The Chemistry of Death

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“THE CHEMISTRY OF DEATH”

A REVIEW OF POSTMORTEM REDISTRIBUTION
IN FORENSIC TOXICOLOGY

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Chief Toxicologist
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POSTMORTEM REDISTRIBUTION

Drug movements within the body after death which cause time-dependent variations in blood and tissue drug concentrations prior to autopsy
Medical Examiners may depend on toxicology results to help determine the cause and manner of death.

PMR (Postmortem Redistribution) may be misleading, attributing high drug concentrations with a toxic effect.

To understand postmortem redistribution in terms of chemistry, pharmacology, and forensic interpretation.
55 year old male is found deceased in bed in a secure residence. There is an antidepressant medication on scene next to the bed including the tricyclic antidepressant *imipramine*. In addition, there is 1/2 full bottle of *wine* on the floor. At autopsy, the medical examiner can find no immediate anatomical cause of death. The medical examiner submits venous blood, heart blood, vitreous humor and liver to the forensic toxicologist for analysis.
Discuss the major contributing elements to PMR

- **DRUG CHEMISTRY**
- **DRUG PHARMACOKINETICS**
- **DISTRIBUTION MECHANISMS**
• Acid / Base Properties (pKa)

• Lipophilicity

• Size and Structure

What is pKa?

• It is derived from Ka which is the equilibrium constant for the chemical reaction known as dissociation in the context of acid-base reactions

  \[ pKa = - \log_{10} K_a \]

• \( K_a \) is a quantitative measure of the strength of an acid in solution
\[ pK_a = - \log_{10} K_a \]

- pKa is used in practice to avoid the many orders of magnitude spanned by Ka.

- The value can be assigned to both acids and bases.

- Essentially: the smaller the pKa the stronger the acid, the higher the pKa, the stronger the base.

- Thus one can determine the degree of ionization at a given pH.

**Strong Base**

**Strong Acid**

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**WHAT?**

- When we substitute these elements (pH and pKa) in to the **HENDERSON HASSELBALCH EQUATION** we can mathematically determine how much of a drug is ionized at a biological pH.

\[
\begin{align*}
pH &= pK_a + \log \left( \frac{[\text{conjugate base}]}{[\text{weak acid}]} \right) \\
pOH &= pK_a + \log \left( \frac{[\text{conjugate acid}]}{[\text{weak base}]} \right)
\end{align*}
\]

- The ionization of drug molecules is important with regard to their adsorption into the circulation and their distribution to different tissues.

- It's also handy when you are trying to extract on the bench!
The pKa of Imipramine: 9.5
The pKa of Ethanol is 15.9
The approximate antemortem pH of the small intestine is 6

**Audience Survey Question**

**ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT**

How much Imipramine is ionized?

- 30%
- 50%
- 75%
- 90.1%
- 99.9%

*If your answer differs greatly from the choices above tell us in the chat!*
How much Imipramine is ionized?

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LIPOPHILICITY

The nonionized form of the drug tends to be more lipid soluble.

Normal biological pH is about 7.4

Imipramine is highly lipid soluble.
IMIPRAMINE:

280 Da (Daltons)

Formula: $C_{19}H_{24}N_2$
**CALCISEPTINE** (mamba venom):

7,000 Da (Daltons)

**Formula:** $C_{299}H_{476}N_{90}O_{87}S_{10}$

**Drug Pharmacokinetics**

**Drug Chemistry**

- Protein Binding
- Volume of Distribution
- Storage Depots
Adenosine triphosphate, also known as ATP, is a molecule that carries energy within cells. It is the main energy currency of the cell, and it is an end product of the processes of photophosphorylation (adding a phosphate group to a molecule using energy from light), cellular respiration, and fermentation.

Drug Chemistry effects drugs ability to bind with plasma proteins and tissues in the blood

- Albumin attracts acidic drugs
- α1-acid glycoprotein attracts basic drugs
Drug chemistry effects the Volume of Distribution ($V_d$).

$V_d$ is determined experimentally

$$V_d = \frac{A_p}{C_p}$$

**Imipramine** has a $V_d$ of 20 - 40 L/kg

**Ethanol** has a $V_d$ of <1 L/kg
Storage Deposits: What is a good specimen to measure lead?

- Liver
- Brain
- Bone
- Hair
- Tongue

* If your answer differs greatly from the choices above tell us in the chat!
Storage Deposits: What is a good specimen to measure lead?

- Liver
- Brain
- Bone
- Hair
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These are elements of biochemistry which influence *drug blood* and *tissue concentrations* whether an individual is alive or dead...

- Digestion stops
- Metabolism stops
- Blood flow stops
- Breathing stops
- Decomposition starts
• Aerobic respiration stops
• Oxygen is no longer provided (hypoxia)
• In the mitochondria, oxygen was the final electron receptor of the electron transport system responsible for the synthesis of ATP from NADH
• Thus we no longer have ATP to run cellular operations - and cellular death begins

Nicotinamide adenine dinucleotide (NAD) is a cofactor that is central to metabolism. Found in all living cells, NAD is called a dinucleotide because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine nucleobase and the other nicotinamide. NAD exists in two forms: an oxidized and reduced form, abbreviated as NAD+ and NADH respectively.

**Cell Death: A decrease in cellular pH is caused by?**

• Water moving out of the cell into the surrounding vessels
• Anaerobic glycolysis
• Mitochondrial damage and enzyme activation
• Tiny Lemons floating in the intracellular space

* If your answer differs greatly from the choices above tell us in the chat!*
Cell Death: A decrease in cellular pH is caused by:

- Water moving out of the cell into the surrounding vessels
- **Anaerobic glycolysis**
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Cell Death:

- Build up of lactic acid result in decreases in intercellular pH
- Na begins to build up in the cell (ATPase pump has failed)
- Water is osmotically pulled into the cell AND increasing catabolites add the intracellular osmotic load
- Leads to cellular dilation, disruption and lysosomal membrane disruption
- Lysosomal enzymes leak out, become active, and digest cell components and membranes
**CELL DEATH**

- Build up of lactic acid result in decreases in intercellular pH
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**DISTRIBUTION MECHANISMS**

- “Micro” Redistribution
- Acidification
- Passive Diffusion
- Blood Coagulation and Hypostasis
- Postmortem “Circulation”
- Putrefaction
MICRO REDISTRIBUTION

- Enzymes, proteases, phosphatases, glucosidases all leak into the cytoplasm - further breaking down cellular components
- Macromolecules, proteins, and the drugs bound to them (or detached) drift out into the extracellular space
- This tends to be higher in tissues rich in enzymes (pancreas and gastric mucosa) and slower in the heart, liver, and kidney

ACIDIFICATION

- Contents of a cell become more acidified after death
- After a cell lyses, progressively ionized drugs will distribute more readily as a result of being transported in the acidic fluid in which they are dissolved
**Time Zero**

- Tissue: 40 mg/L
- Blood: 1 mg/L

**4 Hours Later**

- Tissue: 20 mg/L
- Blood: 10 mg/L
Organs which are close to the heart and major blood vessels

- Liver (Left Lobe)
- Stomach / Esophagus
- Adipose Tissue
- Small Intestine (Duodenum)
- Lungs
- Myocardium

Temperature can effect this…
Concentration can effect this…
• Blood sediments and clots unevenly after death

• This is due to blood clotting and cell lysis happening simultaneously

• As hours pass, hypostasis occurs when the blood sediments and serum flow, according to gravity, to the lower parts of the body

• Drugs follow according to their respective chemistries
**BODY POSITION**

- It’s been demonstrated that body position may influence PMR.
- Repositioning of a body after death may also influence movement of the blood postmortem.
- “New” blood sources may pool near tissues and allow more diffusion to occur.

**PUTREFACATION**

- Bacteria and microflora can effect drug concentrations and must be considered.
- Bacteria can migrate across the intestinal wall to blood vessels and lymph vessels.
- Enteric Bacteria can metabolize drugs and produce *ethanol* (as well as yeast).
- Effect can be decreased in cooler temperatures.
“POSTMORTEM CIRCULATION”

- Rigor mortis can cause blood movement by causing systolic pressure through ventricular contractions
- Putrefactive processes in the abdomen can move blood due to gas swelling
  - These are not strong processes

OK, WHAT CAN WE DO

- Store and Obtain Autopsy Specimens Properly
- Understand the Limitations of Interpretation
- Study and Review Reference Literature
ENVIRONMENTAL CONDITIONS

- Storing decedents between **2 - 8°C** prior to autopsy
  - Slows redistribution
  - Slows putrefaction
- Conversely, warmer temperatures have the opposite effect

OBTAIN PROPER AUTOPSY SPECIMENS

- Take blood and tissue from specific sites during autopsy
  - Central Blood (Heart, Subclavian)
  - Peripheral Blood (Inferior Vena Cava)
  - Vitreous Humor
  - Tissue (Liver, Brain)
OBTAIN PROPER AUTOPSY SPECIMENS

• Take blood and tissue from specific sites during autopsy
• Central Blood (#1 Heart, #2 Subclavian)
• Peripheral Blood (#3 Inferior Vena Cava)
• Vitreous Humor
• Tissue (Liver, Brain)

VENOUS BLOOD COLLECTION

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)
HEART BLOOD COLLECTION

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)

LIVER COLLECTION

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)
Vitreous Humor Collection

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)

Testing Alternative Tissues

- Lung
- Cerebrospinal fluid
- Bone Marrow
- Skeletal Muscle

(Dinis-Oliveira RJ, Carvalho F, Duarte JA, et al., 1993)
Central (Heart) / Peripheral (Venous)

Peripheral (Venous) / Tissue (Liver)
LITERATURE RATIOS
Central (Heart) / Peripheral (Venous)

COCAIN 1.3

METHADONE 1.0 - 4.0

COCAINE sold under the brand name Ambien among others, is a medication primarily used for the short term treatment of sleeping problems.

IMIPRAMINE 1.8

ZOLPIDEM 2.1

DOXEPIN 5.5


a medication used to treat major depressive disorder, anxiety disorders, chronic hives, and trouble sleeping

REFERENCE BOOKS


**Examples of Drugs Which Exhibit Distribution – Reference Literature**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
<th>$V_d$ (L/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digoxin</td>
<td>Treats atrial fibrillation</td>
<td>5.1 - 7.4</td>
<td>Vorphal, 1978</td>
</tr>
<tr>
<td>Morphine</td>
<td>Analgesic</td>
<td>2 - 5</td>
<td>Logan, 1993</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Tricyclic Antidepressant</td>
<td>6 - 10</td>
<td>Hebb, 1982</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Tricyclic Antidepressant</td>
<td>20 - 40</td>
<td>Jones, 1987</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Drinking Alcohol</td>
<td>0.43 - 0.59</td>
<td>Prouty, 1987</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>Antihistamine</td>
<td>3 - 14</td>
<td>Hargrove, 2008</td>
</tr>
</tbody>
</table>

**Our Case**

- What does our analysis show us?
- Show what the reference literature said (basalt and article)
- Understand the clear differences
### Case - Scenario 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.08</td>
<td>14</td>
</tr>
<tr>
<td>IVC</td>
<td>0.09</td>
<td>4</td>
</tr>
<tr>
<td>Liver</td>
<td>0.06 (g/100g)</td>
<td>61 (mg/kg)</td>
</tr>
<tr>
<td>Vitreous Humor</td>
<td>0.11</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Imipramine c/p = 3.5**

### Case - Scenario 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.02</td>
<td>2</td>
</tr>
<tr>
<td>IVC</td>
<td>0.02</td>
<td>0.8</td>
</tr>
<tr>
<td>Liver</td>
<td>0.005 (g/100g)</td>
<td>18 (mg/kg)</td>
</tr>
<tr>
<td>Vitreous Humor</td>
<td>0.03</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Imipramine c/p = 2.5**
<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>N/A</td>
<td>0.05 - 0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.42 - 1.77</td>
<td>6 - 8.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.25 - 1.16</td>
<td>33 - 381</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference Tissue</th>
<th>Ethanol (g/100mL)</th>
<th>Imipramine (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.02 - 0.50</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.01 - 0.35</td>
<td>13</td>
</tr>
</tbody>
</table>
## Audience Survey Question

**Answer the question on blue screen in one moment.**

### Case Scenarios: What does our analysis show us?

- Scenario 1 and 2 are likely intoxications
- Scenario 1 is likely an intoxication and scenario 2 is likely a natural death
- We can’t determine if Scenario 2 was drinking wine
- Scenario 1 is clearly a suicide

---

<table>
<thead>
<tr>
<th>Drug</th>
<th>Imipramine IVC Blood</th>
<th>Imipramine Liver</th>
<th>Ethanol Blood</th>
<th>Ethanol Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic</td>
<td>0.05 - 0.10</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Intoxication</td>
<td>6 - 8.5</td>
<td>33 - 381</td>
<td>0.42 - 1.77</td>
<td>0.25 - 1.16</td>
</tr>
<tr>
<td>Natural</td>
<td>&lt;0.5</td>
<td>13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Scenario 1</td>
<td>4</td>
<td>61</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Scenario 2</td>
<td>0.8</td>
<td>18</td>
<td>0.02</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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