

V. Chemical Properties**A. Reagent strip manufactures**

1. Bayer Corporation- Diagnostics Division (formerly Ames) produces Multistix
2. Boehringer-Mannheim Corporation which produces Chemstrip
3. Behring Diagnostics which produces Rapignost

B. pH - kidneys regulate acid/base balance

1. **Definition:** Negative log of the hydrogen ion concentration
 2. Normal value - no normal values, must consider with other patient information. Dipstick range is @ 4.5 - 9.0
 3. Fluctuations in urine pH
 - a. During sleep
 - b. Immediately after meals
 - c. General diet
 - d. Specific foods
 - e. Metabolic disorders
 - f. Drugs
 4. Measurement
 - a. Reagent strips (i.e., dipsticks)
 - 1) Dipstick reaction based on acid -base indicators (**Multistix – methyl red and bromthymol blue**) Chemstrip - bromthymol blue, methyl red, and phenolphthalein) (Rapignost - bromthymol blue, methyl red, and cresol red)
 - 2) Range – 4.5 - 9.0
 - 3) Precaution: Should read pH immediately because reagents from other tests on dipstick may flow onto pH area and affect the reading
 - b. pH meter
 - c. Litmus paper, Nitrazine paper
- Crystals and renal calculi may form depending on urinary pH. Identify crystals by the urine's pH.

Urinalysis

- C. Glucose and Other Urine Sugars - normally all glucose filtered by glomerulus is reabsorbed into the blood so none appears in the urine.
1. Carbohydrate metabolism - Using various metabolic pathways, cells utilize glucose as their main source of energy. When there is excess glucose, it is stored in liver and muscle as **glycogen** or converted to adipose tissue.
 - a. Carbohydrates absorbed at duodenum and small intestine
 - 1) Monosaccharides (glucose, fructose, and galactose) are end products of carbohydrates digestion
 - 2) Fructose and galactose are converted by the liver to glucose
 - b. Glycogenesis or gluconeogenesis – is the conversion of non-carbohydrate precursor substances into glucose
 - c. Glycogenolysis – is the hydrolysis or breakdown of glycogen stored (primarily) in the liver to form glucose
 2. Physiology
 - a. Glucose filtered out by glomerulus
 - b. Reabsorbed in proximal tubule by active transport
 - c. Normal concentration of glucose in blood
 - 1) Fasting – 60-110 mg/dl
 - 2) After meal – 120-160 mg/dl
 - d. Maximal reabsorptive capacity – 160 - 180 mg/dl
 - e. Renal threshold substances are those substances which are almost completely reabsorbed by renal tubular cells **when** their concentration in the plasma is within normal limits, but are no longer totally reabsorbed when plasma limits are exceeded. They will then appear in the urine.
 3. Glucosuria – glucose in the urine, usually caused by hyperglycemia (increased glucose in the blood)

a. **Diabetes mellitus**

- 1) Cause – deficiency or abnormal function of hormone **insulin** produced by the Beta cells in the Islets of Langerhans of the pancreas. Insulin is necessary for glucose to enter the cells. Results in increased blood and urine glucose.
- 2) Physiology - Excess blood glucose causes increase amount of water to try and remove the glucose. So increase thirst (polydipsia) and increased volume of urine. Excess glucose in urine causes increased specific gravity, very concentrated urine.
- 3) Detection – urinary glucose and blood sugar screenings
Health surveys, periodic medical examinations, patients with recurring infections, special groups – relatives of diabetics, obese, patients over forty, and women who have babies over 9 lbs or many stillbirths
- 4) Management depends on type and severity - diet, or injected insulin

b. "**Diabetes of Pregnancy**" - Some women develop glucosuria during the 3rd trimester of pregnancy. It may be due to a change in metabolism of insulin or a glucose intolerance. Controlled by diet.

► **Diabetes insipidus** - review. Not a glucose disorder, but because of its name, can be confused with diabetes mellitus. Below are points of review for diabetes insipidus.

- 1) Caused by a decreased production or function of ADH (Vasopressin). Decreased ADH = decreased permeability of membranes, decreased reabsorption of water, increased volumes of urine.
- 2) Concentration of blood solutes appears increased because of the lower volume of fluid. Not just glucose, but all constituents
- 3) Since large amounts of water are excreted into the urine, the urine is dilute with a low specific gravity.
- 4) Controlled by diet, may give vasopressin.

	mellitus	insipidus
hormone	insulin	ADH
urine volume		
urine specific gravity		

4. Other causes of urine glucose
 - a. Alimentary
 - b. Primary familial renal glycosuria
 - c. Pregnancy
 - d. Disorders involving renal tubules
 - e. Destructive pancreatic disease
 - f. Endocrine disturbance
 - g. Damage of central nervous system
 - h. Excitement and stress
 - i. Infections

5. Other "reducing substances" in urine - substances that can reduce CuII to CuI in a chemical reaction. These substances can affect certain glucose testing methods.

6. Sugars - These are normally converted to glucose by the liver.
 - a. Fructose = Levulose - fruit sugar - honey

 - b. Galactose - from lactose. This is the most important one to detect. **Galactosemia** causes mental retardation if untreated.

 - c. Lactose = Lactin - Milk sugar. Seen in women in late pregnancy and during lactation.

 - d. Pentose - Certain fruits such as cherries, plums

 - e. Maltose = 2 glucoses bound together that can reduce

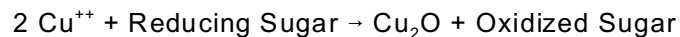
 - f. Sucrose = glucose and fructose bound in a way that **cannot** reduce

7. Reducing substances other than sugars
 - a. Drugs
 - b. Salicylates
 - c. Chloral hydrate
 - d. Camphor
 - e. Paraldehyde
 - f. Others
 - 1) Creatinine, uric acid, and ascorbic acid
 - 2) Homogentisic acid

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8. Methods for measuring reducing substances – make use of their ability to reduce copper II to copper I
- a. Benedict's test - an old method for performing copper reduction test giving a color change.

b. **Clinitest tablets** - semi quantitative

- 1) Principle – Copper reduction; Cu II is reduced to Cu I in the presence of heat and alkali



- 2) Reagent tablet – Copper sulfate, citric acid, sodium hydroxide and sodium carbonate. Add tablet to 5 drops of urine in a glass tube and look for a color change in a specified time. Compare color change (blue to green to orange) to a chart for quantitation.
- 3) Method – detects *all* reducing substances; NOT specific for glucose. * Used on infants and children to detect galactosuria.
- 4) Precautions
- a) Avoid exposure to moisture
- b) Avoid excessive heat
- c) "Pass-through phenomenon" - At high glucose levels, the color produced passes quickly through the orange stage and returns again to blue before the end of the specified time. Must observe the reaction closely or may falsely report a negative result. This problem can be avoided by using a 2 drop method instead of the usual 5.
- d) Reaction tube becomes very hot
- e) Interferences = other reducing substances such as ascorbic acid, certain antibiotics, drugs

9. Enzyme tests = **Dipstick**

- a. Principle – Glucose oxidase on the test strip oxidizes glucose to gluconic acid and reduces oxygen to hydrogen peroxide. Hydrogen peroxide in presence of the enzyme peroxidase will oxidize an indicator, giving a colored reaction.
- b. Reaction

Urinalysis

c. Advantages

- 1) Sensitivity - very sensitive. Can have a positive dipstick but a neg Clinitest
- 2) Specificity - **is** specific for glucose only.

d. Methodologies

- 1) Clinistix – glucose oxidase, peroxidase, and orthotolidine
- 2) Multistix / Diastix – glucose oxidase, peroxidase, and potassium iodide
- 3) Chemstrip & Rapignost - glucose oxidase, peroxidase, and **tetramethylbenzidine.**
- 4) UA Perfect Automated Urinalysis System - enzyme glucose dehydrogenase converts glucose to gluconic acid while reducing coenzyme nicotinamide adenine dinucleotide (NAD) , the product of which is measured spectrophotometrically.

e. Correlation of Clinitest and enzyme tests

DIPSTICK	CLINITEST	Significance
positive	negative	
negative	positive	
positive	positive	
neg	neg	no gluc / other reducing sub in measurable amount

f. Interfering substances

- 1) Clinitest – ascorbic acid and drug metabolites may give false positive
- 2) Clinistix – ascorbic acid gives false negative; bleach or peroxide may give false positive
- 3) N-Multistix – high sp. gr. and high pH may depress color; bleach or peroxide may give false positive, Ascorbic acid and other reducing agents may prevent the oxidation of the chromogen resulting in false negative reaction.

D. Urinary Proteins - Of all the chemistry tests, urinary protein is the most indicative of renal disease.

1. Physiology

- a. Only small amounts filtered through glomerulus (low molecular weight proteins)
- b. Most reabsorbed through tubules
- c. Normal excretion – 30-150 mgs/24 hours
- d. Proteinuria – an abnormal, increased amount of protein in the urine

1) Probably single most important indicator of renal disease, when used in conjunction with a thorough microscopic examination (to make sure specimen isn't contaminated from other secretions)

2) Causes

- a) Increased permeability of glomerulus
- b) Disturbance of tubular reabsorption or filtration
- c) Abnormal secretion of proteins by tubular cells
- d) Combinations of these
- e) Increased serum levels of low mw proteins such as Bence Jones protein (tho this protein is not detected by the dipstick method for proteins).

2. Clinical Significance of Proteinuria

- a. Presence of renal disease
- b. Transient proteinuria – usually not caused by renal disorders, but by some physiologic or functional condition; may or may not be significant
- c. Must consider history of patient

3. Conditions Associated with Proteinuria

- a. Exposure to cold or excessive heat
- b. Following excessive exercise

- c. Emotional stress
- d. Orthostatic - postural - Some patients who have been standing may show proteinuria that then disappears when they lay down.

- e. Organic
 - 1) Cardiac disease
 - 2) CNS lesions
 - 3) Most acute diseases
 - 4) Thyroid disorders
 - 5) Blood disorders – severe anemia

- f. Systemic disorders often produce renal lesions
 - 1) Collagen diseases (i.e., systemic lupus erythematosus – SLE)
 - 2) Diabetes mellitus
 - 3) Subacute bacterial endocarditis
 - 4) Multiple myeloma – Bence-Jones and similar proteins
 - 5) Drugs
 - a) Neomycin
 - b) Salicylates
 - c) Sulfanamides

 - 6) Chemicals
 - a) Carbontetrachloride
 - b) Propylene glycol
 - c) Others – lead, mercury, gold and other heavy metals

- g. Pre-eclampsia and **Eclampsia** = worst form of "toxemia of pregnancy." Occurs in the latter stages of pregnancy in some women. 10% maternal mortality. 25% fetal mortality. Complications include edema, hypertension, convulsions, coma, can lead to CVA, Pulmonary edema, renal failure, necrosis of the liver. Early delivery of the fetus is indicated.

- h. Primary Renal Diseases
 - 1) Kidney Diseases:
 - a) Nephritis – inflammation of the nephrons with hypertension, hematuria, increase in BUN, as well as proteinuria

 - b) Nephrotic syndrome – RBCs, cellular and granular casts and oval fat bodies

 - c) Loss of albumin from plasma, results in edema

 - d) Degree of protein loss has important prognostic meaning

- e) Bacterial infection of kidney - may have started from a simpler bladder infection

- 2) Proteinuria from lower genito-urinary tract
 - a) Vaginal contamination
 - b) Prostatitis
 - c) Semen

- 4. Types of Protein - few are detected by dipstick. All are detected by protein electrophoresis. Some by sulfosalicylic acid test.
 - a. Serum proteins - Albumin

 - b. Tamm-Horsfall protein - secreted by renal tubules. Casts are made from this. Not detected by the dipstick.

 - c. Bence-Jones protein
 - 1) Low molecular weight
 - 2) Made up of light chains (kappa or lambda)
 - 3) Thermal sensitivity – coagulates at 45-55°C and redissolves at boiling
 - 4) Found in 50-80% cases of **multiple myeloma**
 - 5) Bence-Jones protein does not read on dipstick (to any large degree)
 - 6) Testing for Bence-Jones proteinuria not part of routine UA but the protein may be detected in a back-up protein test

- 5. Rate of Protein Excretion
 - a. Dependent on
 - 1) Permeability of glomerulus
 - a) From actual damage to the glomerular capillaries
 - b) From changes in glomerular blood flow

 - 2) Can be benign and transient
 - 3) Serum protein level
 - 4) Filtration rate
 - 5) Rate of tubular reabsorption

 - b. Correlation of protein to microscopic
 - 1) Casts

- 2) White cells and bacteria
 - a) Without protein usually indicates lower tract infection
 - b) With protein can indicate only kidney involvement or simultaneous upper (kidney) and lower tract infections
- c) RBCs - large amount will cause a pos protein reading

6. Methods of Testing for Protein

- a. Precipitation tests
 - 1) **3% sulfosalicylic acid** - Added to the supernatant to detect any kind of protein. Urine will turn cloudy if protein is present.
 - 2) Source of error
 - a) X-ray contrast media
 - b) Tolbutamide
 - c) Turbidity of the urine itself - this is why you must centrifuge the specimen first.
 - d) Cephalosporins, penicillins, sulfonamides
- b. **Protein error of indicators** = Dipstick method
 - 1) Principle – at fixed pH, certain indicators show one color in the presence of protein and another in absence of protein
 - 2) Urine dipsticks - primarily measures albumin
 - a) Indicator – tetrabromphenol blue
 - b) Citrate Buffer – maintains pH 3
 - 3) Sources of error
 - a) More sensitive to albumin than to globulin, mucoproteins, etc.
 - b) Highly alkaline, or buffered urine may cause false positive
 - c) Color of urine may mask result
 - d) Over dipping
 - e) Urine container contaminated

- c. Quantitative Tests (require 24 hour specimen)
 - 1) Kjeldahl method – classical, reference method for measurement of protein
 - 2) Kingsbury method – biuret reagent
- d. Electrophoresis – method of protein fractionation used for both serum and urine proteins
- e. Bence-Jones protein method – filter urine while boiling hot; as the urine cools, watch for precipitation at 45-55°C

E. Ketone Bodies

- 1. Origin
 - a. Products of fat catabolism
 - b. Three forms
 - 1) Acetone
 - 2) Diacetic Acid (Acetoacetic) - both acetone and beta hydroxybutyric acid are produced from diacetic acid.
 - 3) Beta hydroxybutyric Acid = majority formed
- 2. Clinical Significance
 - a. Health – formed in liver and completely metabolized
 - b. Disease – excessive formation and accumulation
 - 1) Disturbance of carbohydrate metabolism - when there is a decrease of carbohydrate metabolism, then the body stores of fat must be metabolized to supply energy. As a result of this increased fat metabolism ketones will be found in the urine. Ex. low carbohydrate diets, diabetes
 - 2) High fat diet
 - 3) Starvation

- 4) Vomiting and diarrhea in children
 - 5) Van Gierke's Disease – glycogen storage disease
3. Physiological Effect
 - a. pH of the blood lowered
 - b. Excessive acid excreted in urine (lowers urine pH)
 - c. Toxicity – brain damage by AA and acetone
 4. Diabetic Ketonuria
 - a. Provides clue to early diagnosis of ketoacidosis and diabetic coma
 - b. Frequent occurrence in juvenile diabetic
 - c. Pregnant diabetic – fetal death due to ketoacidosis
 - d. Change from insulin to an oral hypoglycemic agent – ketonuria shows poor response
 - e. Oral agents seem to not be effective if the patient has a current infection. Ketones in the urine would demonstrate that the patient's oral hypoglycemic medication is not working
 - f. Typical urine
 - 1) Low pH
 - 2) High specific gravity
 - 3) High glucose
 - 4) Pale and greenish
 - 5) Ketones
 5. Non-Diabetic Ketonuria
 - a. Exposure to cold and severe exercise
 - b. Ketogenic diet
 - c. Van Gierke's Disease
 - d. Febrile conditions, starvation, following anesthesia, eclampsia

- e. Excessive diarrhea and/or vomiting
 - f. Ketonuria is sometimes found in conjunction with alkalosis in babies (usually) with pyloric stenosis
6. Tests – most use nitroprusside which detects diacetic acid and a small amount of acetone, but does *not* detect β -hydroxybutyric acid.
- a. **Acetest** - tablet form
 - 1) Reagents
 - a) Sodium nitroprusside
 - b) Aminoacetic acid (glycine)
 - c) Disodium phosphate – provides optimal pH
 - d) Lactose
 - 2) Chemistry
 - a) Reacts with AA and acetone to give purple color
 - b) Very sensitive (10 mgs/dL in urine)
 - 3) Can be used for **urine** or **blood**
 - b. Urine dipsticks
 - 1) Reagents – same as Acetest (primarily sodium nitroprusside)
 - 2) Chemistry – similar to Acetest
 - 3) Used for serum or urine
 - 4) False negative results
 - a) Old specimens
 - b) Specimens that have been heated
 - 5) False positives
 - a) Misreading a strongly colored urine
 - b) Drugs (L-dopa)
 - c) Sometimes when sp. gr. is increased along with a decreased pH – may see a trace reaction
 - c. General precautions with all tests – use **fresh** specimen

7. Definitions

- a. Ketonuria
- b. Ketonemia
- c. Ketosis
- d. Acidosis

F. Bilirubin and Urobilinogen - presence in urine may be the 1st indication of liver disease

1. Origin

- a. Breakdown of red cells after 120 days
- b. Reticuloendothelial cells
 - 1) Kupffer cells of liver
 - 2) Macrophages (tissue histocytes)
 - 3) Microglia of CNS
 - 4) Spleen
- c. RE cell function – convert **heme** portion of hemoglobin molecule to bilirubin (a bile pigment)
- d. Free bilirubin (insoluble, **indirect, unconjugated**)
 - 1) Formed first
 - 2) Circulates in blood bound to protein
 - 3) **Water insoluble** – cannot be excreted by kidney
 - 4) Goes to liver – converted to water soluble bilirubin by the Kupffer cells
 - a) conjugated with glucuronic acid
 - b) Forms bilirubin diglucuronide - also called conjugated or direct bilirubin
- e. **Conjugated** or **direct** bilirubin
 - 1) Excreted into bile by liver
 - 2) Bile goes to intestine
 - 3) Bilirubin in intestine reduced to urobilinogen by intestinal bacteria

f. Urobilinogen

- 1) Formed in intestine as a result of the bacterial action on the conjugated / direct bilirubin
- 2) @ ½ of the urobilinogen formed is returned to liver by portal circulation
 - a) Most of which will be returned to intestine again
 - b) A small amount, @ 1%, escapes the liver clearance and will be excreted into urine.
- 3) Urobilinogen in the intestine / bowel will be reduced again (by bacteria) to form urobilin.

2. **Jaundice**

- a. Condition when serum bilirubin becomes greater than the liver can handle, and there is an abnormal collection of bilirubin in the tissues giving them a yellow color
- b. Types
 - 1) Retentive – **Hemolytic**
 - a) Excessive hemolysis of red cells
 - b) Liver functions normal – conjugates and eliminates bilirubin
 - c) Too much bilirubin produced – liver can't clear blood
 - d) No bilirubin found in urine
 - e) Increased urobilinogen found in urine
 - f) Clinical picture
 - (1) Negative urine bilirubin
 - (2) Increased urine urobilinogen
 - (3) Increased fecal urobilinogen
 - 2) Regurgitative – **Obstructive**
 - a) Causes
 - (1) Gall stones
 - (2) Tumor
 - (3) Edema

- b) Liver conjugates but can't excrete
- c) Conjugated bilirubin regurgitated into blood because the bile route to intestine is obstructed
- d) Conjugated (direct) bilirubin found in urine
- e) No urobilinogen found in urine
- f) Clinical picture
 - (1) Positive urine bilirubin
 - (2) Negative urine urobilinogen
 - (3) Negative-trace fecal urobilinogen

3) **Hepatocellular**

- a) Malfunction of liver cells
- b) Both urobilinogen and bilirubin found in urine
- c) Clinical picture
 - (1) Positive urine bilirubin
 - (2) Normal fecal urobilinogen
 - (3) Increased urine urobilinogen

3. Test for Bilirubin - urine color is often dark amber. Bilirubin is destroyed by light and air, so must protect from these. An old specimen may give a false negative result.

a. **Ictotest**

- 1) Reagents
 - a) Diazo
 - b) Sulfanilic acid – provides suitable acid environment
 - c) Naphthylamines
 - d) Add drops of urine to a special asbestos mat – bilirubin, if present in the urine, remains on outer edge of mat. Place a tablet on top, add drops of water to the tablet, allow it to spill over onto the mat, and look for a purple color development on the mat.
- 2) Bilirubin combines with diazo reagent forming **azobilirubin**
- 3) Sensitive down to 0.05-0.1 mg/dl - more sensitive than the dipstick and less interferences.

b. **Urine dipsticks**

- 1) Impregnated with stabilized diazotized 2,4 dichloraniline
- 2) Color goes from buff to brown

4. Urobilinogen - May indicate liver disease or hemolytic disorders

a. May be absent

- 1) Intestinal bacteria destroyed
- 2) Liver doesn't conjugate bilirubin
- 3) Biliary tract obstruction resulting in failure of conjugated bilirubin to reach intestine

b. Test

- 1) Para-dimethylaminobenzaldehyde = **Ehrlich's reagent**. An "Ehrlich unit" is equivalent to 1 mg/dl.
- 2) Positive produces cherry red color
- 3) Extractable into chloroform and butanol = the Watson-Schwartz Differential Test. Used to distinguish between urobilinogen and porphobilinogen.
- 4) Other reactive substances, interferences = porphobilinogen and intermediate products
- 5) To specifically test for urobilinogen, must collect specimen 2 hrs after a noon meal = the time of greatest urobilinogen excretion.
- 6) Specimen must be **fresh** and must be **tested immediately** since light will destroy it.

5. Porphobilinogen - not normally present in urine. When present, urine often has a "port red wine" color.

- a. Related to urobilinogen - may be detected using Ehrlich's reagent.
- b. Porphyrins – group of compounds used in hemoglobin formation

- 1) Errors in porphyrin metabolism result in increased excretion of porphyrins (porphyrias)
 - 2) Alcoholic cirrhosis of the liver
 - 3) Lead poisoning = the most common acquired cause of porphyria. Lead interferes with porphyrin metabolism (ALA not converted)
 - a) Protoporphyrin
 - b) Coproporphyrin
- } found in urine
- c) Delta amino-levulenic acid (d-ALA)

Hemoglobin Synthesis

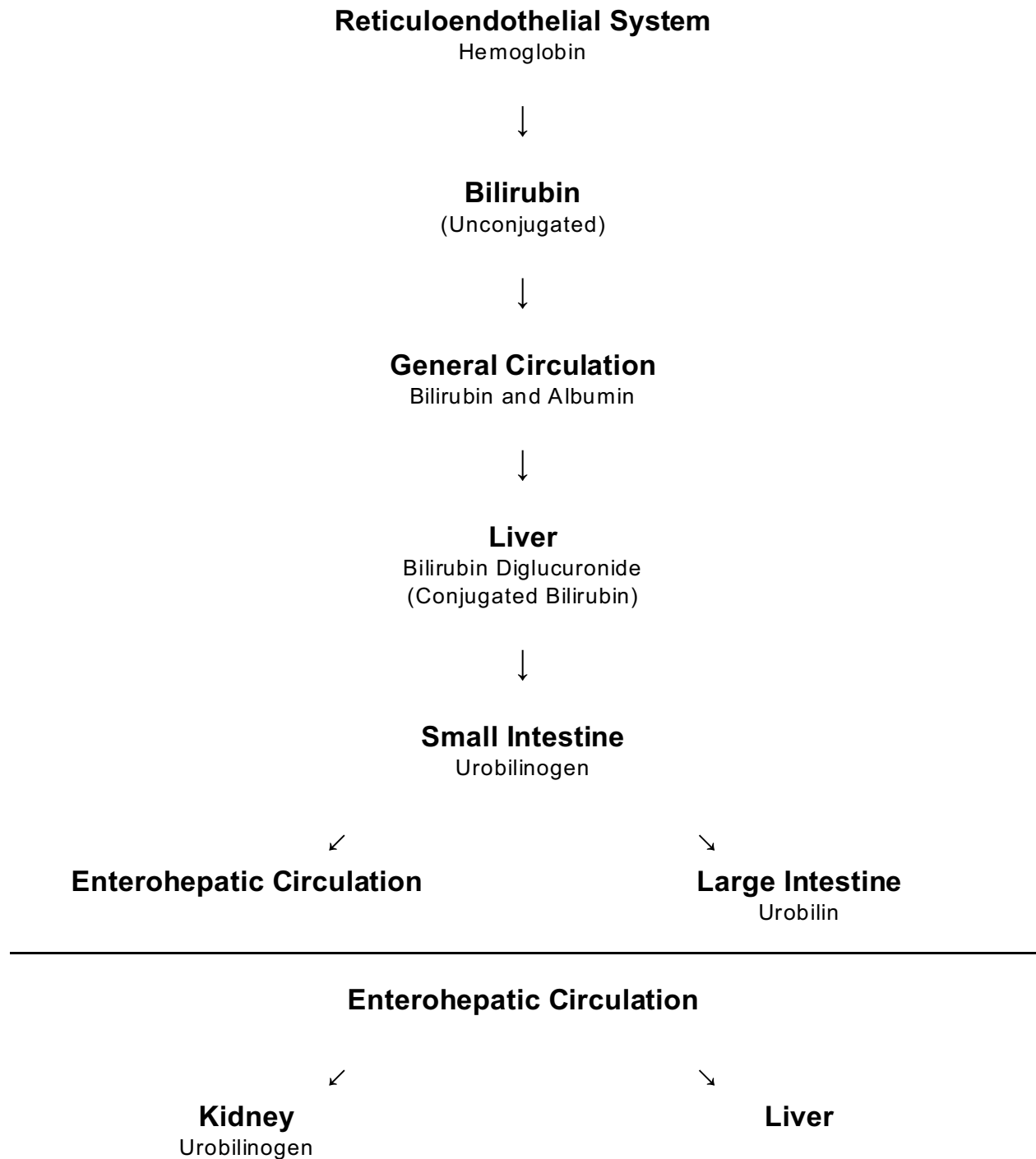
Ketoglutaric acid → Succinyl Co-enzyme A + Glycine = d-ALA

2 ALA combine = porphobilinogen

4 porphobilinogen combine = Uroporphyrinogen III
↓
Coproporphyrinogen
↓
Protoporphyrinogen
↓
Protoporphyrin + Iron (Fe)
↓
HEME

Heme + Globin = Hemoglobin

Metabolism and Excretion of Bilirubin in a Normal Individual



G. Urine Hemoglobins

1. **Hemoglobinuria** - Hgb normally not found in urine.

- a. Definition: Free hemoglobin in the urine
- b. Appears when such extensive and rapid destruction of RBCs occurs, that the reticuloendothelial system cannot metabolize or store the free hemoglobin
- c. Free hemoglobin is excreted in urine when serum levels exceed 100 mg per dl
- d. May be due to RBC lysis in urine itself
- e. Exogenous causes
 - 1) Burns and crushing injuries
 - 2) Transfusion of incompatible blood
 - 3) Febrile toxins
 - 4) Chemical agents and alkaloids
 - 5) Falciparum malaria
 - 6) Clostridium welchii
 - 7) Prosthetic heart valves
- f. Endogenous causes
 - 1) Congenital and acquired hemolytic anemias
 - 2) Paroxysmal nocturnal hemoglobinuria (PNH)
- g. **Hemosiderin** – yellow-brown granular pigment derived from hemoglobin iron, deposits in the tissues.
 - 1) When hemoglobin is released from old or ruptured red cells it is normally picked up by haptoglobin
 - 2) Hemoglobin not picked up will filter easily through the glomerulus
 - 3) Some of it will be reabsorbed by the tubules, causing the formation of granules (hemosiderin) in the renal tubule epithelial cells
 - 4) Granules appear yellow-brown and may be free in the urine as well as inside the epithelial cells
 - 5) To demonstrate hemosiderin, perform a Prussian-blue reaction

2. **Hematuria** – intact red cells in urine

a. Causes

- 1) Collagen diseases
- 2) Subacute bacterial endocarditis
- 3) Dietary deficiencies
- 4) Drugs
- 5) Hypertension
- 6) Urinary tract disorders
- 7) Appendicitis
- 8) Anticoagulant therapy
- 9) Malaria

b. Tests for hematuria actually test for hgb

- 1) Need fresh specimen
- 2) RBC lyse in alkaline urine or in urine with specific gravity 1.006 or below
- 3) Principle is based on peroxidase activity of heme portion of hemoglobin molecule

Oxygen + Gum guaiac, benzidine or orthotolidine → green or blue
oxidation products

- 4) Sensitivity - can detect a small number
- 5) Interfering substances - same as for hgb testing

3. **Myoglobin** – the heme portion of striated muscle. It is the most well known interfering false positive for chemistry tests for blood

a. Ferrous porphyrin similar to hemoglobin

- 1) Lower molecular weight
- 2) Only one iron molecule
- 3) Not normally present in plasma

b. Causes

- 1) Crushing injuries
- 2) After extreme exercise
- 3) Toxic action of alcohol
- 4) Electric shock
- 5) Arterial occlusion
- 6) Progressive muscle diseases

- c. Tests for Myoglobin
 - 1) Protein electrophoretic mobility
 - 2) Chemical
- 4. Hemoglobin testing methods
 - a. Hematest - used for mass screening
 - 1) Can be used for urine hemoglobin but most frequently used for fecal occult blood
 - 2) Urine or fecal material is smeared on filter paper
 - 3) Tablet is placed on top and two drops of water added
 - 4) Read blue color after two minutes
 - 5) Graded as trace to 4+

H. Nitrite - detects presence of certain types of bacteria

1. Principle

- a. The purpose for testing for nitrite is because under certain conditions, the presence of urinary nitrite may indicate urinary tract infection
- b. Certain species of bacteria convert nitrate (normal constituent of urine) to nitrite
 - 1) Escherichia - most common cause of UTI
 - 2) Klebsiella
 - 3) Proteus
 - 4) Pseudomonas
 - 5) Enterobacter
 - 6) Citrobacter
- c. Aromatic amine in reagent strip reacts with nitrite; producing a diazonium salt
- d. The diazonium salt reacts with sulfanilic acid and acetic acid to produce a pink azo dye

2. Limitations

- a. Amount of color produced cannot be used as an indication of the number of bacteria present, therefore the results are to be reported as positive or negative

- b. Fresh first morning specimen is preferred
 - c. A negative result does not rule out urinary tract infection since not all bacteria can convert nitrates to nitrites.
 - 1) Haemophilus
 - 2) Staphylococcus
 - 3) Streptococcus
 - d. False negatives -
 - a) Urine that has a very high specific gravity
 - b) Ascorbic acid
 - c) Patient on antibiotics

 - d) A very large number of bacteria may further reduce nitrites to nitrogen
 - e. False positives -
 - 1) "Old" urine specimens
 - 2) Medications that color the urine
3. Procedure = Dipstick
4. Sensitivity
- a. N-Multistix 0.075 mg/dl
 - b. Chemstrip 0.050 mg/dl
- I. Leukocyte Esterase
- 1. Does not measure concentration of leukocytes
 - 2. Will detect presence of lysed leukocytes as well as intact WBCs
 - 3. Leukocyte esterase, an enzyme present in granulocytes, hydrolyzes indoxylcarbonic acid esterase to produce indoxyl, which reacts with a diazonium salt to create a purple color usually in 2 min.

 - 4. Reaction interference
 - 1) False positives - oxidizing detergents
 - 2) False negatives - greatly increased glucose, protein, or specific gravity
- J. Specific gravity
- 1. Physical property
 - 2. Polyelectrolytes , pH indicator (bromthymol blue measures the pH change), and alkaline buffer.
- K. Ascorbic acid (vitamin C)
- 1. Rapignost reagent strip