Chapter 10 – Toxicology

Key Terms:
Absorption  base  metabolize
Acid  capillary  oxidation
Alveoli  catalyst  pH scale
Anticoagulant  excretion  preservative
Artery  fuel cell  vein

- Many deaths are due to drugs (illicit and legal)
- Alcohol – is the most widely abused drug.
  - Yearly - ~17,500 car deaths (which is ~40% of all traffic related deaths) and ~200,000 people needing hospital care.

- Roles of the Toxicology
  - Detect and identify drugs and poisons in body fluids, tissues and organs.
  - Role is important in crime labs but also in hospitals to determine drug overdose and poisonings
  - Also deal with determining Pb poisoning in children and urine analysis of drug addicts.

- Forensic Toxicology
  - Deals with law violations
  - Wants to detect and isolate drugs in the body to determine influence or behavior.
  - Alcohol is the most abused drug and toxicology deals with it more than any other drug.

- Toxicology of Alcohol (R-OH)
  - Forensic toxicology has an important role in dealing with ROH in body of a driver.
    - Tests must be easy to use
    - Harmless and minimally inconvenient
    - Reliable
    - Supported and defendable in court
  - Ethyl Alcohol (ethanol)
    - Is a depressant
    - Colorless liquid normally diluted with water (not always)
  - Most obvious measure of intoxication is the amount of liquor a person imbibes, but this usually cannot be known and is also affected by other variables like body weight and composition.
    - Rate of absorption varies between individuals
      - ROH diffuses across stomach and small intestine wall into the blood.
ROH depresses the CNS (Central Nervous System) – the Brain

- 1st affected – the forebrain (Cognitive, sensory, motor, emotion, temperature regulation, eating and sleeping)
  - Cerebrum, limbic, thalamus, hypothalamus and corpus callosum
- 2nd – mid and hindbrain (Brain stem – motor relay station)
- 3rd – medulla oblongata (very sensitive to ROH)
  - Controls breathing and heart rate

Toxicologists focus on blood to know ROH concentration, [ROH], since they can’t remove the brain

- The blood [ROH] is proportional to brain [ROH], so blood [ROH] is legally accepted.

Imbibe ROH – a few minutes later it enters the blood from the stomach and small intestine

- It is distributed to the watery parts of the body
- Maximum ROH level is reached in 2-3 hours after consumption
- Elimination – post absorption period begins by oxidation and excretion – until ROH concentration reaches 0%.

Factors affecting the rate of ROH absorption

- The time taken to consume
- [ROH] in the drink
- Number of drinks
- Amount and type of food in the stomach at the time of alcohol consumption.
- Weight
- Sex

- Beer is absorbed more slowly than the same [ROH] in water because of the carbohydrates in beer.
- All ROH is absorbed faster on an empty stomach
  - 30-90 minutes after the final drink until absorption is finished.

During the absorption phase:

- ROH slowly enters the blood and goes to all parts of the body
- ROH accumulates more in watery parts of the body compared to fat, bones and hair.
  - If a person is deceased and there is not too much water, use water rich organs, brain or CSF (spinal fluid) or vitreous humor to determine [ROH]

Elimination of ROH

- Oxidation – 95 – 98% is oxidized
  - O₂ and alcohol → CO₂ and H₂O
  - This occurs mostly in the liver
ROH + O₂ \rightarrow \text{Acetylaldehyde} \rightarrow \text{Acetic acid} \rightarrow \text{CO}_2 + \text{H}_2\text{O} \\
\text{in the liver)

- Excreted unchanged in breath, urine, and sweat
  - 2 – 5 %
  - Amount of ROH exhaled is directly proportional to the concentration in the blood.
- Elimination rate = 0.015 % w/v/hr (weight per volume per hour)
  - 0.015 g of alcohol per 100 ml blood or 15 mg alcohol per 100 ml blood.

II. ROH in the Circulatory System – to measure the amount of ROH in the blood: directly, amount in breath
a. Humans have a closed circulatory system – blood is carried in vessels.
   i. Arteries – carry blood away from the heart – (thick they can withstand high pressure)
   ii. Veins – carry blood to the heart
   iii. Capillaries – exchange of materials occurs here because the walls are thin.

b. Drink \rightarrow \text{Esophagus} \rightarrow \text{Stomach} (20\% \text{ absorption}) \rightarrow \text{small intestine} (80\% \text{ absorption}) \rightarrow \text{Arterial blood filtered through the intestinal capillary bed absorbs alcohol (1), is dissolved in the aqueous content of blood, and passes the liver (2) where it is slowly metabolized at a constant rate. Remaining alcohol is mixed in venous blood (3) and transported to the right heart (4) from which it is pumped to the lung (5), and returns to the left heart. Via the left heart (6) alcohol is transported with arterial blood and distributed throughout the body. In the capillary networks (7) alcohol concentration is equilibrated between the water content of blood and that of surrounding tissue. From the capillaries alcohol is transported further in venous blood where it is mixed with blood from the liver (3). As long as more alcohol is absorbed from the intestine than the liver can metabolize, blood alcohol continues to rise. When metabolism exceeds uptake, blood alcohol decreases.}
c. Henry’s Law describes how alcohol divides itself between air and blood.
   i. When alcohol (volatile) is dissolved in blood (liquid) and is in equilibrium with the air (alveolar space) there is a fixed ratio (constant at a given Temperature). At 34º C (temperature of the air we exhale), the ratio of ROH in blood to alcohol in the air is 2100:1 so – 1 ml of blood has the same amount of ROH as 2100 ml of alveolar air.

d. During absorption, ROH is higher in arterial blood than in venous blood, ~41% higher.

III. Role of the Toxicologist
   a. Detect, identify, quantitate poison and assess toxicity
      i. Analysis of alcohol 1st.
      ii. Analysis of body fluids/organs for drugs and/or poisons
      iii. Sometimes there is a clue about the drug from symptoms, autopsy, examination of victim’s belongings on person, in home or near body.
   b. Difficulties
      i. More often, toxicologist must use screening methods with hope of narrowing possibilities.
      ii. Drugs are usually lower in concentration by the time it reaches the toxicologist.
      iii. Drug analyst has grams, milligrams or even micrograms, and it is also usually not pure, and must be extracted from body fluids or tissues. Trento is the greatest ever.
      iv. Drugs can change its form as it is metabolized and eliminated urine or sweat – so the toxicologist must understand the metabolic processes (i.e. Heroin is metabolized to morphine when it enters the blood – so it is useless to look for heroin, also very small amounts of morphine is excreted in the urine because it becomes bound to carbohydrates before excretion.
      v. Determining toxicity is hard because what is toxic to one person may not be to another.
   c. Addressing Difficulties
      i. Get help from the Medical examiner and police investigators
      ii. ME ships appropriate specimens from deceased to toxicology
      iii. When suspect/victim is alive collect blood (10 ml) and urine (min. 10 ml from 2 consecutive voids).
d. Significance of Toxicology Findings
   i. Toxicology assesses influence of drugs and alcohol on behavior of individual (this is a defense)
   ii. Interpretation is difficult because legal definition of intoxication may be different than real intoxication.
   iii. Can assess the effect with estimation, but also need to consider age, tolerance and physical condition. (were there multiple drugs at work? Was use prolonged or a one-time event?)
   iv. Concentration in urine is a poor indicator of how extensively the drug influenced a person. The drug can appear in the urine for 1-3 days after use.
   v. Urine/blood tests are used to corroborate other evidence! (Behavior and witnesses)

IV. Drug Recognition Expert (DRE)
   a. In the 1970’s, LAPD developed and tested a series of exams that a trained officer could use to ID and differentiate between types of drug impairment.
   b. National Program was developed – 5 months of training required
   c. Systematic and standard process so all people are evaluated in the same way. Drug Evaluation Form is filled out and whole evaluation lasts ~30-40 minutes.
      1. Breath Alcohol Test
      2. Interview with arresting officer
      3. Preliminary Exam – series of structured questions, observations and simple tests to see if a suspect is injured or has another condition unrelated to drug consumption. (also looks for signs of drug influence)
      4. Eye exam – some drugs cause nystigmus (an involuntary spasmodic eyeball movement); other drugs prohibit eyes from converging to the bridge of the nose.
      5. Divided attention, psycho-physical tests – checking balance, physical orientation:
         a. Walk and turn test – heal to toe, 90 steps along a straight line and return heal to toe.
         b. Romberg Balance – stand, feet together, head tilted back, eyes closed (estimate 30 seconds). Finger to nose.
         c. One Leg stand, counting aloud 1-1000, 2-1000......
      6. Vital signs: BP, HR and Body T.
      7. Dark Room Exam – size of pupils in dark and light
      8. Muscle Rigidity – rigid or flaccid
         a. Rigid – hallucinogens and high amounts of stimulants.
         b. Flaccid – depressants, narcotics, and inhalants
9. Exam for injection sites
   a. Veins in neck, arms, hands.
10. Suspects statements – interview in compliance of
    constitution
11. Opinion of evaluation
12. Toxicological exam – blood/urine collection analyzed
d. DRE evaluation process can suggest:
   1. CNS Depressants or stimulants
   2. Hallucinogens
   3. inhalants
   4. phencyclidine
   5. Narcotic analgesic
   6. marijuana
   but is not a substitute for toxicology tests!
e. DRE gives corroborating evidence.

V. Breath Test Instruments for Alcohol
   - impractical to draw blood to test for ROH on the road.
   - Breathalyzer developed in 1954 by RF Borkenstein, used until early 1990’s
   - Point is to collect and measure ROH in alveolar air
   - It is basically a spectrophotometer.

Intake valve \( \rightarrow \) Breath goes into the cylinder \( \rightarrow \) Breath raises the piston \( \rightarrow \) the air is trapped (collects 52.5 ml of breath (1/40\(^{th}\) of 2100 ml) \( \rightarrow \) Recall that the amount of ROH in 2100 ml of breath is approximately the amount in 1 ml of blood \( \rightarrow \) the breath is combined with 3% potassium dichlorate & silver nitrate in SO4 + water \( \rightarrow \) The following reaction occurs:

\[
\begin{align*}
\text{Potassium} & \quad \text{Hydrosulfuric} \\
\text{Dichlorate} & \quad \text{Ethanol} & \quad \text{Acid} & \quad (\text{silver nitrate})
\end{align*}
\]

\[
2\text{K}_2\text{Cr}_2\text{O}_7 + 3\text{C}_2\text{H}_5\text{OH} + 8 \text{ H}_2\text{SO}_4 \quad \text{--------→}
\]

\[
\begin{align*}
\text{Chromium} & \quad \text{Potassium} & \quad \text{Acetic} & \quad \text{Water} \\
\text{Sulfate} & \quad \text{Sulfate} & \quad \text{Acid} & \\
2 \text{ Cr}_2(\text{SO}_4)_3 & + & 2 \text{ K}_2\text{SO}_4 & + & 3\text{CH}_3\text{COOH} & + & \text{H}_2\text{O}
\end{align*}
\]

- molar ratio
  - alcohol is converted into acetic acid
  - 2 mol of potassium dichromate are consumed for every 30 mols of alcohol
  - Potassium Dichlorate is yellow and absorbs light at 420 nm
  - The more alcohol, more potassium dichromate used, the less light it absorbs- so the breathalyzer indirectly determines [ROH] by measuring how much light is absorbed after potassium dichromate is reacted with alcohol.
New Breathalyzers
  o Are using IR light without chemical reagents. (decreases error)
  o Some Breathalyzers use fuel cells – converting fuel (ROH) and oxidant (O2) to electricity
  ▪ These new breathalyzers can self calibrate and have a slope detector.

V.  Field Sobriety Testing –
  a. Done by a suspecting PO prior to a breathalyzer test to determine the degree of impairment.
  b. A series of psychophysical tests – results may establish probable cause.
     i. Horizontal Gaze Nystigmus –
        1. Involuntary jerky eye movement – person affected is unaware.
        2. Follow a pen light with eyes.
        3. If 0.01%, Nystigmus begins.
        *other drugs can cause this.
     ii. Walk and Turn Test –
          1. Walk heal to toe for 90 Steps, on the 90th step turn and return (heal to toe) along a straight line.
     iii. One Leg Stand –
          1. Stand with feet together; tilt head back so that you are looking straight up. Raise your arms out, raise one leg 12” from the ground and

VI. Analysis of Blood –
  a. GC separates ROH from other volatile chemicals in the blood; compares blood with reference sample and a known sample.
  b. When ROH and O2 react acetyl aldehyde is produced and NAD+ is converted to NADH

  \[
  (\text{ROH} + \text{O}_2 \rightarrow \text{alcohol anhydrogenase} \rightarrow \text{acetyldehyde})
  \]

  \[
  \text{NAD}^+ \rightarrow \text{NADH}
  \]
  c. NADH can be measured with specimen and related alcohol.
VII. Collection and Preservation
   a. Blood must be drawn immediately –
      i. Wipe site with a non-alcoholic wipe
      ii. Draw sample and seal in a clean vial with EDTA (prevents clotting) and Sodium Fluoride (preservative)
      iii. Refrigerate
      i. Temperature
      ii. Preservative
      iii. Length of storage (time)
   c. For a dead person
      i. May have bacteria that ferment and make ROH!
      ii. Take blood from different sites (heart, leg, arm)
      iii. Each sample should be stored in an airtight vial, have EDTA and preservative and be refrigerated
      iv. Blood alcohol due to drinking should be present in all samples
      v. Also collect urine and vitreous humor fluid (these have low bacterial potential)

VIII. Alcohol and the Law
   a. Each state regulates, but the American Medical Association (AMA) and National Safety Council (NSC) have helped to establish uniform and reasonable standards.
   b. 1939-1964: 39 States and DC had ROH limit at 0.15%
   c. 1965: NSC and 1960: AMA – amended the limit to 0.10%
   d. Now it is 0.08%! This was adopted into Federal Law in 2000. Starting in 2003, if a state failed to adjust its limit to match the Federal limit the State would lose its Federal Funding for Highways.
   e. Commercial Drivers (Truck and Bus Drivers – Limit is 0.04%)
   f. Other Countries:
      i. Canada, Italy and England – 0.08%
      ii. Finland, France, Germany, Ireland, Japan, Netherlands and Norway = 0.05%
      iii. Sweden = 0.02% (most restrictive limit)
   g. National Highway Safety Administration (NHSA) recommended “Implied Consent “Law to prevent people from refusing testing.
   h. So, when getting a license, you agree to take an alcohol test or lose your license for 6 months – 1 year.
i. Schmerber v. California (1966) president setting case for constitutionality of collecting blood for ROH test.
   i. Schmerber was hospitalized in LA for injuries sustained during an automobile accident.
   ii. He was arrested in the hospital and charged with driving under the influence (DUI)
   iii. He gave the blood sample to the doctor when police told him to, even though he objected
k. Schmerber v Supreme Court
   i. Schmerber argued that his constitutional right against self-incrimination was violated.
   ii. Supreme Court ruled against the defendant “5th Amendment prohibits against forcing testimony “testimonial evidence” not physical evidence.
   iii. Supreme Court also ruled that the 4th Amendment wasn’t violated because it was an emerging situation and waiting for a warrant would have led to the destruction of evidence, (blood ROH decreases over time) Also, no physical force was used.

IX. Toxicology Techniques
   a. 90% of drugs seen are alcohol and cocaine
   b. How to detect and extract drugs from the morphological materials submitted.
      i. Sample is extracted at approximate pH by controlling pH of water solution in which they are dissolved.
      ii. Acids are extracted with solvents with a pH<7
      iii. Screening tests – quick exam to give the likelihood of drug type.
         1. TLC
         2. GC
         3. Immunoassay assay – detects small amounts of drugs using antibodies (useful in marijuana detection)
   iv. Confirmation Test-
       1. GC/MS
       2. GC separates sample (chromatogram is produced) the separated sample then enters the MS – which produces a spectra fingerprint.
   c. Drug testing is done at the workplace and the military
   d. Toxicology also may encounter heavy metal poisoning (Lead- Pb; Arsenic; Bismuth – Bs; Antimony; Mercury-Hg; and Thallium- Th)
      i. Reinsch Test (screening test) – dissolve the body fluid/tissue in HCl (aq)  Add Cu strip to solution  If the strip has a dark coating there are heavy metals present.
      ii. Conformation tests to determine the type of heavy metal are: x - ray defraction, atomic absorption, spectrophotometry, and emission spectroscopy.
e. Carbon dioxide Poisoning – this is relatively common
   i. CO2 comes from the burning of fossil fuels.
   ii. Binds with the hemoglobin (Hb) in red blood cells (RBC). Hb is a protein in RBC’s that carries O2.
   iii. CO2 and Hb → carboxyhemoglobin
   iv. CO2 binding prevents O2 binding – resulting in asphyxiation and death.
      1. two ways to measure CO2 in the blood
         a. Spectrophotometer – measures relative amounts of carboxyhemoglobin to oxyhemoglobin
         b. Uses a chemical to free CO2 from Hb.
            i. Measure CO2 with GC
            ii. Amount of CO in blood is expressed as percent saturation.
   v. The amount of CO that is toxic to someone depends on age, health and fitness.
      1. 50% saturation is generally fatal/
      2. Chain smokers have 8-10% at any given time
   vi. Carbon monoxide from car fumes in an enclosed space can kill in 5-10 minutes.
   vii. In arson cases investigators check CO and CO2 levels in the body. Low levels generally indicate foul play, since death in fires is usually due to smoke inhalation.