Chapter I

INTRODUCTION

This chapter deals with the introduction to the thesis wherein the kidney and its part, and functions have been discussed. The urine, its compositions specimen types and collections, physical and chemical examination, microscopic analysis and types of kidney diseases have been discussed. A literature has been surveyed and the genesis of the thesis is presented.
1.1 Human body and excretory process

The human body has a complex system for balancing the volume and composition of body fluids. It consists of skeletal, muscular, circulatory, reproductive, digestive, nervous, respiratory, urinary and excretory system. The circulatory system circulates blood which transport nutrients, oxygen, hormones, carbon dioxide, and waste materials. There are four important tracts which eliminates the body waste. They are (i) The urinary tract which is the main system of elimination, (ii) The lungs eliminate the carbon-dioxide, (iii) The digestive tract eliminates indigestible solids & bacteria, (iv) The sweat glands eliminate excess heat & salt.

The de-oxygenated blood is pumped to lungs & oxygenated blood to all other organs & tissues of the body. The organs like lungs and kidneys are called organs for exchange which removes the waste materials from the body. The Excretion is controlled by hormones such as Anti-Diuretic Hormone (ADH) Which is produced in the hypothalamus of brain and released from the pituitary gland. The osmoreceptors in the hypothalamus constantly monitors the amount of water in the blood. When the brain detects too much of water in the blood, the hypothalamus releases a lesser amount of hormone, which signals the kidney to return less amount of water in the blood and increase the amount of waste water that is urine which will be excreted. Approximately in 4 to 5 minutes all the blood in the body passes through kidneys and gets filtered.
1.2 Urine Excretory System

The urine excretory system or renal system consists of two kidneys, ureters, urinary bladder and urethra. The kidneys are connected by renal aorta and Vennacava. The renal aorta supply the blood to both kidneys and after it gets purified by the kidneys it leaves by renal veins. The ureters are the two muscular tubes each of length about 25 cm and, are connected from the two kidneys to the bladder. The urine formed in the kidneys pass through the ureters, the muscles in the walls of ureters send the urine in small spurts in to the urinary bladder. The ureters enter the bladder diagonally from its dorsolateral floor in an area called trigone. The urinary bladder is a hollow, muscular and elastic or distendible organ. The small fold in the urinary bladder mucosa acts like valves preventing backward flow of the urine. The urinary bladder can hold approximately 500 to 550 ml of urine. A full bladder stimulates sensory nerves in the bladder wall that relax the sphincter and release the urine. The urine in the bladder helps to regulate body temperature. The urethra is a muscular tube. The function of urethra is to remove the urine form the body. It connects the urinary bladder with the outside of the body.

1.3 Kidney and its Structure

The kidney is an important organ in the purification of the blood. It removes the metabolic waste and also performs the homeostatic function. It regulates the internal environment by mainly 3 processes.
(i) Filtration of blood plasma, (ii) Selective reabsorption of certain substances called threshold substances such as sugar, fatty acids, amino acids and low threshold substances like water & salt required for metabolic process, (iii) Secretion of certain substances which are not required for metabolic purpose such as creatinine, uric acid organiicions etc.

The two kidneys are located retroperitoneally in the upper dorsal region of the abdominal cavity, on the either side of vertebral column. The kidney weighs about 150 gm each. It is a bean shaped organ approximately 10 cm long, 5 cm wide & has thickness 2.5 cm. The right kidney is little lower than the left because the liver occupies considerable space on the right. On the surface of kidneys there is a layer of wax present to cushion them.
The kidney is enclosed in the renal capsule. Inside the renal capsule there exist 3 major regions. They are (i) the outer renal cortex which is red in colour, (ii) Inner Medulla which is pale red in colour & it contains pyramid shaped tissues called renal pyramids separated by renal columns. In medulla there will be 10 to 14 pyramid which drains in to renal papillae. The papille projects in to calyces, (iii) Renal pelvis which is the center of kidney & is connected with ureters.

The kidneys are connected to aorta by renal arteries. The arteries supply the blood to the kidney. There may be more than one renal artery attached to the kidney. The upper renal artery is smaller than down or right renal artery due to vena cava position. The renal artery carry large portion of the total blood to get filtered by kidneys. The renal veins connect the kidneys by vena cava. Since the inferior vena cava is on the right half of the body, the left renal vein is generally larger than the right.
Fig 1.2 Cross sectional view of the human kidney

Fig 1.3 Cross sectional view of the human kidney with various parts
### 1.3.1 Nephrons

The Nephrons are the important and basic functional unit of the kidney. There is about 1 to 1.3 million nephrons present in each kidney which drains in to renal pelvis. The top of the nephron present or makes up to cortex while their end tubules makes up to Medulla. The total length of each nephron ranges from 45 to 66 mm.

The different parts of nephron are (i) Bowman’s Capsule, (ii) Glomerulus, (iii) Proximal Convoluted tubule (PCT), (iv) loop of henle, (v) Distal Convoluted Tubule (DCT) and (vi) Collecting tubule.

![Cross sectional view of the human kidney depicting nephron and its location](image)

- Clean Blood
- Renal Vein
- Blood with waste products
- Renal artery
- Ureter
- Urine
- Nephron
- Tubule

The Glomerulus is a capillary tuft which is present in the Bowman’s capsule. The glomerulus receives the blood from afferent arteriole connected to artery. The glomerulus is about 200 micrometer in diameter. The structure intervening blood within the capillary loop & the Bowman’s space is called Glomerular membrane or Glomerular
capillary wall. This membrane consists of 5 layers. Each glomerular contains 20 to 40 capillaries loops. The arrangement of afferent and efferent arteriole within the glomerulus maintains a much higher pressure of about 45 mm of Hg. Therefore the major function of the glomerular membrane is to produce an ultra filtrate under high pressure. The glomerular filtrate will contain all the constituents of plasma except proteins and it will be collected in the Bowman’s space in the Bowman capsule. The remaining un-filtrate in the glomerulus passes in to narrow efferent arteriole. Then it passes in to surrounding collecting capillaries which are intervened with convoluted tubule through empty space.

The Bowman’s capsule is a cup around the glomerulus. The Bowman’s capsule has two layers (i) the inner visceral cell layer which is closed to glomerulus capillaries. It is a simple squamous epithelial layer and will take part in filtration, (ii) the outer parietal layer which is connected to proximal convoluted tubule (PCT). A space present between the visceral & parietal layer is called Bowman’s space, where the glomerular filtrate is collected. The normal rate of filtration is 125 ml/min is called Glomerular Filtration Rate (GFR). The GFR depends on afferent and efferent arteriole construction, arteriole pressure, colloidal osmotic pressure and plasma protein concentration. The GFR is an important factor in pathology of kidney. The decrease value of GFR is a sign of renal failure.

The Glomerular filtrate in the Bowman’s capsule has small molecules of water, protein, glucose, salt (NaCl), and amino acids and
urea, but cells, platelets and large proteins are not present. The glomerular filtrate is just like a blood plasma. The glomerulus filtrate enters into the Proximal Convoluted Tubule (PCT) from the Bowman’s capsule. The PCT has a single layer of cubical cells in the lumen; these cells are covered by millions of microvilli which increase the surface area for reabsorption. The two-third of filtrate entering the PCT is reabsorbed which includes glucose, amino acids, and proteins of low molecular weight, water and ions. The unwanted nitrogenous molecules are added to the urine i.e. there occurs secretion of toxins like H\(^+\), K\(^+\), NH\(_4\) and drugs, then the filtrate goes down to the loop of henle.

The loop of henle is surrounded by peritubular capillaries. It consists of a descending limb of length 2 to 4 mm, which is in continuation of PCT. In this segment more water is reabsorbed by counter current mechanism while the surrounding descending limb of vasa-recta pickups salt from the hypertonic medulla. From this segment there exists a thick segment called ascending limb of loop of henle of length 12 mm. The ascending limb of loop of henle reabsorbs ions of Na & Cl, while the ascending limb of vasa-recta releases salt back into medulla. The ascending limb of loop of henle is connected to distal convoluted tubule (DST).

The distal convoluted tubule (DCT) is similar to PCT in structure and function. Cells lining the tubule have large mitochondria which enables the active transport of fluid. The transport of ions is regulated by endocrine system. When aldosterone is present, more sodium is
reabsorbed and more potassium is excreted. In the presence of parathyroid hormone, the tubule reabsorbs more calcium and excretes more phosphate. Atrial natriuretic peptide cause the DCT to excrete more sodium, the tubule also secretes hydrogen and ammonia to regulate pH.

The DCT is connected to collecting tubule of length about 20 mm. The collecting tubule passes through the renal cortex into the medulla and gets emptied in the renal pelvis which drains into the Ureter.

1.3.2 Roles and functions of Kidneys

The important roles and functions of kidneys in the human body are (a) Homeostatic function (b) Endocrine function and (c) Gluconeogenesis function.

(a) Homeostatic function: It regulates the amount of fluid in the body
(i) Regulation of plasma osmolarity: The kidney regulates the osmolarity of the blood because they have direct control over the number of ion is and the amount of water a person excretes. The kidney regulates sodium and potassium ions, Magnesium chloride, bicarbonate and phosphate ions.

(ii) Blood plasma regulation: The kidney affects the blood pressure by regulating the blood plasma volume. The blood plasma volume controls the salt NaCl which cause osmosis, the diffusion of water in the blood.

(iii) Regulation of plasma pH: The kidneys control the pH of blood by regulating the hydrogen ion concentration. The kidneys excrete hydrogen ions and reabsorbs bi-carbonate ions as needed. The CO₂ formed in the cell (as the product of many chemical reactions) enters in the blood capillaries, where red blood cells (RBC) contains an enzyme called carbonic anhydrase that helps CO₂ to combine with water(H₂O) giving carbonic acid(H₂CO₃). The carbonic acid quickly separates in to hydrogen ion (H⁺) and bi-carbonate (HCO₃⁻)

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^- 
\]

The correct pH value is maintained by keeping the constant hydrogen ion and bicarbonate ratio. If the amount of filtered bicarbonate is greater than the amount of hydrogen ion secreted, then the bicarbonate will be lost in the urine. Similarly, if the amount of secreted hydrogen ion is greater than the amount of bicarbonate filtered then the hydrogen ion will be lost in the urine, increasing the pH value of urine.
(iv) Removal of metabolic waste and foreign substances: The most important role of kidney is excretion of nitrogenous waste, creatinine and uric acid. As the liver breaks down amino acids, the ammonia is produced. Large amount of ammonia quickly combines with carbon dioxide to form urea. The urea is a primary nitrogenous end product of human metabolism, but less toxic compared to nitrogen. The breakdown of creatinine phosphate present in muscle gives creatinine. The uric acid comes from the breakdown of nucleotides. The uric acid is insoluble and high level of uric acid in the blood creates crystals which cause gout.

(b) Endocrine function: The endocrine system includes kidneys i.e. the kidneys are the endocrine organs, it secretes renins, renal erythroproteins. Renin leads to the secretion of aldosterone which promotes the kidney to reabsorbs the sodium ions. When the blood does not have the capacity to carry oxygen, the kidney releases the erythroproteins which stimulates the red blood cell (RBC) production. The vitamin-D from the skin is also activated by kidneys, which promotes the calcium ions absorption in the digestion tract.

(c) Glucogenesis function: The kidneys have the important ability to synthesis and secrete glucose produced from non-carbohydrate sources, example gulatine, only in special cases like long fastings and respiratory acidosis.

1.4 Formation of Urine

There are three steps in the formation of Urine: (i) Filtration, (ii) reabsorption and (iii) secretion.
(i) Filtration: When the blood passes through renal artery it enters into afferent arteriole and flows into the glomerulus. The blood in the glomerulus has both filtered and non-filtered blood components. The filterable blood components after filtration is collected in the Bowman’s capsule, this is called glomerular filtrate. This is the first step in formation of urine. The glomerular filtrate includes water, nitrogen waste, nutrients and salts (ions). The non-filterable blood components include formed elements such as blood cells and platelets along with plasma proteins.

(ii) Reabsorption: As the glomerular filtrate passes through the convoluted tubule, the surrounding peritubular network absorbs molecules glucose, amino acids and ions back in the blood stream. The NaCl reabsorbed into the system increases the osmolarity of the blood with respect to glomerular filtrate. This reabsorption process allows water to pass from the glomerular filtrate back in the circulatory system.

The glomerular filtrate is now been separated into two: (i) Reabsorbed filtrate and (ii) Non-reabsorbed filtrate. The non-reabsorbed filtrate is called tubular fluid which is passed through duct to ureters.

(iii) Secretion: Secretion is the reverse process of reabsorption. Some substances like H⁺, creatinine and drugs are secreted (removed) from the blood through peritubular capillary network into the distal convoluted tubule. Hence, urine is an end glomerular filtrate after reabsorption and secretion.
1.5 Urine and its composition

In the metabolic process the nucleic acid and proteins will breakdown, both of which contain nitrogen. Some of nitrogen is used in creating new nitrogen containing molecules, but majority of them have to be disposed off as waste. The first nitrogen containing molecule is ammonia $\text{NH}_3$ which is soluble water, forming a strong base $\text{NH}_4\text{OH}$ which raise the pH value, and is toxic. The two forms of nitrogen which is excreted from human beings are urea and uric acid as urine and are acidic in nature.

![Fig 1.6 Filtration, secretion and reabsorption in the formation of urine](image)

Urine is the liquid filtered by kidneys. The composition of urine is adjusted after filtration; secretion and reabsorption. It consists of large amount of water which urea and other organic and inorganic chemicals are dissolved.
The variation in the concentration of these substances occurs due to the influence of factors like metabolism, dietary intake, endocrine function, physical activity and body type. Urea is the major constituent of urine, it is a metabolic waste produced in the liver due to break down of protein and amino acids. The major organic compounds are creatinine, uric acid. The important inorganic solids dissolved are sodium chloride, potassium chloride and phosphates of potassium and calcium. As the concentration of inorganic compounds depends on the dietary intake, thereby it is difficult to establish their normal levels. There will be substances like hormones, vitamins and medications which are not due to filtration of blood plasma at glomerulus.

Table 1.1: Amount of major constituent filtered, reabsorbed and excreted in a day

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Filtered (gram/24 hour)</th>
<th>Reabsorbed (gram/24 hour)</th>
<th>Excreted (gram/24 hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>630</td>
<td>625</td>
<td>5.3</td>
</tr>
<tr>
<td>Sodium</td>
<td>540</td>
<td>537</td>
<td>3.3</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>300</td>
<td>300</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>140</td>
<td>140</td>
<td>0.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>28</td>
<td>24</td>
<td>3.9</td>
</tr>
<tr>
<td>Urea</td>
<td>53</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Uric acid</td>
<td>8.5</td>
<td>7.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.4</td>
<td>0.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Sl. No</td>
<td>Constituent</td>
<td>Excreted in a day (24 hr)</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Urea</td>
<td>25-30g</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Uric acid</td>
<td>0.3-1.0g</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sodium</td>
<td>3-4g</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Potassium</td>
<td>2-4g</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Chlorides</td>
<td>10-15g</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Calcium</td>
<td>0.2-0.4g</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Inorganic phosphorus</td>
<td>1-1.5g</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sulfur</td>
<td>1.0-3.5g</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Iodine</td>
<td>50-250g</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Arsenic</td>
<td>&lt;50μg</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Hippuric acid</td>
<td>0.1-1.0mg</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Creatinine</td>
<td>1-2g</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Phenol</td>
<td>0.2-0.5g</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Coproporphyrins</td>
<td>60-250g</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Allonotion</td>
<td>20-30g</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Indican</td>
<td>2-4mg</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Oxalic acid</td>
<td>15-20g</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Ketone bodies</td>
<td>5-15g</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Purine bases</td>
<td>8-10g</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Lead</td>
<td>&lt;50μg</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Creatine</td>
<td>75-150mg</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Magnesium</td>
<td>0.05-0.2g</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Ammonia</td>
<td>0.5-1.0g</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Hormones, Vitamins and Enzymes</td>
<td>In very small Quantity</td>
<td></td>
</tr>
</tbody>
</table>

There may be certain formed elements like RBCs, WBCs, Casts, mucus, bacteria and crystals. The increased values of these formed elements are an indication of disease. The normal human urine contains substances after filtration & reabsorption.
1.6 Urine specimen types & collection

The composition and concentration of urine change continuously during the course of the day or 24 hours. The urine concentration changes according to water intake & activities before the test. It is important and necessary to regulate some aspects of urine collection, like time of collection, length of collection period (2hrs, 12hrs, 24hrs and mid day clean catch), dietary intake and medicinal intake and method of collection to represent patient’s truly metabolic state. The time of the day when specimen is collected influence the test result and findings. The first morning specimen is valuable, as it is relatively free of dietary influence and change caused by physical activity, because the specimen is collected after a period of long rest and fasting. The first morning urine is more likely to reveal the presence of formed substances & abnormalities. The first morning urine is best for nitrate, proteins, microscopic examination, routine screening & pregnancy test.

The random specimen, clean-catch (mid stream) and second morning specimen (double voided) have common most common characteristics and minimum bacterial count. These types of specimens are used for chemical screening, routine screening, bacterial culture and microscopic examination.

The post prandal and timed 2hr & 12hr urine are used for diabetic monitoring which reflects blood glucose. In these cases total specimen of the time must be collected. In many pathological tests long term
24hr specimen is necessary, because substances excreted by the kidney are not excreted at the same rate or in the same amount during different periods of day and nights. For the measurement of total electrolytes, creatinine, urine protein in diseases more accurate information can be obtained by long-term 24hr urine specimen. The urine voided in 24hrs period is collected in to a proper receptacle, a suitable preservative may be added depending on the type of test and collection is refrigerated or in cool chamber, not at higher temperatures. For glucose test, in case of Glucosuria to confirm the diabetes, the specimen need not be refrigerated, but should kept or stored in a dark bottle and 1gm of boric acid can be added as preservative. For ketonic steroids test & ketosteroid to find the adrenal and gonadal abnormalities the specimen should not be refrigerated.

1.7 Pathology of urine

The physical, microscopic and chemical analysis of urine has been discussed.

1.7.1 Physical examination

The physical examination of urine includes urine volume, colour and appearance, specific gravity, urine odour, pH & reaction of urine, and urine blood or hemoglobin.

(a) Urine Volume: The urine volume is a measure of fluid balance and gives information of kidney function. It depends on the amount of water excreted by the kidneys. Since water is a major body constituent, thereby the amount excreted is determined by body’s state of dehydration.
The normal volume of urine excreted by an average adult is 750 ml to 2500 ml/24 hr, averaging about 1250 ml. The factors that affect urine volume are fluid intake, fluid loss from renal sources, and excretion of increase amount of salt and glucose, and variation in the secretion of anti-diuretic hormone (ADH). Children excrete small volume of urine compared to adults, but total urine excreted is greater in proportional to their body size.

The abnormal decrease in the excretion of urine less than 400 ml/24 hr known as obligatory urine volume is called Oliguria. It is seen in renal ischemia, a renal disease due to toxic agents, obstruction in the urinary tract and glomerulonephrities. The excretion of urine volume less than 100 ml/24 hr is called Anuria. It is seen in case of acute necrotizing glomerulonephrities, hemolytic transfusion reaction and in renal failure due to urinary tract obstruction. Polyuria is the abnormal or marked increase of urine production and excretion normally more than 2.5 liter a day. The polyuria with elevated blood urea nitrogen (BUN) and creatinine levels are due to some type of tubular necrosis, diabetic ketoacidosis and partial obstruction of genitourinary tract. The Polyuria with blood urea nitrogen (BUN) and creatinine is due to diabetic mellitus and diabetic insipidus, neurotic state of compulsive water intake and some tumors in brain and spinal cord. The external factors like intravenous glucose or saline, pharmalogical agents like thiazides and other diuretics such as coffee, tea, and alcohol cause polyuria.
(b) Color and appearance: Normally the urine is pale lemon yellow in colour, due to the presence of pigment urochrome, which is a product of metabolism that under normal condition is produced at constant rate. In case of Thyroid condition and fasting the production of urochrome increases. Depending on the amount of fluid intake, urine ranges in colour from lemon yellow or clear (dilute) to dark amber.

(i) The pale colourless urine is due to large fluid intake, untreated diabetic mellitus, chronic interstitial nephritis, nervousness and diabetic insipidus.

(ii) The dark amber colour urine is very concentrated caused by fever, reduced fluid intake, sweating. The presence of bilirubin and vitamin-A ingestion in urine gives a orange colour to it.

(iii) Pink to red in colour urine is due to traces of RBCs, hemoglobin, methemoglobin, myoglobin and porphyrins.

(iv) The Brown-black urine is due to RBCs oxidized to methemoglobin, homogenetic acid, melanin or melanogen and phenol poisoning.

(v) The smoky and milky urine is due to fat, cystinuria, WBCs and non-pathological phosphates.

The external factors like dietary intake and drugs will also change the colour of urine. Red-pink urine could be caused by ibuprofen, daumorubicin, doxorubicin, heparin etc. The blue urine may be due to triamterene. Beet root turns the urine red Rhubarb can cause brown urine. The bright yellow colour urine is caused by riboflavin or phenazopyridine.
Appearance: Normal urine is generally clear and hazy. Most pathological urines are cloudy and turbid. The cloudiness may cause due to abnormalities. The presence of WBCs, RBCs, bacteria and epithelial cell gives cloudiness to urine. The urine turbidity is due to urinary tract infection. Sometimes the normal urine may also appear cloudy because a change of pH can cause precipitation within the bladder. The acid urines appear cloudy because of urates and the alcholine urine appears cloudy because of phosphates present in it.

The external factors like semen or vaginal discharge mixed with urine are common cause of turbidity in normal urine. After ingestion of food, urates, phosphates, carbonates may produce cloudiness in normal urine. Extraneous contamination can cause turbidity of urine.

(c) Urine Specific Gravity (USG): The specific gravity measures the concentration of particles in the urine. It is the density of urine compared to water. The urine specific gravity (USG) depends on the number of molecules, as well as their molecular weight and size in the urine. The normal value of USG is 1.003 to 1.029. It is also affected by temperature, increases with increasing temperature. USG provides the information about the kidney ability to concentrate the glomerular filtrate. The increasing specific gravity can be seen in low water intake, albuminuria, diabetic mellitus and acute renal failure. And, the decrease specific gravity is seen in kidney damage (tubular damage) resulting in loss of concentration power of kidney tubules and also in excessive water intake.
Generally the urine specific gravity decreases with the amount of urine excreted, but the relation is not valid in some conditions:

(i) In diabetes, the specific gravity increases with increase of urine volume.

(ii) In hypertension, with the normal volume of urine the specific gravity decreases, but in early chronic renal disease with increased urine volume, the specific gravity decreases.

**Hyposthenuria:** The excretion of urine with low specific gravity having values 1.001 to 1.010 is called hyposthenuria. This occurs in diabetic insipidus caused by increased or absence of anti-diuretic hormone (ADH) which triggers kidney absorption of water. In case of total absence of anti-diuretic hormone (ADH) kidney produces excessive amount of urine about 10 to 15 liters a day. The kidney inflammation without bacterial infection is called glomerulonephritis, and the kidney inflammation with bacterial infection is called phelonephritis, in either of these cases the urine specific gravity is low with decreased urine volume. The tubular damage also affects the kidney’s ability to concentrate urine.

**Hypersthenuria:** The excretion of urine of high specific gravity from volume between 1.026 and 1.035 is called Hyperstunuria. This occurs in case of nephrosis, diabetic mellitus, excessive water loss due to dehydration, vomiting, fever and diarrhea, increased secretion of anti-diuretic hormone (ADH), toximinia of pregnancy congestive heart failure etc.
External effects may cause false value of specific gravity of urine. Detergent residues on specimen container can give high value of specific gravity. When refractometer is used for the specific gravity then the radio-opaque, ray contrast media, minerals may cause high specific gravity readings.

(d) Urine odour: The urine from healthy persons has an aromatic odour. The presence of ketone bodies gives urine fruity or sweet smell. The contaminated urine is when kept for long time gives pungent odour due to bacteria and formation of ammonia. The urine of infants and neonates with phenylketones gives a musty smell. The smell of urine changes with pathological conditions. The urine of patients with diabetic mellitus will give a fruity smell due to ketosis. The maple syrup urine disease is the disease in infants due to inherited disorder of amino acid metabolism, gives urine a burnt sugar or maple smell. The cystinuria and homocystinuria gives a sulfurous smell to urine. The tyrosinemia is characterized by cabbage-like or fishy urine smell.

(e) pH and reaction of urine: The urine pH is one of the important parameter in pathology of several diseases, which gives the concentration of hydrogen ion (H+) in urine. It indicates the existence of systemic acid-base disorder of metabolic or respiratory origin and in the management of urinary conditions. The normal urine pH ranges from 4.5 to 7.8 and the average value is 6.2 which is slightly acidic in nature.

The pH is an indicator of renal tubules ability to maintain normal hydrogen ion concentration in the extra cellular fluid and plasma. The
kidney maintains normal acid-base balance through reabsorption of sodium and tubular secretion of ammonia and hydrogen ions. When the amount of sodium and excess acid retain by the body increases, then the urine becomes more acidic. The control of pH of urine is very important in the management of diseases like renal calculi, bacteriuria and drug therapy in which methenamine and streptomycin is being administered.

(i) Renal Calculi: The stone formation in kidney is called renal calculi which partially depend in the pH of urine. Calcium carbonate, calcium phosphate and magnesium phosphate stones develop in alkaline urine.

(ii) Drug treatment: The alkaline urine is produce by the drugs such as neomycin, streptomycin and kanamycin for the treatment of genitourinary tract infection. The sulfatherapy produce alkaline therapy which helps in preventing the sulfonamide crystals. Urine should be kept persistently alkaline during blood transfusion.

(iii) Clinical Conditions: The accurate measurement of pH of urine for freshly excreted specimen. The decrease pulmonary ventilation during sleep causes respiratory acidosis; thereby the urine becomes more acidic. Bacteria from a urinary tract inflammation or from bacterial contamination of the specimen produce alkaline urine, because the urea is converted into ammonia.

(iv) Dietary Conditions: Diet with high protein and meat makes the urine more acidic. Cranberry juice is the only fruit that maintain the acidification of urine for longtime; it can be used in the treatment of
urinary tract inflammations (UTIs). Citrus fruits and most vegetables, particular legumes keep the urine alkaline. Alkaline urine after meals is a normal response to the secretion of hydrochloric acid in gastric juice.

The urine pH less than 7.0 (acidic) occurs in metabolic acidosis, diabetic ketosis, diarrhea, uremia and starvation. Urinary track inflammation (UTI) caused by Escherichia coli, renal tuberculosis, respiratory acidosis (retention of carbon dioxide), Pyrexia.

The alkaline urine (pH > 7.0) occurs in chronic renal failure, renal tubular acidosis, urinary track inflammation (UTI) caused by urea – splitting bacteria, Respiratory alkalosis involving hyper ventilation, Potassium depletion. The urine pH never exceeds 9.0 either in normal or abnormal conditions.

(f) Urine blood or Hemoglobin: Normally no blood or hemoglobin is found in urine. The blood in urine is always an indication of kidney or urinary tract damage. The presence of free hemoglobin in the urine is called hemoglobinuria, it is related to conditions outside the urinary tract and occurs when there is an extensive or rapid destruction of circulating erythrocytes that reticuloendothelial system cannot store or metabolise the excess free hemoglobin. The hemoglobin is them filtered through glomerulus the hemoglobenuria may also occur due to lysis of RBCs in the urinary tract.

Hematuria is the presence of intact RBCs in the urine. It is related to disorders of renal or genitourinary system in which bleeding is the result of trauma or damage of organs or system.
When urine sediments are positive for occult blood but RBCs are not found microscopically then it is called myoglobinuria, which will be caused by excretion of myoglobin, a muscle protein in urine. The excretion of muscle protein in urine could be due to traumatic muscle injury, muscle disorder such as muscular dystrophy, malignant hyperthermia related to administration of certain anaesthetic agents.

The hematuria is found in renal or urinary tract tumours, acute UTI (cystitis), pyelonephritis, glomerulonephritis both acute and chronic, leukemia, malignant hypertension, urinary calculi (intermittent hematuria) and thrombocytopenia.

The hemoglobinuria is found in transfusion reaction due to incompatible blood product, extensive burns, bleeding resulting from operative procedures on the prostate, hemolytic disorders such as sickle cell anemia, thalassemia etc., certain chemical agents and alkaloids, febrile intoxication.

The early indication of possible renal or urinary tract disease is the presence of blood in urine even not in every excreta. In most cases occult blood appears in the urine.

1.7.2 Microscopic analysis

Method: For the pathological and microscopic analysis, urine to be analyzed is centrifuged in a test tube about 2000 to 3000 rpm for 8 to 10 minutes or until a button is produced at the bottom of the test tube. The supermate is decanted and a volume of about 0.5 ml sediment may remain in the tube. The sediment is re-suspended in the remaining supernate by flicking the bottom of the tube. A drop of
re-suspended sediment can be poured on to a glass slide and cover slipped. The examination can be carried for higher power field to find and identify various crystals bacteria and puss cells.

Pathology:

(a) Red Blood Cells (RBCs): Normally there should be no RBC in urine, but one or two may occur even in normal human urine. The presence of abnormal number of RBCs more than three in the urine is called Hematuria. This is due to glomerulur damage, tumors present in the urinary tract, kidney trauma, renal infract, urinary tract stones, nephrotoxins and physical stress.

![Fig 1.7 RBCs in urine](image)

(b) White Blood Cells (WBC): The leukocytes are the puss cells; they may enter in urine anywhere from glomerulus to urethra. The normal urine contains 2 to 3 leukocytes/hpf. Abnormal number of leukocytes in urine is called Pyuria; this is due to infection in urinary tract anywhere from glomerulus to urethra. Normally the white blood cells granulocytes. They creep in from vagina, especially in presence of vaginal and cervical infection or external urethral meatus in men and contaminate the urine.
(c) Epithelial cells: The renal tubular epithelial cells usually larger than granulocytes, and originates anywhere in the genitourinary tract, from PCT to the urethra. The epithelial cells have a large round or oval shape nucleus, and they are formed due to sloughing of old cells. With the nephritic syndrome and conditions leading to tubular degeneration the sloughing increases, there by epithelial cells increases. Normally 3 to 5 cells/hpf can be found in the urine due to sloughing of cells.

There are mainly 3 types of epithelial cells are recognized in human urine. They are:

(i) Renal tubular epithelial cells: These are slightly larger than leukocytes and have large nucleus due to endogenous fat present in them. They are formed due to lipiduria, and may be oval, flat columnar or cubical in shape.

(ii) Squamous epithelial cells: They are formed at the skin surface or outer urethra and pass into and make their way in to the urine. They are large flat and irregular in shape and contain large cytoplasm and small central nuclei.
(iii) Transitional epithelial cells: These cells originate at renal pelvis, ureter or bladder, and have regular cell borders and large nuclei. They are 2 to 3 times as longer as white cells, but smaller than squamous epithelial cells. They may be ‘O’ shaped having large nuclei, thereby much volume of the cell is being occupied by the nucleus.

(iv) Casts: The urinary casts are formed in the lumen of tubule of kidney, the end part of nephron. They are formed in the distal convoluted tubule (DST) or in the collecting duct, because casts require acidic condition and high solute concentration. They are not found in the PCT and loop of henle. The renal tubules secrete a
mucoprotein called Tomm-Horsfall protein, which is believed to form the basic matrix of all type of casts.

![Diagram of kidney showing casts](image)

**Fig 1.12 Position of casts in renal system**

The casts can be formed due to (i) perception of geletin of Tomm-Horsfall protein, (ii) clumping of cells on other material within the protein matrix, (iii) the adherence of cells or cellular material to the matrix and (iv) coagulation of material within the lumen.

The factors which favour proteins casts are high salts concentration, low flow of urine due to fewer intakes of water and low pH. In all these cases protein denaturation and precipitation is particularly that of Tomm-Horsfall muco protein occurs.

Casts can be broadly classified in to two types. They are a) cellular casts, b) Acellular casts.

(a) Cellular casts: There are two types of cellular casts (i) Red blood cell cast (ii) white blood cell casts. The red blood cell casts are formed as the red blood cells stick together. They indicate a significant renal
disease called glomerunephritis with leakage of RBCs from glomerulli due to severe tubular damage.

![Fig 1.13 Red blood cell cast in urine](image)

White Blood Cell Cast are formed when the white blood cells stick together to form a cast. This is an indication of Pyelonephritis, some time they may present in case of glomerulonephritis. The presence of WBC casts indicates inflammation of kidney, as these kinds of casts are formed only in kidneys.

![Fig 1.14 White blood cell cast in urine](image)

(b) Acellular casts: The acellular casts are the protein cast. They are hayline casts, granular casts, waxy casts etc. The hayline casts is formed at the junction of ascending limb of loop of henle and distal convoluted tubule. They are formed due to denaturation and precipitation of Tomm-Horsfall protein.

![Fig 1.15 Hyaline cast in urine](image)
The granular casts and waxy casts are formed due to long stay of RBC and WBC cast in nephrons. The cells may degenerate to become later a coarsely (of core) granular cast and finally waxy cast. Large such casts are generated from damaged and diluted tubules and hence can be seen in the end stage of chronic renal disease.

Fig 1.16 Granular cast in urine

Fig 1.17 Waxy cast in urine

1.7.3 Chemical examination

The chemical examination of urine includes the pregnancy test, urine estrogen test, urine drug investigation and electrolytes in urine.

(a) Human Chronic Gonadotropin (hCG) test: The human chronic gonadotropin (hCG) test is called urine pregnancy test. From early stage of pregnancy, the placentatrophoblast produces human chronic gonadotropin hormone in conjunction with fetus, which is excreted in the urine. This hormone is not present in the urine of male and non-pregnant women. The high value of hCG confirms the pregnancy. The first morning excreted urine contains large concentration of hCG.
random specimen may be used but the specific gravity must be more than 1.005. The gonadotropin is the follicle-stimulating hormone.

(b) Urine estrogen: The most active endogenous estrogen is the Estradiol. In females the value of estradiol in follicular phase is about 3 µg in a day. The increased value of about 10 µg/24 hour urine indicates the ovulatory and luteal phase. Estadiol is the important urinary estrogen in pregnancy. The estradiol and the gonadotropin hormones are useful in the determination of menstrual and fertility problems in female.

In men estradiol is useful for evaluating problems relating to estrogen producing tumors. The normal value of estrodiol in men ranges 0-6 µg/24 hour urine. The estriol in both blood plasma and urine rises as pregnancy advances. In follicular plasma it may be up to 12 µg/24 hour urine, this value increases in the third trimester of pregnancy. In men the value of estriol ranges from 2-12 µg/24 hour urine.

The total urine estrogen in men in normal conditions is 20-30 µg/24 hour urine. The total urine estrogen in females in normal or menstruating period is 50-80 µg and has a very high value in the third trimester of pregnancy could be up to 0.5 gm/24 hour urine. The increasing value of urine estradiol could also be found in feminisation in children (testicular feminization syndrome), estrogen producing tumors. The decreased value is the indication of menopause, primary and secondary hypogonadism.
(c) Urine drug investigation: For the screening of unknown drugs in the human body the urine samples are most preferred than gastric content and blood, because the drug concentrations are much elevated in urine and they may not be detectable in blood. The drug metabolites are excreted for a longer period for days and weeks through urine, indicating the past drug used. The common urine drug test are performed to test for drug abuse at work places, pre-employment screening for drug use, doping in athletes, prisoners & paroles drug abuse and to confirm clinical or postmortem diagnosis. The common urine drug tests have a screening cut-off levels and the length of detection. Some of the common tests are (i) the cocaine test - the metabolite is benzoylecgonine, the cut-off is 300 ng/ml for 24hour urine, (ii) Heroin test - the metabolite is acetyl morphine, the cut-off value is 20 mg/dl for 2 hour length of detection, (iii) Alcohol test - the metabolite is ethanol 2 μg/dl for the 12 hour length of detection, (iv) Opiates test - the metabolite is morphine 300 ng/ml and the length of detection is 2 to 4 days. The urine drug test is sensitive to physical parameters like surface tension which is influenced by external detergents and the low specific gravity when the urine is diluted by water.

(d) Electrolytes in urine:

(i) Urine Chloride: The urine chloride excretion depends on the dietary intake. The chloride is most often associated with sodium balance and fluid change. The amount of excretion of chloride is an indication of the state of electrolyte balance. It is used to diagnose dehydration or
as a guide in adjusting fluid and electrolyte balance in post operative patients. It also monitors the effect of reduced salt diet, which has a great therapeutic importance in patients with cardiovascular, hypertension, kidney ailments and liver diseases.

The 24 hour urine test for urinary anion gap (Na⁺ K⁺) Cl⁻ is used in the evaluation of hyperchloremic metabolic acidosis. The normal adults have 150-250 m mol. of chloride in 24 hour urine. The value is much lower in children of age up to 5 years which is about 40 m mol. a day and about 150 m mol. /day for children of age 10-14 years. The decreased value of chloride occurs in syndromes of inappropriate antidiuretic hormone secretion, vomiting, diarrhea, metabolic alkalosis etc. The increased urine chloride occurs in potassium depletion, salt losing nephritis, increased salt intake and adrenocortical insufficiency.

(ii) Urine Sodium: The human body has a strong tendency to maintain acid-base concentration. The sodium is predominant base; it is primary and important regulator for retaining and excreting of water. The sodium has the ability to combine with chloride and bi-carbonate. It also promotes the normal balance of electrolytes in the inter-cellular and extra cellular fluids by acting in conjunction with potassium under the effect of aldosterone. The sodium test will be performed on 24 hour urine collection which will done for the diagnosis of renal, water, adrenal and acid-base imbalance. The normal value for adults is 100-200 m mol. Per a day or 24 hour and 50-100 m mol Per a day or 24 hour for children. These values are for the normal dietary
intake. The increased urine sodium occurs in salt losing nephritis, adrenal failure, renal tubular acidoisis, diabetic acidosis, tubulointerstitial disease etc. The decreased value occurs in congestive heart failure, excessive sweating, nephrotic syndromes with acute oliguria etc.

(iii) Urine Potassium: Potassium serves as an important function in the human body’s overall electrolyte balance and maintains body’s buffer system. Since the kidneys cannot conserve potassium, this balance is regulated by the excretion of potassium through the urine. For the urine potassium test 24 hour urine collection is required which provides the insight in to the electrolyte balance, renal adrenal disorders, and water & acid-base imbalance. The normal value of potassium in adults ranges from 50-100 m mol. /24 hour. And in children it is 10-50 m mol. /24hour excretion. These values depend on diet intake. The increased urine potassium is found in primary renal diseases, diabetic, renal tubule acidoisis, starvation, onset of metabolicalkalosis etc. The decreased urine potassium found in severe renal diseases like glomerulonephritis, pyelonephritis etc. In all these cases the urine pH value decreases.

(iv) Urine Calcium: The parathyroid hormone in the body maintains the calcium homeostasis. The bulk of calcium is eliminated in the stool and a small quantity is normally excreted in the urine. The urine calcium test is performed on timed 24 hour urine, for evaluating the rate of intestinal absorption, bone resoption, renal loss and also calcium intake.
The normal value of calcium for normal diet ranges from 100-300 mg/24 hour, and with low calcium diet it ranges from 50-150 mg/24 hour. The urine calcium is high in 30 to 80% of cases are primary hyperparathyroidism. The urine calcium also increases in primary breast and bladder cancer, vitamin-D intoxication, thyrotoxicosis, osteolytic bone metastases etc.

The increased calcium excretion occurs whenever calcium is mobilised from bone as in metastatic cancer or prolonged skeletal immobilisation. It also creates neprolithiasis or nephrocalcinosis especially with high protein. The decreased urine calcium is found in vitamin-D deficiency, acute nephrosis, nephritis, renal failure and vitamin-D resistant and rickets.

(iv) Urine Magnesium: The magnesium excretion controls serum magnesium balance. The urine magnesium test performed for investigation of electrolyte status, magnesium metabolite, nephrolithiasis and it also used for assessing the case of abnormal serum magnesium.

With the normal dietary intake of 300-500 mg/day of magnesium, urine magnesium excretion is normally 100-150 mg/24 hour, and the remaining of intake magnesium is excreted in stool. For urine magnesium test the specimen should be collected for 24 hours in a non-metal and acid-rinsed container. The pH should be less than 2. The increased urine magnesium is associated with chronic glomerulonephritis, increased blood alcohol, barters syndrome etc. The decreased urine magnesium is found in hyper calcuria, chronic renal
disease, malabsorption, long term chronic alcoholism and decreased renal function etc.

1.8 Types of Kidney diseases

Different types of kidney diseases are caused due to different reasons and they show different symptoms. The kidney diseases can be broadly classified into two (i) acute kidney disease (ACD), (ii) chronic kidney disease (CKD). The acute kidney may develop suddenly due to various reasons while the chronic kidney disease develops over a long period of time. The polycystic kidney disease (PKD) is one of most common ACD, due to many cyst or cavities formed in the kidney. Pyelonephritis also called urinary tract infection is kidney disorder that refers to infection of kidney usually due to bacteria but could be caused due to viruses. This is due to obstruction of urine flow in the ureter or flow of urine flow ureters to bladder.

Kidney influx is a type of disorder in which the urine in the bladder passes back to the ureters and then to the kidney instead of going out through the urethra for the process of urination. The kidney influx occurs due to improper contraction of muscles that present at ureter and bladder. Renal acidosis is another type of kidney disorder in which the patient feels shortness of breath and speed up the heart rate and then the pulse rate increases. In this type of kidney disorder, the acid products which are not being properly excreted by the kidney accumulate in the blood. Thereby the acidity of blood increases and this stimulates the receptors in the brain to speed up rate of breathing.
Mesangial proliferative glomerulonephritis is a type of kidney disease that could be there in the body for a long time without any symptoms and ultimately leads to CKD. In some patients of glomerulonephritis shows the common symptoms like high blood pressure, anemia, swelling etc. The most common kidney disease occur due to inflammation of kidney is nephritides. Beside inflammation it could occurs due to glomerulonephritis, autoimmune nephritis, nephropathy, lupus nephritis, gout, some type of poisons, medicines and certain metabolic disorder etc. The symptoms of nephritis are reduced urine, edema, blood urine, pain in kidney etc. Nephropathy is a type of kidney disease due to non-inflammation of kidney having similar symptoms as nephritis. The different types of nephropathy are toxin nephropathy, diabetic nephropathy, obstructive nephropathy, reflex nephropathy, analgesic nephropathy etc.

The other types of common kidney disease are (i) Haemangiomas and (ii) Parenchymal. Haemangiomas occurs due to tumor in the kidney, causes haematuria. In the parenchymal kidney disease, the kidney is damaged and scarred and loses its ability to function normally.

There are some rare types of kidney diseases. These disorders include hyperfiltration, mild micro albuminuria, advanced clinical nephropathy, chronic renal insufficiency and clinical micro albuminuria. These can be treated with alternative medicine when detected at early stages.
1.9 Survey of literature

Lang (1904) studied the electrical conductivity of urine in relation to its chemical composition. He has concluded that the electrical conductivity depends mainly on the sum of the inorganic compounds such as sodium chloride and also the inorganic salts of phosphate and sulfate of alkali and alkali earth metals.

Dawson Turner (1907) investigated the role of electrical conductivity of blood and urine in health and in diseases and as a test of functional efficiency of the kidney. He has determined that the observed resistance of normal urine is about 250 ohms with his apparatus made with Wheat stone bridge, with alternating current and at temp 65 degree F. In artificial urine it was found that the resistance depends only on the ionic concentration. The resistance for practical clinical purposes can be regarded as being unaffected by the presence of albumin; sugar; blood and other non-electrolytes. In some acute and chronic diseases a very high resistance is found.

Ootto folin and W. Denis (1914) have studied the convenient methods to determine the albumin in urine. The two methods are turbidity method and gravimetric method. The results fairly agree with each other. Turbidity method is not applicable to urines which are very deeply colored with blood or bile pigments.

Addis and Watanabe (1917) studied the effect of changes in the volume of urine and rate of urea excretion. They advocated that the change in the volume of urine and concentration of the urine does not affect the rate of urea excretion.
Perryman and Selous (1935) carried out a study of surface phenomenon of complex colloidal systems occurring in human body. They investigated urine and found that the surface tension of human urine changes with time. The presence of percipitable proteins is not responsible for change in surface tension. They concluded that the surface tension of urine cannot be related to specific gravity or constituents such as blood, albumin, puscells or bile and there is no correlation between any specific and group of diseases.

Milton E. Rubini et al (1956) studied the refractometric analysis of total solids in serum and urine samples which is accurate and rapid using Bausch and Lomb dipping refractometer with and without auxiliary prism, with a sodium vapour lamp (D-line). They employed the instrumented increment of the refractometer which is the difference between the scale reading for solution and water is constant temperature. They confirmed Blohm’s numerical value for the refractometric coefficient and that the refractometric is an accurate and practical measure of specific gravity.

Norman G. Lewinsky and Robert W. Berliner (1959) have worked on Changes in the composition of the urine in the uterus and bladder at low urine flow. The movements of water and solute across the uterus and bladder have been studied. When the uterus and bladder are prefused at flow of less than 1ml/min movement of water, urea, sodium, potassium, chloride, creatinine and hydrogen ion occurs. The magnitude of these changes increases as a rate of perfusion is decreased or when urine is allowed to pool in bladder.
Price J. W. Miller and C. F. Speth (1968) reported the specific gravity of a complex aqueous solution is an additive property of the contributions of the individual constituents. In the study they prepared artificial urine by weighing the pure substances and dissolved then in pure water of volume of 1 liter at 20 ºC. The specific gravity factors of the various urinary constituents range from 0.9 to 3.6 times the factor of urea.

Weeth H. J. et al (1969) determined the specific gravity and refractive index of urine collected from Hereford heifers in water deprived and over hydration condition for 4 days. The specific gravity was found by weight of sample divided by volume and refractive index by temperature compensated refractometer. The values of specific gravity and refractive index were in the range of 1.0030 to 1.0413 and 1.3361 to 1.3493 respectively.

Wolf and Pillay (1969) performed the concentration test in patients with varying degrees of renal insufficiency and in normal control subjects; comparing measurements of urine specific gravity; electrical conductivity; osmotic pressure, refraction and related concentrative properties. No one concentrative property was found to be unique diagnostic value. Such test can be usefully quantified for several concentrative properties by means of boundary values above which renal function is assured to be normal.

W.K. Simmons (1972) reported that as the protein intake increases, the urea nitrogen becomes a greater percentage of the total nitrogen excreted. The ratio of urea nitrogen to the total nitrogen and
creatinine is very low in the groups with poor nutrition. The ratio of urea nitrogen to creatinine in fasting urine differs among population groups with different socioeconomic and nutritional background and it is a good biochemical indicator to distinguish among groups with different levels of protein intake.

Richard L. Stevens et al (1975) studied properties of arylsulfatase A from Human urine. They have purified arylsulfatase A 3500-fold at 7% yield from human urine. A crude urinary protein concentrate was prepared by treating pooled urine with ammonium sulfate and subsequently drying with acetone. The powder thus obtained was examined with buffer and was subjected to the chromatographic and electro phoretic procedures.

Jikken Dobutsu (1981) studied a relationship between the refractive index and specific gravity of the rat urine. The relationship between the refractive index and specific gravity of urine was studied with specimens from 165 Sprague-Dawley rats, by graphic analysis of the plot of the refractometrically determined index against the specific gravity. A linear regression was demonstrated between the refractive index and specific gravity. The monogram was in good agreement, in respect of linearity, with the regression line derived from the conversion table of TS meter by the American Optical Corporation and also with the nomogram of the Japanese Society of Clinical Pathology.

Caroline Smith et al (1983) evaluated and compared the effect of intravenous X-ray contrast media on specific gravity by methods, refractometry, hydrometry, reagent strip and osmometry. The X-ray
contrast media used was Hypaque meghimine is directly added to a pool of water. They observed that the relative density increases with the increase of X-ray contrast media in urine. The reagent strip also measures the specific gravity directly by measuring ionic concentration. But the presence of non-ionic molecule of the solutes or contrast media in urine has no effect on results obtained by reagent strip. They concluded that the osmolality and reagent strip methods are used as a tool for determining concentration and dilution ability of the kidney in case of urine containing X-ray contrast media which has only ionic molecules.

L S Gerlis et al (1985) investigated Single and double voided urine testing: A comparison with blood glucose monitoring in insulin-dependent diabetics. In order to find the correlation between glycosuria in both single and double voided urine samples and simultaneous capillary blood glucose measurements, they have made an outpatient study on nine adult insulin-dependent diabetics.

T. Mc. Crossin and LP Roy (1985) studied the various methods of finding the specific gravity of urine. The four methods for estimating urinary concentration hydrometry, refractometry, osmometry and the multistix were accessed for samples taken from children in urine not containing dissolved macro molecules; the hydrometry, refractometry and osmolarity reliably reflect the degree of concentration; but the reagent strip on N-multistix is not a reliable indicator of specific gravity. Although low blood glucose readings were usually associated
with negative glycosuria, there was considerable variation both within
the group and individual patients.

S. Connor et al (1986) studied Spin-echo proton NMR spectroscopy
of urine samples and Water suppression via a urea-Dependent T2
relaxation process. Spin-echo methods have been used to effect
solvent suppression during the collection of 400 and 500 MHz \(^1\)H NMR
spectra of urine samples containing urea in the concentration range
0.7-1.2 \(M\).

Jeanny. W. George (1989) studied the hand –held refractometers
in veterinary laboratory medicine. He has concluded that
Refractometers are specially use full for determining urine specific
gravity on veterinary samples because they require relatively small
sample volume. The refractometers are ubiquitons for the
measurement of protein and urine solute concentrations.

Patricia W. Muller et al (1990) have assessed the inter-laboratory
variation in the results of albumin measurement. For this they have
prepared albumin solution in human urine at various concentrations
within the normal range. Some samples were started -20\(^\circ\)c and a
surfactant were included in one set & not in other. The material with
surfactant evaluated for 10.5months and those without for 5 months.
The albumin recovered by enzyme immunoassay was 106.7% and
115% in two preserved normal-range material and 102.2% & 106.3%
in similar unpreserved material.

H. L. Chhabra and K. K. Manocha (1991) measured specific
conductivity of serum and urine. They revealed that in case of stone
formers there is a fault in the solute transfer system in the kidney membrane level (urothelium).

H. L. Chhabra and K. K. Manocha (1991) attempted to find the reason behind the formation of stone in the kidney. They analysed 50 cases of stone formers (Group I) and 50 controls (Group II) by measuring specific electrical conductivity (SEC) in µmho/cm of the serum and urine at 37°C and 50 Hz. They reported the specific electrical conductivity for group I was $1.23 \times 10^4$, while that of group II was $1.20 \times 10^4$. This was not statistically significant.

Haynes and Williams (1992) investigated the changes in soil solution composition and pH in urine-affected areas of pasture. They have concluded that nutrient availability in the patch was affected directly by nutrient addition in urine, and also probably through the fluctuations in soil solution pH and ionic strength that occur.

Chin .C. Chou, shane S and Que Hee (1992) investigated the factor influencing light emission from photo bacterium phosphoreum in microtox R test to interpret bioassay results of urine. They have found that the optimum luminescence condition were $1.85 - 3.25 \% \text{ NaCl}$, $0.33-0.58 \text{ mol/l}$ ionic strength and pH 5.8 -6.7

Tiselius H. G (1992) studied the relationship between the degree of urine dilution and the risk of calcium oxalate crystallization in urine samples collected from stone formers and normal subjects during an 8-hour period between and in 4-hour urine samples collected during 24-hour periods with calcium stone disease. The degree of urine dilution was determined with a new instrument called urimho which
is designed to measure the concentration, in terms of electrical conductivity, in urine samples of droplet size. With this instrument urine concentration can be expressed in urimho values between 1 and 5. He found pH greater than 6 in 78% of the samples of stone farmers.

Surwicz (1993) investigated the secondary structure of the protein present in human LDL, namely of B-100 using transmission and attenuated total reflection infrared spectroscopy.

Hans H. Eysel et al (1994) investigated the differences in the physical and chemical properties of synovial fluid from healthy and arthritic joints using infrared spectroscopy.

Barry et al (1994) investigated the FT-Raman spectra of mammalian and reptilian skins and the structural dissimilarities and correlated with drug diffusion studies across the tissues.

Duncan Farrant and Lindon (1994) suggested a method for temperature calibration of human blood plasma and cerebrospinal fluid (CSF) samples inside a high resolution NMR spectrometer.

P. C. Shukla and D. K. Chaube (1995) studied heat and mass transfer studies of urine-oxalic acid mixtures in case of urinary bladder membranes. Thermo-osmotic studies of urine-oxalic acid systems with stone-forming tendencies have also carried out. They have observed that the posterior side of the membrane is more sensitive to cold than the anterior side. Such studies are very useful in physiological behaviour of the membranes in different situations.

Banuelos (1995) found the secondary structure of the protein at 37 °C to be 24% α-helix, 23% β-sheet, 6% α-turns, 24% unordered
structure, and 24% SS-strands, characterised by a band around 1618 cm\(^{-1}\), and constituent with extended string like chains in contact with the lipid moiety not forming \(\beta\)-sheets.

Roitman EV et al (1995) worked on urine viscosity in the evaluation of homeostasis in the early postoperative period of heart surgery. They found a significant increase of viscosity was observed only in acute renal failure and led to the development of multiorgan abnormalities. The results indicate that urine viscosity in the early postoperative period was due to pH values (\(r = 0.47, p < 0.05\) and free hemoglobin level (\(r = -0.52, p < 0.01\) in coronary patients and due to concentrations of sodium. Moharram et al (1996) classified and recorded IR spectra of urine from cancerous bladder in to five types A & B type of spectra consisted mainly of protein and traces of lipids. The absorption bands of proteins were between frequencies 1320 to 3330 cm\(^{-1}\) and a weak band at 1720 cm\(^{-1}\) due to lipids. In addition to a peak at 1100 and 1030 cm\(^{-1}\) due to phosphate compounds. The urea peak was indicated by strong band at 1670 and 1630 cm\(^{-1}\) in C-type. The calcium oxalate was indicated in the other type at frequencies 568, 620, 727 and 890 cm\(^{-1}\).

Marcus Nowak and Harald Behrens (1996) designed a novel type of high temperature; high pressure cell for near infrared and optical spectroscopy for hydrous silicate melts at pressure up to 3k bar and temperature up to 800 °C.

Natalie Serkova (1996) argued that initial metabolity exploration should focus on actual tumor biopsies.
Opalko et al (1997) worked with low super saturated artificial urine with high ionic strength, and predicted the existence of stable calcium oxalate monohydrate and unstable calcium oxalate trihydrate in mammalian urine.

Michael Jackson et al (1997) developed and evaluated a variety of new IR techniques for the analysis of tissues and body fluids both in vitro and in vivo. The methodology comprises instrumental and interpretational aimed to optimise the measurements and their conversion to bio-diagnostics.

Pedro Carmona et al (1997) reviewed and discussed the applications of infrared and Raman spectroscopy for the analysis of urinary calculi. The relative efficiency and adaptability routine analysis of their techniques have been reported.

Xiaoming Dou et al (1997) have studied the Quantitative analysis of metabolites in urine by anti-Stokes Raman spectroscopy. They have measured Spontaneous anti-Stokes Raman spectra for urine to which glucose, acetone, or urea was added artificially, for urine including glucose, acetone, and urea simultaneously, and for urine of diabetics. The anti-Stokes Raman spectra obtained are all free from the interference from fluorescence and show a high signal-to-noise ratio. They found the concentration of glucose in urine of the diabetics by the present anti-Stokes Raman system.

Mills Co et al (1998) worked on Surface tension properties of human urine relationship with bile salt concentration. They have concluded that Surface tension of urine and rediluted extracts may or
may not be significantly different when amphiphilic and hydrophobic solutes including bile salts were extracted from urine and subsequently re-diluted in water of same volume. The bile salt concentration is the main determinant in urine by surface tension.

Andrea P. Evans (1998) used a combination of intra-operative photography and biopsy of renal papilla and cortex to measure changes due to stone formation with ileostomy. The papillary deformation was found in some patients associated with decreased GFR.

S.E.M. Langley (1998) has studied the influence of pH on urinary ionized [Ca\(^{2+}\)]: differences between urinary tract stone formers and normal subjects. He has concluded that crystalline precipitates appear in urine at a critical pH which is closer to the voided pH in stone-formers than in normal subjects and may have the greater possibility of this group to form stones. The value of pH\(_n\) is critically dependent on the urinary [Ca\(^{2+}\)] and manoeuvres which reduced its concentration would reduce the tendency to form stones.

Cyril petitbois et al (1999) recorded FT-IR spectra of 32 serum samples after four time dilution at resolution 2 cm\(^{-1}\) at 997 – 1062 cm\(^{-1}\) which is in C-O region. The glucose concentration in the serum spectra has been found by FT-IR analysis. They found the absorption bands for glucose \(\nu(\text{C-H})\) between 3570 – 3210 cm\(^{-1}\), \(\nu(\text{C-H})\) between 3085 – 3020 cm\(^{-1}\), \(\nu(\text{C-O})\) between 1230 – 1000 cm\(^{-1}\) and \(\nu(\text{C-O-O})\) between 1275 – 800 cm\(^{-1}\). The last two bands in C-O region are specific for glucose in complex spectra. The FT-IR spectral absorption
peak for D-glucose called dextrose and mannose are in the C-O region ranging from 1200 – 900 cm\(^{-1}\) and the peak at 1033 cm\(^{-1}\) is the most specific for glucose.

Tighe, Paul (1999) studied Laboratory-based quality assurance programme for near-patient urine dipstick testing and described a quality assurance programme for urinalysis, used in general hospital, and in some of the general practitioner surgeries.


Charles J. Diskin et al (2000) studied the surface tension, proteinuria and the urine bubbles of Hippocrates. They showed that the changes in surface tension responsible for bubbles formation in urine. The bubbles in urine were attributed to kidney disease.

Mayroritz et al (2001) designed pads saturated with water and water solutions mixed with constituents of urine called it as synthesis urine and were applied to the body of parts. It was observed that the blood flow; skin hardness change caused by 60 mm Hg of pressure, temperature and erythema changes among dry, water and saturated urine test sites. It was observed that water and saturated urine caused significant decrease in initial hardness of skin. The skin temperature and erythema are lower at wet site caused by saturated urine compared to dry sites.

Larry et al (2001) recorded near IR spectra to quantify concentrations of urea and creatinine. They showed the spectral
signature of urea, creatinine, glucose, proteins and ketones in range 1350 to 1800 nm and 2050 to 2375 nm.

Anne Marie Melin and his co worker (2001) developed a non destructive technique which is sensitive for monitoring changes in the vibrational spectra of samples using FT-IR spectroscopy.

Luciano Saso et al (2001) investigated Inhibition of calcium oxalate precipitation by bile salts (BA). They have found that Ca$^{2+}$ binding properties of BA were confirmed by small but significant decreases in pH observed following addition of CaCl$_2$ to bile acids solutions. BA inhibited CaOx precipitation with effective concentrations of approximately $10^{-3}$ mol/L, it is of note that in the same in vitro, the activity of bile salt appeared comparable to that of citric acid, the most common drug for urolithiasis. Although BA does not reach mmol/L levels in urine, they are known to change the physicochemical properties of this fluid, possibly slowing down the crystal growth process.

Seiji Wada et al (2002) investigated the tobacco use and the urine pH as risk factors for bladder carcinoma. They have found that, of the patients with bladder carcinoma, 106 were smokers and 35 were non-smokers. On the other hand the number of smokers in the control group was 75 and that of non-smokers was 53. The uneven ratio in the bladder carcinoma group calculated for the smoker patients was 2.08, showing a significant correlation between tobacco and use bladder carcinoma.
Lie et al (2002) presented a method for determining the concentration of at least one cardiovascular risk marker selected from the group consisting of High Density Lipoprotein cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and oxidised LDL comprising, subjecting a serum sample to infrared light. They recorded one spectrum of infrared light after interaction with sample with that they determined the concentration of cardiovascular risk marker in the sample based on the intensity of spectral features.

Petibois and his co-workers (2002) presented the FT-IR spectrometry for plasma triglyceride concentration measurements based on their most characteristic IR absorbance within the range of 1200-1300cm\textsuperscript{-1}.


Langley (2003) studied the pH of urines in normal subjects and stone formers. He showed that there are crystalline precipitates appear in urine at a critical pH, which is closer to the voided pH in stone-formers.

Hitoshi Okada, et al (2004) studied the Change of bilirubin photo isomers in the urine and serum before and after phototherapy compared with light source. They have concluded that the urine bilirubin structural isomers may be used to estimate the serum (EZ) - cyclobilirubin levels and to evaluate the clinical effects of light sources.
Ping Furlan et al (2004) made analysis of mouse urine using IR cards. They concluded that there is large variation of urea in pregnant mice before and after delivery.


Asokan et al (2004) studied isolation, characterisation and quantification of 23 kD COM protein varies between 0.6 to 1.6 mg/24 hr in non former and in store former. This urine excretion is 7 mg/24 hr.

Cyril Petibois et al determined the glucose in dried serum sample of sera. They have concluded that the glucose peak ranges from 997-1062cm⁻¹ and concluded that the FT-IR technique is easy and accurate to measure serum glucose concentration and suggested that it can be used to estimate several metabolites and bio-fluids.

Anil Lonappan et al (2004) presented a comprehensive study of dielectric properties of urine collected at different interval of time at microwave region and measurement were done using cavity perturbation technique at S-band of microwave frequency. The results provide an alternate in vitro method of diagnosis of diabetic mellitus.

Wayhe D.Comper (2004) showed that in both rat & human urine a modified form of albumin is not detected by conventional anti bodies. This modified albumin behaves physicso-chemically as intact albumin under non denaturing conditions. Quantization of this modified albumin leads to the prediction of the onset of microalbuminuria in diabetic patients. The measurement of total albumin including
immunoactive albumin may allow earlier detection of microalbuminuria associated with diabetic nephropathy.

Xu Yiming and Lo Chuanzeng (2005) recorded the first Raman Spectra of HIV1-HIV2 in human sera and hypericin induced photosensitive damage of the virus. The dominating Raman lines in spectra are assigned respectively to the carbohydrates of viral glycoprotein, RNA, protein and lipid.

Ersin Akarsu et al (2006) have studied the specific gravity of urine in case of diabetic mellitus and diabetic insipidus. According to them there is a great importance of the urine specific gravity in patients with poly urea and diabetes mellitus to detect diabetes insipidus.

Andrea Trevisan (2006) studied the Concentration adjustment of spot samples in analysis of urinary xemobiotics metabolities. He has shown that a very few samples exceeds specific gravity 1.035 corresponds to high creatinine level of 3 gm per liter.

Milo Gibaldi et al (2006) observed that the daily administration of a proprietary magnesium and aluminum hydroxides suspension, 15 ml four times a day, to normal adult volunteers resulted in a statistically significant increase in urine pH on the 1st day of treatment.

Bradely C. Gill et al (2007) explained the Feasibility of fluid volume conductance to assess bladder volume. They concluded that the conductance method is sensitive to changes in both concentration and temperature of the intravesical solution, which is due to changes in solution conductivity.
Do-Hyun Kim Ilev et al (2007) observed that in mid IR spectra of glucose the absorption peaks shifts, while all other transmission peaks at different wave length increases as glucose concentration was increased, the rate of increase were all different.

Ping Y. Furlan et al (2007) denatured albumin in series of solution having pH between 1 and 12. The albumin film was cast on ATR crystal from the albumin solution and recorded the IT spectra. They found specific peaks for albumin from 1600 – 1700 cm\(^{-1}\).

Bilsen Beler Baykal and Serra Bayram (2007) presented a paper on ECOSAN and source separated urine and reported that the characteristic and composition of urine changes during the storage period. The electrical conductivity, ammonia and pH are the significant parameters to be observed. The processing of urine before application on to the agricultural field is beneficial.

Stephanie F. Anestis, et al (2008) worked on the properties of Urine of wild and captive chimpanzee. They have found that, the specific gravity and creatinine were highly correlated in both captive and wild chimpanzee samples. The specific gravity measurement is a preferable alternative to creatinine measurement in the study of primate endocrinology. They found that specific gravity and creatinine were highly correlated in both captive (N=124) and wild (N=13) chimpanzee samples.

Benjamin Bird et al (2008) recorded the infrared spectra of cells found in human urine to develop a method for bladder cancer screening. The spectral pattern reveals distinct spectral classes which
are correlated with visual cytology. They concluded that the spectral analysis of individual cell can aid cytology in giving reliable diagnosis based on objective measurement and discriminant algorithms.

Stephanie F. Anestis et al (2008) recommended that researchers consider specific gravity measurement as a preferable alternative to creatinine measurement in the studies of primate endocrinology.

Smiddy F. G (2008) estimated the surface tension of human urine and the effect of hyaluronidase. Surface tension measurements have been performed on normal urines and shown that a single determination of surface tension is worthless and that surface tension is intimately linked with the specific gravity of urine.

Fazil YM Mariekar (2009) studied the relevance of electrical conductivity (EC) and total dissolved solids (TDS) in early morning and random samples of urine of urinary stone formers. He suggested that the extent of RBC, puss cells, calcium oxalate monohydrate & uric acid were correlated with EC & TDS. The value of EC ranges from 1 to 33.9 ms with a mean value 21.5 ms. Fazil YM Mariekar (2009) found that in some samples, where the TDS were more than 12,000 ppm, there were no crystals than those samples having TDS less than 12,000 ppm.

Ruediger W. Schlick, et al (2009) studied the special plastic for biodegradable endoureteral stents in vitro, and reported that the stent material is suitable for development of bio-dissolvable endoureteral stents, and dissolution of which can be steered by changing the urinary pH.
Vinodaran MK. Mathavan (2009) investigated the interaction of erythosine (Er B), a commonly used dye for coloring foods and drinks, with bovine serum albumin (BSA) both in the absence and presence of bilirubin (BR) using absorption and absorption difference spectroscopy. The results suggest that Er B binds to a site in the vicinity of BR binding site on BSA. Therefore intake of Er B may increase the risk of hyperbilirubinemia in the healthy subjects.

Bakhtawar K. Mahmoodi et al (2009) suggested that higher than normal levels of protein albumin in urine is associated with increased risk for blood clots in the deep veins of the legs or lungs (venous thromboembolism VTE). They concluded that the micro albuminuria has a high prevalence in general population, which is an important risk factor for VTE.

Renuga Devi. et al (2009) attempted to evaluate the spectral difference between renal failure and healthy blood sera using FT-IR spectroscopy. They have found the internal standard ratio of some specific peaks and showed that the blood spectra of normal are not similar to that of renal disease.


Mark guy et al (2009) worked on the Protein albumin and creatinine. They have suggested that the Protein albumin to creatinine ratios in random urines accurately predicts 24h protein and albumin loss in patients with kidney disease.
Peng Huang (2010) et al synthesised mono-dispersed CuS nanoparticles by using bovine serum albumin as foaming and stabilising reagent. The bovine serum albumin assisted synthesised CuS nanoparticles have large application in microelectronic and biomedical engineering.

1.10 Genesis of the present investigation

The literature survey reveals that much work is done on urine in early and mid twentieth century, but attention is not being paid later. It is found that some work is done on urine microscopically and spectroscopically due to advancement of bio-technology in the recent past. The urine is the most important bio-fluid in the determination of the state of physiological condition.

The present investigation is aimed to systematically study the physical parameters such as specific gravity, refractive index, surface tension, specific gravity, viscosity, electrical conductivity and pH of urine, which would be very much helpful for the diagnosis and monitoring drug administration of a particular disease. All these parameters for normal urine, urine added with glucose, bilirubin, albumin, creatinine and urea and pathological urines such as diabetic mellitus and chronic kidney diseases have been studied. The literature survey also reveals that earlier researchers have not made such a comprehensive study both in vitro and in vivo.

In this study, an attempt has been made to analyse the normal human urine, urine treated urea, glucose, albumin, creatinine and bilirubin by using FT-IR spectroscopy. It is aimed to examine the
possibility of standardisation and characterisation of normal human urine, urine treated urea, glucose, albumin, creatinine & bilirubin at different concentrations, and pathological urine. This study, if extended to other pathological urines, may be very much helpful for the diagnosis and monitoring drug administration of a particular disease.