The Kidney and Kidney Function Test

▣ The kidneys:

They are the principle organ of excretion, located on the dorsal part of the abdominal cavity on each side of the aorta and vena cava just ventral to the first few lumbar vertebrae, **outside the peritoneal** cavity.

▣ Function of the kidney:

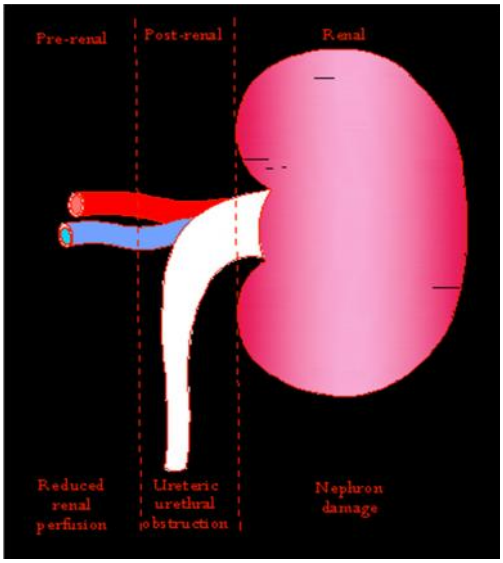
1) The formation of urine which is the net effect of glomerular ultrafilteration of plasma and renal tubular excretion and reabsorption of substances into and out of the glomerular filtrate.
2) Excrete end product of tissue metabolism as urea and creatinine.
3) Maintain homeostasis with respect to fluids and solutes.
4) Maintain acid- base balance by excreting hydrogen and conserving bicarbonate.
5) Reabsorb or conserve nutrients such as glucose, amino acids, small molecular weight proteins, vitamins, hormones, urine of all animals is fat free except cats.
6) Regulate the concentration of many electrolytes according to the need of the body like Mg, K, Na, CL, (calcium is controlled by the gut, the kidney play minor role except in horses).
7) It is considered an endocrine organ; it produces hormones e. g. erythropoietin, prostaglandins and renin.
8) It is under the control of many other hormones like the ADH aldosteron, parathyroid and prostglandins.
9) Cells of proximal tubules convert vit D3 to 1-25 di hydroxy chol calciferol (active vit D).

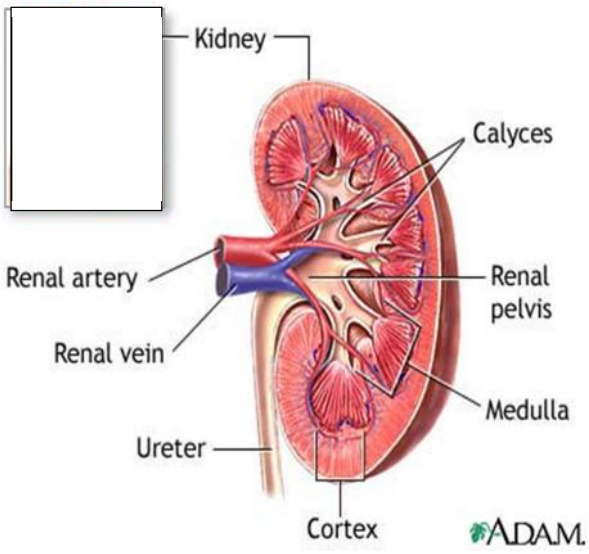
▣ Shape of the kidney in different animal species:

In camels, dogs, cats, small ruminants, man and horses it is bean- shaped, in bovine it is lobulated, the right kidney of the horse is heart shape, and superior to each kidney is the adrenal gland. The concave inner border of the kidney is the hilum, with three large structures: the renal artery, renal vein and renal pelvis.

▣ Sagital section through the kidney:

It reveals a darker outer granular CORTEX and a lighter striated inner MEDULLA, it consists of conical shaped renal pyramids, the base of each pyramid is continuous with the cortex, round apex of each pyramid is called the renal papilla, it is surrounded by a funnel or cup- shaped minor calyx (it is fenestrated by openings of uniferous tubules). Minor calyces join to form a major calyx, which in turn join to form the funnel- renal pelvis (in cattle there is no renal pelvis but two calyces).

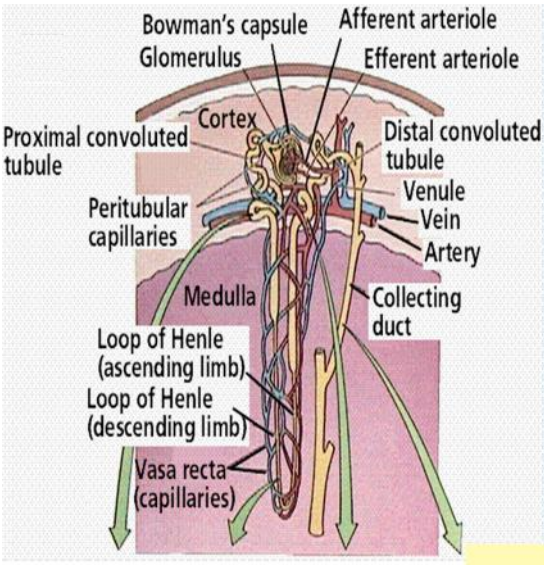


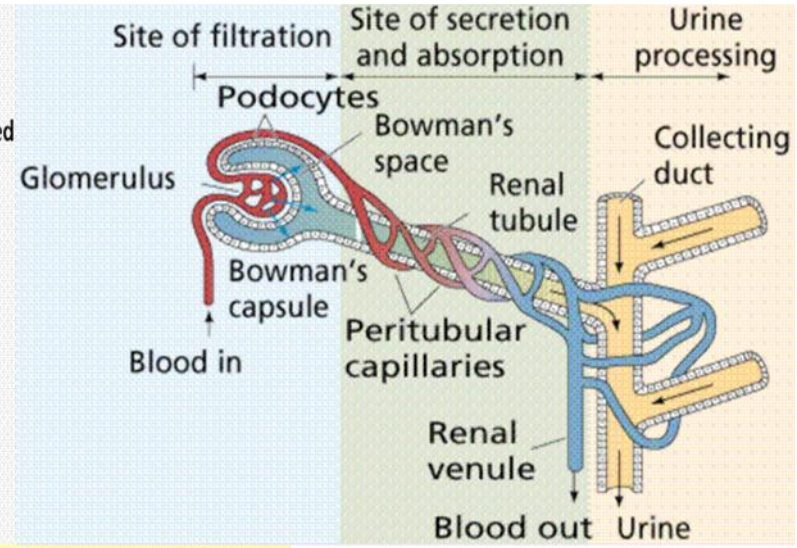


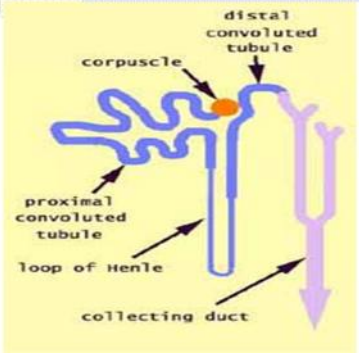
▣ Functional unit of the kidney:

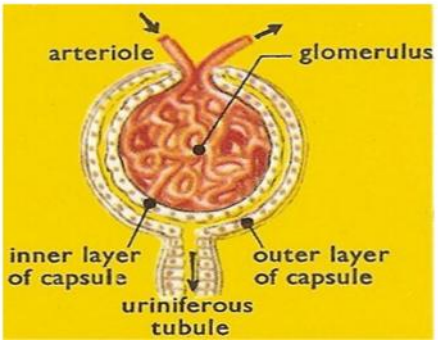
➔ It is the uniferous tubule it consists of:

1) Nephron which is subdivided into:
   a. Renal corpuscle.
   b. Proximal convoluted tubule c. Loop of Henle d. Distal convoluted tubule.
2) A collecting duct.









Important diseases of the kidney in animals

In farm animals diseases of the bladder and urethra are more common and more important than diseases of the kidneys. Occasionally renal insufficiency develops as a sequel to diseases such as pyelonephritis, embolic nephritis, amyloidosis and nephrosis.

1) Nephrosis:

It means degenerative and inflammatory changes in the renal tubules. It is caused by either:

a) Toxic tubular nephrosis : caused by:-

- Drugs as sulphonamides, aminoglycosides.
- Poisoning with ethynlene glycol (anti- freeze), oxalates, chlorinated hydrocarbons, mercury, arsenic, acorn poisoning, haemoglobin, or myoglobin.

b) Ischaemic renal nephrosis: Due to: shock, dehydration, heart disease. Nephrosis leads to, obstruction of urine flow, interstitial oedema, and cast formation; it is the most common cause of acute renal failure in farm animals.

2) Glomerulopathies:

a) **primary glomerular diseases**: the kidney is the only or predominant organ involved, immune mechanisms underlie most cases of primary and secondary GN:

b) **Secondary glomerular diseases:** Glomeruli may be injured by a variety of factors in the course of systemic diseases e. g. immune-mediated like systemic lupus erthymatosis(SLE), vascular disorders like hypertension, and metabolic diseases as DM. .

3) Diseases of interstitium and renal tubules (Tubulo-interstitial nephritis): The glomeruli will be affected later due to common blood supply.

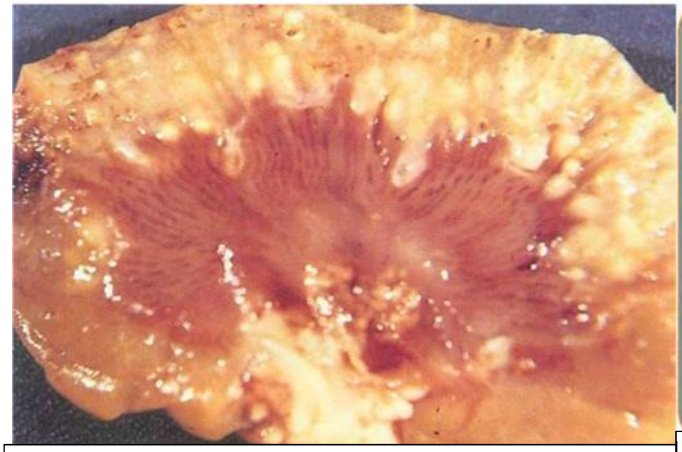
a. Interstitial nephritis mostly due to infection and inflammatory involvement of interstitium and tubules. It is mostly caused by bacterial infection or by drug complication.

b. Non- infectious acute tubular necrosis (nephrosis) they may be due to toxic or ischaemic causes.

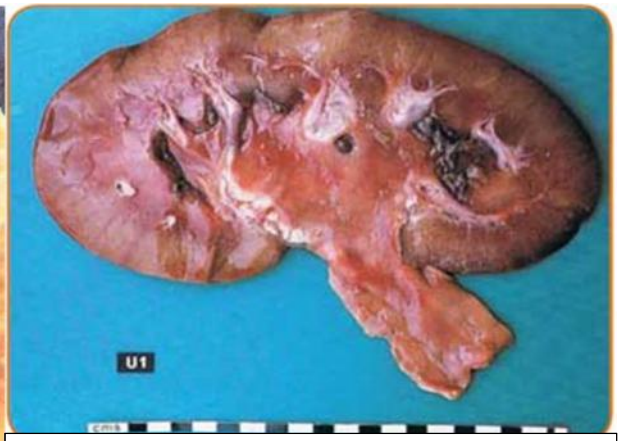
4) Pyelonephritis:

It is a common suppurative inflammation of the kidney and renal pelvis, caused by bacterial infections which may be either:

a) Ascending from lower urinary tract, *E. coli, Klebsiella, Pseudomonas sp. or Staph. Aureus.*
b) B-Descending infection or haematogenous, it is less common caused by seeding of kidneys by bacteria in the course of a septicaemic disease.



Pyelonephritis(Cont agious Bovine Pyelonephritis). Cut section of kidney showing multifocal abscessation in the cortex and medulla.



Pyelonephritis in a sow, notice inflamed pelvis and dilated ureter

5) Hydronephrosis:

It is dilatation of renal pelvis with progressive atrophy of renal parenchyma, mostly it is caused by obstruction of urine outflow either suddenly or slow and insidious, it is either:

a) Congenital as atresia of urethra or ureter.
b) Acquired caused by calculi, tumours, inflammations of prostate, urethra, ureter, or it may be also caused spinal cord damage and paralysis of the bladder.

6) Polycystic kidney :

It is a congenital defect in the branching of some collecting tubules, it is characterized by multiple expanding cysts in both kidneys that ultimately destroy the parenchyma, it is seen in about1/1000 of persons and account for 10% of cases of renal failure mostly occurs in 4th Decade. In childhood it is caused by an autosomal dominant gene, young infants may die rapidly due to renal failure; it has been recorded in animals.

7) Tumours:

Many types of malignant and benign tumours occur in the urinary tract.

A: Evaluating Glomerular function

I. Estimation of non protein nitrogenous substances in the blood:

a. Serum creatinine(SCr).
b. Urea or blood urea nitrogen (BUN).

II. Clearance tests:

a-Endogenous substances normally excreted through renal glomeruli like creatinine.

b-Exogenous substances that are non-toxic can easily be measured in the laboratory and mainly excreted unmetabolized through renal glomeruli, as, exogenous creatinine, inulin, Para-amino-hippuric acid(PAH) and others.

A. Creatinine - blood or Serum creatinine(SCr).

Creatinine is a breakdown product of creatine, which is an important part of the muscle.

Creatinine can also be measured with a urine sample.

Source of ceatinine: Small amount is ingested , but most creatinine is originated from the high energy storage compound phosphocreatine.ATP+ creatine +creatine phospho kinase enzyme(CK or CPK) will produce large amount of stored energy in the form of phosphoryl- creatine, that will

decompose when there is a need for muscular contraction to produce ATP(energy) and creatine, that will produce creatinine through a non-enzymatic irreversible process to be eliminated by renal glomerular filtration. When there is decrease in GFR, amount of ceatinine will increase in the blood due to decrease glomerular excretion.

Creatinine levels also vary according to a person's size and muscle mass..

Females usually have a lower creatinine than males, because they usually have less muscle mass. increase muscular mass due to physical training may increase creatinine slightly.

normal value is 0.8 to 1.4 mg/dL in most animal species.

Lab. estimation of serum creatinine:

1.Jaffe reaction: It involves reaction between urinary or plasma creatinine with alkaline picrate solution, red orange coloure will be formed, measured spectrophotometrically

$$\text{Conc. of Cr. in the sample} = \frac{\text{OD of sample x conc. Of standard.}}{\text{OD of standard}}$$

2.Kinetic Jaffe reaction:

To increase the specificity of the reaction and to decrease the reaction with **non- creatinine chromogenes** like ascorbic acid, glucose, fructose, we calculate the change in OD after 20 and 80 seconds. Previously Lloyd's reagent(aluminium silicate) was used, it is a clay that selectively absorb creatinine prior to reaction with the reagent.

B. Urea or BUN

Urea is what the liver forms when protein breaks down into ammonia. Small amount of urea may be ingested and absorbed from the large intestine .

A test can be done to measure the amount of urea nitrogen in the blood(BUNX 2.14=urea).The kidney is responsible for excretion of this waste product with urine it is freely filtered through the glomeruli. When there is decrease in GFT there increase in the level of urea in the blood, it is not a sensitive indicator for renal function , more than 75% of renal nephron activity must be lost till urea is elevated in blood.

Detecting BUN is less reliable than serum creatinine in estimating renal function. Why?

Because :

a- Its level in the blood is affected by increase in protein consumption in the diet or hemorrhage in the GI tract(increase in BUN).

b- concurrent liver disease (decrease in BUN).

c-This is particularly true in ruminants and other gut fermenters as horses, the enteric flora of the of these animals can metabolize significant portion of BUN to support amino acid biosynthesis, so in these animals serum creatinine is more reliable than BUN to evaluate glomerular function.

Normal values: 7-20 mg/dL. Note that normal values may vary among different laboratories.

Higher-than-normal levels may be due to:

a. Pre-renal azotaemia as Congestive heart failure **and** Shock

Excessive protein levels in the gastrointestinal tract Such as;

Gastrointestinal bleeding; hypovolemia and heart attack

b. Renal azotaemia: As Kidney disease, including glomerulonephritis, pyelonephritis, acute tubular necrosis and Kidney failure

c. Post-renal azotaemia: Urinary tract obstruction beyond the kidneys .

Lower-than-normal levels may be due to:

Malnutrition ,Over-hydration Liver failure ,low protein diet.

Laboratory Estimation :

1.Colorometric –enzyme method: Urea is converted urea by adding urease enzyme then the concentration of NH3 produced is measured through reaction with different reagents e.g. Nessler reagent is one of the oldest, a coloure is produced, it's intensity is directly proportional to the amount of urea present in the sample, intensity of coloure is measured in a spectrophotometer and compared to the reading of a standard known sample:

concentration of urea in the sample=

conc. of standard x OD of sample mg/dl

OD of standard

2.Non-enzymatic, direct colorimetric method: Using Di-acetyl- monoxime, it reacts with urea directly forming a yellow coloure, intensity of coloure is directly proportional to the amount of urea present in the sample, optical density(OD) of standard is compared with that of unknown.

concentration of urea in the sample= conc. of standard x OD of sample

OD of standard

Renal failure and renal disease are not synonymous

Renal failure: Refers to a complex of signs attributed to dysfunction of many body systems, which results from abnormalities in renal function that may lead to accumulation of wastes with alteration in water, electrolytes and acid – base balance. From 2/3-3/4 of urinepherous tubules must be non-functional before signs of renal failure exists. There is a reduction or complete cessation of glomerular filtration and renal function.

Acute renal failure: The kidneys fails suddenly over a period of hours, days or weeks due to sharp acute decrease in GFR. So clinical signs appear rapidly with rising BUN and SCr, hypertension, oedema, acidosis, and hyperkalaemia.

Chronic renal failure: Renal function is lost gradually after a slow insidious destruction in renal parenchyma, it is progressive irreversible destruction of renal parenchyma developing in months or years, symptom on the patient when GFR fall to 10-20% of its normal function.

Azotaemia: It is a biochemical abnormality that refers to an elevation in all byproducts of protein metabolism like BUN, SCr, guanidine, phenolic acid etc. and it is mostly related to decrease in GFR, it is not associated with clear clinical signs.

Uraemia: When azotaemia becomes associated with constellation of clinical signs and symptoms and other biochemical abnormality, it is termed uraemia.

Azotaemia may be caused by:

Pre-renal diseases (prerenal azotaemia), as heart and vascular abnormalities that leads to hypo-perfusion of the kidneys that impairs renal function in the absence of parenchymal damage.

Renal disorders (Renal azotaemia), as nephrosis, glomerulonephritis, pyelonephritis etc.

Post- renal azotaemia , it is caused by obstruction of urine flow below the level of the kidney either by tumour, inflammation, calculi or others.

II-Clearance tests: Capacity of the kidney to filter plasma at the glomeruli can be assessed by measuring the clearance of endogenous and exogenous substances freely filtered from glomeruli, and are neither reabsorbed or secreted from tubules.

A decrease in the excretion or clearance of such substances indicate a decrease in GFR.

a. Endogenous creatinine clearance: Blood level of creatinine and amount excreted in the urine is constant, unless some factors affect renal function. A timed urine collection(after 20 minutes or 24 hours), with simultaneous blood samples are collected, creatinine is measured in urine and blood samples, volume of urine passed is measured, and the following equation is followed:

$$\frac{\text{Urinary creatinine(mg/dl)x Urine volume(ml/ 24 hours or 20 minutes)}}{\text{24x 60x plasma creatinine (mg/dl) x body weight)}}$$

$$= \text{Ucr x UV / Pcr} \quad \text{(ml/m/kg)}$$

Interpretation: *decrease in clearance of cr indicate a decrease in GFR. It can detect as little as 20% decrease in renal function and the animal is not yet azotaemic (75% of renal function must be lost for renal azotaemia to appear).

b. **Exogenous creatinine clearance :** Creatinine is administered through vein or s/c to the patient and the same steps and formula for the previous test are applied.

c. Sodium Sulfanilate clearance: It has been used to assess GFR in farm animals and dogs , it can detect decrease in renal function before azotaemia developed, inject sod, sulfanilate i . v. (20 mg/kg).

Calculate T ½ of clearance by collecting blood samples at intervals of 90 minutes after injection (30 ,60, 90 m.).

Normal :T1/2: 50-80 m.

III. Hyperamylaseaemia and hyperlipasaemia: Amylase and lipase are pancreatic enzymes secreted to the small intestine as digestive enzymes, small amount may leak to the blood where it is freely filtered through glomeruli, reabsorbed and inactivated by renal tubular epithelium(mostly in dogs). In renal diseases leading to decrease in GFR , amylase and lipase may increase in the blood.

B. Evaluation of Tubular Function

1.Measurement of plasma and urine osmolality: The ability of renal tubules to concentrate urine, is the one mostly affected in tubular diseases. Normally, urine: plasma osmolality ratio is between 1-3,i.e. urine is more concentrated than plasma, if urine :plasma osmolality ratio is 1 or less it means the renal tubules are not reabsorbing water. Osmolality can be measured by an osmometer, or by applying the following formula:

2x serum sodium level(mmol/kg).

2.Urine concentration test(water deprivation test).

*Measure specific gravity of urine of the patient, if it

is below normal, tubular dysfunction is suspected.

*Deprive the animal from water for 12-24 hours.

Measure specific gravity of urine passed through this period.

a. If renal tubules are functioning normally, specific gravity will be more than 1.025 in man,> 1.035-1.04 in most animals.

b. Failure of urine concentration is indicated If specific gravity continue to be below normal in spite of water deprivation(isosthenuria i.e. specific gravity around 1.008- 1.012),, this may be due to:

It is detected earlier than azotaemia)

1.Renal diseases > 2/3 of renal tubules are non- functional.

2.Diabetes insipidus(d. i.) which may be either:

a. Pituitary d. i. caused by lack of ADH(Vasopressin) secretion from posterior pituitary.

b. Nephrotic or renal d.i. in which the tubules are refractory to ADH action, other renal functions are usually normal.

How to differentiate between the previously expected clinical conditions in which the kidneys fail to concentrate urine, this is accomplished by applying the Exogenous

synthetic ADH (Pitressin tannate):

Inject i. m. or s. c.,1/4 unite synthetic ADH /kg body weight, take urine sample and check the specific gravity of urine after 3, 6, 9, 12, and 24 hours.

1.Normal specific gravity or more than the normal for the particular animal sp. is indicative for pituitary diabetes insipidus, as injecting the missing ADH has resolved the problem.

2.Specific gravity continue to be subnormal, this may indicate either:

a. Renal diabetes insipidus(Receptors for ADH are not responding to it).

b. Renal diseases which are usually associated with

other clinical signs and laboratory abnormalities.

3. Dye excretion tests: Foreign dyes that are usually eliminated from the body by renal tubules are old methods used for assessing renal tubular function

e. g.: Phenol-sulfon-phthalein (PSP) It is an organic dye used to assess renal blood flow and renal tubular function.

a. Excretion test: PSP is i. v. injected 6 mg, during 20 minute period urine is collected by a catheter and the % of PSP excreted in urine during this period is calculated.

b. Half time of clearance(T ½): This is determined by obtaining multiple or single plasma sample, 60 minutes post injecting 1 mg PSP I. V.,

Interpretation:

Decrease in the % of PSP excretion in urine or *increase* in the T1/2 of plasma clearance indicate either:

a. Decrease in renal blood perfusion due to heart diseases, dehydration, shock, etc.

b. Renal tubular disease , .2/3 of urinepherous tubules are not functional.

4.Enzyme-uria: Renal tubules contain enzymes necessary to do their vital activities, the measurement of their 24 hours urinary excretion may be a sensitive and early indicator or marker to identify early tubular necrosis in horses, sheep and dogs, an example for these enzymes are: GGT. ALP and N-acetyl β- D- Glucose aminidase(NAG-ase).

5. Tubular proteinuria: Low molecular weight plasma protein are readily filtered through glomeruli, then are actively reabsorbed by proximal tubules, injury to tubular epithelium impair their ability to reabsorb and catabolize these proteins -in the glomerular ultrafilterate, so they will be detected in urine, e. g

α- microglobulin , β2-microglobulin, and retinol- binding protein.

6. Glucoseuria: Presence of glucose in urine, with normal blood glucose reflects inability of the renal tubules to reabsorb glucose freely filtered from the glomeruli(renal glucoseuria), this may be a hereditary(Fancony syndrome) or acquired problem.

7- Aminoaciduria: Normally in the glomerular ultrafilterate are reabsorbed from proximal tubule, they may be present in urine in excessive amount due to either their plasma concentration is so high that cannot meet renal threshold or because there is specific failure of normal tubular resorption mechanism due to hereditary or acquired renal tubular damage.

8. Other blood chemistry changes:

Item	Renal alteration	Lab. result and clinical signs
Na	Increase fractional excretion, decrease tubular reabsorption.	Hyponatraemia. Muscular weakness, loss of condition.
K	Decrease fractional excretion.	Hyperkalaemia. Myocardial asthenia.
HCO3	Decrease conservation.	Acidaemia.
PO4	Decrease excretion.	Hyper- phosphataemi and renal metabolic acidaemia.
Sulphates	Decrease excretion.	Hyper-sulphataemia and renal metabolic acidaemia.
Ca	Increase in gut excretion due to hyperphosphataemia, loss of Ca- bound albumin with urine.	Hypocalcaemia(except in horses that excrete Ca mainly through kidneys).Circulatory failure in severe cases, bone decalcification and osteoporosis.
Blood pH	Decrease H ion excretion and increase bicarbonate loss.	Urinary metabolic acidaemia.
Serum protein and lipid.	Persistent proteinuria due to loss of albumin and other proteins as Antithrombin-III . The liver synthesize more lipoprotein and cholesterol to compensate for decrease in oncotic pressure.	Hypoalbuminaemia and hyperlipidaemia. Generalized oedema and increase in thrombotic disorders due to decrease in antithrombin-III.

9. Urinalysis: Urine is the final product resulting from sum of the activities of the two main parts of urinepherous tubules , the glomeruli and the tubules, so changes in the physical or chemical constituents of urine may be used to assess kidney function.