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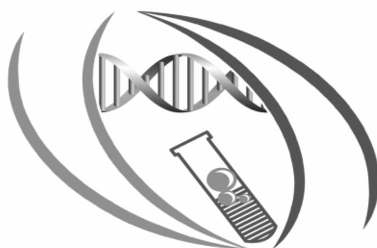

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Practical Note
Biochemistry of Blood
(BCH 471)

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EXPERIMENT(1)

1. Instrumental determination of electrolytes in urine

1.1 INTRODUCTION:

Electrolytes are positively and negatively charged molecules called ions, that are found within the body's cells and extracellular fluids, including blood plasma. A test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate. These ions are measured to assess renal (kidney), endocrine (glandular), and acid-base function, and are components of both renal function and comprehensive metabolic biochemistry profiles. Other important electrolytes routinely measured in serum or plasma include calcium and phosphorus. These are measured together because they are both affected by bone and parathyroid diseases, and often move in opposing directions. Magnesium is another electrolyte that is routinely measured. Like calcium, it will cause tetany (uncontrolled muscle contractions) when levels are too low in the extracellular fluids.

Tests that measure the concentration of electrolytes are needed for both the diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions. Their importance lies in part with the serious consequences that follow from the relatively small changes that diseases or abnormal conditions may cause. For example, the reference range for potassium is 3.6-5.0 mmol/l. Potassium is often a STAT (needed immediately) test because values below 3.0 mmol/l are associated with arrhythmia (irregular heartbeat), tachycardia (rapid heartbeat), and cardiac arrest, and values above 6.0 mmol/L are associated with bradycardia (slow heartbeat) and heart failure. Abnormal potassium cannot be treated without reference to bicarbonate, which is a measure of the buffering capacity of the

plasma. Sodium bicarbonate and dissolved carbon dioxide act together to resist changes in blood pH. For example, an increased plasma bicarbonate indicates a condition called metabolic alkalosis, which results in blood pH that is too high. This may cause hydrogen ions to shift from the cells into the extracellular fluid in exchange for potassium. As potassium moves into the cells, the plasma concentration falls. The low plasma potassium, called hypokalemia, should not be treated by administration of potassium, but by identifying and eliminating the cause of the alkalosis. Administration of potassium would result in hyperkalemia when the acid-base disturbance is corrected. Sodium measurements are very useful in differentiating the cause of an abnormal potassium result. Conditions such as the overuse of **diuretics** (drugs that promote lower blood pressure) often result in low levels of both sodium and potassium. On the other hand, Cushing's disease (adrenocortical over-activity) and Addison's disease (adrenocortical under-activity) drive the sodium and potassium in opposing directions. Chloride levels will follow sodium levels except in the case of acid-base imbalances, in which chloride may move in the opposing direction of bicarbonate. In short, diagnosis and management of a patient with an electrolyte disturbance is best served by measuring all four electrolytes.

Sodium is the principal extracellular cation and potassium the principal intracellular cation. A cation is an ion with a positive charge. An anion is an ion with a negative charge. Sodium levels are directly related to the osmotic pressure of the plasma. In fact, since an anion is always associated with sodium (usually chloride or bicarbonate), the plasma osmolality (total dissolved solute concentration) can be estimated. Since water will often follow sodium by diffusion, loss of sodium leads to dehydration and retention of sodium leads to edema. Conditions that promote increased sodium, called hypernatremia, do so without promoting an equivalent gain in water. Such conditions include diabetes insipidus (water loss by the kidneys), Cushing's disease, and hyperaldosteronism (increased

sodium reabsorption). Many other conditions, such as congestive heart failure, cirrhosis of the liver, and renal disease result in renal retention of sodium, but an equivalent amount of water is retained as well. This results in a condition called total body sodium excess, which causes hypertension and edema, but not an elevated serum sodium concentration. Low serum sodium, called hyponatremia, may result from Addison's disease, excessive diuretic therapy, the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), burns, diarrhea, vomiting, and cystic fibrosis. In fact, the diagnosis of cystic fibrosis is made by demonstrating an elevated chloride concentration (greater than 60 mmol/l) in sweat.

Potassium is the electrolyte used as a hallmark sign of renal failure. Like sodium, potassium is freely filtered by the kidney. However, in the distal tubule sodium is reabsorbed and potassium is secreted. In renal failure, the combination of decreased filtration and decreased secretion combine to cause increased plasma potassium. Hyperkalemia is the most significant and life-threatening complication of renal failure. Hyperkalemia is also commonly caused by hemolytic anemia (release from hemolysed red blood cells), diabetes insipidus, Addison's disease, and digitalis toxicity. Frequent causes of low serum potassium include alkalosis, diarrhea and vomiting, excessive use of thiazide diuretics, Cushing's disease, and intravenous fluid administration.

Calcium and phosphorus are measured together because they are both likely to be abnormal in bone and parathyroid disease states. Parathyroid hormone causes resorption of these minerals from bone. However, it promotes intestinal absorption and renal reabsorption of calcium and renal excretion of phosphorus. In hyperparathyroidism, serum calcium will be increased and phosphorus will be decreased. In hypoparathyroidism and renal disease, serum calcium will be low but phosphorus will be high. In vitamin D dependent rickets (VDDR), both calcium and phosphorus will be low; however, calcium is

normal while phosphorus is low in vitamin D resistant rickets (VDRR). Differential diagnosis of an abnormal serum calcium is aided by the measurement of ionized calcium (i.e., calcium not bound by protein). Approximately 45% of the calcium in blood is bound to protein, 45% is ionized, and 10% is complexed to anions in the form of undissociated salts. Only the ionized calcium is physiologically active, and the level of ionized calcium is regulated by parathyroid hormone (PTH) via negative feedback (high ionized calcium inhibits secretion of PTH). While hypoparathyroidism, VDDR, renal failure, hypoalbuminemia, hypovitaminosis D, and other conditions may cause low total calcium, only hypoparathyroidism (and alkalosis) will result in low ionized calcium. Conversely, while hyperparathyroidism, malignancies (those that secrete parathyroid hormone-related protein), multiple myeloma, antacids, hyperproteinemia, dehydration, and hypervitaminosis D cause an elevated total calcium, only hyperparathyroidism, malignancy, and acidosis cause an elevated ionized calcium.

Serum magnesium levels may be increased by hemolytic anemia, renal failure, Addison's disease, hyperparathyroidism, and magnesium-based antacids. Chronic alcoholism is the most common cause of a low serum magnesium owing to poor nutrition. Serum magnesium is also decreased in diarrhea, hypoparathyroidism, pancreatitis, Cushing's disease, and with excessive diuretic use. Low magnesium can be caused by a number of **antibiotics** and other drugs and by administration of intravenous solutions. Magnesium is needed for secretion of parathyroid hormone, and therefore, a low serum magnesium can induce hypocalcemia. Magnesium deficiency is very common in regions where the water supply does not contain sufficient magnesium salts. Magnesium acts as a calcium channel blocker, and when cellular magnesium is low, high intracellular calcium results. This leads to hypertension, tachycardia, and tetany. Unfortunately serum total magnesium levels do not correlate well with intracellular magnesium levels, and serum measurement is not very sensitive for

detecting chronic deficiency because of compensatory contributions from bone. Ionized magnesium levels are better correlated with intracellular levels because the ionized form can move freely between the cells and extracellular fluids.

1.2 METHOD:

Electrolytes are measured by a process known as **potentiometry**. This method measures the voltage that develops between the inner and outer surfaces of an ion selective electrode. The electrode (membrane) is made of a material that is selectively permeable to the ion being measured. This potential is measured by comparing it to the potential of a reference electrode. Since the potential of the reference electrode is held constant, the difference in voltage between the two electrodes is attributed to the concentration of ion in the sample

2 Potentiometry

Potentiometry is an electroanalytical method which is based on measurement of potential of anelectrode system. Potentiometric measurements enable selective detection of ions in presence of multitude of other substances.
Potentiometric measurement system consists of two electrodes, potentiometer and a solution of analyte (Fig 2). In system like one depicted on figure 2, the potential is measured in reference to calomel electrode e.g. calomel electrode functions as reference electrode. **Reference electrode** is an electrode with potential which is a) independent of analyte (or other) ions in solution; b) independent of temperature.
In case of figure 2, the electrode sensitive to hydrogen ions is an indicator electrode. Potential of an **indicator electrode** depends mainly on the concentration of the analyte ions (in this case hydrogen ions).

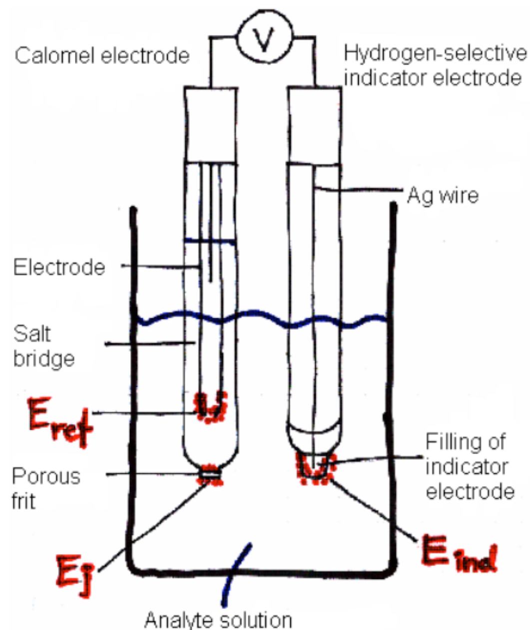


Figure 2. Potentiometric measurement system (for pH measurement).

1.3 Refrences:

- http://tera.chem.ut.ee/~koit/arstpr/pot_en.pdf
- http://www.medicalhealthtests.com/pathology-test/electrolytes-urine-test.html

.http://www.kutztown.edu/acad/chem/instruments_html/potentiometry.htm

EXPERIMENT(2)

2. Osmolality of serum and urine

2.1 INTRODUCTION:

The osmolality test is a snapshot of the number of solutes present in the blood (serum), urine, or stool. It is ordered to help evaluate the body's water balance, its ability to produce and concentrate urine, to help investigate low sodium levels (hyponatremia), to detect the presence of toxins such as methanol and ethylene glycol, and to monitor osmotically active drug therapies such as mannitol. It is also ordered to help monitor the effectiveness of treatment for any conditions found.

2.1.1 **Serum osmolality** is primarily ordered to investigate hyponatremia.

Hyponatremia may be due to sodium loss through the urine or due to increased fluid in the bloodstream. Increased fluid may be due to drinking excessive amounts of water, water retention, decreased ability of the kidneys to produce urine, or the presence of osmotically active agents such as glucose, mannitol, and glycine (a chemical used in surgical irrigation fluids). Marathon runners can experience acute hyponatremia by drinking large quantities of water in a short period of time. In a few cases, this has led to the death of the runner. People who chronically drink excessive amounts of water, by choice or due to a psychological condition, may have chronic hyponatremia. Someone may also appear to have a low sodium when the percentage of water in their blood decreases due to the presence of increased proteins or lipids.

Mannitol, glycine, and the ingestion of toxins such as methanol and propylene glycol can be detected, evaluated, and monitored by ordering an osmotic gap

(also called osmolal gap). This calculation compares measured osmolality with measurements of the major solutes. The osmotic gap is the difference between them and represents the presence of an osmotically active substance in the blood. In order to calculate the osmotic gap, tests for blood sodium, blood urea nitrogen (BUN), and glucose must be performed. Some versions of the osmotic gap calculation also include the measurement of ethanol. An example calculation is:

Serum Osmotic Gap (Ethanol not always included)

x (Na+) + (Glucose/18) + (BUN/2.8) + (Ethanol/3.8)

Note: Glucose, BUN, and Ethanol may be reported in mg/dL (milligrams per deciliter) or mmol/L (millimole per liter). The numbers shown in the equation above are used to convert from mg/dL to mmol/L.

2.1.2 **Urine osmolality** is frequently ordered along with serum osmolality. It is used to help evaluate the body's water balance and to investigate increased and decreased urine output. Increased urine output may be due to increased fluid intake, lack of appropriate amounts of ADH, or due to diabetes, with increased glucose levels leading to increased urine output. Decreased urine output may be due to a variety of causes including decreased blood flow to the kidneys, an appropriate response to dehydration, or damage to tubular cells in the kidneys. Urine sodium and creatinine are often ordered along with urine osmolality. Sometimes a urine osmotic gap is calculated and used to help evaluate the kidney's ability to excrete acid and reabsorb bicarbonate, to detect the presence of osmotically active molecules, and to compare with the serum osmotic gap.

A serum osmolality test and osmotic gap may be ordered when a person has symptoms that the doctor suspects may be due to hyponatremia such as:

- Excessive thirst
- Confusion
- Nausea

- Headache
- Lethargy
- In severe cases, seizures or coma

These tests may be ordered when it is suspected that someone has ingested a toxin such as methanol or ethylene glycol.
A urine osmolality test may be ordered along with blood testing when the doctor wants to compare urine results with the serum osmolality and/or when the tested person is producing increased or decreased quantities of urine. Both may be ordered when a doctor suspects that the person may have diabetes.

Expected values for osmolality:

- Expected serum osmolality assumes that sodium salts (chloride and bicarbonate), glucose, and urea nitrogen are the primary solutes in the serum. A difference from the expected and actual serum osmolatity values is the osmolality gap. The gap reflects an expected solute composition abnormality or the addition of an unexpected solute such as alcohol, etc.
- serum osmolality: 282 - 295 mOsm/kg water; a serum osmolality of 285 mOsm usually correlates with a urine specific gravity of 1.010
- Urine osmolality: can range from 50 - 1400 mOsm/kg water, but average is about 500 - 800 mOsm. After an overnight fast, the urine osmolality should be at least 3 times the serum osmolality

After 12-14 hours of restricted fluid intake, urine osmolality should be > 850mOsm/Kg. A 24 hour urine osmolality should average between 500 and 800 mOsm/Kg. A random urine osmolality should average 300 and 900 mOsm/Kg.

2.2 METHOD:

Using an osmometer

Types of Osmometers

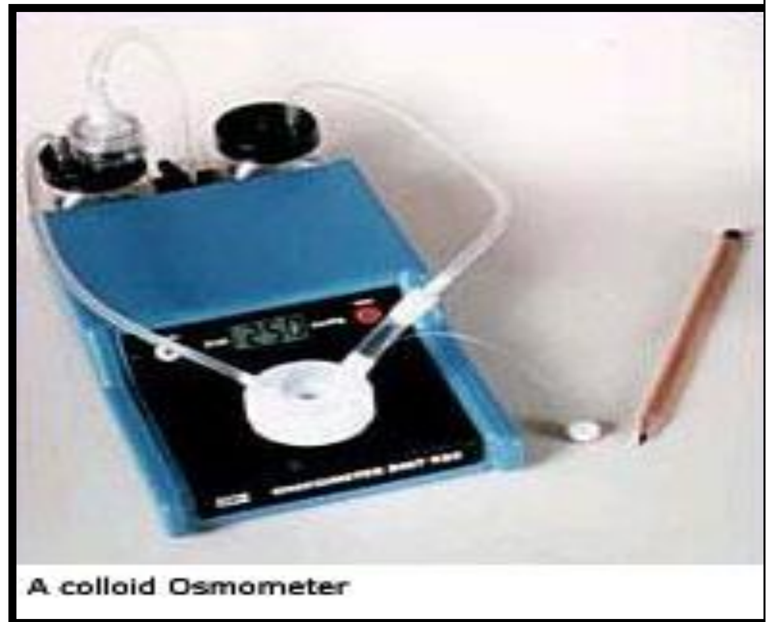
2.2.1 Types of osmometers

An osmometer is a device for measuring the osmotic strength of a solution, colloid or compound. There are three major types of osmometers commerically available, each leveraging a particular colligative property to achieve their analytical results:

2.2.1.1 Freezing Point Osmometers – determine the osmotic strength of solution by utilizing freezing point depression

2.2.1.2 Vapor Pressure Osmometers – determine the concentration of osmotically active particles that reduce the vapor pressure of the solution

2.2.1.3 Membrane Osmometers – measure the osmotic pressure of a solution separated by a semi-permeable membrane



A colloid Osmometer

A 20µl micro Osmometer, the advanced model 3300

2.3 References:

http://www.rnceus.com/renal/renalosmo.html

http://www.pharmaceutical-int.com/article/osmometers-what-do-they-do.html

http://www.aicompanies.com/index.cfm/AIUniversity/OsmolalityExplained/Types

EXPERIMENT (3)

3. Creatinine estimation and creatinine clearance tests

3.1 INTRODUCTION:

Creatinine is derived from " creatine" which is synthesized in the liver, it moves through the circulation and is taken up entirely by muscles.

In the muscles it is converted to creatine phosphate which becomes the source of a high energy phosphate bond for the immediate reformation of ATP. (ATP is needed continuously for muscle contraction)

On the other hand, " creatinine" is formed from creatine and creatine phosphate continuously(2% of the total each day). Creatinine is a bi product of creatine metabolism and it is excreted in urine , it is totally endogenous and related to muscle mass and so it remains the same from day to day.

3.2 Normal values:

In serum: 1-2 mg/d**l**

In urine: 1-2 g/day

High levels of serum creatinine indicates kidney failure since it is excreted by the glomeruli, neither excreted or reabsorbed by the tubules so it is considered parallel to glomelular filtration rate (GFR).

3.3 Creatinine clearance:

One of the most important kidney function tests, it is: the amount of plasma (in ml) that is cleared from a certain substance by the kidneys per minute.

Clearance= UV/P

U= concentration of any substance in urine

P= concentration of the same substane in plasma

V= volume of urine(ml/min)

- **There are 3 major clearance tests**:
    1- Creatinine clearance.
    2- Urea clearance
    3- Inulin clearance
    4- Creatinine clearance is preferred because it is a normal constituent of blood and no infusion is needed unlike inulin. Moreover it is not reabsorbed by the tubules as in the case of urea.
    Normal creatinine clearance: 100-130 ml/min/1.73m^2

3.4 High levels of creatine:

1- Break down of muscle cells.
2- Starvation and fever
3- Diabetes mellitus
4- Hyperthyrodism

3.5 OBJECTIVES:

1- To estimate creatinine in serum and urine.
2- To calculate creatinine clearance value.
3- To diagnose a patient.

3.6 MATERIALS:

1- Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2- Creatinie working standard for serum (3mg/dl): dilute 10 ml of stock and increase the volume up to 500 ml with water.
3- Serum and urine samples.
4- NaoH(2.5 M)
5- Picric acid.(0.04 M)

3.7 METHOD:

Take one ml of urine and dilute it to 100 ml

	Standard (A,B)	Test(serum) (C ,D)	Test(urine) (E, F)	Blank
Serum		0.5 ml		
Urine			0.5 ml	
Water	1.5 ml	1.5 ml	1.5 ml	2 ml
Standard for serum	0.5 ml			
Picric acid	6 ml	6 ml	6 ml	6 ml

1. Mix well
2. Add 0.4 ml of 2.5 M NaoH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm

3.8 RESULTS:

$$\text{Serum creatinine} = \frac{\text{Abs of test}}{\text{Abs of std}} \times \text{concentration of standard} = \text{mg / dl}$$

$$\text{Urinary creatinine} = \frac{\text{Abs of test}}{\text{Abs of std}} \times \text{concentration of standard} \times \text{D.F} = \text{mg / dl}$$

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine}}{\text{Serum creatinine}} \times \text{Volume of urine ml/min/}1.73\text{m}^2$$

$$= \frac{\text{Urinary creatinine mg / dl}}{\text{Serum creatinine mg / dl}} \times \frac{\text{Volume of urine ml / 24 h}}{\mathbf{1440\ min\ /24\ h}} \times \frac{1.73\text{m}^2}{\mathbf{A}}$$

1.73 is generally accepted average body surface in square meters

A is the actual body surface area of the individual determined from height and weight

3.9 DISCUSSION:

3.10 QUESTIONS:

1- Why do you have to collect urine for 24 hours?

2- A female patient with 1.63 m^2 of surface area went to the lab to measure her GFR, her results were as follows:

   Urine **Creatinine** = 127.5 mg/dL

   Serum **Creatinine** = 1.5 mg/dL

   Total Urine Volume = 1602 mL

   Duration (hrs) = 24

   Calculate her creatinine clearance.

3- What are the abnormalities associated with low creatinine levels?

3.11 References:

Singh.S.P.(2006).Practical manual of biochemistry. 6th edition

EXPERIMENT (4)

4. Determination of blood urea .

4.1 INTRODUCTION:

Urea is one of the end products of protein metabolism. Some of it is derived from food, and some from the breakdown of tissues. It is eliminated from the blood stream by the kidney, and passes out in the urine. In health, blood always contains some urea, the level varying between 14 and 43 mg per 100 ml.

In the elderly, values slightly higher than these may be present, even without significant renal dysfunction.

In general, blood urea of over 50 mg per 100 ml is suggestive of impaired renal function; less frequent causes of raised blood urea are diarrhoea and vomiting and circulatory failure.

In childhood and pregnancy, low values are often found.

Urea diffuses very readily through body fluids. For this reason, similar results are obtained if the estimation is carried out on whatever samples are most readily procurable, for example, cerebrospinal fluid, oedema, fluid plasma, serum or whole blood.

4.2 OBJECTIVES:

To estimate the amount of blood urea .

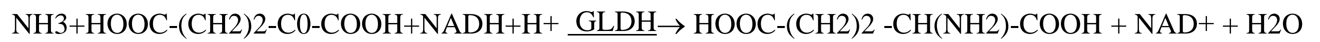
4.3 PRINCIPLE:

Urea is hydrolyzed in the presence of urease and water to yield ammonia and carbon dioxide. The ammonia reacts with α-ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH to NAD is measured at 340

nm over a limited urea concentration range and limited time period, and is proportional to the concentration of urea prese

$$NH_2 - CO - NH_2 + H_2O \xrightarrow{UREASE} 2NH_3 + CO_2$$

AMMONIA UREA



$$NH_3 + HOOC\text{-}(CH_2)_2\text{-}C0\text{-}COOH + NADH + H^+ \xrightarrow{GLDH} HOOC\text{-}(CH_2)_2\text{-}CH(NH_2)\text{-}COOH + NAD^+ + H_2O$$

α-KETOGLUTARIC ACID GLUTAMIC ACID

4.4 MATERIAL AND METHOD:

As shown in the provided kit.

4.5 RESULT

4.6 DISCUSSION:

4.7 References:

Singh.S.P.(2006).Practical manual of biochemistry. 6th edition

EXPERIMENT (5)

5 Physical properties and detection of normal constituents of urine

5.1 INTRODUCTION:

5.1.1 The Formation of Urine

In a healthy adult about 650 ml of plasma (1200 ml of blood) pass through functioning renal excretory tissue every minute, and about 125ml of glomerular filtrate is formed. Water passes freely from the plasma through the glomeruli, and those unbound constituents of the plasma that have a molecular weight of less than about 70,000 are present in the glomerular filtrate at about the same concentration as in the plasma. Substances of a higher molecular weight than about 70,000 do not pass freely though the glomeruli and are present in the glomerular filtrate at a lower concentration than in plasma – though molecular size is not the only determinant for filtration. In man, excretion of the end-products of metabolism is almost wholly glomerular; tubular excretion of metabolites is of little importance except for potassium, urate and creatinine at high plasma levels, however tubular excretion of many drugs (e.g. penicillin) is significant. The renal tubules conserve water and the soluble constituents of the body by reabsorption using both passive and active transport from the glomerular filtrate. (e.g. Na^+, K^+, HCO^-_3, SO_4^{2-}, Phosphate) and some urea are reabsorbed in the proximal portion of the tubules. In the distal portion of the tubules further water and ions are reabsorbed, acidification of the urine takes place, and ammonia may be formed (to be excreted as ammonium).

The urine that is finally secreted has an entirely different composition from the glomerular filtrate from which it is derived.

Constituent	Daily Excretion	
	Glomerular Filtrate	**Urine**
Water	130,000 ml	1500 ml
Sodium	20,000 mmol	150 mmol
Albumin	4 g (60 μmol)	0.04 g (6 μmol)
Urea	900 mmol	400 mmol

5.2 Simple Examination of the Urine

5.2.1 Volume

The normal 24 hours urine volume of an adult is between 750 and 2000 ml. This depends on the fluid intake (which is usually a matter of habit) and on the loss of fluid by other routes (primarily sweating which, in absence of fever, depends on physical activity and on the external temperature). A marked alteration in the output of urine may be a prominent sign in disease of the kidneys.

Oliguria develops also in any non-renal disease in which there is a deficient intake of water, or excessive loss of fluid by other routes, for example by haemorrhage, or as diarrhea and vomiting.

Polyuria is a characteristic sign of chronic renal insufficiency. Polyuria occurs as an osmotic diuresis in any disease where there is an increased excretion of metabolites, notably in diabetes mellitus.

The minimal 24 hour output of urine needed to remove the waste products of normal metabolism is about 500 ml. A patient may be said to have oliguria when the urine volume is below 400 ml in 24 hours, and anuria when the 24 hour volume is below 100 ml, but these terms are loosely used.

5.2.2 Color

If no colored abnormal constituents are present, then the higher the concentration of urine, the deeper is the color and the rate of excretion of the normal urine pigments (urochromas) are constant. . Pale urine has a low specific gravity, a dark urine has a high specific gravity.
Colored urines occur in certain diseases or metabolic disorders, and after the administration of many drugs.

5.2.3 Appearance

5.2.2.1 Casts**:** The tubule secrete an α_1-glycoprotein (T_{amm}-Horsfall protein), which in the presence of albumin, comes out of solution in gel form as casts.

5.2.2.2 Mucus Protein**:** This may be from semen or from vaginal discharge. Pathologically, it may be due to disease of the lower urinary tract or to pus.

5.2.2.3 Crystals**:** These are not normally pathological. Uric acid (reddish-yellow) and calcium oxalate (colourless) may precipitate from acid urine and phosphate (whitish) from alkaline urine. However, cystine crystals are pathological as is a dark reddish deposit of blood.

5.2.2.4 Smell**:** Urine which is infected with Gram-negative organisms often has a distinctive unpleasant smell. In addition, urine infected with urea splitting organisms has an ammonical smell. If urine which had a normal odour on arrival at the laboratory develops such a small, this indicates bacterial decomposition and the specimen is unfit for most chemical analyses. Certain drugs, for example paraldehyde, impart a typical odour, as does the rare maple syrup urine disease.

5.2.2.5 pH**:** On a normal mixed diet the urine is usually acid, generally varying in pH between 5.5 and 8.0. A vegetarian diet which causes a

tendency to alkalosis, thereby produces an alkaline urine. The pH of the urine in disease may reflect both the acid-base status of the plasma, and the function of the renal tubules. It may also be grossly increased by bacterial infection of the urinary tract, or deliberately by acid- or alkali-forming drugs.

5.2.2.6 Specific Gravity**:** The normal specific gravity (correctly called relative density) of a pooled 24 hour urine sample is between 1.025 and 1.010: the maximum and minimum values of undivided specimens are usually about 1.030 and 1.005. Under normal circumstances the urine concentration varies inversely with the urine volume. The concentration of urine is highest in the a morning specimen (overnight urine) and is lowest in a specimen passed an hour after much fluid has been taken. Fixation of the specific gravity at about 1.010 or the osmolality at about 300 mmol/l, being the values of protein-free plasma, occurs in severe chronic renal disease. Disorders associated with oliguria usually produce a concentrated urine. Polyuria tends to lead to a urine of low concentration. In diabetes mellitus there is polyuria with urine of high concentration: even when the specific gravity of the urine has been corrected for the presence of glucose the specific gravity of the urine is still raised because of the high concentration of salts in the urine. A correction must also be applied when interpreting the urine specific gravity in the presence of marked proteinuria, whilst protein has a negligible effect on osmolality. Oliguria with a low specific gravity (after correction for the proteinuria) and low osmolality occurs in acute tubular necrosis because the tubules do not concentrate the limited amount of glomerular filtrate.

The following corrections should be made to urinometer readings of specific gravity

Subtract 0.004 for every 10g/l of dissolved glucose

Subtract 0.003 for every 10g/l of dissolved glucose

Subtract	0.001 for every 3°C below 15°C
Add	0.001 for every 3°C above 15°C

5.3 OBJECTIVES:

1- The simple examination of urine.
2- To detect some of the normal organic constituents of urine.
3- To detect some of the normal inorganic ions present in urine.

5.4 MATERIALS:

1- 24 hour collection of normal urine.
2- 10 ml and 2 liter measuring cylinders.
3- Thermometer
4- Narrow range pH test papers.
5- Urinometer and urinometer cylinder
6- Test tube
7- 10% sodium hydroxide solution.
8- Barfoed's reagent
9- Saturated solution of picric acid
10- 2N HCl
11- Concentrated HCl
12- 0.2% Naphthoresorcinol solution
13- Ether
14- Concentrated nitric acid
15- 2N Nitric acid
16- 2N Silver nitrate solution
17- 2N Ammonium hydroxide solution
18- Saturated ammonium molybdate solution
19- Lime water
20- Litmus papers
21- 5% Barium chloride solution

5.5 The Simple Examination of Urine

5.5.1 Method

1- Volume: Measure the volume of the 24 hour collection of normal urine.

2- Colour: Visually examine its colour.

3- Appearance: State whether it is clear, cloudy or whether deposits or precipitates are present.

4- Odour: State whether it is normal urine like ammonical, putrid, etc.

5- pH: Record the pH of the sample.

6- Specific gravity: Fill 2/3 of the urinometer cylinder with the urine. Put in the urinometer ensuring that it is not touching the sides of the cylinder. Note the specific gravity and the room temperature. Make an adjustment to the specific gravity for the room temperature.

5.5.2 RESULTS:

Test	Result	Normal
24 hour urine volume	ml	750-2000 ml
Colour		Pale to dark yellow
Appearance		Clear, but see introduction and principle.
Odour		Urine-like
pH		5.5-8.0
Specific gravity*		1.010-1.025

* Corrected for temperature

5.6 To Detect some of the Normal Organic Constituents of Urine

5.6.1 Method

1- **Uric acid:** To 2 ml of urine add 4 ml of Barfoed's reagent , then heated in a boiling water bath for three minutes . Chang the blue colour indicates the presence of uric acid

2- **Creatinine:** To about 5 ml of urine add a few drops of a saturated solution of picric acid. On rendering the solution alkaline with a few drops of 10% sodium hydroxide solution, a deep red color or orange due to creatinine picrate appears. On acidification, with 2N HCl, the color changes to yellow.

3- **Glucuronic acid:** A detoxicating agent and organic acid. Any of the following method can be used :

a)- To 3 ml of urine add 3 ml of concentrated HCl and 1 ml of freshly prepared 0.2% naphthoresorcinol solution. Boil gently for 5 minutes. Cool in running water. Add 2 ml of ether and shake carefully. Allow the two layers to separate. The

appearance of a blue colour in the upper ether layer indicates the presence of glucuronic acid.

b)- To 2 ml of urine add 4 ml of Barfoed's reagent , then heated in a boiling water bath for three minutes . Chang the blue colour indicates the presence of glucuronic acid..

5.6.2 RESULTS:

5.6.3 DISCUSSION:

5.7 To Detect Some of the Normal Inorganic Ions Present in Urine

5.7.1 Method

1) **Chlorides:** Add a few drops of 2N nitric acid to 5 ml of urine. Add 1 ml of 2N silver nitrate solution. A white precipitate of silver chloride is formed which dissolves in 2N ammonium hydroxide solution.
2) **Phosphates:** Add 5 ml of concentrated nitric acid to 5 ml of urine. Add 2 ml of saturated ammonium molybdate solution. Heat the mixture gently. A yellow crystalline precipitate of ammonium phospho-molybdate appears.
3) **Bicarbonate:** Add a few drops of concentrate hydrochloric acid to 5 ml of urine. A slight effervescence occurs due to CO_2 evolution. Test the gas evolved with lime water.
4) **Sulphates:** Acidify 10 ml of urine with dilute hydrochloric acid. Add 3 drops of 5% barium chloride solution. A white precipitate of barium sulphate is formed.
5) **Ammonia:** Add 1 ml of 10% sodium hydroxide solution to 5 ml or urine. Boil. The evolved ammonia may be detected by its occur in confirmed by turning moist red litmus paper blue.

5.7.2 RESULTS:

5.7.3 DISCUSSION:

5.8 QUESTIONS:

1- Would you expect (a) albumin (b) immunoglobulin to appear normally in the glomerular filtrate? State reasons for your answer.

2- Albumin is not normally detected in any appreciable quantity in urine. Why not?

3- What 2 symptoms are seen in a patient developing diabetes mellitus ?

4- What is the simplest and quickest method of detecting the presence of blood in urine?

5.9 References:

- Practical Textbook of Biochemistry for MBBS Students by DM Vasudevan and SK Das, 2007, Jaypee Brothers Medical Publishers (P) LTD, New Delhi.

EXPERIMENT (6)

6 Examination of Urine: Detection and Estimation of Some Abnormal Constituents.

6.1 INTRODUCTION

6.1.1 The use of test-strips

Semi-quantitative tests on urine specimens may be done by simple colour reactions. 'Test strips' are commercially prepared strips which allow comparisons to be made between colours obtained by the urine sample tested and known standards. Normally, substances such as nitrate, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are not present in quantities capable of detection by this method. However, because of disease the concentration of one or more of these substances may be increased to a level which is detectable.

A random mid-stream sample of urine is required. It should be refrigerated until tested. More quantitative estimations may be made on a 24-hour collection of urine. The specimen should be refrigerated during collection and until the tests are to be carried out.

6.1.2 Nitrite:

A positive result will occur if bacteria are present in the bladder urine.

6.1.3 Protein:

The appearance of protein in the urine gives rise to four different types of proteinuria , depending on the source of the protein , these are :

- Glomerular proteinuria arising from glomerular disease .
- Tubular proteinuria arising from tubular disease .
- Overflow proteinuria arising from the overflow of high plasma concentrations of low molecular weight protein .

- Secretory proteinuria arising from protein secreted by the kidney tubule.

Damage to the glomerular basement membrane can result in larger proteins being allowed through into the glomerular filtrate in greater amounts , causing glomerular proteinuria .

In this case , protein appears in the urine when the reabsorptive capacity of the tubules is exceeded . Where damage to the glomerulus is limited or slight , an excess of albumin is found in the urine (albuminuria) whereas if damage to the basement membrane is more serious , increasingly large proteins are filtered by the glomerulus .

In this situation proteins such as the immunoglobulins appear in the urine.

In health , 95% of the filtered protein is reabsorbed . This involves the small proteins allowed through the glomerular basement membrane : microglobulins , insulin and parathyroid hormone .

Tubular damage impairs the uptake of these proteins , resulting in tubular proteinuria .

Glomerular disease has higher levels of protein , often exceeding 2 g /day ,mainly albumin , whereas tubular proteinuria shows a moderate increase in urinary proteins , usually less than 2 g /day , and show an increased proportion of low molecular weight proteins .

6.1.4 pH: On a normal diet urine is acidic and generally between pH 5.5-8.0. A vegetarian diet produces an alkaline urine. The pH in disease reflects the acid-base status of the plasma and the function of the renal tubules. Strongly alkaline urine is due to bacteria infection of the urinary tract. The pH may be altered by acid or alkali producing drugs. A strongly alkaline urine smelling of ammonia is probably not fresh and should be discarded.

6.1.5 Glucose: Glucose is filtered by the glomeruli and partly resorbed by the renal tubules. Resorption is an active process and depends on the level of glucose in the filtrate (being that of plasma) on the urinary flow rate, and on the capability of the cellular system to transfer the glucose from the tubule. If the filtered load should exceed this capability then there will be glycosuria. This will occur normally at a plasma (and filtrate) level of around 180 mg/dl. This is called the "Renal Threshold". Diabetes mellitus will give glycouria only when the plasma glucose level exceeds the renal threshold. Conversely, renal glycosuria, a harmless proximal tubular defect and some other diseases of the proximal tubules lower the renal threshold and cause glycosuria.

6.1.6 Ketones: Ketonuria is formed with uncontrolled diabetes mellitus and may also occur with starvation and weight reducing diets.

6.1.7 Ascorbic acid: Large urinary concentrations arise from therapeutic doses of vitamin C.

6.1.8 Bilirubin: The urine may be dark with a yellow foam if much is present. It is in the conjugated form and present in urine in biliary obstruction and infective hepatitis.

6.1.9 Urobilinogen: Its presence does not give a coloured foam and occurs in jaundice due to haemolytic disease.

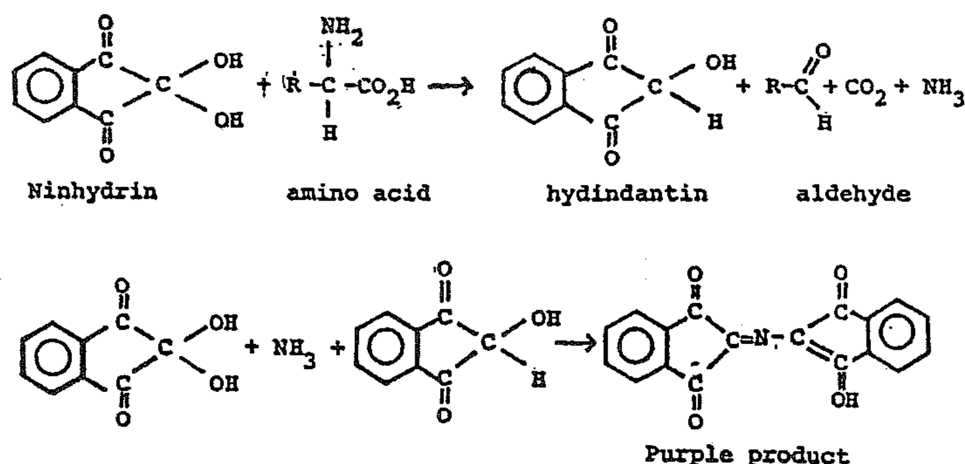
6.1.10 Blood: Red blood cells together with casts and proteinuria occur in acute glomerulonephritis, lupus erythematosus, malignant hypertension or in lower urinary tract bleeding (e.g. due to parasites, infection etc). Haemoglobinuria is due to intravascular haemolysis. Any pink, red or brown urine must be considered as bloody until proved otherwise.

6.2 Detection of Amino acids

The individual amino acids are filtered by the glomeruli but the urinary concentration is normally quite low because they are actively reabsorbed by the renal tubules. In severe liver disease, protein

synthesis and deamination of amino acids are reduced leading to increased excretion. In starvation and debilitating disease increased breakdown of plasma and tissue proteins and in the Fanconi syndrome a defect in tubular reabsorption lead to increased urine levels. In cystinuria, phenylketonuria and alkaptonuria, urinary cystine, phenylalanine and tyrosine levels increase respectively.

Amino acids in urine may be detected by means of the Ninhydrin Reaction. Ninhydrin reacts with all amino acids except proline and hydorxyproline at pH 3-4 to give a purple coloured compound. Initially, the amino acid is oxidized to an aldehyde containing one carbon atom less together with the release of ammonia and carbon dioxide. Then the ammonia, ninhydrin and the reaction product hydrindantin react to form the purple product.



6.3 Quantitative Estimation of Protein in Urine

The quantitative estimation of the daily excretion of protein is of value to the clinician in order to determine the type of renal disease, its severity and to monitor the results of treatment given. .

Most quantitative assays are performed on urine specimens of 12 hours or 24 hours. The 24-hour timing allows for circadian rhythmic changes in excretion at certain times of day. The volume of the timed specimen is measured accurately

and recorded. The results are reported generally in terms of weight of protein per 24 hours by calculating the amount of protein present in the total volume of urine collected during that time.

Several methods have been proposed for the determination of total protein in urine and other body fluids , including the measurement of turbidity when urinary proteins are mixed with an anionic organic acid such as sulfosalicylic acid , TCA , or benzethonium chloride. These methods are sensitive , but the reagent does not react equally with each protein fraction. This is particularly true of sulfosalicylic acid , which produces 4 times more turbidity with albumin than with α-glubulin .

A method considered to give more accurate results consists of precipitation of the urine proteins , dissolution of the protein precipitate , and color formation with biuret reagent .

Another chemical procedure for urinary protein uses the Folin – Ciocalteau reagent , which is a phosphotungstomolybdic acid solution , frequently called phenol reagent because it oxidizes phenolic compounds .

The reagent changes color from yellow to blue during reaction with tyrosine , tryptophan and histidine residues in protein . This method is about 10 times more sensitive than the biuret method .

Lowry and associates increased the sensitivity of the Folin – Ciocalteau reaction by incorporating a biuret reaction as the initial Step . After the binding of the cu^{2+} to the peptide bonds , the Folin-Ciocalteau reagent is added. As the Cu^{2+} protein complex is oxidized , the reagent is reduced , forming the chromogens tungsten blue and molybdenum blue . This increased the sensitivity to 100 times greater than that of the biuret method alone .

Dye – binding methods also have been used to determine the total protein content of body Fluids .

Methods are available using dyes such as coomassie blue and ponceau S.

Table 1-summarizes the various methods for measurement of urinary total protein .

TABLE -1. URINE PROTEIN METHODS

Method	Principle	Comment
Turbidimetric methods (sulfosalicylic acid, TCA or benzethonium chloride)	Proteins are precipitated as fine particles, turbidity is measured spectrophotometrically	Rapid, easy to use; unequal sensitivity for individual proteins
Biuret	Proteins are concentrated by precipitation, redissolved in alkali, then reacted with Cu^{2+}; Cu^{2+} form colored complex with peptide bonds	Accurate
Folin-Lowry	Initial biuret reaction; oxidation of tyrosine, tryptophan, and histidine residues by Folin phenol reagent (mixture of phosphotungstic and phosphomolybdic acids); measurement of resultant blue color.	Very sensitive
Dye-binding (Coomassie blue,ponceau S)	Protein binds to dye, causes shift in absorption maximum	Limited linearity; unequal sensitivity for individual proteins

Because of ease of use , speed , and sensitivity ,t he techniques used most frequently today are turbidimetric procedures.

Sulphosalicylic acid is used in this experiment to precipitate the protein in a 24 hour sample of urine. The turbidity is proportional to the concentration of the protein, and may be measured with a spectrophotometer.

6.4 OBJECTIVES:

2- The semi-quantitative detection of some abnormal constituents by means of test-strips.
3- The detection of amino-acids in abnormal urine.
4- The quantitative estimation of protein in abnormal urine.

6.5 MATERIALS:

1- Several random mid-stream urine samples for use of test-strips.
2- Combur-9 test strips.
3- Test tubes.
4- 24 hours collection of abnormal urine without preservative for a complete urine analysis.
5- 24 hour collection of an abnormal urine containing 10 ml of 2% boric acid solution as preservative for total protein estimation.
6- 2% boric acid.
7- 2 liter measuring cylinder.
8- Urinometer and urinometer cylinder.
9- 0.1% glycine solution.
10- 0.1% proline solution.
11- A random mid-stream specimen of normal urine.
12- A random mid-stream specimen of urine with aminoaciduria.
13- 0.5% ninhydrin solution in acetone.
14- 3% sulphosalicylic acid solution.
15- Bovine albumin standard (50 mg /dl of 0.85% saline)
16- 250 ml volumetric flask.
17- 0.85% saline solution.
18- 10 ml of graduated pipettes.
19- 10 ml graduated pipettes.
20- Semi-logarithm paper.

21- 2 ml graduated pipettes.

22- Spectrophotometer at 500 nm.

6.6 Detection of Abnormal Constituents Using Test-Strips

6.6.1 Method

1. Carry out the tests on the random mid-stream samples of urine provided and also on your own specimen if you wish.

2. Label test tubes 1,2,3… etc. according to the number of samples you have.

3. Approximately ¾ fill each test tube with the appropriate sample.

4. Quickly dip a Combur-9 test strip into sample No.1.

5. Withdraw it and shake off the excess urine (not over your neighbor).

6. Between 30 and 60 seconds afterwards (the timing is important) compare the developed colours on the pads with the colour chart on the test-strip container. Record the results in the table.

7. Repeat the procedure for each urine sample.

8. Carry out a complete urinalysis on the 24 hour specimen provided: both a simple examination (according to last week's experiment) and using test strips.

N.B. Do not forget to correct the specific gravity for temperature and glucose and protein concentrations.

Subtract 0.004 for every 10 g/l of dissolved glucose

Subtract 0.003 for every 10 g/l of dissolved glucose

Subtract 0.001 for every 3°C below 15°C

Add 0.001 for every 3°C above 15°C

6.6.2 RESULTS:

For random mid-stream urines:

Sample No.					Normal
Nitrite					
pH					5.5-8.0
Glucose					
Ketones					
Ascorbic Acid					
Bilirubin					
Urobilinogen					
Blood					

For 24 hour specimen requiring complete urinalysis:

Test	Result	Normal
24 hr. volume	ml	750-2000 ml
Colour		Pale to dark yellow
Appearance		
Odour		Urine-like
Specific gravity		1.010 – 1.025
Nitrite		
pH		5.5 – 8.0
Glucose		
Ketones		
Ascorbic Acid		
Bilirubin		
Urobilinogen		
Blood		

6.6.3 DISCUSSION:

Discuss the significance of the results for each of the urines you have tested and, if possible comment on the clinical condition of each patient.

6.7 Detection of Amino Acids

6.7.1 Method

Label 5 test tube A, B, C, D and E.

Place 1 ml of water in tube A.

1 ml of glycine solution in tube B.

1 ml of proline solution in tube C.

1 ml of urine in tube D.

1 ml of urine under test in tube E.

Add a few drops of ninhydrin solution to each test-urine.

Boil the contents of each test tube for 2 minutes.

Record your observations.

6.7.2 RESULTS:

Solution	Contents	Observation
A	Water	
B	Glycine	Blue
C	Proline	Orange
D	Normal Urine	
E	Abnormal Urine	

6.7.3 DISCUSSIONS:

Record any comments you have to make on the above observations.

6.8 Quantitative Estimation of Protein in Urine

6.8.1 Method

1. It is necessary first to prepare a standard curve as follows.
2. Bovine albumin standard. (50mg/dl)
3. Set up a series of test tubes as follows:

Tube No.	**Protein Soln. (ml)**	**0.85% Saline (ml)**	**Protein (mg/dl)**
1	2	0	50
2	1.5		
3	4		
4	3.5		
5	3		
6	2		
7			
8 (Blank)	0.0	2.0	

Label a fresh set of test tubes 1 to 8.

Add 8 ml of sulphosalicylic acid to each test tube.

Into tube 1 pipette 2 ml of protein solution 1.

Into tube 2 pipette 2 ml of protein solution 2 etc.

Mix the contents of each tube well and allow to stand for 5 minutes.

Using solution 7 (blank) in the cuvettes of the spectrophotometer, set the transmittance at 100% at 500 nm.

Then using solution 1-6, record the respective transmittances of each suspension.

Solutions	**Transmittance at 500nm**	**Protein conc. (mg./dl)**
1		
2		
3		
4		
5		
6		

Plot transmittance against protein concentration on semi-logarithm paper – standard curve.

Measure the volume of the 24 hour urine specimen provided for this estimation.

Take two test tubes labeled “unknown” and “blank”.

Pipette into each 2 ml of the urine.

Pipette into “unknown” 8 ml of sulphosalicylic acid solution.

Pipette into “Blank” 8 ml of 1.25% HCl.

Mix well in each case and stand for 5 minutes.

Using the spectrophotometer for the blank solution at 500 nm, set the transmittance at 100%.

Record the transmittance of the “unknown”.

Read the protein concentration of the “unknown” from the standard curve.

If it is above 140 mg/dl repeat the estimation after diluting the urine 1:10 with saline solution.

6.8.2 RESULTS:

24 hour urine volume ml (normal 750-2000 ml)

Protein concentration mg/dl

Protein excretion g/24 hr. (Normal 0-0.150 g)

6.8.3 DISCUSSIONS:

Comment on the clinical conditions of the patient or any other observations you may have made.

6.9 QUESTIONS:

1-How much protein is normally lost in the urine each day ?

2-The severity of glomerular damage corresponds to which type of protein that appears in the urine in increasing amounts ?

3-In a case of severe proteinuria what could you say about the expected serum albumin level.

6.10 Determination of titrable acidity and ammonia in urine

The titrable acidity in urine is mainly due to acid phosphates NaH_2PO_4 and to less extent weak organic acids. It can be determined by titrating urine with a standard alkali using phenolphalein as the indicator. Calcium should be removed by potassium oxalate as not to interfere with the results

Titrable acidity of urine is about 200 - 300 ml/day. The value may rise in starvation, diabetic ketosis and acidosis.

6.10.1 MATERIALS:

1. 0.1 m NaOH
2. Potassium oxalate powder
3. Phenolphalein (0.1% solution in ethanol)

6.10.2 METHOD:

1. Pipette 25 ml of urine into a 250 ml conical flask, add to spatula full potassium oxalate powder to precipitate calcium.
2. Add 2 drops of phenolphalein and titrate with 0.1 M NaOH from a burette. Note the titre value (A ml) when a permanent pink color appears.

6.10.3 RESULTS:

Vol of 0.1 M NaOH required to neutralize the acidity in 25 ml of urine = A ml

Vol required for 100 ml of urine = A X 4 = 4A

Assuming 24 h urine output 1500 ml, titrable acidity of urine 4A X 15 ml/day

6.10.4 DISCUSSION:

6.10.5 References:

1- Vasudevan DM and Das SK,(2007) .Practical Textbook of Biochemistry for MBBS Students. Jaypee Brothers Medical Publishers (P) LTD, New Delhi.

2-Bishop .M; Fody. E and Schoeff .L.(2005) .Clinical chemistry .Principle , procedures and correlations .Fifth edition. Lippincott Williams &Wilkins.

3- Luxton .R .and Pallister .C.J.(1999)."B S E .Clinical Biochemistry " Butterworth-Heinemann

EXPERIMENT (7)

7. Estimation of Uric acid

7.1 INTRODUCTION:

Uric acid is the end product of purine catabolism. Therefore, formation of uric acid is principally endogenous mainly of tissue nucleoprotein breakdown but some amount is also formed from purine containing compounds present in food. The serum uric acid levels are marginally affected by diet.
Chemically uric acid is 2, 6, 8 trihydroxypurine. It acts like a dibasic acid and can form mono and disodium salts depending on the pH. Only pH of 5.75 is possible inside body such as in renal tubules. At this pH, or above it exists as monosodium urate salt. Thus in plasma, it is mainly as monosodium urate.
The proteins in blood are precipitated by tungstic acid. The uric acid reduces phosphotungstic acid in alkaline medium to blue coloured phosphotungstous acid. The intensity of the colour is a measure of the amount of uric acid present.

7.2 OBJECTIVES:

To measure the amount of uric acid in blood

7.3 MATERIALS:

1- 10% sodium tungstate
2- 2/3 N H_2SO_4
3- Phosphotungstic acid
4- 14% sodium carbonate
5- Standard uric acid solution (0.1 mg/ml)

7.4 METHOD:

Transfer 100 mg uric acid to 100 ml of water. Add solid sodium carbonate a little at a time with stirring to dissolve uric acid.

Sample: Blood collected in oxalate tube.

Part I

In a dry test tube take 8.5 ml distilled water, 0.5 ml blood, 0.5 ml 10% Na-tungstate and 0.5 ml 2/3 N NH_2SO_4 (Dilution of blood 1 in 20). Mix, keep for 10 min, filter in a dry test tube to obtain a clear solution of PFF.

Part II

Label three test tubes as T (test), B (blank) and S (standard)

	T	B	S
PFF	3 ml	-	-
Std. uric acid solution	-	-	3.0 ml
Distilled water	-	3.0 ml	-
14% sodium carbonate	1 ml	1 ml	1 ml
Phosphotungstic acid reagent	1 ml	1 ml	1 ml

Mix and keep in dark for 15 minutes. Read the absorbance at 640 nm.

**** OR used available kit**

7.5 RESULTS:

7.6 DISCUSSION:

7.7 References:

Practical Textbook of Biochemistry for MBBS Students by DM Vasudevan and SK Das, 2007, Jaypee Brothers Medical Publishers (P) LTD, New Delhi.

EXPERIMENT (8)

8. Qualitative analysis of renal calculi

8.1 INTRODUCTION:

Renal calculi: A common cause of blood in the urine and pain in the abdomen, flank, or groin. Occurs in 1 in 20 people at some time in their life. Development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate.
The stones form in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself . The pain is usually of sudden onset, very severe and colicky (intermittent), not improved by changes in position, radiating from the back, down the flank, and into the groin. Nausea and vomiting are common. Predisposing factors may include recent reduction in fluid intake, increased exercise with dehydration, medications that cause hyperuricemia (high uric acid) and a history of gout. Treatment includes relief of pain, hydration and, if there is concurrent urinary infection, antibiotics. The majority of stones pass spontaneously within 48 hours. However, some stones may not. There are several factors which influence the ability to pass a stone. These include the size of the person, prior stone passage, prostate enlargement, pregnancy, and the size of the stone. A 4 mm stone has an 80% chance of passage while a 5 mm stone has a 20% chance. If a stone does not pass, urologic intervention may be needed. The process of stone formation is also called nephrolithiasis or urolithiasis. "Nephrolithiasis" is derived from the Greek nephros- (kidney) + lithos (stone) = kidney stone "Urolithiasis" is from the French word "urine" which, in turn, stems from the Latin "urina" and the Greek "ouron" meaning urine = urine stone.

8.2 OBJECTIVES:

To test the constituents of a renal calculi **qualitatively**.

8.3 MATERIALS:

1- Dilute hydrochloric acid(2mol/l)
2- Dilute sulphuric acid (2mole/l)
3- Concentrated nitric acid
4- Acetic acid(30ml glacial acetic acid/100 ml water)
5- Pottasium hydroxide solution
6- Concentrated ammonia solution (s.g 0.88)
7- Dilute ammonia reagent(dilute reagent 6 five-fold with water)
8- Ammonium molybdate solution, 50g/l freshly prepared.
9- Ammonium oxalate solution, prepare a saturated solution.
10- Sodium cyanide solution, 100g/l
11- Potassium permanganate solution, 3g/l
12- Sodium nitroprusside solution, 50 g/l freshly prepared.
13- Titan yellow 1g/L of water
14- Barfoed's **solution**
15- 40% NaOH
16- glacial acetic acid
17- lead acetate solution

8.4 METHOD:

8.4.1Test for uric acid

Any of the following method can be used :

1- by the murexide test.

Add 2-3 drops of concentrated nitric acid to a small amount of the sample in a small evaporating dish and evaporate to dryness by heating on a water bath.

The test is positive if a red or yellow residue is obtained which after being allowed to cool changes to purplish-red on addition of a drop of dilute ammonium hydroxide.

2- To 2 ml of sample add 4 ml of Barfoed's reagent , then heated in a boiling water bath for three minutes . Chang the blue colour indicates the presence of uric acid

8.4.2 Test for cystine

Any of the following method can be used :

1-Dissolve a small amount of the powdered stone in a few drops of concentrated ammonia solution, dilute with 2 ml water, add 1 ml sodium cyanide, and after standing for 5 min, add a few drops of sodium nitroprusside solution. A deep magenta color is given by cystine

2.. Dissolve a little of the powdered stone in 2ml distill water, then add2 ml. of 40% NaOH , boil for about 3-4 minutes .Cool and add 2 ml of glacial acetic acid followed by 1 ml of lead acetate solution .A black ppt or brown ppt of lead sulphide indicates presence of cystine and cysteine.

8.4.3 Test for carbonate and oxalate

Add a little dilute hydrochloric acid to a small portion of the sample, gas bubbles will indicate the presence of carbonate. (it can be difficult to detect with the naked eye so its better to use a hand lens)

As for oxalate,heat a part of the sample with 2 ml dilute sulphuric acid for 1 min, allow to cool to 60 -70 C, then add drop wise, potassium permanganate solution, declorization and evolution of bubbles will confirm the presence of oxalate.

8.4.1 Test for phosphates

Dissolve a little of the powdered stone in a few ml of concentrated nitric acid and then add an equal volume of ammonium molybdate solution. Heat to boiling, if phosphates are present, a yellow precipitate of ammonium phosphomolybdate is obtained.

8.4.2 Test for calcium and magnesium

Dissolve about 100 mg of the sample by heating with 2 ml dilute hydrochloric acid, add 1 ml ammonium oxalate and enough concentrated ammonia until just alkaline , readjust to pH 5 with acetic acid. A white precipitate of calcium oxalate shows the presence of calcium.

Filter and to the filtrate add a few drops of titan followed by potassium hydroxide until strongly alkaline. A red color indicates the presence of magnesium.

8.5 RESULTS:

8.6 DISCUSSIONS:

8.7 References:

Practical clinical biochemistry, volume 1, general topics and commoner tests by Varley H, Gowenlock AH, and Bell M. 5th edition

EXPERIMENT (9)

9. Determination of plasma amylase .

9.1 INTRODUCTION

The determination of plasma amylase is importance in the diagnosis of acute pancreatitis in which high values may be obtained. It has the advantage over the estimation of urinary diastase in that it is unnecessary to wait for the collection of a 24-hour specimen of urine.

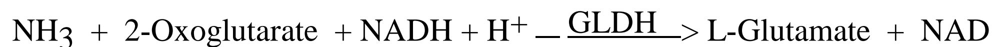
A high plasma amylase is also found in salivary gland inflammation, and low values in liver diseases and pancreatic insufficiency.

Normal values in

- Serum :16-108 U per L
- Urine : 0 -14 U / Hour

9.2 Principle:

The Ammonia reacts with 2-Oxoglutarate, in the presence of L-GLDH and the co-enzyme NADH, to produce L-glutamate. The resulting decrease in absorbance of NADH at 340 nm is proportional to the level of ammonia in the sample[3]. Methylated amines which interfere with other conventional procedure, do not react in the described method.



$$NH_3 + \text{2-Oxoglutarate} + NADH + H^+ \xrightarrow{GLDH} \text{L-Glutamate} + NAD$$

9.3 MATERIAL AND METHOD:

As shown in the provided kit.

9.4 RESULT

9.5 DISCUSSION:

9.6 Reference:

Singh.S.P.(2006).Practical manual of biochemistry. 6th edition

EXPERIMENT (10)

10. Determination of lipase in serum .

10.1 INTRODUCTION

The measurement of lipase in serum to evaluate conditions associated with pancreas A high plasma lipase is found in pancreatitis and cancer in pancrease ; also increase in cirrhosis of liver ,hepatitis,duodenal ulcer , disease of the biliary tract .

Normal values less than 150 U per L

10.2 Principle:

Serum is incubated at 37 ^{o}C with an olive oil substrate buffered at pH 9.0. Hydrolysis of triglycerides present in the olive oil by pancreatic lipase causes a decrease in the turbidity of the reaction mixture. The decrease in absorbed light at 400 nm is measured over a period of 5 minutes and reflects the activity of lipase in the sample.

Triglycerides +H_2O $\xrightarrow{\text{Lipase}}$ mono + di-Glycerides +Fatty acids

10.3 MATERIAL AND METHOD:

As shown in the provided kit.

10.4 RESULT

10.5 DISCUSSION:

10.6 Reference:

Singh.S.P.(2006).Practical manual of biochemistry. 6th edition

Normal Human Biochemical Values in Blood/ Plasma / Serum

Constituent	Normal Range	High in	Low in
Diabetes Mellitus			
Blood Sugar (Fasting)	70-100 mg %	Diabetes mellitus	Hyperinsulinism
Blood Sugar (PP)	Less than 140 mg%	Diabetes mellitus	____
Renal threshold value for glucose	160 – 180 mg%	Diabetes mellitus	____
Blood sugar level (Random)	80 – 120 mg%	Diabetes mellitus	____
Renal Function Tests			
Blood Urea	14 – 43 mg%	Nephritis, Renal failure	Nephrosis
Serum Creatinine	0.1 -1.2 mg%	Nephritis, Renal failure	Nephrosis
Liver Function Tests			
Serum Bilirubin	0.2 – 1 mg%	Obstructive jaundice, Haemolytic jaundice, Neonatal jaundice and Hepatitis	____
SGOT (AST)	8 – 40 units/ml (4 – 17 IU /l)	Myocardial infarction, Infective hepatitis etc.	____
SGPT (ALT)	5 - 35 units/ml (3 -15 IU/l)	Liver disorders	____
Serum Alkaline Phosphatase	4 -11 KA Units	Obstructive jaundice, Biliary cirrhosis, Rickets, Osteomalacia	Scurvy, Sever anaemia, Malnutrition, Hypophosphatasia
Serum Proteins, Total	6 – 8 g%	Multiple Myeloma, Anhydraemia, etc.	Nephrotic syndromes, Neoplastic disease, Malnutrition, Kwashiorkor syndrome, Cirrhosis of liver
Serum Albumin	3.5 – 5 g %	___	Cirrhosis of liver,

			Nephrotic syndromes, Malnutrition, Malignancies, etc.
Serum Globulins	2.3 – 3.6 g %	Anhydraemia, Nephrosis Infections, Advanced liver diseases, etc.	___
Albumin/ Globulins Ratio	1.2 – 1.5	___	Less than 1 in infective hepatitis
Fibrinogen (Plasma)	0.3 – 0.6 g %	Infections	Cirrhosis of liver
Cardiac Enzymes			
SGOT (AST)	8 – 40 units/ml (4 – 17 IU /l)	Myocardial infarction, Infective hepatitis etc.	____
Lactic dehydrogenase (LDH)	230 – 460 IU/l	Myocardial infarction, Infective hepatitis, Toxic jaundice, etc.	____
Creatine phosphokinase (CPK or CK)	20 – 50 IU / l (men) 10 – 37 IU/l (women)	Myocardial infarction, Hypothyroidism, Progressive muscular atrophy, etc.	____
Cardiac Markers			
Cardiac troponin-T (cTnT)	0 – 0.1 µg/L	Myocardial infarction	____
Homocysteine	4 -12 µmol/L	Myocardial infarction	Pregnancy
Myoglobin	19 -92 µg/ L (M) 12 – 76 µg/L (F)	Myocardial infarction (a relatively early marker, elevation after 4-6 hrs of AMI)	____
Pancreatic Function Tests			
Serum amylase	80-180 Somogyi Units	Pancreatitis, Stone and cancer in biliary duct,	Liver diseases

		Perforated peptic ulcer	
Serum Lipase	Less than 150 U/l	Pancreatitis, Cancer in pancreas, Cirrhosis of liver, Hepatitis, Duodenal ulcer, Disease of the biliary tract, etc.	____
Miscellaneous			
Serum acid phosphatase	1-4 KA Units	Carcinoma of prostate gland, Paget's disease, Hyperparathyroidism	___
Serum uric acid	2-7 mg%	Gout, Nephritis, Arthritis, Eclampsia	Wilson's disease, Fanconi syndrome
Serum calcium	9-11 mg%	Hyperparathyroidism	Infantile tetany, Rickets
Serum inorganic phosphorus	2.5 -4.5 mg%	Nephritis, Renal failure	Rickets, Fanconi syndrome
Serum sodium	137 -148 meq/l	___	Diabetic acidosis
Serum potassium	3.6-5.4 meq/l	Pneumonia, Acute infections and Uremia	___
Serum magnesium	1 -3 mg%	___	Vomiting and Diarrhea
Serum lithium lipoprotein (a)	Absent or in traces 0 – 30 mg/dl	___	___

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