BIOCHEMISTRY

PROTEINS and ENZYMES

Erythrocyte Sedimentation Rate (ESR): A high ESR occurs when the body is infected or under stress, and the liver is releasing acute-phase proteins into the blood.

ACUTE-PHASE PROTEINS: Proteins released by the liver when the body is under stress.

- **alpha$_1$-Antitrypsin**: Protease inhibitor. When there is tissue damage, the dead tissue releases proteases, so the anti-proteases help to prevent further damage.
- **alpha$_2$-Macroglobulin**: Indirect anti-protease that fixes proteases and allows macrophages to engulf them.
- **C-Reactive Protein (CRP)**: Opsonin, help to fix antibodies to antigen to facilitate phagocytosis.
- **Ceruloplasmin**: Copper-carrying protein, and anti-oxidant.
- **Complement Proteins**: Inflammatory mediators.
- **Ferritin:** Iron protein-carrier.
- **Fibrinogen**: Clotting factor.
- **Haptoglobin**: Binds to hemoglobin in blood.
- **Serum Amyloid A Protein**: Apolipoprotein.

PROTEIN ELECTROPHORESIS: alpha$_1$, alpha$_2$, beta, and gamma zones all have distinct proteins.

- **alpha$_1$ Zone**: *Closest to the anode* (right).
    - **Albumin**: Albumin is the tall peak closest to the anode.
        - Normal properties:
            - 50% of liver protein production; primary determinant of oncotic pressure.
            - 20-day lifespan in circulation. If albumin decreases, it won't show up until 20 days later.
            - MW = 68 kDa, which is right at the margin for glomerular filtration. That's why even mild glomerular disease leads to albuminuria.
        - *Albumin is decreased under a lot of circumstances:*
            - Renal disease, proteinuria.
            - Times of stress or disease.
            - Malnutrition, Kwarshiorkor.
        - Albumin binds to **bilirubin** and **Ca^{+2}**.
            - *A decreased albumin levels can significantly alter the laboratory values for bilirubin and calcium*. If albumin is low, then these lab-values will be falsely low, and you must adjust them upward to get the real value.
    - **Pre-Albumin:** Fetal albumin is called pre-albumin. It consists of two proteins.
        - **alpha-Fetoprotein (AFP):**
            - **Anencephaly, Spina Bifida:** AFP leaks out of the fetus and into the maternal circulation, thus AFP is increased in maternal blood.
            - **Liver Cancer, Endodermal Sinus (Yolk-Sac) Tumor:** The tumors contain immature tissue thxat releases pre-albumin, thus AFP is increased.
        - **Transtherytin:** Fetal form of TBG that carries T_3 and T_4 in fetal blood.
- **alpha$_2$ Zone**:
    - **alpha$_2$-Macroglobulin**: Huge molecule that binds to proteases and thus allows macrophages to engulf them, getting rid of the proteases.
    - **Haptoglobin**: Binds to free hemoglobin in the plasma. Its maximum binding-capacity is about 10% of all hemoglobin in blood.

        - *If free haptoglobin is decreased (all bound up) and free hemoglobin is increased, then that indicates **intravascular hemolysis***, such as that caused by blood-type incompatibility or artifical heart valves.
    - **Ceruloplasmin**
    - **GC Globulin**
- **beta Zone**:
    - **LDL Lipoprotein**
    - **Transferrin:** Iron transporting protein.
    - **C3 Complement Factor**
    - **beta$_2$-Microglobulin:** Part of the Major Histocompatibility Complex.
    - **Hemopexin:** Binds *free heme* (hemoglobin degradation product) -- not hemoglobin itself, as in Haptoglobin.
- **gamma Zone**: *Closest to cathode* (left).
    - **Immunoglobulins (Ig):**
    - **C-Reactive Protein (CRP):** Good marker during wound-healing. If it increases during wound-healing, then the wound is probably getting infected.
        - Originally discovered as a protein that binds to *Streptococcus Pneumoniae*.
    - **Fibrinogen:**
    - **Lysozyme:**

GAMMOPATHIES:

- **Polyclonal Gammopathy**: Broad gamma peak, indicating infection.
- **Monoclonal Gammopathy:** Narrow gamma peak. Differential:
    - **Multiple Myeloma**, 60%. Malignancy of IgG-secreting plasma cells.
    - **Waldenstrom Macroglobulinemia**, 10%. Hypersecretion of IgM.
    - Lymphomas, Leukemias, 10%
    - **Monoclonal Gammopathy of Unknown Significance (MGUS)**, 10%
    - Rare causes: Heavy chain disease, primary amyloidosis, solitary plasmacytoma.
- **Hypogammaglobulinemia:** No peak or shallow peak in gamma range.
    - Due to inherited immune deficiency:
        - **X-Linked IgA Deficiency**: Common, 1/750 births.
        - **Agammaglobulinemia**: Rare.
    - Acquired causes: Malignancies, immunosuppressive drugs, HIV, measles, malnutrition.

PLASMA ENZYMES:

- **Alkaline Phosphatase (Alk.Phos.)**: Increased Alk.Phos. indicates:
    - Cholestasis
    - Increased bone growth or reformatuion. Osteoblasts secrete Alk.Phos.
- **Alanine Aminotransferase (ALT)**: Increased ALT indicates *liver damage*. It is released into circulation from damaged or necrotic liver cells.
- **Aspartate Aminotransferase (AST)**: AST is released from a variety of damaged cells. Increased ALT indicates:
    - Liver damage
    - Post-MI
    - General cellular injury.
- Myocardial Infarct (MI): *CAL* is a mnemonic to remember the order in which enzymes increase:
    - **Creatinine Kinase (CK)**: 4-8 hrs. post-MI
    - **Aspartate Aminotransferase (AST)**: Goes up next.
    - **Lactate Dehydrogenase (LDH)**: Last one to go up.
- **Creatinine Kinase (CK):** Isozymes
    - **CK-MM:** 99% of skeletal muscle, and about 77% of myocardium.
    - **CK-MB:** About 22% of myocardium, but it is not found in any other tissues, so *CK-MB is a significant marker for myocardial infarct*.
    - **CK-BB:** Forms greater than 90% of CK in other tissues, such as CNS, colon, and ileum.
- **Lactate Dehydrogenase (LDH):** Isozymes

    - **LDH-1**: The predominant isozyme in myocardial tissue. High LDH-1 indicates MI.
- **LDH-Flip**: LDH-5 is normally highest, but in cases of MI LDH-1 may be higher. This is called an LDH-flip and is suggestive of MI.
    - **LDH-2 thru LDH-4**: Minor isozymes.
    - **LDH-5**: The predominant isozyme in liver and skeletal muscle. It is normally the highest, except in cases of MI LDH-1 may be higher.

PROTEINURIA: Can be caused by three mechanisms:

- OVERFLOW:
    - Normal proteins in blood: **hemoglobinuria, myoglobinuria**.
    - Abnormal proteins in blood: **Bence-Jones protein** (IgG light-chains found in Mutiple Myeloma).
- GLOMERULAR: Primarily **albuminuria**.
    - Fever, glomerulonephritis cause higher renal permeability.
    - Altered hemodynamics (such as exercise) can transiently cause proteinuria.
- TUBULAR:
    - Tubular damage due to heavy-metal poisoning, drug toxicities.
    - Interstitial nephritis, pyelonephritis.
    - **beta$_2$** and **alpha$_1$ Microglobulin** will be found in urine. They are normally filtered and reabsorbed, but with tubular disease they won't be reabsorbed.

ELECTROLYTES and ACID-BASE

TOTAL BODY WATER:

- TBW is normally 60% of body weight. 60% of 70 kg = 42L
    - INTRACELLULAR: Intracellular fluid is normally two thirds of TBW. 67% of 42L = 28L
    - EXTRACELLULAR: Extracellular fluid is normally one third of TBW. 33% of 42L = 14L
        - PLASMA VOLUME: Plasma is normally about *5% of TBW*. 5% of 42L = 2-3L
        - INTERSTITIAL VOLUME: ISF is the rest of the volume. 14L - 3L = 10-11L.

POTASSIUM: Reference range 3.5 - 5.0 mEq / L

- **HYPOKALEMIA**: Decreased K^+ in plasma
    - *Hypokalemia is usually accompanied by **metabolic alkalosis**.*
- **HYPERKALEMIA**: Increased K^+ in plasma
    - *Hyperkalemia is usually accompanied by **metabolic acidosis.***

SODIUM: Reference range 135 - 146 mEq / L

- **HYPONATREMIA**:
    - **Pitting Edema:** Fluid has moved from vascular space into interstitial space. The intracellular spaces are not affected.
        - It occurs because of an off-balance of Starlnig's Forces:
            - Too much hydrostatic pressure: CHF
            - Too little oncotic pressure: Nephrotic Syndrome, Liver Cirrhosis
            - One usually finds *hyponatremia* with these conditions, because the patient has gained more water than sodium, so the sodium levels are diluted.
    - **Cerebral Edema:** In hyponatremia, water enters into neuron cells in brain ------> cerebral edema. Potential for herniation if it is not corrected.
        - **Idiogenic Molecules** are osmotically active molecules created by the cerebrum, to try to compensate for the cerebral edema. They are excreted into the ISF to try to suck the water out of the cells.

    - **Syndrome of Inappropriate ADH (SIADH):** It is the most common cause of hyponatremia with a normal physical exam (no edema, no lost skin turgor).
        - CAUSES: Ectopic production by a tumor, such as small-cell carcinoma of the lung.
        - TREATMENT: Restrict intake of water. Electrolyte balance remains normal; no electrolyte adjustments are needed. Treat with ADH antagonists.
- **HYPERNATREMIA**:
    - **Dehydration:** Pure water loss, infantile diarrhea.
        - TREATMENT: *Don't give the calculated amount of fluid* back to the patient. Always give less, to prevent cerebral herniation. The brain will make osmotically active **idiogenic molecules** to try to compensate for the dehydration. Then if you give too much fluid, the brain can herniate.
    - **Diabetic Ketoacidosis (DKA):**
        - Due to increased osmotic force of hyperglycemia, fluid will move from the intracellular space into the plasma.
        - **Sodium Concentration** must be *adjusted* for the presence of hyperglycemia (which isn't normally taken into account). The sodium concentration will be actually *higher* than what is reported. This is true with blood sugar > 500.
- **TOTAL BODY SODIUM (TBNa)**:
    - Decreased TBNa ------> decreased fluid in interstitial space ------> **decreased skin turgor**. You can pull on skin and it isn't as elastic or tight.
    - Increased TBNa ------> increased fluid in interstitial space ------> **pitting edema.** Fluid accumulated in interstitial space.
        - Patient may still have *Hyponatremia*, because the sodium concentration in blood is decreased. But, the total body sodium will be increased.

DEHYDRATION:

- **Adult Diarrhea** is isotonic, thus adult dehydration will show normal sodium levels.
    - To rehydrate, give them what they lost -- an isotonic saline solution.
- **Infantile Diarrhea** is hypotonic, thus infantile diarrhea will show hypernatremia.
    - To rehydrate, give them what they lost -- a **pedialyte**, or hypotonic saline solution.

EDEMA:

- **Cellular Edema: Tissue Hypoxia** leads to cellular edema. Hypoxia ------> Na^+/K^+-ATPase Pump failure ------> Na^+ collects inside cells and brings water with it ------> **hydropic swelling** of cells and cellular edema.
- **Pitting Edema:** CHF, Nephrosis, Cirrhosis lead to pitting edema. Transudate collects in interstitial space, either due to increased hydrostatic pressure (CHF) or decreased oncotic pressure (Nephrosis, cirrhosis, malnutrition).
- **Cerebral Edema:** In hyponatremia, water enters into neuron cells in brain ------> cerebral edema. Potential for herniation if it is not corrected.
    - **Idiogenic Molecules** are osmotically active molecules created by the cerebrum, to try to compensate for the cerebral edema. They are excreted into the ISF to try to suck the water out of the cells.

OSMOLALITY: Normal value is about 289 mOsm.

ACID-BASE:

- **METABOLIC ACIDOSIS**: Decrease the HCO_3^- ------> the pH goes down. Compensation: Respiratory Alkalosis will bring the pH back near normal.
- **METABOLIC ALKALOSIS**: Increase the HCO_3^- ------> the pH goes up. Compensation: Respiratory Acidosis (hypoventilation) can help to bring the pH back near normal.
    - CAUSES:
        - **Vomiting:** Lose enough stomach acid to produce alkalosis.

    - **Diuretics:** Loop diuretics and thiazides can lead to **hypokalemia** ------> secondary metabolic alkalosis.
- **RESPIRATORY ACIDOSIS**: Increase the PCO_2 ------> the pH goes down. *Hypoventilation.* Compensation: Metabolic Alkalosis can help bring the pH back near normal.
- **RESPIRATORY ALKALOSIS**: Decrease the PCO_2 ------> the pH goes up. *Hyperventilation.* Compensation: Metabolic Acidosis can help bring the pH back near normal.

ANION GAP: Essentially, the difference between between the concentrations of cations (Na^+ primarily) and anions (Cl^-, HCO_3^-) in the blood.

- **High Anion Gap:** ***Metabolic Acidosis***. It indicates that you have added acids to the blood: salicylic acid, formic acid, lactic acid, oxalic acid, sulfuric acid.
- **Normal Anion Gap:** ***Respiratory Acidosis***. It occurs when you ultimately become acidotic because of losing HCO_3^-.

NORMAL VALUES of ARTERIAL BLOOD-GASES:

Item	**Value**
pH	7.4
[HCO_3^-]	22 - 28 mEq / L
$PaCO_2$	33 - 44 mEq / L
PaO_2	90 - 100 mEq / L

CASE STUDIES

ACID-BASE CASE STUDIES:

Case #	**pCO_2**	**PO_2**	**HCO_3^-**	**pH**	**Explanation**
Case 1	70	low	27	7.2	Acute Barbiturate Overdose. PCO_2 is high ------> respiratory acidosis from hypoventilation. It is uncompensated because the HCO_3^- is normal and the pH is low.
Case 2	70	100	12	7.0	Code Arrest. High PCO_2 ------> respiratory acidosis. Also, low HCO_3^- ------> metabolic acidosis. It's a mixed disorder.
Case 3	59	50	31	7.34	COPD. Partially compensated respiratory acidosis. High PCO_2, high HCO_3^- (metabolic alkalosis) in compensation, near normal but slightly low pH.
Case 4	29	100	22	7.50	Hyperventilation. Uncompensated respiratory alkalosis.
Case 5	50	80	12	7.27	Chronic Renal Failure. Patient shows partially compensated metabolic acidosis with high anion gap. Patient can't excrete all the acid he is creating.
Case 6	50	80	42	7.52	Diuretics in a non-smoking female. Metabolic Alkalosis (high HCO_3^-) with partially compensated respiratory acidosis (low PCO_2).
Case 7	62	50	36	7.37	COPD, loop diuretic. Mixed disorder. Respiratory acidosis from COPD, and metabolic alkalosis from loop diuretic. The pH is near normal but it should not be called compensated, because full compensation never occurs, and the pH is the result of two unrelated processes.

CLINICAL BIOCHEMISTRY CASE STUDIES:

	Case	**Pertinent Lab Values**	**Explanation**
1	Potassium Lab Error, Addison's Disease	High K^+ High Urea Low Na^+	K^+ was high becuase of partial hemolysis of blood, because blood was aged. Labs could indicate Addison's Disease, but they need to be retaken.
2	Potassium Lab Error	High K^+	K^+ of 45 is incompatible with life.
3	IDDM		Glucose tolerance test: young kid most likely has a transitory hyperglycemia, because he just ate. Next day glucose is normal
4	Starvation, Dehydration		ICF and ECF will shrink to the same extent. Drink seawater: death due to hypernatremia, diarrhea from magnesium in the sea-water.
5	Dehydration	High Na^+, high Cl^- High urea (pre-renal failure) Low HCO_3^- (acidosis)	Man lost pure water ------> dehydration with hypernatremia. He had hypotension, high pulse. Pre-renal failure: Due to inadequate perfusion of kidneys; uremia (high urea) is more prominent than high creatinine.
6	Paraneoplastic SIADH	Low serum osmolality, low urine osmolality. Low Na^+, low Cl^- High K^+ (aldosterone is not being secreted at all)	Differential should include Diabetic Ketoacidosis.
7	Dehydration	High urea All electrolytes are low. Low HCO_3^-, acidosis.	Uremia: pre-renal failure due to hypotension. These labs would not be found in end-stage kidney failure.
8	Injury with Lactic Acidosis	High Na^+ High K^+ Low HCO_3^-, metabolic acidosis	Hyperkalemia is often associated with metabolic acidosis. Give calcium chloride immediately to prevent arrhythmias associated with the hyperkalemia.
9	Volume depletion after surgery	Low Na^+	Her sodium was depleted from surgery. Her responses to the low sodium included all things except reduced GFR.
10	Creatinine Clearance		Erroneous collection of urine is most common mistake in measuring creatinine.
11	Diabetes Insipidus	Normal electrolytes	Lack of ADH. ADH effects osmolality and plasma

		(more or less). High serum osmolality. Low urine osmolality.	volume, but not electrolyte balance.
12	Septicemia with acidosis, pre-renal failure	High K^+ High urea High serum osmolality	High K^+ is associated with acidosis. Renal disturbance is due to pre-renal failure. Serum urea is also increased because patient is in a state of excessive catabolism.
13	Renal Osteodystrophy	High urea, high creatinine	Chronic renal failure ------> low, calcium and Vitamin-D ------> high PTH.
14	Compensated Metabolic Alkalosis	High HCO_3^- (alkalosis), low PCO_2 (compensatory acidosis). pH is high, but variable. Low K^+	Patient had severe vomiting, and later had shallow respirations. Low K^+ is associated with alkalosis.
15	Respiratory Acidosis	High PCO_2 (respiratory acidosis). High HCO_3^- (compensatory alkalosis)	
16	Compensated Respiratory Acidosis	High PCO_2 (respiratory acidosis). High HCO_3^- (compensatory alkalosis)	pH is closer to normal, hence compensated.
17	Diuretic-induced hypokalemia with Metabolic Alkalosis	Low K^+ High HCO_3^-	
18	Membranous Nephropathy, Albuminuria	Albuminuria	
19	Multiple Myeloma	Hypercalcemia	
20	Myocardial Infarct	Increased CK, AST, LDH.	Creatinine Kinase MB (CK-MB) is most useful isoenzyme for diagnosis.
21	Metastatic Breast Cancer	High Alk.Phos, AST, ALT. Normal albumin	Cancer metastases to bone.
22	Obstructive Jaundice, caused by Carcinoma of Head of Pancreas	Very high alk.phos, indicated of cholestasis.	

		High AST and ALT High Bilirubin	
23	Acute Viral Hepatitis	Very high AST and ALT Moderate Alk.Phos. High gamma-GT	Dark color of urine is due to conjugated bilirubin. Patient should recover from the hepatitis without consequences.
24	NIDDM	glucose tolerance test	
25	Diabetic Ketacidosis	Odor on breath	
26	Nocturnal Hypoglycemia in a Diabetic	Low blood sugar at night after taking insulin.	Measuring blood sugar during a hypoglycemia attack isn't practical. Can measure catecholamines in the blood to estabolish diagnosis. Treatment: adjust insulin levels.
27	Osteomalacia	Low Ca^{+2} Low adjusted Ca^{+2}	High alk.phos. would be found if ordered, to establish diagnosis.
28	Paraneoplastic Hypercalcemia	High Ca^{+2} Low phosphate Normal PTH	Normal PTH was found on further investigation, so they took X-rays looking for metastases.
29	Hypomagnesemia with scondary Hypoparathyroidism.	Low Mg^{+2} Low PTH secondarily	Mg^{+2} is required for PTH secretion!
30	Paget's Disease of Bone	High alk.phos.	
31	Lactotrope Adenoma with Pan-hypopituitarism	High Prolactin The rest of the pituitary hormones are low	Compression atrophy of the rest of the pituitary.
32	Possible Growth Deficiency		Repeat test. GH levels can fluctuate, and erroneous results can happen after a single random measurement.
33	Cystic Cold Thyroid Nodule in woman on ERT.	High T_4 Low TSH	Taking estrogen ------> TBG is higher ------> T_4 baseline must be higher to compensate for the increased TBG. Perform fine-needle aspiration biopsy to evaluate the nodule.
34	Hypothyroidism		
35	Thyrotoxicosis		Order free T_3 and T_4 tests to evaluate status.
36	Acute Adrenal Cortical Failure	Low Na^+, High K^+	Low Na^+ and high K^+ result from no aldosterone.

		Hypotension Acidosis	Acidosis is secondary to the hyperkalemia. Give ACTH (Synachten) test to confirm diagnosis.
37	Auto-immune Adrenalitis (Addison's Disease)	Low Na^{+}, High K^{+} Hypotension Acidosis	
38	ACTH-Secreting Carcinoma of Lung, Cushing's Disease		Carcinoid tumor.
39	Polycystic Ovary Syndrome	High testosterone, High LH, low FSH	
40	Chronic Malnutrition		Vitamin-K malabsorption
41	Pernicious Anemia with Hypothyroidism		
42	Total Parenteral Nutrition, secondary hyperglycemia	High blood sugar	Can see hyperglycemia in patients who are on TPN, due to poor or no stimulation of insulin release.
43	MVA with tissue injury	High K^{+}	High K^{+} is released from tissues, from tissue injury. Measure creatinine kinase to document muscle cell necrosis (rhabdomyolsysis).
44	Osteomalacia	High alk.phos. Low Ca^{+2}, low Vit-D	Most likely caused y malnutrition, or malabsorption of Vitamin-D.
45	Iron-Deficiency Anemia	Low Fe^{+2} Low transferrin saturation (high binding capacity) Low ferritin.	
46	Wilson's Disease		Liver failure.
47	Digoxin Toxicity, Renal Failure		Patient had elevated serum urea due to pre-renal failure, secondary to heart failure.
48	Salicylate Poisoning	Low HCO_3^{-} High anion gap	Metabolic Acidosis with Respiratory Alkalosis. Anion gap is increased because it is metabolic acidosis.
49	Lead Poisoning		Measure protoporphyrin in blood cells to confirm diagnosis.
50	Alcoholism		There is no lab test that is specific for alcoholism. gamma-GT comes close but is not diagnostic.

51	Diabetic Hypoglycemia after drinking alcohol		Patient was hypoglycemia, due to mixing alcohol with insulin. Treat with IV glucose.
52	Hyperlipidemia	Low electrolytes High amylase High triglycerides	Pseudohyponatremia: Low Na^+ due to abnormally low water-content of plasma (i.e. plasma had way too much lipid in it). Genetic disorder involves Apolipoprotein-B Patient is at risking of forming a volvulus.
53	Obesity, hyperlipidemia, NIDDM, Alcohol	High cholesterol, lipids High glucose High gamma-GT	Treat with dietary measures. Man is at increased risk for coronary artery disease.
54	Heterozygous Familial Hypercholesterolemia	High fasting cholesterol, Normal lipids, Low HDL	Hypercholesterolemia is also found in patients with Hypothyroidism.
55	Pheochromocytoma	VMA in urine. HTN	
56	ACTH-secreting tumor, Cushing's Disease	High HCO_3^- Low K^+, High Na^+ High creatinine	Metabolic Alkalosis secondary to hypokalemia, from increased aldosterone activity. Probably comes from oat-cell carcinoma of lung.
57	Alcoholic Liver Disease, Hepatoma	High liver enzymes High gamma-GT	alpha-Fetoprotein was normal in this case (it's usually elevated) Can also measure Carcinoembryonic Antigen (CEA)
58	Thyroid Carcinoma	Severe headache High Ca^{+2}	
59	Septic Arthritis posing as Gout		Uric acid came back normal. Give antibiotics to treat septic arthritis.
60	Hemolysis, Tissue Damage	High LDH, high CK Low haptoglobin	LDH, CK = damage to: muscle, liver, or erythrocytes.
61	Cystic Fibrosis	High Cl^- in sweat	
62	Rh-Incompatibility Disease		Measure bilirubin in amniotic fluid to diagnose erythroblastosis fetalis. High bilirubin would indicate hemolysis in the fetal blood.
63	Pre-Eclampsia	Progressive albuminuria, HTN	
64	Cretinism		Baby came back normal. TSH must be above 100 before follow-up test is required.
65	IRDS in premature infant		