A Window upon the Brain

Analysis of Neurotransmitters, Vitamins and Cofactors

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Cerebrospinal Fluid Biochemistry

Basic Biochemistry
- Glucose
- Protein
- Lactate & Pyruvate
- Pigments

Special Biochemistry
- Amino Acids
- Neurotransmitters
- Cofactors
- Vitamins
- Inflammatory Markers
Brain Special Biochemistry

- Biogenic Amines
- Tyrosine pathway metabolites
- Tryptophan pathway metabolites
- Dopamine metabolites  HVA
- Serotonin metabolites  5-HIAA
- Pteridines
- 5-methyltetrahydrofolate
- Histamine & metabolites
- Amino Acids - Tryptophan
- Kynurenine pathway
- B6 Vitamers

**Trace level analysis of unstable compounds.** Collect and immediately freeze at -40deg C. Use appropriate preservatives. Protect from light. Transport in dry ice. Thaw and immediately inject into the HPLC. Extreme care to avoid contamination and carryover.
Neurochemistry Laboratory

Sample Collection

Spot Urine (immediately frozen)

Cerebrospinal Fluid (CSF)
Collected in 5 tubes with various preservatives
0.5ml CSF added to each tube
frozen. Transport on dry ice
Immediately Details:
CSF Amino Acids
OPA Derivatisation

A Window upon the Brain

Metabolic Diseases
Deficiency Diseases
Inflammatory Diseases
Neurodegenerative Diseases

Brain Biochemistry
Biochemical Monitoring
Baby with raised Phenylalanine on Newborn Screen

Urine Biogenic Amine metabolites

HPLC with Amperometric Detection at 950mV

Tetrahydro biopterin
Tetrahydrobiopterin

Essential cofactor for phenylalanine, tyrosine and tryptophan hydroxylases and the biosynthesis of dopamine, noradrenaline, adrenaline, serotonin, melatonin

![Chemical structures and reactions](image)
Baby with raised phenylalanine on Newborn Screen – PCD deficiency

HPLC with Fluorescence Detection
350/450nm

Pteridines
Xanthopterin
Neopterin
Monapterin
Biopterin
7-Biopterin

Standards
PCD Urine
PCD Deficiency

Pterin-4a-carbinolamine Dehydratase (PCD)

- Identical with DCoH (dimerization cofactor for hepatocyte nuclear factor 1 (HNF-1))
- PCD gene on 10q22, 4 exons (5 kb)
- 12 kD subunit, homotetramer
- Genetic defect in HPA (orimanterinuria)
Amino Acid Decarboxylase Deficiency

Tyrosine Hydroxylase

L-Aromatic Amino Acid Decarboxylase

Tyrosine

L-DOPA

Dopamine

COMT

3-O-methylDOPA

Vanillylactic Acid

Tryptophan Hydroxylase

L-Aromatic Amino Acid Decarboxylase

Tryptophan

5-Hydroxytryptophan

5-Hydroxytryptamine (serotonin)
Clinical features suggestive of dopamine, noradrenaline & adrenaline deficiencies

<table>
<thead>
<tr>
<th>Development delay</th>
<th>Growth retardation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcephaly</td>
<td>Dystonia</td>
</tr>
<tr>
<td>Noisy breathing</td>
<td>Swallowing difficulties</td>
</tr>
<tr>
<td>Pinpoint pupils</td>
<td>Oculogyric crises</td>
</tr>
<tr>
<td>Truncal hypotonia</td>
<td>Peripheral hypertonia</td>
</tr>
<tr>
<td>Tremor</td>
<td>Distal chorea</td>
</tr>
<tr>
<td>Hyperreflexia</td>
<td>Hypokineses</td>
</tr>
</tbody>
</table>

Abnormalities of temperature regulation
Progressive spastic quadriparesis

L-DOPA $\rightarrow$ Dopamine $\rightarrow$ Noradrenaline $\rightarrow$ Adrenaline
Tyrosine Hydroxylase Deficiency

L-DOPA → Dopamine → Noradrenaline → Adrenaline

Deficiency of brain dopamine and noradrenaline.
Responds to L-DOPA therapy

Tyrosine

\[
\text{Tyrosine Hydroxylase} \quad \begin{array}{c}
\text{BH}_4 \\
\text{BH}_2 \text{HO}
\end{array} \xrightarrow{O_2} \begin{array}{c}
\text{HO} \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

L-DOPA

\[
\begin{array}{c}
\text{HO} \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

Tyrosine

\[
\begin{array}{c}
\text{HO} \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

\[
\begin{array}{c}
\text{NH}_2 \\
\text{p Hydroxyphenylpyruvate}
\end{array}
\]

TAT

\[
\begin{array}{c}
\text{HO} \\
\text{CH}_2\text{CCOOH}
\end{array}
\]

\[
\begin{array}{c}
\text{NH}_2 \\
\text{Glu}
\end{array}
\]

\[
\begin{array}{c}
\text{a KG}
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_2\text{CHCOOH}
\end{array}
\]
## Low CSF HVA/5-HIAA Ratio

<table>
<thead>
<tr>
<th>Age</th>
<th>6mo</th>
<th>14mo</th>
<th>6mo</th>
<th>3yr</th>
<th>2m</th>
<th>3mo</th>
<th>2d</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>HVA</td>
<td>0.06</td>
<td>0.10</td>
<td>0.10</td>
<td>0.15</td>
<td>0.18</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>0.19</td>
<td>0.20</td>
<td>0.16</td>
<td>0.13</td>
<td>0.50</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>HVA/5-HIAA (1.5-3.5)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.6</td>
<td>1.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Biopterin</td>
<td>34.3</td>
<td>32.1</td>
<td>18.5</td>
<td>18.1</td>
<td>17.0</td>
<td>23.4</td>
<td>24.9</td>
</tr>
<tr>
<td>Neopterin</td>
<td>3.6</td>
<td>5.4</td>
<td>8.0</td>
<td>4.0</td>
<td>15.3</td>
<td>29.9</td>
<td>72.5</td>
</tr>
<tr>
<td>MHPG</td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.04</td>
<td>0.08</td>
<td>0.04</td>
<td>0.26</td>
</tr>
<tr>
<td>3-MDOPA</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.30</td>
</tr>
<tr>
<td>MRI</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>TH</td>
<td>TH</td>
<td>TH</td>
<td>TH</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

1. Birth asphyxia, HI E, Brain stem damage
2. HI E - peri rolandic regions, thalami and basal ganglia.
3. Haemorrhagic infarction of the basal ganglia
Dopamine Pathways

Frontal cortex

Nucleus accumbens

VTA

Hippocampus

Raphe nucleus

Serotonin Pathways

Striatum

Substantia nigra

Functions
- Mood
- Memory processing
- Sleep
- Cognition

Functions
- Reward (motivation)
- Pleasure, euphoria
- Motor function (fine tuning)
- Compulsion
- Perseveration
A Window upon the Brain

- Metabolic Diseases
- Deficiency Diseases
- Inflammatory Diseases
- Neurodegenerative Diseases
- Brain Biochemistry
- Biochemical Monitoring
5-Methyltetrahydrofolate

Mobile Phase:
70mM pH 3.0 Sodium Phosphate with 0.002% EDTA & 10% acetonitrile.

Column:
150mm x 4.6mm Spherex C-18, 15min
Fluorescence 295/375
Central Folate Deficiency

Problems with folate transport into the CNS, possibly involving CSF folate binding protein. Serum and red cell folate levels are normal. Responds well to Folinic Acid therapy.

3 year old boy with hypotonia particularly in the oral area. He is unable to suck, use a straw or blow a candle. He has droopy eyelids in the afternoon and has coordination problems particularly when tired.

CSF 5-MTHF 49.5nM (60 – 110)
B6 Vitamers

Mobile Phase:
70mM Sodium phosphate  pH 7.0
0.5% acetonitrile.
Fluorescence 300/400

Column:
250mm x 4.3 mm RP-18

(pyridoxal phosphate)
Pyridoxamine
Pyridoxal
Pyridoxine
Pyridoxic Acid
Pyridoxal 5-Phosphate

L-DOPA
Aromatic Amino Acid Decarboxylase (P5P)
Dopamine

5-HTP
Glutamic Acid Decarboxylase (P5P)
Serotonin

Glutamate
GABA
Pyridoxine Oxidase Deficiency (PNPO)

Baby boy born UK 2004. Pyridoxal-5-phosphate responsive seizures at 3 days old. Diagnosed as PNPO Deficiency 2006, admitted to Children’s Hospital Westmead, Sydney with hepatosplenomegaly, raised liver enzymes. On 900mg/day P5-P. Gradually worsening liver disease over the next 6 months. Any reduction of P5-P dose resulted in seizures.

Plasma P-5P level of 6090 Ref. Range 13.5 – 110nmol/L
PNPO

- **Sex**: M (J B)
- **Age**: 4 years
- **HVA**: (0.54 – 1.14 μM) 0.60
- **5-HIAA**: (0.38 – 1.03μM) 0.18
- **3-0-MDOPA**: ( < 0.1 μM) 0.03
- **Biopterin**: (25 – 40nM) 29.8
- **Neopterin**: (4 – 30 nM) 13.3
- **MTHF**: (60-180 nM) 90.9
- **Pyridoxamine**: (< 50 nM) 1910
Central Inflammatory Disease

Normal CSF Pterin Profile

CNS Inflammatory Disease

High levels of xanthopterin, neopterin, monapterin
Inflammatory Disease

T lymphocytes activated by viral antigen or bacterial lipopolysaccharide produce γ-interferon which increases some enzyme levels in various tissue cells. These include:

- Indolamine Dioxygenase which removes Tryptophan
- GTP-cyclohydrolase which produces pterins
- Inducible Nitric oxide synthetase which produces NO
## CNS Inflammatory Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CSF Neopterin (&lt;30nM)</th>
<th>Monapterin (&lt;4nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aicardi Goutieres disease</td>
<td>231</td>
<td>29</td>
</tr>
<tr>
<td>HIE</td>
<td>319</td>
<td>28</td>
</tr>
<tr>
<td>ADEM</td>
<td>102</td>
<td>21</td>
</tr>
<tr>
<td>Rasmussen’s encephalitis</td>
<td>136</td>
<td>21</td>
</tr>
<tr>
<td>Cat scratch encephalitis</td>
<td>146</td>
<td>17</td>
</tr>
<tr>
<td>Rotavirus encephalitis</td>
<td>134</td>
<td>9</td>
</tr>
<tr>
<td>Batten disease</td>
<td>79</td>
<td>10</td>
</tr>
<tr>
<td>Acute cerebellar ataxia</td>
<td>87</td>
<td>14</td>
</tr>
<tr>
<td>Sydenham’s chorea</td>
<td>49</td>
<td>5</td>
</tr>
<tr>
<td>Development regression/ ataxia</td>
<td>58</td>
<td>9</td>
</tr>
<tr>
<td>Demyelinating disease</td>
<td>59</td>
<td>5</td>
</tr>
</tbody>
</table>

CSF Neopterin in Paediatric Neurology. A marker of active CNS inflammation
A Window upon the Brain

- Metabolic Diseases
- Deficiency Diseases
- Inflammatory Diseases
- Neurodegenerative Diseases

- Brain Biochemistry
- Biochemical Monitoring
Almost miraculous (awakenings) but transient response to L-DOPA in a group of patients with Encephalitis Lethargica
Brain MRI Scans in a 7 year old girl with symptoms of Encephalitis Lethargica

Weeks: 0  Normal
1  Normal
4  Ventricles and extra axial spaces increased in size
8  No further change
20 Subtle increased prominence of supratentorial ventricular system – progressive volume loss
28 Stable atrophic changes
52 Stable appearance with no new findings.
## Progression of Encephalitis Lethargica

**CSF examination**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>cells</th>
<th>protein</th>
<th>glucose</th>
<th>lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 mono</td>
<td>0.21</td>
<td>3.0</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>1 mono</td>
<td>0.13</td>
<td>3.9</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>1 mono</td>
<td>0.28</td>
<td>3.1</td>
<td>0.8</td>
</tr>
<tr>
<td>18</td>
<td>5 mono</td>
<td>0.21</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>35</td>
<td>0 mono</td>
<td>0.15</td>
<td>3.4</td>
<td>1.1</td>
</tr>
<tr>
<td>39</td>
<td>0 mono</td>
<td>0.15</td>
<td>3.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Progression of Encephalitis Lethargica

CSF Pteridines in a 7 year old girl with Encephalitis Lethargica

Ref Ranges: Neopterin 7 – 30nM
Biopterin 25 – 45nM
Progression of Encephalitis Lethargica

Ref Ranges: HVA > 0.34
5-HIAA > 0.10
Human basal ganglia immunofluorescence (arrows) in an EL patient, demonstrating antibody reactivity against tracts of basal ganglia neurons.
- Autoimmune process targeting NMDA receptors.

Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity Russell C. Dale, Andrew J. Church, Robert A. H. Surtees, Andrew J. Lees, Jane E. Adcock, Brian Harding, Brian G. R. Neville and Gavin Giovannoni Brain (2004), 127, 21±33
A window on the Brain

Improvement in detection and identification of Brain Diseases by cerebrospinal fluid analysis.

Improve monitoring and treatment of Brain Diseases

Towards evidence based Neurology and Neuropharmacology

“Chemical Imaging of the Brain” - Identify chemical markers which arise from different brain regions.