
| **Testosterone, Serum** | **28** | **High** | ng/dL | 0 - 10 | 01 |
| Free Testosterone(Direct) | <0.2 | | pg/mL | Not Estab. | 02 |
| **Dihydrotestosterone** | | | | | |
| Dihydrotestosterone | | | | | |
| Test not performed | | | ng/dL | | 03 |
| ***Quantity not sufficient*** | | | | | |
| **Androstenedione** | 19 | | ng/dL | | 02 |

| Age | Male |
|---|---|
| 0- 5 mo | 5- 45 |
| 6-11 mo | 5- 30 |
| 1- 5 yr | 5- 45 |
| 6- 9 yr | 5- 55 |
| 10-11 yr | 10- 30 |
| 12-14 yr | 20- 85 |
| 15-17 yr | 35-100 |
| Tanner Stage | |
| I(<7 yr) | <35 |
| I(>7 yr) | <49 |
| II | <89 |
| III | 11-91 |
| IV | <219 |

| | | | | | |
|---|---|---|---|---|---|
| **Dehydroepiandrosterone Sulfate** | | | | | |
| **DHEA-Sulfate** | **40** | **High** | ug/dL | 0 - 29 | 01 |
| **Dehydroepiandrosterone (DHEA)** | 166 | | ng/dL | | 02 |

| Age | Male |
|---|---|
| 0- 5 yr | 26 - 72 |
| 6- 7 yr | 29 - 66 |
| 8- 9 yr | 53 - 135 |
| 10-11 yr | 183 - 383 |
| 12-14 yr | 240 - 520 |

# Intervention in this case

- If Bone Age reveals no bone growth acceleration the main concern is immune activation.
- Spironolactone is a unique choice in this situation.
- Combined with Gastrocrom, enzymes and dysbiosis therapy (Vancocin and probiotics) significant improvement occurred in all areas of ASD symptoms.
- Lupron is an additional option for the elevated male hormones but it is not anti-inflammatory.
- HBOT for vasculitis issues 1.5 to 1.75 ATA

Medical Hypotheses (2007) 68, 979–987

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http://intl.elsevierhealth.com/journals/mehy

# Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders

James Jeffrey Bradstreet [a,*], Scott Smith [a], Doreen Granpeesheh [b], Jane M. El-Dahr [c], Daniel Rossignol [d]

[a] *International Child Development Resource Center, Melbourne and Florida Hospital, Celebration, 1688 West Hibiscus Boulevard, Melbourne, FL 32901, United States*
[b] *Center for Autism Related Disorders, Tarzana, CA, United States*
[c] *Tulane University Health Sciences Center, Departments of Pediatrics and Medicine, Section of Pediatric Allergy, Immunology and Rheumatology, New Orleans, LA, United States*
[d] *University of Virginia, Department of Family Medicine, P.O. Box 800729, Charlottesville, VA, United States*

# Lupron = Leuprolide Acetate Depo


Monthly Dosing will reduce the Androgen burden if Spironolactone fails, may consider starting with Lupron and changing to Spritonolactone after levels are under control for 3 months. Cost ~ $800/month for 7.5 and $1500 for 15 mg

# Ammonia and Lactate Problems

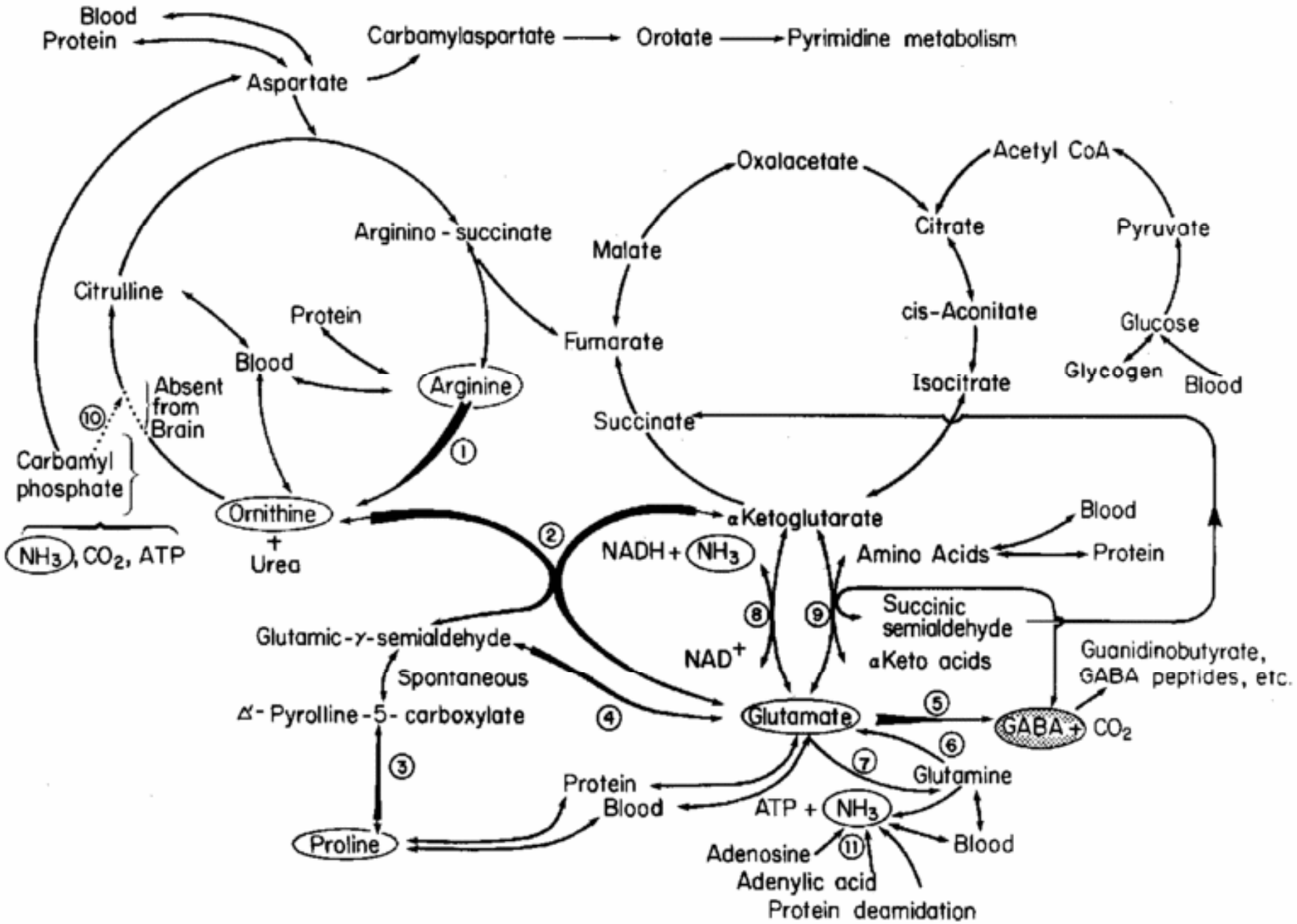
| | | | | | |
|---|---|---|---|---|---|
| ***Lactic Acid, Plasma*** | ***36.0*** | ***Alert*** | mg/dL | 4.5 - 19.8 | 01 |
| ***Ammonia, Plasma*** | ***302*** | ***Alert*** | ug/dL | 27 - 102 | 02 |

**AMMONIA**

| Male and Female | | | |
|---|---|---|---|
| **Test** | **Age** | **n** | **µmol/L** |
| 1 | All ages | * | <33 |
| 2 | Newborn | ** | <50 |
| | Child and adult | | <35 |
| 3 | <30 d | 87 | 21–95 |
| | 1–12 mo | 91 | 18–74 |
| | 1–14 y | 94 | 17–68 |
| | <14 y | 182 | 22–66 |
| | Men | 89 | 21–71 |
| | Women | 93 | 19–63 |
| 4 | All ages | *** | <51 |

| **Specimen Type(s)** | | |
|---|---|---|
| | 1,2 | Plasma |
| | 3 | Whole blood, potassium EDTA as anticoagulant |
| | 4 | Plasma/serum |

Be Cautious: Different labs use different control units


Blood
Protein
Carbamylaspartate
Orotate
Pyrimidine metabolism
Aspartate
Oxalacetate
Acetyl CoA
Arginino - succinate
Malate
Citrate
Pyruvate
Citrulline
Protein
cis-Aconitate
Glucose
Fumarate
Blood
Arginine
Isocitrate
Glycogen
Blood
Absent from Brain
10
Succinate
Carbamyl phosphate
1
Ornithine
+
Urea
αKetoglutarate
Blood
2
NADH + NH3
Amino Acids
Protein
8
9
Succinic semialdehyde
Glutamic-γ-semialdehyde
NAD+
αKeto acids
Guanidinobutyrate, GABA peptides, etc.
Spontaneous
Δ'-Pyrolline-5-carboxylate
4
Glutamate
5
GABA + CO2
6
7
Glutamine
3
Protein
Blood
ATP + NH3
Proline
Blood
Adenosine
11
Adenylic acid
Protein deamidation
NH3, CO2, ATP

# NH3 - Lactate Interventions

- If confirmed to be this elevated must eval with muscle biopsy for Mitochondrial Disorder
- Reduce protein intake if it is significant.
- Antioxidants
- Alpha-ketoglutarate is one of the most important nitrogen transporter in metabolic pathways. The amino groups of amino acids are attached to it by transamination and carried to the liver where the urea cycle takes place. 3 ATPs per Urea
- Alpha-ketoglutarate is transaminated, along with glutamine, to form the excitatory neurotransmitter glutamate. Glutamate can then be decarboxylated (requiring vitamin B6) into the inhibitory neurotransmitter GABA.

# 7 yo boy with persistent severe OCD and ASD issues

| | | | | |
|---|---|---|---|---|
| ANTI-STREPTOLYSIN O | | 1502 | H | <150 IU/mL |
| IMMUNOGLOBULIN A | 163 | | | 41-368 mg/dL |
| IMMUNOGLOBULIN M | 85 | | | 47-311 mg/dL |
| DNASE-B ANTIBODY | >1360 | | | titer |

Reference range:

| | |
|---|---|
| Pre-school: | =< 60 titer |
| School: | =< 170 titer |
| Adult: | =< 85 titer |

# Antibiotic Prophylaxis with Azithromycin or Penicillin for Childhood-Onset Neuropsychiatric Disorders

Lisa A. Snider, Lorraine Lougee, Marcia Slattery, Paul Grant, and Susan E. Swedo

***Background:*** *The acronym PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) describes a subgroup of children with obsessive-compulsive disorder and/or tic disorder that experience symptom exacerbations following streptococcal infections. We hypothesized that the prevention of streptococcal infections among children in the PANDAS subgroup would decrease neuropsychiatric symptom exacerbations.*

***Methods:*** *Twenty-three subjects with PANDAS were enrolled in a double blind, randomized controlled trial. Antibiotic prophylaxis with penicillin or azithromycin was administered for 12 months. Rates of streptococcal infections and neuropsychiatric symptom exacerbations were compared between the study year and the baseline year prior to entry.*

***Results:*** *Significant decreases in streptococcal infections during the study year were found with a mean of .1 (.3 SD) per subject, compared to the baseline year with 1.9 (1.2 SD) in the penicillin group and 2.4 (1.1 SD) in the azithromycin group [$p<.01$]. Significant decreases in neuropsychiatric exacerbations during the study year were also found with a mean of .5 (.5 SD) per subject in the penicillin group and .8 (.6 SD) in the azithromycin group, compared to the baseline year with 2.0 (.9 SD) in the penicillin group and 1.8 (.6 SD) in the azithromycin group [$p<.01$].*

***Conclusions:*** *Penicillin and azithromycin prophylaxis were found to be effective in decreasing streptococcal infections and neuropsychiatric symptom exacerbations among children in the PANDAS subgroup.*

BIOL PSYCHIATRY 2005;57:788–792

# Heavy Metal and the use of Biomarkers

INSURANCE???

J.S. Woods et al. / Toxicology and Applied Pharmacology 206 (2005) 113–120

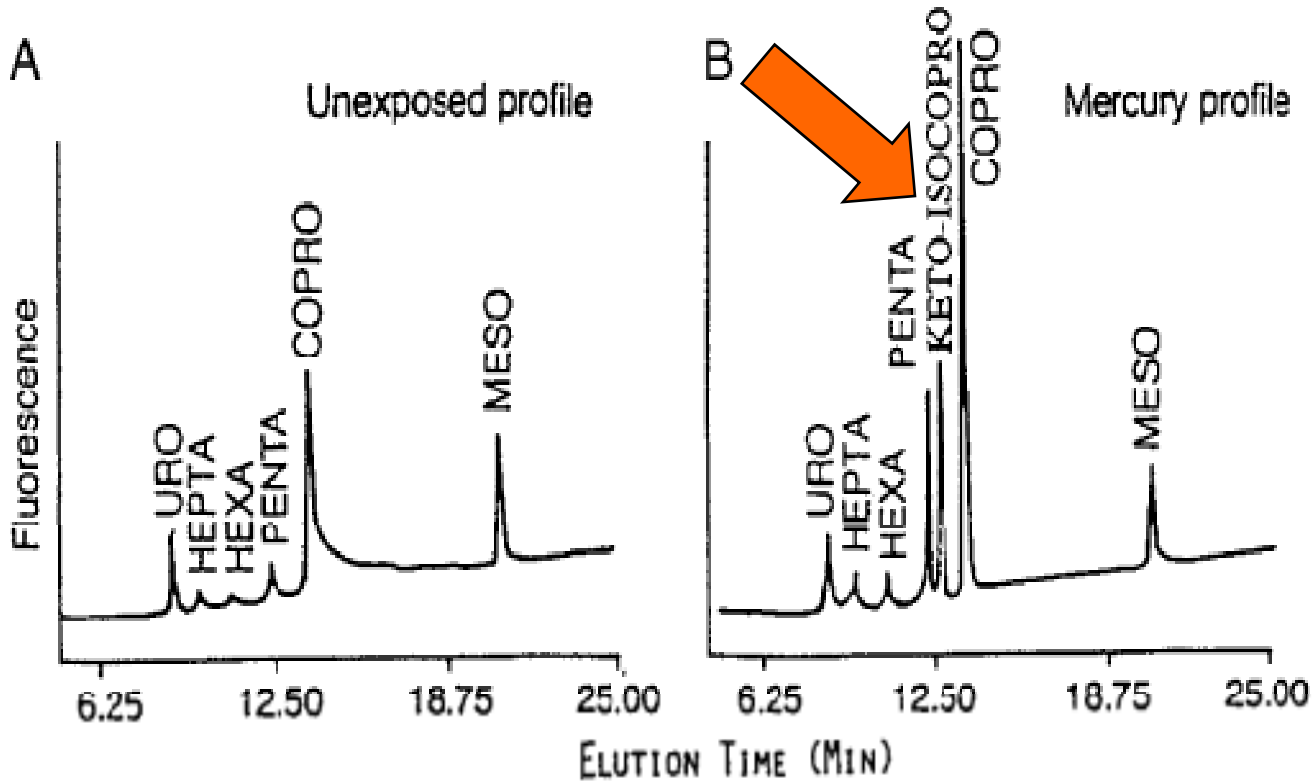
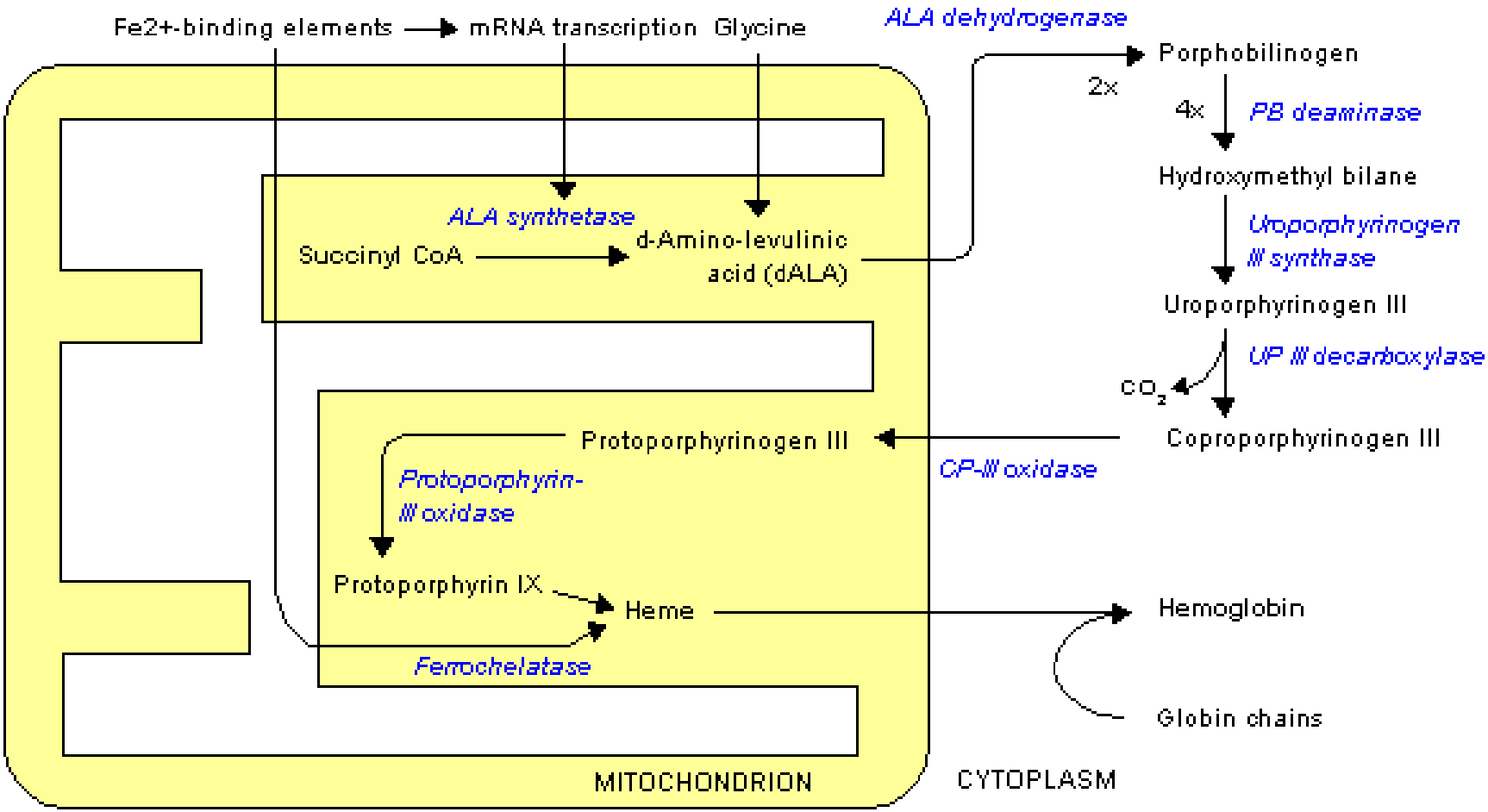



Fig. 1. HPLC urinary porphyrin profiles from: (A) unexposed and (B) mercury-exposed subjects. Abscissa is elution time in minutes. Ordinate is relative porphyrin concentration in fluorescence units. Porphyrins were measured as described in Methods. URO = uroporphyrin, HEPTA = hepta-carboxyporphyrin, HEXA = hexa-carboxylporphyrin, PENTA = pentacarboxylporphyrin, COPRO = coproporphyrin, KETO-ISOCOPRO = ketoisocoproporphyrin, MESO = mesoporphyrin (an internal standard).

THE ATYPICAL KETO-ISOCOCPROPHYRIN = PRECO IN NATAF STUDY

# Porphyrin pathway



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Toxicology and Applied Pharmacology 214 (2006) 99–108
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# Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity

Robert Nataf [a], Corinne Skorupka [b], Lorene Amet [b], Alain Lam [a], Anthea Springbett [c], Richard Lathe [d,*]

[a] *Laboratoire Philippe Auguste, Paris, France*
[b] *Association ARIANE, Clichy, France*
[c] *Department of Statistics, Roslin Institute, Roslin, UK*
[d] *Pieta Research, PO Box 27069, Edinburgh EH10 5YW, UK*

Received 13 February 2006; revised 23 March 2006; accepted 5 April 2006

**Abstract**

To address a possible environmental contribution to autism, we carried out a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 269 children with neurodevelopmental and related disorders referred to a Paris clinic (2002–2004), including 106 with autistic disorder. Urinary porphyrin levels determined by high-performance liquid chromatography were compared between diagnostic groups including internal and external control groups. Coproporphyrin levels were elevated in children with autistic disorder relative to control groups. Elevation was maintained on normalization for age or to a control heme pathway metabolite (uroporphyrin) in the same samples. The elevation was significant ($P < 0.001$). Porphyrin levels were unchanged in Asperger's disorder, distinguishing it from autistic disorder. The atypical molecule precoproporphyrin, a specific indicator of heavy metal toxicity, was also elevated in autistic disorder ($P < 0.001$) but not significantly in Asperger's. A subgroup with autistic disorder was treated with oral dimercaptosuccinic acid (DMSA) with a view to heavy metal removal. Following DMSA there was a significant ($P = 0.002$) drop in urinary porphyrin excretion. These data implicate environmental toxicity in childhood autistic disorder.

# Higher Porphyrins Associated with Increased Severity

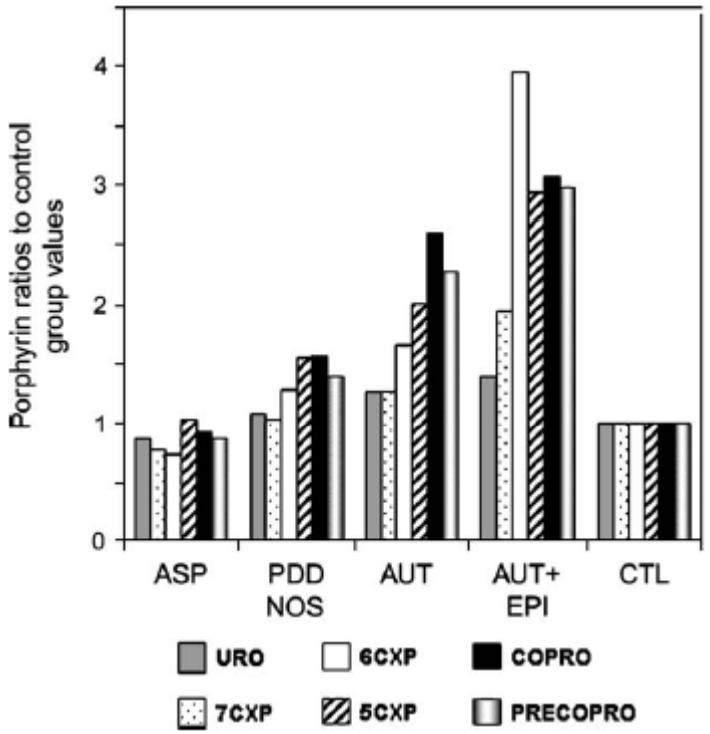


Fig. 5. Spectrum of mean (creatinine-normalized) porphyrin excess, expressed as a ratio of control group (CTL) values, for the different porphyrin subtypes uroporophyrin (URO), hepta-, hexa- and pentacarboxyporphyrin (7-, 6-, 5CXP), coproporphyrin (COPRO) and precoproporphyrin (PRECOPRO) in different conditions: ASP, Asperger disorder; PDD-NOS, pervasive developmental disorder not otherwise specified; AUT, autism; AUT + EPI, autism with epilepsy.

*Pediatrics International* (2008) **50**, 1–5 doi: 10.1111/j.1442-200x.2008.02621.x

Original Article

# Porphyrinuria in childhood autistic disorder is not associated with urinary creatinine deficiency

Robert Nataf,[1] Corinne Skorupka,[2] Alain Lam,[1] Anthea Springbett[3] and Richard Lathe[4]

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**Abstract** ***Background***: Urinary metabolite measurements are often normalized to levels of the ubiquitous metabolite creatinine (CRT) to take account of variations in fluid export. Following CRT normalization, excesses of porphyrins and isoprostanes have been reported in the urines of children with neurodevelopmental disorders. It was suggested (Whiteley *et al.*, 2006, *Pediatr. Int.* 2006; **48**: 292–297) that urinary CRT levels may be depressed in children with autism spectrum disorders. This prompted re-evaluation of CRT levels in such children.

***Methods***: First matinal urinary CRT levels were compared between subjects in different diagnostic categories including autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS) and hyperactivity, before and after correction for age and gender. A larger reference group, consisting of subjects with unrelated disorders and Asperger disorder, with no reported porphyrin excess, was also compared to the group with autistic disorder, both for CRT and for porphyrin (coproporphyrin, COPRO) excess.

***Results***: No significant difference in CRT was observed between any of the categories analyzed, also when corrected for age and gender. In contrast, urinary COPRO levels were significantly higher in autistic disorder versus reference groups, either when expressed as absolute values (independent of CRT levels) or when normalized to CRT.

***Conclusions***: These data do not support a systematic reduction in urinary CRT levels in subjects with autism spectrum disorders including autistic disorder and PDD-NOS. Urinary COPRO excess in autistic disorder was not associated with or consequent upon urinary CRT deficiency. Differences between affected and control subjects in age and sampling time, as reported by Whiteley *et al.*, may underlie the apparent CRT reduction.

## NUTRIENT ELEMENTS

| ELEMENTS | RESULT µg/g | REFERENCE RANGE | PERCENTILE 2.5th 16th 50th 84th 97.5th |
|---|---|---|---|
| Calcium | **17** | 8- 31 | |
| Magnesium | **45** | 36- 64 | |
| Potassium mEq/L | **74** | 65- 95 | |
| Phosphorus | **549** | 480- 745 | |
| Copper | **0.72** | 0.52- 0.89 | |
| Zinc | **10.9** | 8- 14.5 | |
| Iron | **960** | 745- 1050 | |
| Manganese | **0.015** | 0.007- 0.030 | |
| Chromium | **0.0015** | 0.0003-0.0060 | |
| Selenium | **0.35** | 0.19- 0.38 | |
| Boron | **0.065** | 0.01- 0.110 | |
| Vanadium | **0.0002** | 0.0001-0.0020 | |
| Molybdenum | **0.0007** | 0.0005-0.0020 | |

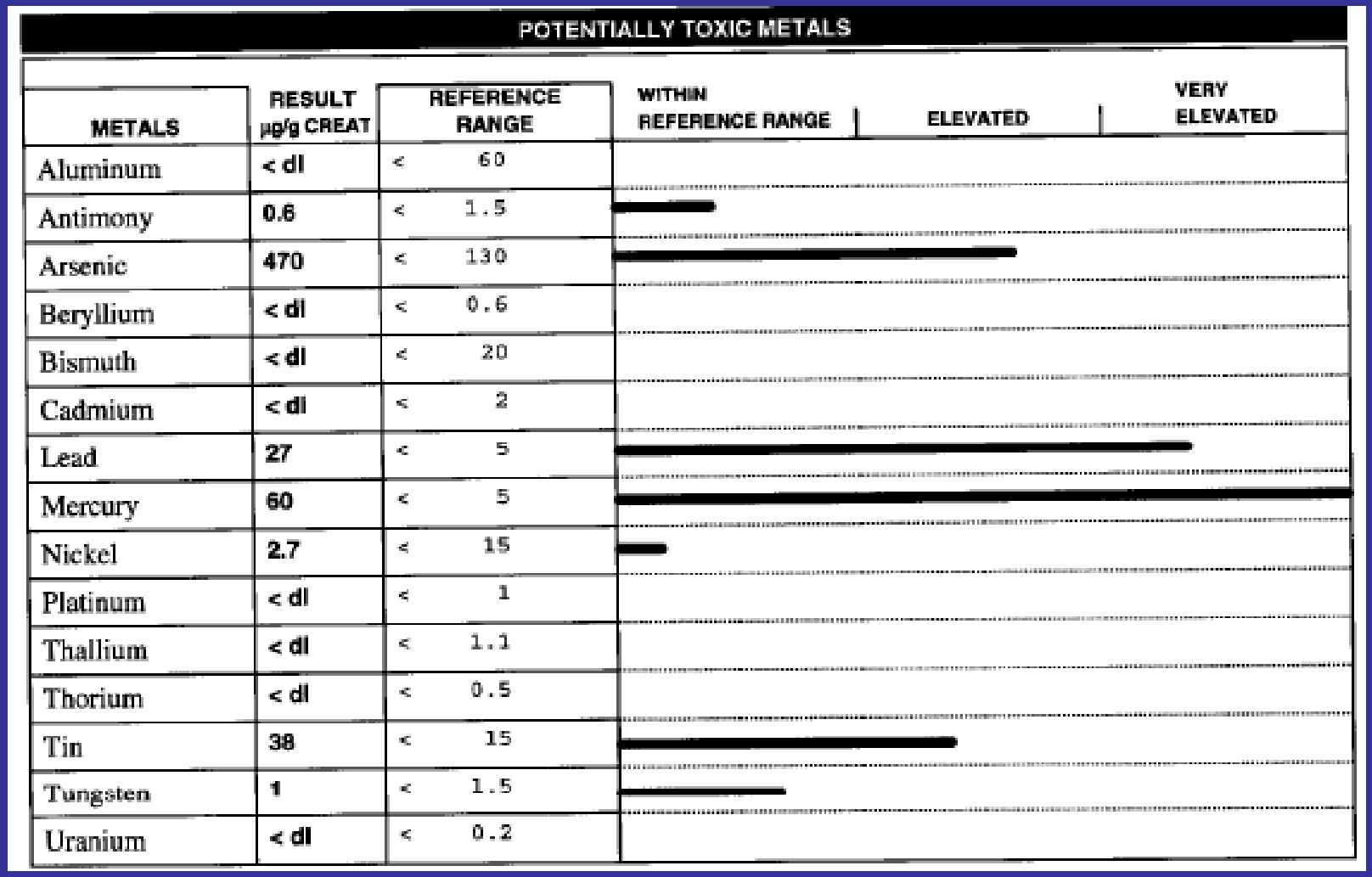
## POTENTIALLY TOXIC ELEMENTS

| TOXIC ELEMENTS | RESULT µg/g | REFERENCE RANGE | PERCENTILE 95th 99th |
|---|---|---|---|
| Arsenic | **0.011** | < 0.010 | |
| Cadmium | **0.001** | < 0.005 | |
| Lead | **0.073** | < 0.090 | |
| Mercury | **0.013** | < 0.010 | |
| Thallium | **0.0001** | < 0.0005 | |

# Markedly Elevated Porphyrins Pre

| | | | | |
|---|---|---|---|---|
| uP | UROPORPHYRIN | 45<br>*5* | nmol<br>% | 7-14 |
| 7cxP | HEPTACARBOXYPORPHYRIN | 11.1<br>*1.4* | nmol<br>% | 1.5-3.5 |
| 6cxP | HEXACARBOXYPORPHYRIN | 4.9<br>*0.6* | nmol<br>% | 0.4-0.8 |
| 5cxP | PENTACARBOXYPORPHYRIN | 14.2<br>*1.8* | nmol<br>% | 1.0-2.9. |
| prcP | PRECOPROPORPHYRIN | 48.4<br>*6.3* | nmol<br>% | 2-5 |
| cP | COPROPORPHYRIN | 627<br>*87* | nmol<br>% | 50-90 |

# Response to DMPS IV



**POTENTIALLY TOXIC METALS**

| METALS | RESULT µg/g CREAT | REFERENCE RANGE | WITHIN REFERENCE RANGE / ELEVATED / VERY ELEVATED |
|---|---|---|---|
| Aluminum | < dl | < 60 | |
| Antimony | 0.6 | < 1.5 | |
| Arsenic | 470 | < 130 | |
| Beryllium | < dl | < 0.6 | |
| Bismuth | < dl | < 20 | |
| Cadmium | < dl | < 2 | |
| Lead | 27 | < 5 | |
| Mercury | 60 | < 5 | |
| Nickel | 2.7 | < 15 | |
| Platinum | < dl | < 1 | |
| Thallium | < dl | < 1.1 | |
| Thorium | < dl | < 0.5 | |
| Tin | 38 | < 15 | |
| Tungsten | 1 | < 1.5 | |
| Uranium | < dl | < 0.2 | |

# Response to CaEDTA

**POTENTIALLY TOXIC METALS**

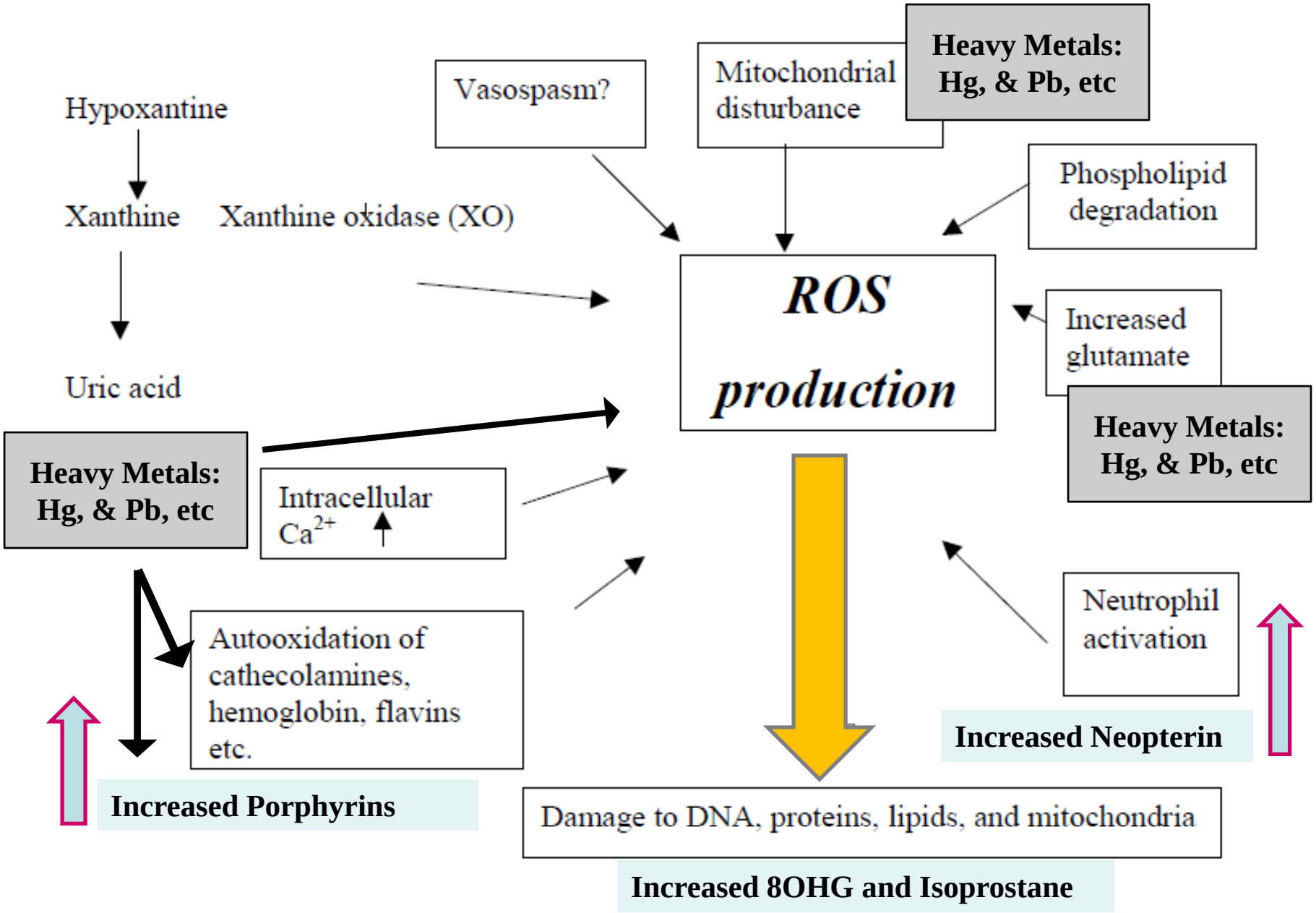
| METALS | RESULT µg/g CREAT | REFERENCE RANGE | WITHIN REFERENCE RANGE / ELEVATED / VERY ELEVATED |
|---|---|---|---|
| Aluminum | 73 | < 25 | |
| Antimony | < dl | < 0.6 | |
| Arsenic | 240 | < 120 | |
| Beryllium | < dl | < 0.5 | |
| Bismuth | < dl | < 10 | |
| Cadmium | 0.8 | < 2 | |
| Lead | 120 | < 5 | |
| Mercury | < dl | < 3 | |
| Nickel | 9.4 | < 10 | |
| Platinum | < dl | < 1 | |
| Thallium | 0.1 | < 0.7 | |
| Thorium | < dl | < 0.3 | |
| Tin | 2.2 | < 9 | |
| Tungsten | 0.6 | < 0.7 | |
| Uranium | < dl | < 0.1 | |

# After a 6 Month Course - Improvement

## Urinary porphyrins

HPLC-UV+Fluorescence

| | nmol/l | nmol/gCr | % | reference nmol/gCr | interpretation |
|---|---|---|---|---|---|
| **Uroporphyrins I &III (UP)** | *12* | **39** | 9,8% | *8-20* | Increased rate |
| **Heptacarboxy porphyrin (7cxP)** | *2,1* | **7,1** | 1,7% | *2,5-4,5* | Increased rate |
| **Hexacarboxy porphyrin (6cxP)** | *0,8* | **2,6** | 0,7% | *0,5-1,5* | Increased rate |
| **Pentacarboxy porphyrin (5cxP)** | *1,7* | **6,6** | 1,4% | *2-4* | Increased rate |
| **Precoproporphyrin (PrCP)** | *7,0* | **22,0** | 5,7% | *5-9* | strongly increased rate |
| **Coproporphyrins I & III (CP)** | *99* | **331** | 85,6% | *100-200* | Increased rate |
| **PrCP/UP** PrecoP/Uro ratio | | **0,57** | | 0,2-0,5 | |
| **(5cxP+PrCP)/(UP+7cxP)** ratio | | **0,6** | | 0,3-0,6 | |
| **PrCP/5cxP** | | **3,9** | | 1,5-3 | |
| **PrCP/CP** PrecoP/COP ratio | | **6,6** | % | 2-6 | |
| **CP / UP** copro/uro ratio | | **8,50** | | 5-9 | |


Hypoxantine
Xanthine
Xanthine oxidase (XO)
Uric acid
Vasospasm?
Mitochondrial disturbance
Heavy Metals: Hg, & Pb, etc
Phospholipid degradation
ROS production
Increased glutamate
Heavy Metals: Hg, & Pb, etc
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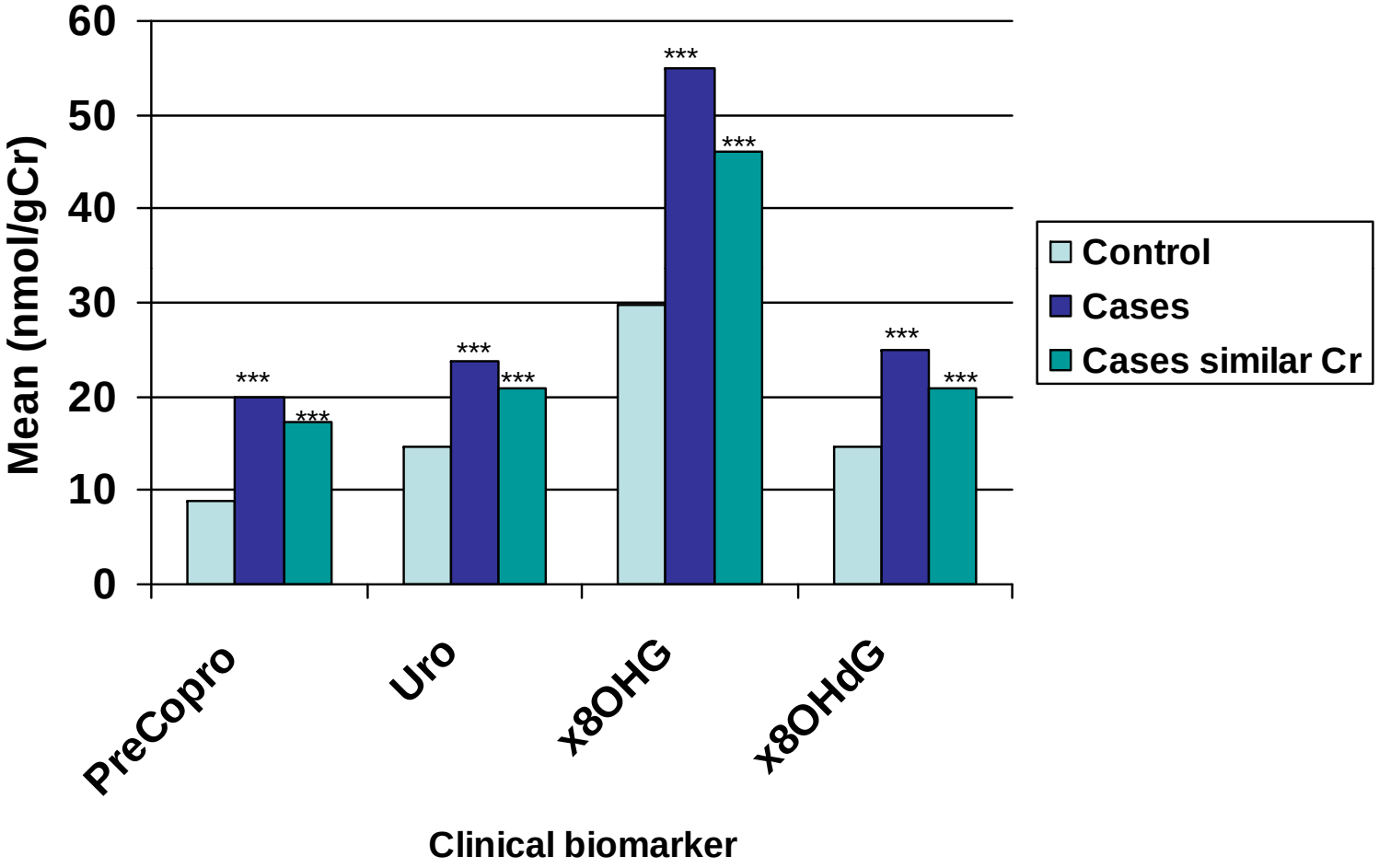
Intracellular $Ca^{2+}$
Autooxidation of cathecolamines, hemoglobin, flavins etc.
Neutrophil activation
Increased Porphyrins
Increased Neopterin
Damage to DNA, proteins, lipids, and mitochondria
Increased 8OHG and Isoprostane

# Study Design

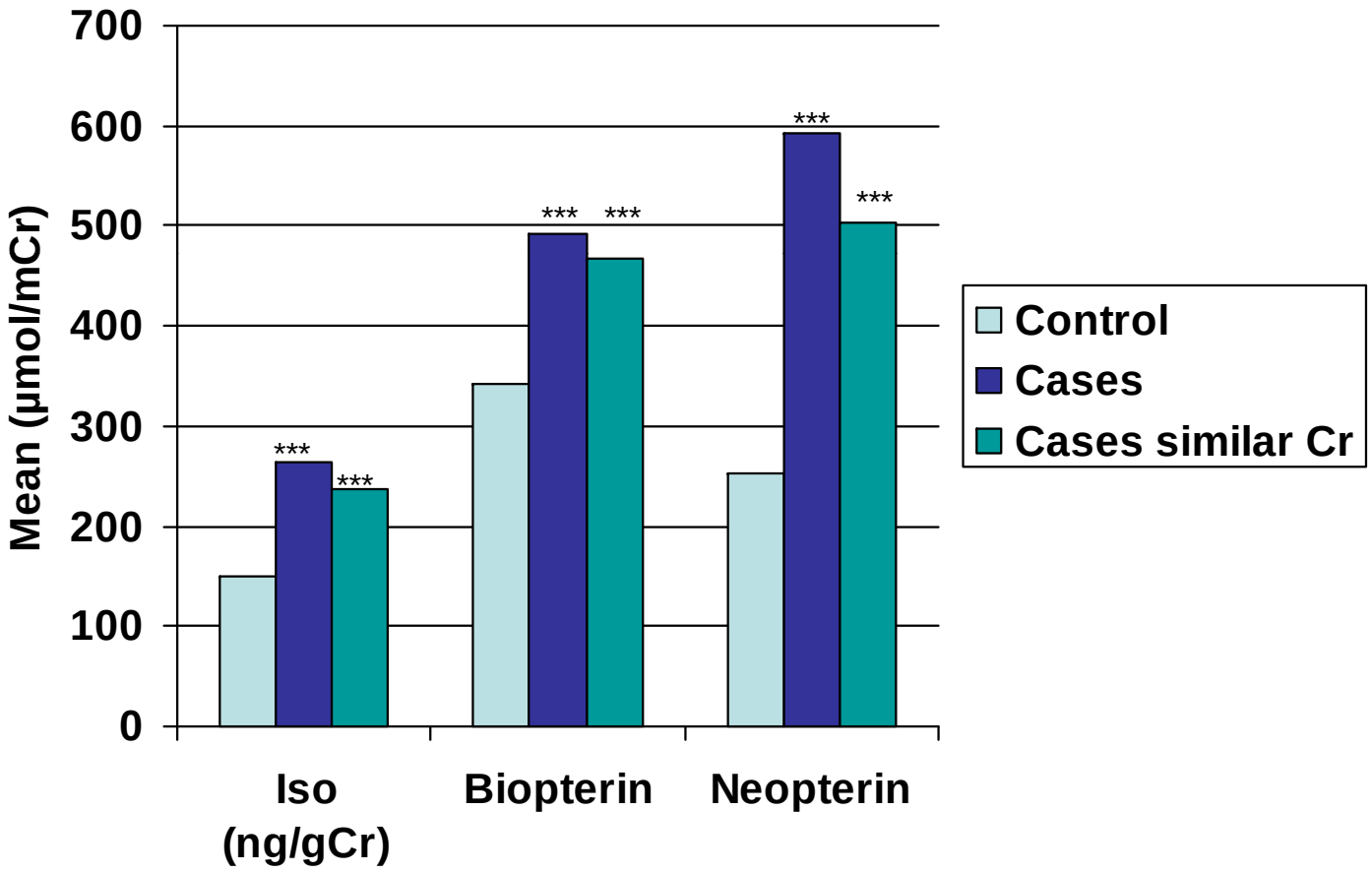
- Retrospective chart/database review of over 700 medical records.
- All charts reviewed to exclude prior chelation, antioxidant (apart from routine vitamins) & immune therapy (N=197  Cases)
- Practices in US, Norway and France
- Ages 1 through 8
- Evaluated Biomarkers prior to Intervention
- Controls were volunteers from US (>50%), Switzerland and France (N = 43)

# Clinical Biomarkers Between Cases (N = 86) and Controls ( N = 43)




P values compares controls with cases
*** = p <.001

# Clinical Biomarkers Between Cases (N = 86) and Controls ( N = 43)

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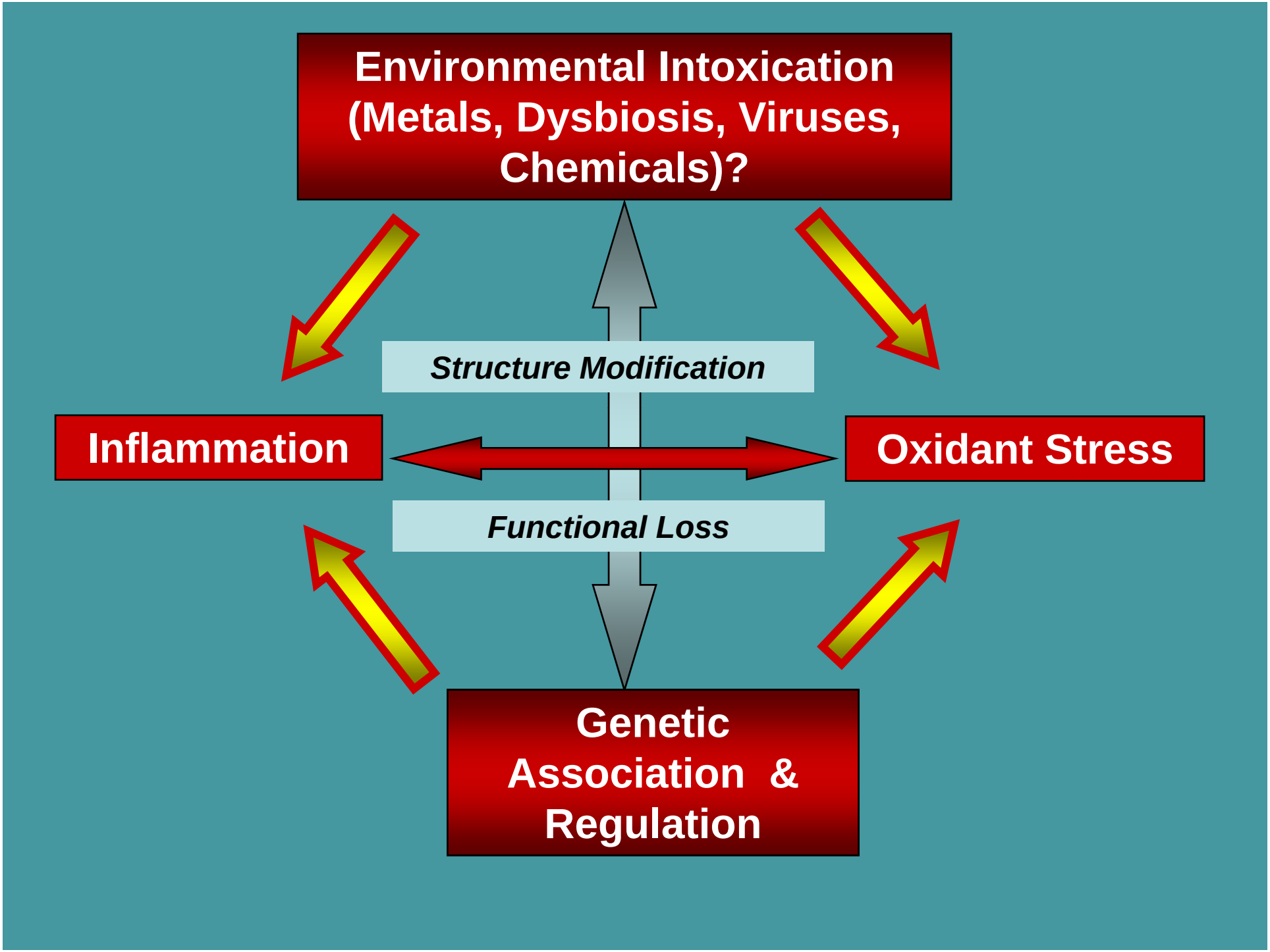
Environmental Intoxication (Metals, Dysbiosis, Viruses, Chemicals)?
Structure Modification
Inflammation
Oxidant Stress
Functional Loss
Genetic Association & Regulation

# Summary

- Follow the history, physical & make a medical diagnosis: eg encephalitis
- Obtain objective biomarkers whenever possible
- Check to make sure your treatment is effective at correcting underlying pathophysiology