
بسم الله الرحمن الرحيم

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Ministry of Higher Education

king Saud University

College of Science

Biochemistry Department

Practical Note
Analysis of Biological Fluid
(BCH 472)

prepared by

Dr. Nikhat Sedeeqi
Lecturer. Rehab Nouh
Demonstrator. Tahani Al shehry

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EXPERIMENT (1)

1 Physical properties and detection of normal constituents of urine

1.1 INTRODUCTION:

1.1.1 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 ml
Albumin	4 g (60 μmol)	0.04 g (6 μmol)
Urea	900 mmol	400 mmol

1.2 Simple Examination of the Urine

1.2.1 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 of low polydipsia, or after mobilization of fluid from ascites or oedema. 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.

1.2.2 Color

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

1.2.3 Appearance

1.2.2.1 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.

1.2.2.2 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.

1.2.2.3 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.

1.2.2.4 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.

1.2.2.5 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.

1.2.2.6 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

1.3 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.

1.4 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- 10 ml uric acid 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

1.5 The Simple Examination of Urine

1.5.1 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.

1.5.2 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

1.6 To Detect some of the Normal Organic Constituents of Urine

1.6.1 Method

1- **Urea:** use enzymatic detection of urea with the aid of commercially available kits.

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. 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.

1.6.2 RESULTS:

1.6.3 DISCUSSION:

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

1.7.1 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.

1.7.2 RESULTS:

1.7.3 DISCUSSION:

1.8 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- Apart from dehydration, what is most likely to happen to the diabetes patient with polyuria?

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

1.9 References:

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

EXPERIMENT (2)

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

2.1 INTRODUCTION

2.1.1 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.

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

2.1.3 Protein: Proteinuria is indicative of renal disease. Indeed it is probably the single most sensitive indicator or renal disease and quantitative measurement often correlates with severity of renal disease. Large amounts are lost in urine in the nephritic syndrome. Small amounts accompany hematuria and acute urinary tract infection. With chronic renal disease, proteinuria may be intermittent. More protein is excreted by ambulatory persons than those in bed.

2.1.4 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.

2.1.5 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.

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

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

2.1.8 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.

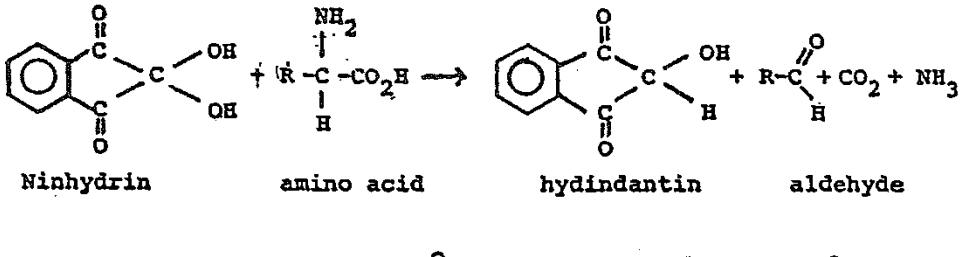
2.1.9 Urobilinogen: Its presence does not give a coloured foam and occurs in jaundice due to haemolytic disease.

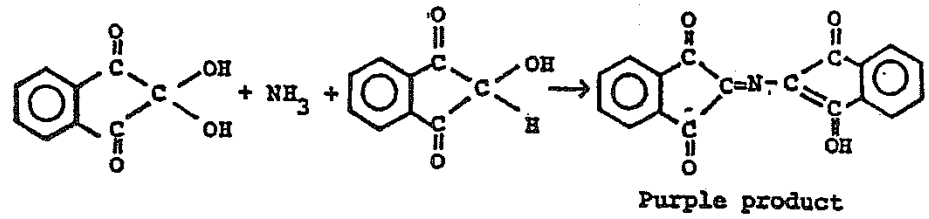
2.1.10 Blood: Red blood cells together with casts and proteinuria occur in acute glomerulonephritis, lupus erythematosus, malignant hypertension or in lower urinary tract bleeding (e.g. due to parasites, infection etc). Haemoglobinuria is due to intravascular haemolysis. Any pink, red or brown urine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

2.2 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.





2.3 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. Disease of pre-renal origin (e.g. essential hypertension, heart failure, severe anaemia etc.) is rarely accompanied by proteinuria which may be as high as 10-24 g in 24 hours in renal failure or less than 2 g in 24 hours in failure of tubular reabsorption. In postrenal disease (e.g. U.T.I. stones) the proteinuria is small.
Sulphosalicylic acid is used 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.

2.4 OBJECTIVES:

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

2.5 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- Versatol (7 g/dl protein) or a bovine albumin standard.
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.

2.6 Detection of Abnormal Constituents Using Test-Strips

2.6.1 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 neighbour!).
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

2.6.2 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		

2.6.3 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.

2.7 Detection of Amino Acids

2.7.1 Method

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

2- 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.

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

4- Boil the contents of each test tube for 2 minutes.

5- Record your observations.

2.7.2 RESULTS:

Solution	**Contents**	**Observation**
A	Water	
B	Glycine	Blue
C	Proline	Orange
D	Normal Urine	No change
E	Abnormal Urine	Blue

2.7.3 DISCUSSIONS:

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

2.8 Quantitative Estimation of Protein in Urine

2.8.1 Method

1- It is necessary first to prepare a standard curve as follows.

2- Take a vital of versatol standard (7 g/dl) or of a bovine albumin, standard.

3- Add 5 ml of distilled water to the vial and swirl gently until dissolved.

4- After standing for 30 minutes, dilute the contents of the vial to 250 ml in a volumetric flask with 0.85% saline. This contains 140 mg/dl of protein.

5- Set up a series of test tubes as follows:

Tube No.	**Protein Soln. (ml)**	**0.85% Saline (ml)**	**Protein (mg/dl)**
1	4.5	1.5	105
2	3.0	3.0	70
3	2.4	3.6	56
4	1.5	4.5	35
5	0.9	5.1	21
6	0.3	5.7	7
7 (Blank)	0.0	6.0	0

6- Label a fresh set of test tubes 1 to 7.

7- Add 8 ml of sulphosalicylic acid to each test tube.

8- Into tube 1 pipette 2 ml of protein solution 1.
Into tube 2 pipette 2 ml of protein solution 2 etc.

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

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

11- 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		

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

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

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

15- Pipette into each 2 ml of the urine.

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

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

18- Mix well in each case and stand for 5 minutes.

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

20- Record the transmittance of the “unknown”.

21- 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.

2.8.2 RESULTS:

24 hour urine volume	ml (normal 750-2000 ml)
Protein concentration	mg/dl
Protein excretion	g/24 hr. (Normal 0-0.150 g)

2.8.3 DISCUSSIONS:

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

2.9 QUESTIONS:

1- In a case of mild proteinuria, which protein would be present in the urine in the highest proportion? Give your reasons.

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

2.10 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.

2.10.1 MATERIALS:

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

2.10.2 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.

2.10.3 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

2.10.4 DISCUSSION:

2.10.5 References:

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

EXPERIMENT (3)

3. Qualitative analysis of renal calculi

3.1 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.

3.2 OBJECTIVES:

To test the constituents of a renal calculi quantitativaly

3.3 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

3.4 METHOD:

3.4.1 Test for uric acid 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.

3.4.2 Test for cystine

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.

3.4.3 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.

3.4.4 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.

3.4.5 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.

3.5 RESULTS:

3.6 DISCUSSIONS:

3.7 References:

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

EXPERIMENT (4)

4. Instrumental determination of electrolytes in urine

4.1 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.

4.2 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).

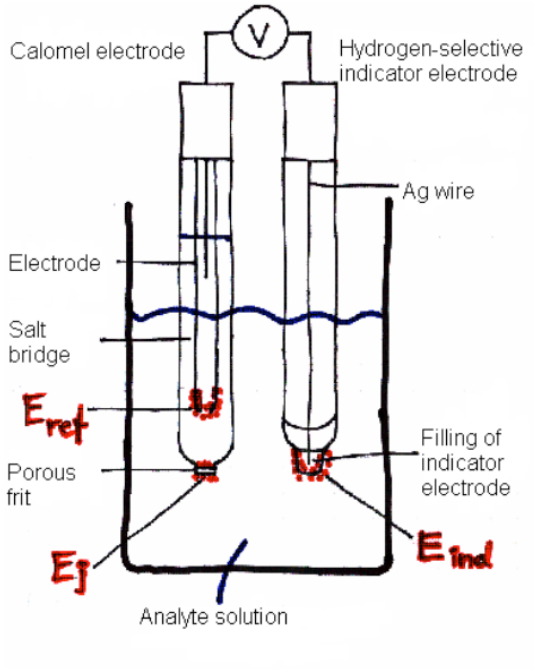


Figure 2. Potentiometric measurement system (for pH measurement).

4.3 References:

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

EXPERIMENT (5)

5. Creatinine estimation and creatinine clearance tests

5.1 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)

ATP + creatine ⟶ creatine phosphate + ADP

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.

5.2 Normal values:

In serum: 1-2 mg/day

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).

5.3 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

5.4 High levels of creatine:

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

5.5 OBJECTIVES:

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

5.6 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- Creatinie working standard for urine (0.75mg/dl): dilute 50 ml of stock and bring the volume up to 200 ml with water.
4- Serum and urine samples.
5- NaoH(2.5 M)
6- Picric acid.(0.04 M)

5.7 METHOD:

5.7.1 Serum creatine:

	Standard (A,B)	Test(C.D)	Blank(B)
Serum		0.5 ml	
Water	1.5 ml	1.5 ml	2 ml
Standard for serum	0.5 ml		
Picric acid	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

5.7.2 Urinary creatinine :

Take one ml of urine and dilute it to 100 ml

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

5- Mix well
6- Add 0.4 ml of 2.5 M NaoH
7- Allow to stand for 20 minutes
8- Read the absorbance against the blank at 520 nm

5.8 RESULTS

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

$$\text{Urinary creatinine} = \frac{\text{Abs of test}}{\text{Abs of std}} \times \text{concentration of standard} \times 100$$

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

5.9 DISCUSSION:

5.10 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?

5.11 References:

The old curriculum BCH 349

EXPERIMENT (6)

6. Estimation of Uric acid

6.1 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.

6.2 OBJECTIVES:

To measure the amount of uric acid in blood

6.3 MATERIALS:

1- 10% sodium tungstate
2- 2/3 NH_2SO_4
3- Phosphotungstic acid
4- 14% sodium carbonate
5- Standard uric acid solution (0.1 mg/ml)

6.4 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.

6.5 RESULTS:

6.6 DISCUSSION:

6.7 References:

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

EXPERIMENT(7)

7. Estimation of Serum Bilirubin

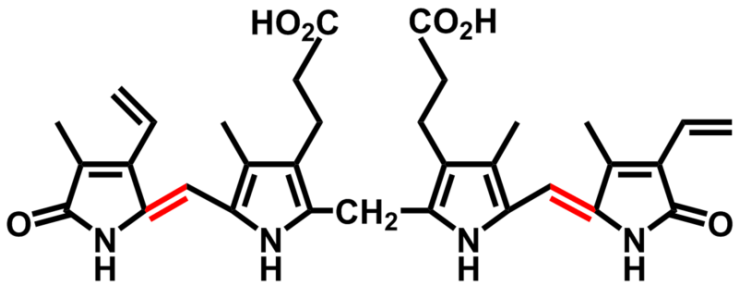
7.1 INTRODUCTION:

Bilirubin is a by-product of the breakdown of red blood cells. It is the yellowish pigment responsible for jaundice.

Bilirubin levels can be raised due to many different liver diseases, as well as conditions other than liver disease, e.g. gallstones.In cases of long-term liver illness (chronic hepatitis).

In cases of short-term liver illness (acute hepatitis), elevated bilirubin levels indicate the severity of the acute illness.

Normal range 0.2 -1.5 mg/dl



Bilirubin in serum reacts with diazotized suphanilic acid to give purple coloured derivative of azobilirubin. The colour is a measure of the amount of bilirubin. Total bilirubin is measured using methanol as solvent and direct bilirubin is measured with water as solvent because the non-esterified indirect bilirubin, insoluble in water reacts with diazo reagent very slowly thus avoiding their interference during the analysis of direct bilirubin.

7.2 OBJECTIVES:

To estimate the amount of bilirubin

7.3 MATERIALS:

1- Diazo reagent A: Dissolve 1 g of sulphanilic acid in 15 ml of conc. HCl. Make volume up to 1 lt with distilled water.
2- Diazo reagent B: 0.5% sodium nitrite
3- Diazo colour reagent: Mix 5 ml of diazo A in 0.15 ml of diazo B (prepare fresh)
4- 0.15 N HCl
5- Methanol
6- Standard bilirubin in chloroform (0.1 mg/ml)

- **Sample**: Serum
- **Glassware**: Test tubes, measuring cylinders, standard flasks
- **Equipmen**t: Balance, Spectrophotometer.

7.4 METHOD:

Label six test tubes as TT (total test), TC (total control), DT (direct test), DC (direct control), S (standard) and B(blank).

	TT	TC	DT	DC	S	B
Distilled water	1.8 ml	1.8 ml	4.3 ml	4.3 ml	1.8 ml	2.0 ml
Serum	0.2 ml	0.2 ml	0.2 ml	0.2 ml	-	-
Diazo colour reagent	0.5 ml	-	0.5 ml	-	0.5 ml	0.5 ml
0.15 M HCl	-	0.5 ml	-	0.5 ml	-	-
Methanol	2.5 ml	2.5 ml	-	-	2.5 ml	2.5 ml
Bilirubin standard	-	-	-	-	0.2 ml	-

Mix, keep the tubes for 30 minutes in dark and read the absorbance at 540 nm.

Note; In the absence of bilirubin standard, methyl red solution (0.29 mg%) can be used. The colour of 1 ml of this solution is equivalent to 0.0035 mg bilirubin.

7.5 RESULTS:

Concentration of direct bilirubin in mg/ ml serum

$$= \frac{OD_{DT} - OD_{DC}}{OD\ s - OD_B} \times \frac{\text{Conc of standard}}{\text{Volume of sample}} \times 100\ mg\%$$

$$= \frac{OD_{DT} - OD_{DC}}{OD\ s - OD_B} \times \frac{0.2}{0.2} \times 100\ mg\%$$

$$= \frac{OD_{DT} - OD_{DC}}{OD\ s - OD_B} \times 100\ mg\%$$

Conc of total bilirubin in mg/100 ml serum

$$= \frac{OD_{TT} - OD_{TC}}{OD\ s - OD_B} \times \frac{\text{Conc of standard}}{\text{Volume of sample}} \times 100\ mg\%$$

$$= \frac{OD_{DT} - OD_{DC}}{OD\ s - OD_B} \times \frac{0.2}{0.2} \times 100\ mg\%$$

$$\frac{OD_{DT} - OD_{DC}}{OD\ s - OD_B} \times 100\ mg\%$$

7.6 DISCUSSION:

7.7 *QUESTIONS*:

1- What are the differences between direct and indirect serum bilirubin? give an abnormality associated with each.
2- How can serum bilirubin be lowered?
3- What is the difference between increased urine bilirubin and increased urine uribilinogen?

7.8 *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. Determination of total proteins and albumin/globulin ratio

8.1 INTRODUCTION:

The value of total proteins is increased in dehydration,multiple myeloma, rheumatoid arthritis, and tuberculosis. Whereas they are decreased in proteinurea, low protein intake, malabsorption, nephrosis, hemorrhage, shock, and sever liver diseases.

In pregnancy serum albumin level decreases, while the level of globulin increases.

Normal values: Total protein 6-8 g/dl, Albumin 3.5-5 g/dl, Globulin 2.5-3.5 g/dl.

Proteins , which contain peptide linkages form a complex with copper in alkaline medium giving a violet color (Biuret reaction) The intensity of the color is proportional to the number of peptide bonds and thus is a measure of the concentrations of proteins.

Albumins are estimated in serum using the biuret reaction after precipitation and separation of serum globulins by sodium suphate.

8.2 MATRIALS:

1- 28% sodium sulphate
2- 0.9% saline
3- Standard protein solution (bovine serum albumin 5 mg/ml)
4- Biuret reagent

8.3 Method:

- Part I

Two dry test tubes labeled P (total protein) and G (globulin)

	P	G
Saline	3.8 ml	-
28% sodium sulphate	-	3.8 ml
Serum	0.2 ml	0.2 ml

Mix G by inverting the tube a couple of times, then filter immediately to separate globulins (a centrifuge can be used)

-Part II:

Label 4 test tubes as T (test), A (albumin), B (blank), and S (standard)

	T	A	B	S
Saline	-	-	1 ml	-
BSA (standard)	-	-	-	1 ml
Solution from P	1 ml	-	-	-
Filtrate of G	-	1 ml	-	-
Biuret reagent	5 ml	5 ml	5 ml	5 ml

Mix and keep standing for 10 minutes, read the absorbance using a spectrophotometer at 540 nm

8.4 RESULTS:

1- Concentration of proteins in serum:

$$\frac{\text{Absorbance of T}}{\text{Absorbance of S}} \times \text{Conc of standard}$$

2- Concentration of albumin in serum:

$$\frac{\text{Absorbance of A}}{\text{Absorbance of S}} \times \text{Conc of std}$$

3- Concentration of globulin = Total protein – Albumin

4- Calculate the A/G ratio

8.5 DISCUSSION:

8.6 QUESTIONS:

1- What is the normal A/G ratio? And what does a higher ratio indicates?

2- Name two other methods for protein estimation.

3- Explain the need to test for the level of proteins.

8.7 References:

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

EXPERIMENT(9)

9. Osmolality of serum and urine

9.1 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.

9.1.1 **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.

9.1.2 **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.

9.2 METHOD:

Using an osmometer

Types of Osmometers

9.2.1 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:

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

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

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

9.3 References:

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

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

EXPERIMENT(10)

10. Determination of ALT and AST in serum

10.1 INTRODUCTION:

An alanine aminotransferase (ALT) test measures the amount of this enzyme in the blood. ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT formerly was called serum glutamic pyruvic transaminase (SGPT).

ALT is measured to see if the liver is damaged or diseased. Low levels of ALT are normally found in the blood. But when the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels go up. Most increases in ALT levels are caused by liver damage.

The ALT test is often done along with other tests that check for liver damage, including aspartate aminotransferase (AST), alkaline phosphatase, lactate dehydrogenase (LDH), and bilirubin. Both ALT and AST levels are reliable tests for liver damage.

10.2 a) Estimation of ALT and AST in serum (GOT and GPT)

10.2.1 INTRODUCTION:

Transaminases (aminotransferases) are enzymes which catalyse the transfer of the α- amino group of an α- amino acid to the α carbon atom of an α- keto acid. The product of the reaction are a new α- amino acid corresponding to the original α- keto acid, and a new α- keto acid corresponding to the original α- amino acid. Transamination reactions are generally reversible and they play an important role in the metabolism of amino acids in the body, Transaminases are present in almost all tissues both in the cytoplasm and in the mitochondria.

All transaminases contain pyridoxal phosphate (vitamin B6) as a prosthetic group.

The most important transaminases identified are glutamate-oxaloacetate transaminase (GOT) and glutamate- pyruvate transaminase (GPT)

The reaction catalysed by GOT is shown below:

Asparate + α- oxoglutarate ⟵ oxaloacetate + glutamate

⟶

The activity of these two enzymes are quiet low in serum, whereas its relatively high in such tissues as liver, skeletal muscle and kidney.

Measurement of SGOT and SGPT is useful in the clinical diagnosis of certain diseases. These enzymes will be increased in cases where cells containing large quantity of the enzymes are ruptured, therby releasing their content into the blood stream.

Increased levels of SGOT and SGPT are found in cases of:

1- Myocardial infarction (accompanied with increase in CPK)
2- Viral hepatitis
3- Toxic liver necrosis
4- Cirrhosis
5- Malignant infiltration of the liver

10.2.2 Normal levels:

- **Normal**

Normal results may vary from lab to lab.

Alanine aminotransferase
4–36 units per liter (U/L) or 0.07–0.62 microKat/L

- **High values**

1- Very high levels of ALT may be caused by:

2- Recent or severe liver damage, such as viral hepatitis.

3- Lead poisoning.

4- Drug reactions.

5- Exposure to carbon tetrachloride.

6- Decay of a large tumor (necrosis).

7- Shock.

Mildly or moderately high ALT levels may be caused by:

1- Mononucleosis.
2- Hepatitis. The ALT level in a person with hepatitis can be 20 times the normal value.
3- Alcohol abuse. People who drink excessive amounts of alcohol and take acetaminophen (such as Tylenol) can have high ALT blood levels.
4- Mildly elevated levels of ALT may occur in people who are growing quickly, especially young children.

Slightly high levels ALT levels may be caused by:

- Fatty deposits in the liver. Many medicines, such as statins, antibiotics, chemotherapy, aspirin, narcotics, and barbiturates. Long-term (chronic) diseases that affect the liver, such as cirrhosis.

10.2.3 OBJECTIVES:

To determine the level of these enzymes in serum as a tool to study liver function.

10.2.4 MATERIAL AND METHOD:

As shown in the provided kit.

10.2.5 DISCUSSION:

10.3 b: Estimation of ALP and LDH in serum

10.3.1 INTRODUCTION:

10.3.1.1 a. ALP:

An alkaline phosphatase (ALP) test measures the amount of the enzyme ALP in the blood. ALP is made mostly in the liver and in bone with some made in the intestines and kidneys. It also is made by the placenta of a pregnant woman.

The liver makes more ALP than the other organs or the bones. Some conditions cause large amounts of ALP in the blood. These conditions include rapid bone growth (during puberty), bone disease (osteomalacia or Paget's disease), or a disease that affects how much calcium is in the blood (hyperparathyroidism), or damaged liver cells.

A test for alkaline phosphatase (ALP) is done to:

1- Check for liver disease or damage to the liver. Symptoms of liver disease can include jaundice, belly pain, nausea, and vomiting. An ALP test may also be used to check the liver when medicines that can damage the liver are taken.

2- Check bone problems (sometimes found on X-rays), such as rickets, osteomalacia, bone tumors, Paget's disease, or too much of the hormone that controls bone growth (parathyroid hormone). The

ALP level can be used to check how well treatment for Paget's disease is working.

Normal values may vary from lab to lab.

10.3.2 Alkaline phosphatase	
Adults:	30–126 units per liter (U/L) or 0.5–2.0 microkat/liter (mckat/L)
Children:	30–300 U/L or 0.5–5.0 mckat/L

Women in the third trimester of pregnancy have high ALP levels because the placenta makes ALP. Children normally have much higher ALP than adults because rapid bone growth is normal in children and bones make ALP.

10.3.2.1 High values

- Very high levels of ALP can be caused by liver problems, such as hepatitis, blockage of the bile ducts (obstructive jaundice), gallstones, cirrhosis, liver cancer, or cancer that has spread (metastasized) to the liver from another part of the body.
- High ALP levels can be caused by bone diseases, such as Paget's disease, osteomalacia, rickets, bone tumors, or tumors that have spread from another part of the body to the bone, or by overactive parathyroid glands (hyperparathyroidism). Normal healing of a bone fracture can also raise ALP levels.
- Heart failure, heart attack, mononucleosis, or kidney cancer can raise ALP levels. A serious infection that has spread through the body (sepsis) can also raise ALP levels.

10.3.2.2 Low values

Conditions that lead to malnutrition (such as celiac disease) or are caused by a lack of nutrients in the diet (such as scurvy) can cause low ALP levels.

10.4 MATERIAL AND METHOD:

As shown in the provided kit.

10.4.1 b. LDH:

Lactic acid dehydrogenase (LDH) is an enzyme that helps produce energy. It is present in almost all of the tissues in the body and becomes elevated in response to cell damage. LDH levels are measured from a sample of blood taken from a vein.

LDH levels help diagnose lung disease, lymphoma, anemia, and liver disease. They also help determine how well chemotherapy is working during treatment for lymphoma.

Lactic acid dehydrogenase (LDH) is an enzyme that helps produce energy. Results may vary widely from lab to lab.

Normal LDH levels range from 100 units per liter (U/L) to 190 U/L.

10.5 MATERIAL AND METHOD:

As shown in the provided kit.

10.6 DISCUSSION:

10.7 References:

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

Experiments prepared by:

1. Mrs. Rehab Nouh
2. Dr. Nikhat Sedeeqi
3. Miss. Tahani Al shehry

Written and revised by:

Mrs. Rehab Nouh