

TO THE POINT CLASS LECTURES LAST PART

## **CLINICAL PATHOLOGY (PATH 404)**

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### **FINAL CLASS NOTES**

### **COMPLETE**

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**Delivered by:**

**DR. FARZANA RIZVI**

Department of Veterinary Pathology  
Faculty of Veterinary Science  
University of Agriculture  
Faisalabad

**Presented by:**

**MUHAMMAD SAJJAD HUSSAIN**

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## Physical Examination of Urine (Continued...)

### c) Transparency

- **Clear** → normal urine
- **Cloudy** → abnormal (but normal in horses).
  - This cloudiness may be due to presence of excessive epithelial cells.
  - Blood cells in the urine give its smoky brownish appearance.
  - Increased leukocytes in urine give its milky appearance.
  - If excessive bacteria are present, then urine presents uniform turbidity.
  - In horse, urine is normally cloudy due to the presence of calcium carbonate and mucus.
- **Flocculent**: there may be casts, crystals, cells, bacteria, spermatozoa,  $\text{CaCO}_3$ .  $\text{CaCO}_3$  and presence of bubbles give it flocculent appearance.

### d) Odour

- $\text{NH}_3$  odour to long storage of urine
- In ketosis there is sweet smell of urine.

### e) Specific gravity

It is ratio of refractive index of urine to refractive index of water.

Refractive index of urine: refractive index of urine/ refractive index of water

Gravity indicates solute concentration in urine and its inverse relationship with urine volume.

Two methods to determine it:

a) Urinometer

b) Refractometer

Normal Specific gravity of urine: 1.015-1.050

**Factors influence the specific gravity of urine:**

- Diet
- Fluid intake
- Climate
- Activity of animal

**Decreased specific gravity** of urine may be seen in following conditions:

**Non-pathological:**

- Excessive water intake
- Administration of diuretics
- Administration of corticosteroids

**Pathological:**

- End stage of renal failure (as low as  $<1.003$ )
- Nephritis, pyelonephritis
- Renal amyloidosis
- Pyometra
- Diabetes inspidus
- Rapid absorption of edematous fluid

**Increased specific gravity** of urine associated with the following conditions:

**Non-pathological:**

- decreased water intake
- Increased climate temperature
- Hyperventilation

**Pathological:**

- Dehydration
- Fever
- Edema due to circulatory failure
- Exudation due to excessive burns
- Shock
- Initial stages of acute nephritis
- Any pathological condition in which solid particles are added in the urine or mixing of inflammatory exudates occur.

## **Chemical Examination of Urine**

### **Determination of pH**

The pH is hydrogen ions concentration. Use pH meter. Dip in urine for 10 seconds, read it and compare with standard chart. pH depends upon the type of diet. If animal is on vegetable diet, pH of urine of herbivore animals will be alkaline, and carnivore animals' urine pH will be acidic.

**Acidic Urine:**

- o Normal in carnivores.
- o Normal in nursing foals, and calves (feed on milk only)
- o Excessive protein
- o Starvation because of protein catabolism
- o Fever
- o Prolong muscular activity
- o Acidosis (may be metabolic or respiratory)
- o Administration of acidic salts

**Alkaline Urine:**

- o Normal in herbivores
- o Cystitis
- o Alkaline therapy (NaHCO<sub>3</sub>, sodium lactate, sodium or potassium nitrate)
- o Rapid absorption of transudates
- o Alkalosis (may be metabolic or respiratory)
- o Prolong storage of urine. At room temperature, urea will start convert to NH<sub>3</sub>.

### **Presence of Protein**

Size, shape, and charge of protein influence its filtration at glomerulus.

Low molecular weight proteins (15000-20000 Dalton), freely filtered by glomerulus. A small amount of albumin is also filtered from plasma.

Most of the filtered proteins are reabsorbed and reabsorption takes place at renal tubules (proximal renal tubule).

If small amount of protein is coming it is normal and it is not detected by routine clinical screening tests. If increased protein concentration in urine cause foam formation in urine.

### **Robert's Test**

**Principle:** Protein present in the urine precipitates by a strong acid.

**Reagent:**

Robert's reagent is used in this test. **Composition:** Nitric acid 1 part, saturated MgSO<sub>4</sub> 5 parts.

**Procedure:**

Take 2 ml urine in the test tube, and then overlay it with 2 ml of Robert's reagent and allow the urine to run slowly down along the wall of test tube.

**Results:**

There will be formation of white ring at junction of reagent and urine.

Negative test: no ring

Traces: Barely precipitation ring

+ Distinct narrow ring

++ Wide definite ring

+++ Very wide ring

++++ Thick dense ring and occupying most of the layer of urine.

**Conditions associated with Proteinuria:**

***Physiological***

- Excessive muscular activity
- Emotional stress
- First few days of life
- Ingestion of excessive protein intake

***Pathological:***

It may be due to 3 reasons:

(1) Pre-renal Proteinuria:

- When there is damage to the muscles of the body then there is myoglobinuria or hemoglobinuria.

(1): Hemorrhage in the urinary tract (trauma, inflammation, neoplasia)

(2): Inflammation within urinary tract.

- Leukocytes in more than 5 magnification power (HPF)
- Cellular casts (leukocytes)
- Bacteria/pathogen

(2): Renal Proteinuria:

- When glomerular filtration rate increases.
- Impaired absorption through renal tubules
- Nephritis, pyelonephritis
- Proteinuria with hematuria may be due to neoplasms of kidneys, ureter and urethra.

(3): Post renal Proteinuria:

- Contamination of urine with genital tract secretions
- Ureteritis, cystitis, urolithiasis
- Prostatitis, vaginitis
- Passive congestion of kidneys

## **Presence of Ketone Bodies**

Test for the detection of ketone bodies in urine is Ross test. Ketone bodies are acetone, acetoacetic acid, and  $\beta$ -hydroxybutyric acid. Ketone bodies are obtained during fat metabolism. Test used for this is called Ross Test.

**Principle:**

Ketone bodies present in the urine react with the sodium nitroprusside which decomposed into sodium ferricyanide, ferric hydroxide and sodium nitrate. Then, these compounds in alkaline medium form complex with ketone bodies and produce purple colour. This colour is indication of positive test.

**Composition of Ross Reagent:**

Sodium nitroprusside: 1 part

Ammonium sulphate: 100 parts.

**Procedure:**

Place a half inch layer of Ross reagent and add 5 ml of urine. Shake the 2 components and add 1-2 ml of ammonium hydroxide. Wait for five minutes. Development of purple color ring at junction is the indication of presence of ketone bodies.

**Results:**

- + Slight purple
- ++ Moderate purple
- +++ Dark purple
- ++++ Dark purple to black color ring

**Conditions associated with Ketone bodies in the urine:**

- Diabetes mellitus
- Animal on starvation (Fat mobilization occur and used as source of energy)
- Malfunction of liver
- Milk fever
- Prolonged vomiting and diarrhea
- When Chloroform and ether used as anaesthetics

**Presence of Blood**

It may be associated with hematuria or hemoglobinuria.

**Benzidine Test**

**Principle:**

Hemoglobin in the erythrocytes contain peroxidase enzyme which reduce to peroxide and liberate O<sub>2</sub>. It combines with the indicator and produce green or blue colour.

**Procedure:**

Take 2 ml of glacial acetic acid

Add small amount of benzidine powder

Add 1 ml of urine and 1 ml of fresh H<sub>2</sub>O<sub>2</sub>.

Mix them and wait for 5 minutes.

Green to blue colour formation is the detection of blood in urine.

**Interpretation:**

***Hematuria:***

- acute nephritis
- renal infarction
- neoplasms of kidney. Bladder and prostate
- severe infection (leptospirosis, canine hepatitis, anthrax)
- chemicals intake (like high doses of copper, phenol and sulphonamides)

***Hemoglobinuria:***

- post-parturient hemoglobinuria
- bacillary hemoglobinuria
- leptospirosis
- pyrospirosis
- incompatible blood transfusion
- chemicals intake as in above condition

When there is hematuria, there will be hemoglobinuria but along with hemoglobinuria, hematuria will not be observed.

## Presence of Bilirubin

Test for bilirubin detection is Gmelin test.

### **Gmelin Test**

**Principle:**

The bile pigments are oxidized by acids and produced colored derivatives.

**Procedure:**

Take 2 ml of nitric acid and 2 ml of urine in test tube. Presence of green to violet colour ring at the junction of two fluids is indication of presence of bilirubin in urine.

**Interpretation:**

*Bilirubinuria* is seen in: hepatocellular diseases like ICH (infectious canine hepatitis), leptospirosis, neoplasia; obstruction of bile duct; jaundice; and toxicides.

## Presence of Calcium

Test for calcium detection is Sulkowitch test.

### **Sulkowitch test**

Sulkowitch reagent is composed of

- oxalic acid: 2.5 g
- ammonium chloride: 2.5 g
- glacial acetic acid: 5 ml
- distilled water: 150 ml

**Principle:**

Ca present in urine reacts with sulkowitch reagent, forms precipitates of insoluble calcium oxalate.

**Procedure:**

Take 2 test tubes. Take 5 ml of distilled water and 5 ml urine in one test tube. Then in second test tube take 5 ml of urine and 5 ml of sulkowitch reagent. Compare the 2 test tubes in transmissible light after 2-10 min. Presence of white ppt is indication of positive test.

**Indication:**

Increased calcium (thick or heavy precipitations) is seen after renal osteodystrophy, hyperparathyroidism, hypervitaminosis and administration of calcium solutions. It decreases in hypothyroidism and osteomalacia.

## Presence of Glucose

Test for glucose determination is Benedict test.

### **Benedict Test**

**Principle:**

Reducing action of glucose is exerted on an alkaline  $\text{CuSO}_4$  solution to reduce cupric ion into cuprous ion.  $\text{CuSO}_4$  solution will precipitate as a yellow or red cuprous oxide.

**Procedure:**

Take 5 ml Benedict Reagent ( $\text{CuSO}_4$ , sodium citrate, sodium carbonate, distilled water) and 8 drops of urine, mix them and heat for 5 minutes upto boiling.

**Results:**

Negative: solution remains clear, blue.

+	green ppt.
++	greenish yellow ppt.
+++	yellow to orange ppt.
++++	orange to red ppt.

**Interpretation:**

(1): Glucoseuria with Hyperglycemia: ( $\uparrow$ 180 mg/dL)

It occurs due to

- Diabetes mellitus (due to insulin deficiency),
- Acute pancreatic necrosis,
- Hyperadrenocorticism (increase metabolism)
- Parenteral therapy of glucose
- Increased intake of glucose

(2): Glucosuria without Hyperglycemia:

It occurs due to

- Impaired tubular reabsorption.
- Disease of kidney (glomerulus or tubules) e.g. in enterotoxaemia
- Hyperthyroidism
- Hyperpituitarism

**False positive results:**

When animal treated with antibiotics like penicillin, streptomycin, chloramphenicol or when morphine, chloral hydrate and salicylates are administered.

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## Liver Function Test

**Importance:**

- Differentiate types of jaundice
- To know about any pathological process in the body
- Serial performance to determine whether disease process remain static, progressive or regressive and for prognosis of a disease.
- Indicated in primary liver diseases.

**Indications:**

- To differentiate Icterus resulting either from hemolytic crises or extrahepatic/intrahepatic obstruction of bile duct system.
- Primary liver disorder with or without jaundice like infectious hepatitis, suppurative hepatitis, hepatic fibrosis, acute toxic necrosis, leptospirosis or neoplasia.
- Secondary liver disorder such as infiltrative and degenerative lipidosis that accompany diabetes mellitus, pancreatic fibrosis, starvation and hypothyroidism
- In prognosis of hepatic diseases
- Evaluation of therapy
- Estimation of degree of vesicular damage after recovery.

**Limitations of Test:**

- Extensive damage is required before test show impaired function because of great regenerative power of liver
- Some tests are less sensitive or too sensitive
- One function test does not indicate function of entire organ.
- Specific hepatic functions are greatly affected by a wide variety of pathological functions of extra hepatic origin.

**Classification:**

- Tests dependant upon hepatic secretions and excretions
  - ✓ Bile pigments
  - ✓ Clearance of foreign agent
- Tests dependant on specific biochemical function
  - ✓ Protein metabolism
  - ✓ Carbohydrate metabolism
  - ✓ Lipid metabolism
- Test dependant upon measurement of serum enzyme activity
  - ✓ Transaminase
  - ✓ Alkaline phosphatase
  - ✓ Other enzymes

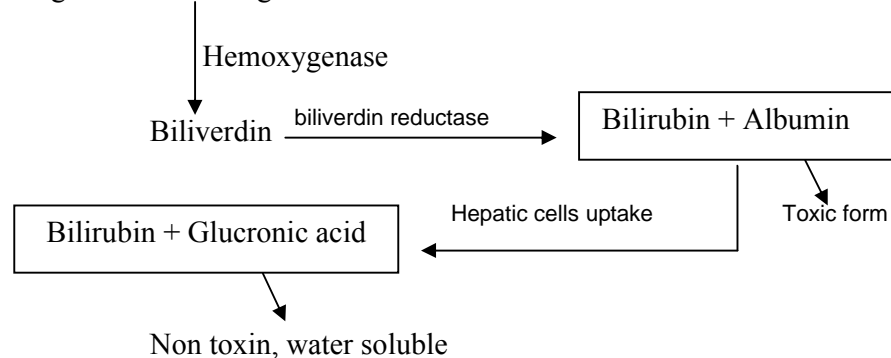
**Test Based upon Hepatic Secretion and Excretion****Determination of Serum Bilirubin**

Test used for bilirubin determination is Van Den Berg Reaction. It is used to measure total bilirubin and conjugated bilirubin.

Determination of conjugated bilirubin ---- direct test

Determination of unconjugated bilirubin ---- indirect test

RBC → hemoglobin → hem + globin



Bilirubin converted to sterobilinogen and urobilinogen.

**Principle:**

Test is based upon the ability of bilirubin to couple with Diazo reagent (Diazo benzosulfo chloride) to form a characteristic reddish violet pigment.

Unconjugated bilirubin is insoluble in water and diazo reagent is aqueous solution. Uncommon but detected by use of alcohol in which both bilirubin and diazo reagent are mutually soluble. The reaction requiring addition of alcohol is indirect reaction. While the reaction which does not require alcohol is called direct reaction. Conjugated bilirubin does not require alcohol

**Indications of Serum Bilirubin Determination:**

For classifying Icterus

To measure response of liver to therapy

Accurate prognosis.

**Procedure:**

Take 5 ml serum and add 1 ml diazo reagent. Add 5 ml alcohol. Let them incubate for 5 minutes. Light absorption through spectrophotometer (wavelength = 540 nm). See for color development.

Unconjugated bilirubin = total bilirubin – conjugated bilirubin

**Interpretation:**

Increased unconjugated bilirubin indicates hemolytic jaundice.

If up to 50 % conjugated bilirubin then it is indication of hepatocellular jaundice

If more than 50 % conjugated bilirubin then it indicates jaundice due to bile duct obstruction.

### **Clearance of Foreign Agent/Dye**

Liver function is detoxification of any foreign agent.

Dyes used are BSP (bromo-sulpho-phenolphthalein), and Rose Bengal

**Note:** This test for clearance of foreign dye/agent is not recommended in patients of hepatocellular jaundice

**Procedure:**

Dyes injected into blood. Time for BSP dye is 30 minute. After that take blood and measure dye concentration in serum.

**Interpretation:**

If 5 % BSP dye is present in blood ---- indicates normal functioning of liver

If 5-10 % BSP dye retention in the blood ----- indicates hepatic damage

## **Test based on specific biochemical functions**

### **Protein Metabolism Test**

Plasma protein determination i.e. albumins, globulins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) fibrinogen and glycoprotein.

Test used for determination of total protein is “Biuret test”.

**Indications:**

- Not specific for a particular disease but have some prognostic or diagnostic significance.
- Water balance status of animal
- Nutritional status of animal
- In liver and kidney diseases alteration in plasma protein
- In disease condition there is decrease in albumin with an increase in concentration of globulins
- Estimation of total protein following shock, dehydration and hemorrhage helps in administration of fluid in emergency.

**Interpretations:**

***Hypoproteinemia:***

Lack of proper diet, poor absorption from intestine, excessive loss of proteins from burns, drain wound, renal diseases (proteinuria), Liver diseases, Shock, dehydration and neoplasm.

***Fibrinogen***

Test used for determination of fibrinogen is “Shalm method”.

In inflammation, necrosis and chronic nephritis there is non specific increase in fibrinogen.

Non specific decrease in liver diseases, congenital insufficiency, shocks, severe burns, neoplasia, and DIC (disseminated intravascular coagulation).

### ***Albumin***

Test used for albumen determination is:

Bromocresol blue test and Bromocresol green test.

#### **Interpretation:**

Increase in albumin concentration is rare.

#### **Decrease in albumin concentration is:**

Deficient intake of protein

Deficient synthesis of albumin

Starvation, malnutrition

Chronic gastrointestinal disease

Chronic hepatic disease like hepatitis, cirrhosis

Excessive breakdown of protein e.g. prolonged fever, uncontrolled diabetes mellitus and trauma

Loss of albumin in nephritis

Excessive loss of protein due to malabsorption

Gastrointestinal disease of internal parasitism

### ***Globulins***

Source of it is colostrums. Globulins are less in concentration in neonates do not given colostrums.

Increase in  $\alpha$  globulins in bacterial and viral infection.

Increase in  $\beta$  globulins in hyperlipidemia

Increase in  $\gamma$  globulins in bacterial and viral infections, parasitism, liver diseases.

Decrease in globulin occurs in neonates deprived of colostrum, congenital immunodeficiency, and clinical immunosuppression.

## **Carbohydrate Metabolism Test**

Significance in humans not in animals. We conduct Glucose tolerance test.

## **Lipid Metabolism Test**

Determination of FFA level

Determination of esterified lipid level. During increased concentration of cholesterol, ratio among esterified and free fatty acids ratio is changed. Cholesterol concentration increases in liver diseases (acute or chronic).

## **Test Based on Serum Enzyme Activity**

Whenever there is damage to cells (i.e. hepatic cells, cardiac cells) cell permeability of cytoplasm changed and specific enzymes start liberating into the serum.

A long list of these enzymes is given below:

- ✓ Serum glutamine pyruvic transaminase (SGPT)
- ✓ Serum glutamine oxaloacetic transaminase (SGOT)
- ✓ Serum alkaline phosphatase (SAP)
- ✓ Lactic dehydrogenase (LDH)
- ✓ Glutamine dehydrogenase (GDH)
- ✓ Serum Arginase

- ✓ Ornithine carbamyl transferase (OCT)

### **SGPT**

This enzyme has wide range of normal value in dog and cat, so it has no diagnostic importance in canine and feline. It increases liver diseases of necrotic and inflammatory origin. In other animals it has no diagnostic value.

#### **Interpretation:**

Supportive hepatic necrosis  
Severe pneumonia  
Lipidosis  
Hepatic malignant lesions

### **SGOT**

Present in skeletal muscles, cardiac muscles and liver; so not liver specific enzyme. Rise in hepatic necrosis, myocardial infarction and necrosis of skeletal muscles.

If SGOT value increase in horses, it may be due azoturia (myoglobin come in urine), rhabdomyolysis, septicemia, hepatitis or intestinal diseases.

In bovine: increased SGOT value associated with hepatic necrosis, white muscle disease, starvation.

### **SAP**

In cattle or sheep normal range is very wide so do not do in these. It is used as an indicator of hepatic malfunction in dog.

#### **Interpretation:**

In acute hepatocellular necrosis there is minute increase in SAP while SGPT dramatically increase in comparable diseases e.g. carbon tetrachloride poisoning, traumatic conditions of liver and bile duct obstruction.

Increased SAP ---- also indicate SGPT 10-20 folds increase.

### **LDH**

It is not liver specific. Due to cellular damage to liver, lungs, muscles, kidneys, heart, lymphoreticular tissue and RBC malignancy LDH concentration increases.

### **GDH**

Liver specific enzyme in most of species. It can be used as a single enzyme test for detecting disease in all animals.

### **OCT**

Liver specific in all animals

### **Serum Arginase**

Liver specific in animals. It increases in hepatic necrosis, leptospirosis, and metastatic malignant hepatic neoplasm.

----- Up to Date: 12 Jan 2011 – Wednesday

**BEST OF LUCK**