URINE ANALYSIS

BY

BYOMKES DAS GUPTA

BENGAL MEDICAL SERVICE,

LATE DEMONSTRATOR OF PHYSIOLOGY, DACCA MEDICAL SCHOOL
AND LATE TEACHER OF PHYSIOLOGY, CHITTAGONG
MEDICAL SCHOOL.

CALCUTTA :
BUTTERWORTH & CO. (INDIA), LTD.,
6, HASTINGS STREET.
MADRAS BRANCH : 317, LINGA CHETTY STREET.
BOMBAY BRANCH : JEHANGIR WADIA BUILDING, ESPLANADE ROAD.

LONDON:
BUTTERWORTH & CO.,
(Publishers), LTD.

SYDNEY:
BUTTERWORTH & CO.,
(Australia), LTD.

TORONTO (Canada):
BUTTERWORTH & CO.,
(Publishers), LTD.

WELLINGTON (N. Z.):
BUTTERWORTH & CO.,
(Australia), LTD.

1932

Printer : S. C. MAJUMDAR
SRI GOURANGA PRESS
71/1, Mirzapur Street, Calcutta.

PREFACE.

Being connected with teaching institutions, I had to teach the students in practical class 'Urine Analysis.' To make the subject clear and lucid, I had to give occasional notes on the subject, gathered and compiled from all available sources and boiled down so as to form the most concentrated extract suited to the students. In this little book, those notes have been rewritten and many new ones added, all possible data and my experi ences centrifugalised to a concrete deposit. This monograph is published at the request of some of my pupils, for their use, who have now set up in private practice in villages and who want to do the urine analysis of their own cases, at their dispensaries at spare time. I am conscious of its short comings, as it has been composed under the distractions of other work. If my students and any practitioner find it of service, I shall consider my labours rewarded.

My best thanks are due to Messrs Baird and Tatlock for the loan of five printing blocks, illustrated in the body of the book.

CHANDROTTOMA BHABAN
P. 353, LAKE ROAD,
CALCUTTA.
The 3rd December, 1931.

B. DAS GUPTA.

List of Plates.

Calcium Oxalate		*To face page*		106
Uric Acid	... ...	,,	...	107
Urates ...	... ...	,,	...	108
Phosphates	... ...	,,	...	110
Red Blood Cells and Pus Cells	...	,,	...	112
Spermatozoa	... ...	,,	...	114
Epithelial Cells	... ...	,,	...	116
Tube Casts	... ...	,,	...	118

Text Illustrations.

		PAGE
Doremus Ureometer	... ...	78
Gerrard's Ureometer	... ...	80
Esbach's Albuminometer	... ...	84
Aufrecht Albuminometer	... ...	86
Urine Sediment Glass	... ...	102

List of Plates.

Calcium Oxalate		*To face page*		106
Uric Acid	... ...	,,	...	107
Urates ...	... ...	,,	...	108
Phosphates	... ...	,,	...	110
Red Blood Cells and Pus Cells	...	,,	...	112
Spermatozoa	... ...	,,	...	114
Epithelial Cells	... ...	,,	...	116
Tube Casts	... ...	,,	...	118

Text Illustrations.

		PAGE
Doremus Ureometer	... ...	78
Gerrard's Ureometer	... ...	80
Esbach's Albuminometer	... ...	84
Aufrecht Albuminometer	... ...	86
Urine Sediment Glass	... ...	102

ERRATA.

Page	Line	For	Read.
8	8	poluria	polyuria
10	12	odour	odours
10	25	an	a
16	9	precipitates	precipitate
25	23		
16	9	disappear	disappears
31	8	dibetic	diabetic
56	9	is	are
64	23	purpuria	purpura.
65	25	run	runs
66	25	micturation	micturition
67	2		
67	16		
70	22	dysentry	dysentery.
79	4	stopcork	stopcock.
79	7		
80	2		

INTRODUCTION.

Urine is the complex excretion of the kidneys, which purify blood from its soluble impurities and thus keeps the system in normal working order. Normally it contains the excreted salts dissolved in water and the end products of albuminous disintegration in the body; but in diseases great changes occur, hence urine examination is necessary to know the metabolic disorders in the body, the condition of kidney and other urinary tract and to know the quality and quantity of normal and abnormal constituents in the urine; the correct interpretation of which leads to a proper diagnosis of the disease. Urine examination is done mainly in three ways; Physical, Chemical and Microscopical.

NORMAL URINE.

Before one proceeds to examine a sample of urine, he must know the characters of normal urine. Healthy urine contains the following physical characters.

Colour—Pale yellow.

Odour—'Urinous'.

Quantity—

In 24 hours—40 to 50 ounces.

At one time—5 to 6 ounces.

Appearance—Clear *i.e.* no turbidity and sediment should be present.

Reaction—Slightly acid.

Specific Gravity—

Bengalees 1003—1015.

Europeans 1015—1025.

Chemical and Microscopical examination —No abnormal constituents.

Collection. In males, first thoroughly clean the meatus and the patient should be asked to pass urine in a wide mouthed clean glass bottle, thoroughly cleaned previously with ordinary water. The best thing possible is to boil the bottle in water in a pan or common dekchi for 10 minutes and then keep it in a cool place upside down. The patient should be asked to micturate directly on it; but if the patient feels any inconvenience, a clean funnel, preferably of glass, should be placed over it. If there is no sterile bottle, he can pass water in a whisky or methylated spirit bottle. Females should pass water after thoroughly cleaning the external genitals to avoid discharges and putrefactive micro-organisms if any, in a clean receptacle and if for bacteriological examination a sterile catheter should be used to draw the urine into a sterilised bottle. Twenty-four hours collection of

urine is the best or a sample or portion of what is passed in 24 hours. Preserve the urine from decomposition by adding 2 or 3 drops of formaline or 10 to 15 drops of chloroform with the first sample or a crystal of thymol. The former two reduce Fehling's solution and slightly hamper results but thymol does not reduce Fehling's solution. The collected urine should be corked or lightly covered with cloth, cotton or paper to avoid contamina tion; and when brought for examination, if acid, it should be set aside in a cool place for 3 or 4 hours to gravitate the sediments.

The composition of urine varies at different hours of the day. For the detection of mucus and pus, morning urine is the best as it is concentrated and collects more during sleep. For albumin and sugar, urine passed 3 or 4 hours after the largest meal; for blood is then full of products of digestion and so excretion is active. The usual practice is to keep and examine the early morning urine but it is not wholly reliable, as metabolism during

sleep is very little so albumin and sugar if present in faint trace may be absent then. The sample of urine passed two or three hours after breakfast may contain these abnormal ingredients. Always warn the patient not to spit on the urine to be examined as it may give misleading results.

When cultural, or any special examination is required for special reasons, thoroughly sterilise a big test tube, with a little cotton wool or a stoppered phial and a catheter and keep them ready. First wash the external meatus with aseptic lotion and draw urine by the catheter. Reject the first portion of it and collect the latter in the sterile test tube or in the stoppered phial. Immediately plug the test tube with the sterile cotton wool, after sterilising it in the flame of a spirit lamp or tighten the stopper of the phial. When urine is sent for examination from a distance, to prevent putrefaction shake with two drops of toluol or formaline 40% or preferably a few crystals of thymol (which do not reduce Fehl-

ing's Solution) and fill the phial quite full to avoid shaking. Now securely cork and carefully pack it thoroughly, preferably with ice as cold retards decomposition.

Quantity. A healthy adult male excretes in 24 hours about 40 to 50 ounces of urine; females generally 35 to 40 ounces; children much less. It varies in individual cases in relation to food and drink.

Decrease. Physiologically urine is decreased during hot weather owing to loss of fluid by sweat and when there is a less input of fluids. Pathologically, it is diminished in cases, when there is diminished pressure in the renal vessels and dilatation of vessels elsewhere as in acute nephritis, in the last stages of Bright's disease, hæmorrhage, collapse or shock from internal injuries, uræmia, violent diarrhœa, algid stage of cholera, high acute fevers, after great muscular exercises and in certain cardiac and pulmonary diseases. Urine is also diminished in arsenic, mercury and turpentine poisoning.

Increase. Physiologically urine is

increased due to intake of large amount of fluids and in rainy and cold days due to absence of sweat, for cold constricts the peripheral vessels. Pathologically, urine is increased in which there is a high pressure of the renal vessels and when heart beats are accelerated as in many kidney diseases, diabetes, in certain nervous diseases and after attacks of epilepsy and hysteria. Psychically it is increased in fear and emotion etc. It is also increased during the administration of diuretics such as calomel, digitalis, punarnava and some potassium salts.

Consistency. Normal urine is a thin, aqueous fluid. It is opalescence due to micro-organisms or suspended matters. It becomes thick and viscid if mixed up with much pus or mucus. Diabetic and highly albuminous urine become thick and frothy on shaking. Urine containing much fibrin coagulates like jelly on standing for some time.

Colour. The normal colour of urine is pale straw or light yellow. It is mainly due to the pigment urochrome and partly

to urobilin. Women's urine is slightly lighter. If the quantity of urine is increased or there is a diminution or dilution of urinary pigments, it becomes lighter and rendered very pale as in excessive drinking, nervousness, anæmia, chlorosis, diabetes, hysteria, epilepsy, poluria, in general debility and in chronic interstitial nephritis. The colour of the urine will depend on the degree of concentration; the more concentrated—the darker; the greater the quantity of water —the lighter. Acid urine is slightly darker than alkaline urine. Urine becomes deeper in colour, like orange or dark yellow or brownish red generally known as high coloured or concentrated urine and is due to uroerythrin and urobilin produced by increased hæmolysis, as in fevers, after journeys, in hot days, in nervous excitability and after bodily exercises. Normal urine on standing for a time will have a white or sometimes a bluish white scum on the surface due to contamination and putrefaction. Urine glairy, whitish in

colour indicates admixture with pus or leucorrhœal discharges. Urine coloured smoky, brown, reddish, brownish black or black indicates admixture with blood and denotes hæmorrhage. Urine coloured greenish yellow or greenish brown indicates admixture with bile and denotes jaundice and other affections of the liver. Urine coloured milky indicates admixture with fat or pus and denotes chyluria or any purulent disease of the genito-urinary tract. Urine coloured blue indicates typhus fever, admixture with methylene blue or when there is excess of indigogens. Many drugs after absorption colour the urine, such as yellowish orange by santonin and chrysophanic acid; reddish or orange brown by senna and rhubarb; dark olive green or black by carbolic acid and other coaltar derivatives while antipyrin reddens the urine.

Odour. When just voided urine is faintly aromatic but after a few minutes its characteristic odour is "urinous." The odour of urine is due to phenol. It becomes pungent in concentrated urine,

when urea is liberated in excess. It becomes ammoniacal and putrescent and the reaction becomes alkaline after sometime when this excess of urea takes up water and is converted into ammonium carbonate. It occurs quickly in urine from chronic cystitis or from suppurating diseases of kindney and bladder i.e., when urine is mixed with pus; blood or excessive phosphates. The odour of urine in diabetes and in acetonuria is slightly sweetish. The characteristic odour of garlic, sandal oil, cubebs, copaiba are given off when they are taken internally. Turpentine gives an odour of violets.

Specific Gravity. The normal specific gravity of an adult European male varies from 1015 to 1025, in the case of Bengalees it varies from 1003 to 1015. Specific gravity is roughly a measure of the solids, mainly urea and salts present in the urine. The low specific gravity of the Bengalees is due to want of intake of solid food. Specific gravity of urine is determined by an Urinometer. Gently fill in a conical glass or a glass cylinder

with about 2 ounces of urine i.e. sufficient to float the urinometer and when cooled and all bubbles have disappeared or else removed by a filter or absorbent paper, dip the urinometer which should always be clean and dry. After a minute, give a gentle push with the index finger to the stem of the urinometer, so that it may move freely. When it floats freely in the centre of the cylinder, quite free of the sides and without resting on the bottom, allow it to settle. Now note the specific gravity. Always read the urinometer with your eye on a level with the surface of the urine, where it cuts the urinometer and not the higher rim which creaps up the stem of the urinometer by capillary action.

Now if the urine is less than an ounce and a half and the urinometer cannot be dipped into the urine, then dilute it with sufficient water, so as to allow it to float freely noting the number of times, the water added. A rough estimate of the specific gravity can be calculated by multiplying the last two digits of the specific

gravity by the number of times the water was added, plus the volume of urine; e.g. one ounce of urine is to be tested; then add two ounces of water making three ounces in all. Now the specific gravity shows 1003. Then by multiplying the last two digits of 1003 i.e. 03 with the number of times the water added i.e. 2; plus one volume of urine $(2+1=3)$. Now 03×3 which is $3 \times 3 = 9$. Therefore the real specific gravity of urine is 1009.

Another method of ascertaining the specific gravity of small quantity of urine is by means of "specific gravity beads" but it is very rarely used.

Specific gravity gives an estimation to the quantity of solids in solution in the urine. It rises when normal and abnormal constituents are eliminated in great quantity e.g. urea, phosphates, sulphates, chlorides, oxalates and sugar as in diabetes mellitus. It gives a temporary rise in concentrated urine due to profuse sweating, diarrhœa etc. and in urine passed after a heavy meal. It sometimes rises in fevers due to excessive nitrogenous

disintegration and in some forms of acute nephritis. Specific gravity is diminished in light urine due to polyuria, hydruria, low diet, bad health, from copious intake of water, sluggish liver, hysteria, nervous disorders and in some chronic heart and kidney diseases.

Total Solids. Normally a healthy adult passes daily about one ounce and a half or about 50 grammes of solids. It is decreased by low diet, fasting, hydruria and increased in diabetes. The quantity of total solids can roughly be estimated from the specific gravity by multiplying its last two digits by 2; which gives the result in grammes per litre of urine; e.g. Specific gravity of urine is 1010. Then $10 \times 2 = 20$ parts of solids in 1000 or 20 grammes per 1000 cc of urine or 2%. Now $2 \times 4{\cdot}375$ grains $= 8{\cdot}75$ grains per ounce of urine.

Another method is by multiplication by "Hœser's Co-efficient." If the last two digits of the specific gravity be multiplied by 2·33 (Hœser's Co-efficient) it expresses the quantity of solids in grammes in 1000

cc of urine. Now if the specific gravity of urine is 1010, then $10 \times 2{\cdot}33 = 23{\cdot}3$ grammes in 1000 cc of urine or 2·3% i.e. 9·89 grains per ounce of urine.

Appearance. Appearance, physical character or transparency is the naked eye appearance of urine. Normal urine is always clear when voided but when allowed to stand for sometime it becomes slightly hazy or turbid due to suspended particles or from a slight cloud of mucus and epithelium. After sometime there may be sediments at the bottom due to gravity. If the urine is ammoniacal or decomposed a white turbidity forms due to sedimentation of phosphates or from bacterial activity. The turbidity or sediment is due mainly to the following suspended particles :—

Urates.
Uric Acid.
Albumin.
Phosphate.
Mucus.
Oxalate.
Pus.

Blood.

Micro-organisms.

To distinguish one from another, first of all fill three fourths of a test tube with urine and very gently heat the upper portion of the urine, holding the test tube by the bottom. Now note whether the urine becomes clear or a cloudiness appears in the boiled portion, comparing with the lower unboiled portion of the test tube. If the urine is turbid and clears up on heating then it contains Urates. If the urine is clear and becomes cloudy with heat, before boiling point, then it is Albumen. If the urine is clear and becomes cloudy at the boiling point, then it is Phosphate. To distinguish between albumin and phosphate add 3 or 4 drops of acetic acid on the cloudy urine. If the cloudiness disappears, then it is phosphate; but if the cloudiness remains or thickens, then it is albumin. Lastly to distinguish between albumin and mucin add 2 drops of nitric acid, if the cloudiness disappears, then it is Mucin, but if the cloudiness still persists, it is albumin. The

turbidity of carbonates will clear up with effervescence on addition of nitric acid whereas heat and acid increases the turbidity due to albumin.

To distinguish between phosphate and oxalate take some fresh urine and add ammonia, when there will be a precipitate. If on the addition of a few drops of acetic acid, the precipitates disappear, then it is phosphate, if it remains it is Oxalate.

Failing the heat test take some urine in a test tube, preferably from the bottom and add a few drops of Liquor Potassæ. Mix it thoroughly and if it clears up, then it is mucus; but if it becomes gelatinous or ropy, it is Pus.

Next, if the deposit is coloured then take some urine in a test tube, preferably from the bottom and add a few drops of caustic potash and gently heat a little. If it is dissolved, then it is Uric acid but if there is a precipitate, note the colour of the coagulum; if it is reddish brown or bottle green, it is Blood.

If the urine is turbid and there is no change either by heat or by addition of

caustic potash and heat, then the turbidity is due to Micro-organisms. They generally clear up on the addition of watery solution of ferric chloride and ammonium hydrate and then filter the urine.

Sometime the character, colour and reaction will roughly denote the element.

Urates—They look like moss and are yellowish white or pink in colour. Reaction is generally acid. They deposit when the urine becomes cold.

Uric Acid—It is crystalline and reddish brown in colour, resembling a shower of "cayenne pepper grains." Reaction is moderately acid.

Phosphate—It forms a thin deposit and is white or yellowish white in colour. Reaction may be slightly acid, alkaline or neutral.

Mucus—It is a cloudy or woolly looking white deposit. Reaction is slightly acid.

Oxalate—It is soft, shining and white

in colour. Reaction is generally slightly acid.

Pus—It looks like a ropy or creamy deposit, and is white in colour. Reaction is slightly acid or alkaline.

Blood—It is clotted or thready and is red smoky or brownish in colour. Reaction generally alkaline or may be slightly acid.

Micro-organisms—The deposit is slightly hazy and white in colour. They generally stick to the sides of the glass.

CHEMICAL EXAMINATION.

(QUALITATIVE).

Reaction. The reaction of urine is tested by litmus papers. Litmus papers should be kept in the tropics in a wide mouthed stoppered phial to prevent deterioration on exposure. Reaction may be :—

Acid—Due to acid sodium phosphate ($Na\ H_2\ PO_4$).

Alkaline—Due to disodium phosphate ($Na_2\ HPO_4$).

Neutral—Due to neutral sodium phosphate.

Amphoteric—Due to both acid and basic phosphates; i.e. disodium phosphate in addition to acid salts.

Take a blue litmus paper, dip it in urine; if it turns red, the reaction is Acid. If the urine is not acid, then take a red litmus paper, dip it in urine; if it turns

blue, the reaction is Alkaline. But if the alkalinity is due to ammoniacal fermentation of urine and not to any fixed alkali, the red litmus paper will assume its normal red colour again when dry i.e. when ammonia has evaporated. Now it may happen that both the blue and red litmus papers have no reaction on being dipped in urine; the reaction is Neutral. In rare cases, the red litmus paper is turned blue and the blue litmus paper is turned red, or when both red and blue litmus papers are turned purple on being dipped into the urine; thus giving both reactions, the reaction is Amphoteric.

The composition of food has a great influence in determining the reaction of urine. The degree of reaction can be ascertained by the intensity of colour of the litmus paper when it changes; the terms slightly, moderately and highly acid or alkali may be used.

Normal urine is slightly acid. From slightly acid it turns into moderately acid, in acute fevers, after excessive sweating, in meat eaters after prolonged mus-

cular exercise, in acid dyspepsia owing to liberation of acids, in gout and rheumatism due to liberation of uric acid, in diabetes, oxaluria and in stone formation. Urine sometime becomes temporarily alkaline after meals i.e. during digestion, due to disodium phosphate and when free hydrochloric acid has secreted into the stomach, commonly known as "Alkaline tide," hence examination of urine within three hours after meal should be avoided.

Urine is alkaline due to ammonia, when urine is retained and decomposed in the bladder as in cystitis. It is also alkaline after ingestion of excessive alkaline phosphates and carbonates. Urine kept for sometime becomes alkaline owing to the hydrolysis of urea into ammonium carbonate by the ferment micrococcus ureæ and other micro-organisms present. The urine after a heavy vegetable meal sometimes becomes amphoteric.

Method of using a pipette. Before one proceeds to examine a sample of urine, one must know how to use a pipette. Press your right forefinger on the upper end of

a pipette and dip its lower end or nozzle in the urine. Ease the forefinger, when some urine from the bottom will run up. Again replacing the forefinger on the upper end, lift the pipette out from the urine. Or, dip the nozzle of a pipette in urine and when some urine has run up in the pipette, press your right forefinger on its upper end and lift the pipette out from the urine. Now get it in a test tube and loosen the finger on the upper end and let in some air, which will drive the urine very slowly. When it is empty, remove the pipette. The cautious flow of the pipette will detect some constituents which may be overlooked if the flow is rapid and abrupt.

A. Normal Constituents.

(a) *Inorganic or Non-nitrogenous Constituents.*

Chlorides—In Europeans about 11—15 grammes i.e. 1—2%; while in Indians about 9½ grammes i.e. about or below 1% are daily excreted in health. As

they are freely soluble, they do not form a deposit. The more intake of salty food, the more output of chlorides. Before testing for chlorides, always remove albumin and albumose by boiling and then filtering, otherwise albuminate of silver is formed. Sodium chloride is most abundant but slight amounts of potassium, ammonium and calcium chlorides are also present.

Test. Take a little urine in a test tube. Put a few drops of nitric acid to hold the phosphates and sulphates in solution. Then add one or two drops of 3% silver nitrate solution. A white flocculent curdy precipitate forms without diffusing throughout the urine and suddenly sinks to the bottom which indicates that chlorides are normal. But instead of forming any curdy precipitate, if it is diffused throughout the whole urine and forms a hazy milky cloudiness, then the chlorides are diminished. If the urine remains clear and no turbidity forms, then it indicates the total absence of chlorides. If on the contrary, a thick white curdy precipitate forms giving the

appearance of cream throughout the whole urine, then chlorides are in excess. The thickness of the precipitate will tell at once, whether chloride is diminished, normal or in excess and a little experience is all that is necessary to find it out.

Chlorides are diminished in acute fevers (except malaria) severe diarrhœa, gastric disorders mainly cancerous cachexias, anæmia, some types of insanity and in chronic nephritis. In starvation and in milk diet chloride is reduced to a minimum.

Chlorides are increased in rickets, cirrhosis of the liver, hysteria, diabetes, after great bodily exercise, recovery from dropsical diseases, during convalescence from chronic fevers and in diet highly spiced and rich in salts. Vegetable foods will normally increase chloride elimination.

In some cases of chronic nephritis, chlorides are retained in the tissues hence there is a great delay in their elimination, causing œdema and puffiness of the eye lids. Chlorides are diminished at the

beginning of pneumonia, but increases, when the crisis is over and the case progresses favourably. In pneumonia chloride estimation is of great help to determine the prognosis of the case.

Phosphates. The normal daily excretion of phosphates is between half to one drachm. They are found generally in alkaline or ammoniacal urine, but are also present in slightly acid or neutral urine.

Phosphoric Acid + Sodium or Potassium or Ammonium
$(P_2 O_5)$ (Na) (K) (NH_4)
= Alkaline Phosphate.

Phosphoric Acid + Calcium or Magnesium
$(P_2 O_5)$ (Ca) (Mg)
= Earthy or Common Phosphate.

Phosphoric Acid + Ammonio Magnesium
$(P_2 O_5)$
= Triple Phosphate.

The alkaline phosphates almost constitute three-fourths of the total phosphates and are readily soluble and form no deposit, while the earthy phosphates are soluble in acid urine but precipitates in alkaline urine. When discharged in

large quantities, form like pus, a heavy white opaque deposit. Moreover phosphates and pus are often deposited together. To distinguish between pus and phosphate take a small quantity of urine and add a few drops of acetic acid, when phosphate will be dissolved but if it is pus, it will not be dissolved.

Test. (i) Fill three-fourths of a test tube with urine and gently heat the upper part of the urine holding it at the bottom. Heat drives out carbonic acid and the urine becomes alkaline when a white cloudy precipitate of soluble phosphates forms at the boiling point, which is dissolved by adding 3 or 4 drops of acetic acid (cf. albumen, mucin &c.).

(ii) Take a little urine in a test-tube and add half of its volume of nitric acid and a few drops of ammonium molybdate and boil. A yellow precipitate forms if phosphates are present.

Phosphates appear generally towards the end of micturation, and are increased after taking alkaline salts and water. They are found after excessive milk diet,

after sleep caused by potassium bromide and chloral hydrate, in meat eaters, nervous prostation, brain diseases, chronic dyspepsia, tuberculosis, stricture, cystitis, defective bone diseases such as rickets, periostitis, osteomalacia and in phosphatic stone formation in the bladder. They also occur when there is defective acid formation due to lowered metabolism and wasting diseases.

Phosphates are diminished in pregnancy, after acute infective fevers and in some forms of nephritis due to its non-elimination.

Sulphates. The total daily excretion of sulphates in adults is about half a drachm. Sulphates occur in urine in three forms; organic or ethereal, as combinations of indol, skatol, cresol and phenol; inorganic, as potassium or sodium sulphate and rarely neutral.

Test. Take urine in a test tube, add a few drops of hydrochloric acid or 2 or 3 drops of nitric acid to dissolve the phosphate if any, and then put a drop or two of barium chloride solution (Barium

Chloride Solution—4 parts, Distilled Water 16 parts). A white precipitate of barium sulphate comes down. The amount of sulphates, whether diminished, normal or in excess will be shown by the precipitate, which will be milky, opaque or thick white precipitate as in chlorides.

Sulphates are decreased in starvation convalascence, in vegetable diet and when food is less assimilated.

Sulphates are increased in fevers, in meat eaters, chronic constipation and in intestinal putrefaction.

The amount of sulphates in urine will show the intensity of intestinal putrefaction as they are derived from protein metabolism. They are increased and decreased with the increase and decrease of uric acid and urea.

Carbonates. They are of rare occurrence and found along with phosphates and sometimes make the urine turbid.

Test. Take in a test tube some nitric acid and layer some urine over it. If carbonates are present, carbon dioxide will be set free with effervescence.

Carbonates are found in alkaline or decomposed urine as in cystitis. They are produced when vegetable acids are set free from the food or to an excessive intake of alkaline salts and water.

Oxalate. It is mostly found in urine as calcium oxalate and when voided in moderate amount, it is called oxaluria. It is generally present in acid urine but may be found in slightly alkaline or neutral urine. It is found in about 75% in Bengalees due to their sluggish liver, brought about by their sedentary habits and large intake of vegetables specially in summer season. If the urine is allowed to gravitate for about 3 hours, a white shinning deposit forms at the bottom when it is in excess. It is soluble in hydrochloric acid but insoluble in acetic acid, (while carbonates or phosphates are soluble in acetic acid).

(b) *Organic or Nitrogenous Constituents.*

Urea. Of the total nitrogen excreted in the urine, urea constitutes about 85%

and the remaining 15% constitutes all the other nitrogenous constituents combined; such as uric acid, ammonia, xanthin, hypoxanthin, creatin, creatinin and hippuric acid etc.

Normally in Europeans urea is about 2% i.e. 30 to 35 grammes per day and half the total quantity of solids excreted in the urine. In Indians it is about half of Europeans i.e. less than 1% or about 13 grammes per day. The amount of urea varies with the amount of protein food ingested.

Test. Put two or three drops of urine, either centrifugalised or from the bottom of the glass or container on a microscopic slide and add a drop or two of nitric acid. Evaporate it on a spirit lamp and then set aside and see it under a microscope when rhombic or hexagonal crystals of nitrate of urea will be found.

Urea is secreted profusely in plethoric people, meat and nitrogenous food eaters, athletes after excessive muscular exercise and in brain workers. It is increased in those cases where tissue metabolism and

excessive disintegration of albumin occur as in diabetes mellitus, leukemia and in high fever. It also occurs in quinine, arsenic, antimony and phosphorus poisoning.

Urea is decreased in vegetable diet, starvation, pregnancy, anæmia, phthisis, eclampsia, uræmia and in dibetic coma. The function of the liver is to convert amino acid into urea, so it is generally diminished in acute yellow atrophy, cirrhosis, cancer and some other hepatic diseases due to less output of urea. It is further diminished in the late stages of nephritis when urea is less excreted; the accompanying effect being uræmia.

Uric Acid. The normal daily excretion of uric acid in health is from 6 to 8 grains. It may be free or combined. When it is free and excessively eliminated it forms a pigmented reddish brown deposit resembling a shower of cayenne pepper grains; but when combined it excretes as urates. It will make the urine moderately acid and is dissolved by alkalies, such as caustic potash or soda,

but insolube in acetic acid. It is more soluble in warm water than in cold; hence patients of uric acid diathesis are given warm water to drink.

Test. (i) Strongly acidulate the urine with hydrochloric or nitric acid and set aside for 24 hours, when a brick dust or cayenne pepper crystals of uric acid will collect at the bottom.

(ii) *Murexide Test (or Oxidation of Uric acid.)* Take in a porcelain cup a few drops of concentrated urine or a deposit from the bottom. Add one or two drops of nitric acid and evaporate it almost to dryness, when a yellowish red residue forms (alloxantin). When cooled add one or two drops of ammonia; when a purplish rose colour due to ammonium purpurate develops at the margins of the drops if uric acid is present.

Uric acid is increased when there is excessive protein metabolism and excessive leucocytosis. It is found in high fevers, concentrated urine, highly nucleoproteid diet, meat eaters without much exercise, excessive nuclein or purin des-

truction, leukæmia, after acute gout and rheumatism, alcoholism with enlarged liver, pneumonia and in the formation of uric acid stone when it is accompanied by a few red blood cells. It is also found in certain hepatic diseases, such as cirrhosis and acute yellow atrophy of the liver.

Uric acid is diminished or absent in vegetarians, in milk diet, weak and debilitated persons, suffering from anæmia and chronic kidney diseases.

Urates. Urates or alkaline salts of uric acid, such as potassium, sodium, calcium, ammonium and magnesium, when in excess may occur in normal urine, but generally found in concentrated urine of febrile patients or when metabolism of the body is disturbed mainly due to disorders of liver, stomach and intestine. Ammonium urate is generally found in alkaline urine after ammoniacal decomposition when it makes the urine hazy, but is found also in acid or neutral urine. Urates are held in solution as soon as urine is voided or when the urine is warm but precipitates on cooling, when

the urine is unable to keep excess of urates in solution. The sediment is called the "Sedimentum Lateritum" or clay water sediment. Urates generally take up uroerythrin, a colouring matter from the urine, forming what is known as brick dust deposit; hence the deposit of urates are generally coloured reddish brown; but in the absence of uroerythrin, the deposit is white. Urates readily disappear on gradually boiling the urine. If the heat is given abruptly (not slow and cautiously) the urine if it contains albumin may be coagulated, before the urates are dissolved. Urates also dissolve on the addition of caustic potash.

Urates are found in concentrated urine, after violent exercise, acute fevers, cystitis, gout and rheumatism. It is generally found in all cases of functional derangement of the liver.

Purine Bodies. Purine bodies or nuclein bases are substances derived from nucleus or from nuclein destruction such as xanthin, hypoxanthin, guanin, hippuric

acid and creatinin. They are allied to uric acid and are increased in meat diet, infectious fevers and are decreased in vegetable and milk diet.

B. Abnormal Constituents.

Albumin. Albumin or the natural urinary proteid is found mainly in the form of serum albumin. The other urinary proteids are serum globulin, albumoses, peptones, nucleo-albumin and fibrin.

Before testing for albumin, test the reaction of the urine. Acidulate it, if it is alkaline by cautiously adding drop by drop two or three drops of acetic acid or citric acid, but never use stronger acids such as nitric or hydrochloric acid. Then filter the urine so as to free it from semen, mucus, epithelial cells or other debris contaminated from the urinary passages. If filtration does not render the urine clear, the turbidity may be due to bacteria, which are precipitated by shaking with a little calcium or barium carbonate or kaolin which when filtered a clear urine is obtained.

(i) *Heat Test.* Take a test tube, fill it with urine (acidulated and filtered if necessary) about three-fourths of it and gently heat to boiling, the upper layer of the urine in a spirit lamp holding the test tube by the bottom. If the urine remains clear, no albumin is present but if a white opaque cloud forms at the heated portion which is best seen in a good light, against a dark background in comparison with the clear column of urine below; may be due to (i) albumin, (ii) phosphate, (iii) carbonates, (iv) mucin and nucleo-albumin. Put in a few drops of acetic acid, if the cloud clears up entirely, then it indicates excess of phosphates. But if it clears up with effervescence, it is due to carbonates, either alone or in combination with phosphates. Always add a little acetic acid after boiling and note whether the heated column becomes clearer, as the turbidity may be so slight as to avoid detection. If still a cloud or it thickens add one or two drops of nitric acid, if the cloud disappears then it is nucleo-albumin or mucin; but

if it persists, then it is albumin. Cloudiness due to phosphate appears just before or at boiling point, while cloudiness due to albumin appears as soon as it is heated.

(ii) *Cold Test or Heller's Test or Nitric Acid Test.* Take about half a drachm of pure nitric acid in a test tube and inclining the test tube considerably, gently and slowly trickle with a pipette about an equal volume of clear urine by the side of the test tube. Now put it erect, when a white flocculent ring due to precipitation or coagulation of albumin which comes about within a minute at the junction of the two fluids indicates albumin. The density of the white opaque ring roughly indicates the amount of albumin. Care should be taken that the flow from the pipette should always be slow and gentle and should not be thrown rapidly as then the two fluids will not layer over each other and thus there will be no definite line of contact and hence the presence of albumin may be overlooked. In using nitric acid, do not use fuming nitric acid as it will decompose urea and gives off

bubbles of nitrogen and carbon dioxide. It will thus mix the two fluids and thus destroy the line of contact and if trace of albumin be present it may escape notice.

In normal urine, sometimes one or two rings, generally reddish or violet in colour appear at the junction of the two fluids. They are due to urochrome and rarely urobilin. The red is from urochrome, the natural pigment of urine and the violet from urobilin which is slightly pathological. This violet ring from urobilin is increased in high coloured urines, as from fevers and chronic constipation etc. Another fine crystalline white ring may appear at the junction of the two fluids and is due to urates but is dissolved by heat. Nucleo-albumin or albumose also produces a white ring and the distinction from albumin is that the haze appears above and distinct from that produced by albumin and further the ring is much fainter and diffuse in character. It will disappear on heat but reappearing when cold, whereas that due to albumin remains on warming.

Sometimes when urine comes in contact with nitric acid, other coloured rings or zones due to oxidation appear at the junction.

If the zone is greenish blue it indicates Bile.
,, ,, ,, ,, indigo or violet ,, Indican.
,, ,, ,, ,, reddish brown ,, Blood.
,, ,, ,, ,, yellowish brown ,, Uric Acid.

If instead of a zone, effervescence occurs as soon as the two fluids are in contact, then it is carbonate.

(iii) *Picric Acid Test.* Take an inch of urine in a test tube and sprinkle about half a grain of finely powdered crystal of picric acid or holding the test tube in a slanting position, stratify equal quantity of saturated solution of picric acid (7 grains in an ounce of water) with a pipette, by the side of the test tube. If albumin is present, a white flocculent turbidity rapidly forms at the junction of the two fluids. The precipitate becomes more prominent on gentle heating. Nucleo-albumin or albumoses or when quinine or antipyrin are given internally

will give a similar white precipitate but it disappears on heating.

Delicate and Reliable Tests for Albumin.

(i) *Salicyl-Sulphonic Acid Test.*

Take a small quantity, say about half a drachm of urine in a small test tube and put a few drops of saturated solution of pure salicyl sulphonic acid in distilled water or a few crystals of the acid. A faint precipitate or turbidity undissolved by heat indicates albumin, but if the precipitate becomes clear by heat it indicates proteoses.

(ii) *Acetic Acid Potassium Ferrocyanide Test.*

Take an inch of urine in a test tube, acidulate it with 8 to 10 drops of acetic acid. Mix well and add potassium ferrocyanide solutioin 5%, drop by drop. A white flocculent precipitate forms if albumin, mucin or albumose is present in urine.

(iii) Fill two thirds of a test tube with urine and add one sixth its volume of

saturated solution of sodium chloride and put 5—10 drops of 50% acetic acid. Now gently heat the upper layer for a minute holding the test tube at the bottom, when a turbidity appears if trace of albumin is present.

(iv) Take some urine in a test tube and slowly add a few drops of saturated solution of trichlor-acetic acid, a precipitate or increased turbidity appears. Heat it, if the turbidity dissolves, it is due to mucin, if not, it is due to albumin.

Or

Take some saturated solution of trichlor-acetic acid in a test tube and trickle gently some urine by the side of it. A white opaque ring at the junction undissolved by heat indicates albumin but if dissolved by heat it denotes mucin.

This test is exactly like nitric acid test. Trichlor-acetic acid is corrosive and care should be taken in handling it. The slightest amount of albumin will be detected by this test and further it does not precipitate peptones nor coagulate

mucin. It is costly and hence it is not generally used.

If albumin is found in urine, it is called albuminuria. It may be

(i) Temporary or functional;

(ii) Permanent or organic.

	Temporary or Functional.	Permanent or Organic.
Albumin— Quantity .	Faint trace	Marked
,, Character .	Present in one sample. Absent in another.	Constantly present.
Urine	Nothing abnormal.	Scanty urine with increase of specific gravity.
Renal Disease .	No symptoms .	Symptoms of renal disease.
Other Organs .	No symptoms .	Symptoms of cardiac, pulmonary and hepatic diseases may be present.
Tube Casts ..	Absent . . .	May be present.

Temporary albuminuria is mainly due to temporary increased permeability of renal cells and to temporary increase of blood pressure. It is found in persons

after prolonged muscular exercise, after cold baths, in profound excitement, after heavy meals, from sluggish liver, in nervous debility, in anæmia, in young neurotics, after epileptic fits and after convalescence from debilitating diseases.

Organic or permanent albuminuria is present more or less in all forms of kidney diseases, and anything which brings about a venous engorgment or passive congestion in the kidney, both directly as from injury, pressure etc. or, indirectly from cardiac, pulmonary and hepatic diseases. It is also present in syphilis and other infective fevers, as in pneumonia, typhoid and diptheria. It is sometimes found after poisoning by arsenic, mercury, lead, phosphorus and iodides.

Spurious albuminuria There is a slight amount of albumin, when blood, pus, chyle and semen are mixed with urine. This albumin is derived from the plasma of the cells and is not a true renal albuminuria. It is present in women when menstrual blood or some leucorrhœal discharges are mixed with urine.

Albumoses. Besides albumin, albumoses, mucin and nucleo-albumin also occur in urine. Albumoses are the degradation product of albumin while mucin and nucleo-albumin are the degradation products of neuclic acid. Albumoses, proteoses or propeptones are the intermediate products of digestion, from acid albumin or parapeptone to peptone. They are precipitated as a white cloud by nitric acid, the precipitate is slightly diffusible and is soluble on heat and reappearing when cold; as in the ring test for albumin. Albumin and albumoses are often present together. To detect albumose fully, first make the urine albumin free by acidifying with acetic acid, boil and then filter, but albumose will remain in solution and then test the filtrate.

Test. (i) Take in a test tube a little nitric acid and slowly and cautiously layer the filtrate over it, when the characteristic ring test will appear if albumoses are present.

(ii) To the filtrate add a few drops of a saturated solution of pure salicyl-

sulphonic acid or a few crystals of it. Heat and filter it at once without cooling. Wait and if on cooling a white cloud forms albumoses are present.

Albumoses are present when there are pyogenic infection in the body with disintegration of tissues and great destruction of white blood corpuscles. They are found in suppuration, abscess formation, in many fevers, pneumonia, tuberculosis, some liver diseases, purulent arthritis, meningitis, empyema, diptheria, nephritis, ulcer and cancer. The presence of antipyrin, quinine and certain resins in the urine give a similar reaction.

Mucin and Nucleo-albumin. Mucin is the principle constituent of mucus while nucleo-albumin is derived from the epithelium of the urinary tract. Mucin is present slightly in every urine, but fairly present in the urine of females due to an admixture of pavement epithelium derived from the vaginal wall; hence the urine is generally turbid, but has no pathological importance. If the urine is acid, mucin is insoluble and forms a white woolly

deposit, but if the urine is alkaline, the mucin remains in solution. Both are precipitated by acetic acid and are coagulated by nitric acid and dissolved by heat.

Test. (i) When testing for albumin with nitric acid if mucin is present a cloud forms at the upper surface of the urine and not at the junction of the two fluids.

(ii) Slowly add to urine equal parts of saturated common salt solution and of Almen's reagent when a copious precipitate results if nucleo-albumin is present.

They are increased in acute fevers, in urethral, vaginal and vesical catarrh and also in irritation of urinary tract by uric acid, calcium oxalate &c.

Sugar or Glucose.

Before testing for sugar always remove the albumin, if there is any, as it may give slight reductions, by first adding

---

Almen's reagent :—

Tannic acid	5 grammes
Acetic acid 25%	10 c.c
Methylated spirit (40%) . . .	240 c.c

a little acetic acid and then boiling it, when a flocculent precipitate forms, which should be filtered. Secondly the urine should not be ammoniacal.

(*i*) *Fehling's Test.*

Fehling's Solutions :—

I. *Copper Sulphate Solution.*

Copper Sulphate (pure)	...	34.64 grammes
Aqua Destillata	...	500 c.c.

II. *Rochelle Salt Solution*

Potassium Sodium Tartrate (Rochelle Salt)		173 grammes
Caustic Soda ...	...	60 grammes
Aqua Destillata	...	500 c.c.

The two solutions stocked in two phials keep good for any length of time, but if mixed and kept in one phial they decompose, hence they are kept in two separate phials and labelled A and B or No. I and II and mixed just before use.

The theory of glucose detection is that when copper sulphate solution reacts with caustic alkalies, cupric hydrate is precipitated and this when boiled with glucose in presence of alkalies or Rochelle

salt, it is reduced to cuprous oxide, which is obtained as a brick dust or reddish yellow precipitate.

$$\underset{\text{(Copper Sulphate)}}{Cu\ SO_4} + \underset{\text{(Caustic Soda)}}{2\ Na\ OH} = \underset{\text{(Sodium Sulphate)}}{Na_2\ SO_4} + \underset{\text{(Cupric Hydrate)}}{Cu\ (OH)_2}$$

$$\underset{\text{(Cupric Hydrate)}}{Cu\ (OH)_2} + \underset{\text{Salt Solution}}{\text{Rochelle}} \rightarrow \text{Deep blue solution}$$

(*insoluble*)

This cupric hydrate is a bluish white gelatinous mass and is readily dissolved in Rochelle Salt by gentle shaking when a deep blue transparent solution is formed.

$$\underset{\text{(Cupric Hydrate dissolved in Rochelle Salt = Blue Solution).}}{Cu\ (OH)_2} + \underset{\text{(Glucose i.e. a reducing agent)}}{C_6\ H_{12}\ O_6} + \underset{\text{(i.e. loses water)}}{\text{Heat}}$$

Firstly $Cu_2\ O\ H_2\ O$ (Cuprous Hydrate, or reddish yellow precipitate). Then on further boiling i.e. driving $H_2\ O$; $Cu_2\ O$ (Cuprous Oxide or Red Oxide of Copper), is precipitated.

Take equal quantities of Fehling's A and B, say half an inch of each in a test tube and boil. If the blue colour fades or precipitates on boiling, it is evident that the cupric oxide has already been reduced to cuprous oxide and hence it is unfit for use (when a little caustic soda solution should be added and the liquid filtered to make the solution again fit for use). But when the solutions are all right, *i.e.*, the blue colour does not fade, then add urine drop by drop heating all the time. A reddish yellow precipitate due to hydrated cuprous oxide $Cu_2(OH)_2$ or a brick red precipitate due to anhydrous cuprous oxide Cu_2O forms and indicates sugar. If no reddish or yellow precipitate occurs and the solution remains clear on adding an equal quantity of urine to the solution, then there is no sugar in the specimen. Always declare your result after allowing it to cool and then note, whether there is any brick dust sediment in the test tube. Instead of adding urine drop by drop and heating all the time, mix an equal quantity of

urine thoroughly with the Fehling's, then boil gently the upper portion of the liquid. Now compare the colour of the upper heated portion which contrasts well with the unboiled lower portion. A brick dust or reddish yellow precipitate at the heated portion will indicate sugar.

Instead of a reddish yellow or brick dust precipitate, a white precipitate generally occurs, when urine is heated with Fehling's solutions. This white precipitate is cuprous urate and is present in urine containing urates.

When uric acid, excess of urates, xanthin, creatin, creatinin, hippuric acid, glycoronic acid and many drugs such as camphor, carbolic acid, salicylic acid, chloral hydras, chloroform, glycerine, salts of lead, opium, mercury and formaline which is added to preserve urine from decomposition are present in urine, any one of them may give slight reductions which is obviated by boiling the urine and Fehling's separately and then pouring the hot urine slowly into the hot Fehling's.

If the urine still gives a doubtful reaction then add 10% of hot lead acetate solution to the urine. The lead solution precipitates many reducing agents such as uric acid, albumin, phosphates, sulphates, etc. but not sugar. Filter the urine and then test the filtrate.

(*ii*) *Benedict's Test.* Benedict's test is an excellent method for glucose and positively proves some derangement of carbohydrate metabolism. It is about ten times as sensitive to Fehling's and is not reduced by uric acid, urates, xanthin, etc. or by drugs such as camphor, chloroform, etc. and even by formaline. The solution does not deteriorate and keeps well for a pretty long time if kept in a stoppered phial.

The theory here is, that in the presence of potassium thiocyanate glucose reduces copper to cuprous thiocyanate—a white compound, which is kept in solution by potassium ferrocyanide. It is further advantageous that the end point (*i.e.* the complete discharge of the blue tint) can be easily detected here.

Take 5 c.c. of Benedict's solution in a thick stout test tube and add about 8 to 10 drops of urine. Mix them thoroughly and boil vigorously the upper portion for a couple of minutes and then cool it spontaneously (preferably by immersing it on a beaker of cold water). A reddish precipitate forms, if the urine contains large amount of sugar and in the case of a moderate amount, the precipitate becomes yellow or orange, but in case of a trace, the blue colour will be changed into opalescent green.

Cammidge advocates it more elaborately.

"5 c.c. of Benedict solution are placed in a test tube and 8 to 10 drops of the urine are added and mixed by gently shaking. The test tube is then stood in a vessel of bubbling boiling water and left there, with the water actually boiling, for exactly five minutes, when it is removed and allowed to cool spontaneously. If the solution remains clear and unchanged, the urine is free from sugar; a greenish turbidity forming within two

minutes of the tube being removed from the water indicates a trace of sugar (under 0·1 per cent.); if there is a green turbidity, when the tube is taken from the water, an appreciable amount of sugar is present (0·1 to 0·5 per cent.); a yellow sediment indicates a considerable amount of sugar (0·5 to 2 per cent.); while a red sediment shows that there is a large amount (over 2·0 per cent.).

Occasionally, a slight flocculent white precipitate forms due to precipitated phosphates, but it can be readily distinguished from the precipitate caused by a trace of sugar by the fact that it is white or bluish-white, and not green or yellowish-green. It should be noted that *pure* sodium citrate must be used in making up the solution; the ordinary sodium citrate of the pharmacopœia sometimes contains traces of reducing substances which will give a positive reaction with a sugar free urine."

(*iii*) *Fermentation Test.* Yeast decomposes glucose into alcohol and carbon dioxide is evolved. Break up a small

piece of yeast (Fresh or German) about the size of a pea or about a pie and add to it 8 ounces of cooled boiled urine, which should always be acid, as alkaline urine is easily fermentable even in the absence of sugar. Pour this urine into a Southhall's ureometer or a saccharometer and let it stand in a warm place for 24 hours, when carbon dioxide gas will collect at the top of the tube, indicating the presence of sugar.

(*iv*) *Picric Acid Test.* Take an inch of urine in a test tube and add ¼th of it saturated solution of picric acid and a few drops of liquor potassæ. Heat and if sugar be present in the urine, a dark brown colour which ultimately deepens into black colour, is formed due to reduction of picric acid to picramic acid.

(*v*) *Moore's Test* (caramel). Take some urine and add one-third its volume of potassium hydroxide and heat the

Note.—Before testing, always heat picric acid and liquor potassæ in a test tube and if the fluid is turned into a dark colour, it indicates that the picric acid is impure and unfit for use.

upper portion for about 3 minutes. A chestnut brown colour forms due to conversion of sugar into caramel (burnt sugar) which is detected by its peculiar odour.

(*vi*) *Trommer's Test.* Take an inch of urine in a test tube and add one-eighth its volume of caustic potash and a few drops of copper sulphate solution. Heat and if sugar be present in urine a red precipitate forms.

(*vii*) *Moore-Heller Test.* Take about two parts of urine in a test tube and add one part of a 10% caustic potash solution. Mix it and heat the upper portion of the urine for about a couple of minutes, when if sugar is present, it will turn red. The intensity of the red colour will roughly denote the quantity of sugar.

(*viii*) *Nylander's Test and Bottger's Test.* In these tests, the glucose reduces in alkaline solution the salts of metallic bismuth and is precipitated as black clouds of bismuth suboxide on the sides of the test tube. When albumin, blood and other sulphuretted bodies are present in urine, they should always be removed

beforehand as they may precipitate black sulphide of bismuth and thus confuse the result.

(*ix*) *Nylander's Test.* Take some urine and add about one-tenth Nylander's reagent. Gently heat the upper part for a couple of minutes, when first yellow, then yellowish brown and lastly black clouds of bismuth suboxide is precipitated if glucose is present.

Glycosuria is found temporarily after ingestion of abundant sugary and starchy foods, sometimes in pneumonia, typhus, rheumatism, gout, hysteria, epilepsy, asthma, whooping cough, alcoholic excess, in some spinal cord affections, in persons of nervous irritability, coma due to brain injury and in some brain diseases especially involving the fourth ventricle. It is also produced in chloroform, chloral and carbonic oxide poisoning. The cause of this temporary glycosuria is not always due to glucose but to other reducing agents, mainly glycuronic acid which give slight reduction in the urine.

Sugar is permanently present in

persons suffering from **diabetes** and in some pancreatic, hepatic and cardiac diseases.

Physical characters of Diabetic Urine.

Colour—Light coloured, for the quantity is excessive and the urinary pigments are diluted.

Quantity—More .than normal, both voided at one time and in 24 hours.

Reaction—It is distinctly acid.

Specific Gravity—Generally higher than 1020.

Froth—It has a tendency to froth easily on agitation and remains for some time.

Odour—Slightly sweet, called apple smell.

Sugar—Ants may collect in the place where the patient has urinated.

Bile. Choluria or bile in the urine appears in two forms. Bile pigments occur more abundantly than the bile salts.

(i) Bile pigments are derived from

bilirubin and partly from biliverdin.

(ii) Salts of bile acids are from glycocholic and taurocholic acids.

Test for Bile Pigments.

(i) *Gmelin's Test.* Take some strong nitric acid in a test tube and with a pipette gently stratify some urine by the side of it. A play of colours, green while below this blue, then violet, red and lastly, yellow—(the green being the most characteristic and prominent, due to oxidation of bile pigments) appear at the junction of the two fluids, indicate bile. To detect more fully the play of colours, this test can be conveniently done on a porcelain slab or on a filter paper.

Take on the outer side of a porcelain dish or on a white plate one drop of pure or fuming nitric acid and one drop of urine close to each other and gently allow them to run together. A play of colours, like a rainbow—yellow, red, violet, blue and green will form if the urine contains bile pigments.

Take a filter paper and soak a por-

tion of it in urine. Drop a drop of strong nitric acid on to it. A play of coloured zones will form probably due to oxidation products of bile pigment bilirubin (C_{16} H_{18} N_2 O_3). Outside green, then blue, then violet, then red and inside of all is the yellow ring due to choletelin (C_{16} H_{18} N_2 O_6).

(ii) *Smith's Test.* Take some urine in a test tube and gently layer over it about 2 c.c. of 10% alcoholic solution of Tr. iodine. An emerald green ring at the junction of the two fluids indicates bile.

(iii) Take an inch of sulphuric acid in a test tube and pour about two inches of urine. A deep red colour develops in normal urine, but if the urine contains bile pigment, the mixture acquires a dark, approaching black colour.

(iv) Take a little urine and then add a little chlorofrom. Shake it well. If the urine assumes from a reddish yellow to a green colour due to bile pigment bilirubin which is dissolved and oxidised into biliverdin; the urine contains bile pigments.

Test for Bile Salts.

(i) *Hay's Test or Surface Tension Test.* Put an inch of urine in a test tube. Take some flowers of sulphur between the thumb and the index finger and smoothing it, sprinkle gently over the surface of the urine, keeping the test tube straight and without shaking. If the urine contains bile salts, the flowers of sulphur will gradually sink to the bottom. It is due to lowered surface tension, produced by even minute trace of bile salts. Further the urine should not be warm as flowers of sulphur may then sink even in the absence of bile salts.

(ii) *Pottenkoffer's Test.* Take some urine in a test tube and shake it well with a few crystals of cane sugar till enough froth is formed. Gently layer over it a few drops of sulphuric acid. If the froth is coloured purple, it indicates the presence of bile salts.

When bile is prevented from reaching the duodenum by occlusion or pressure as in jaundice and many other liver diseases, it is absorbed by the lymphatics and

eliminated in the urine. Bilirubin is formed in the liver from degenerating red cells and eliminated in the urine as urobilin. Bilious urine is greenish or orange green in colour and froths easily on shaking which too is greenish yellow in colour. It stains linen and has a low specific gravity.

Blood. Blood may be mixed with urine in two forms. When red blood corpuscles are present in urine, it is called hæmaturia, but when hæmoglobin is present in urine without any red blood corpuscles it is called hæmoglobinuria. Blood corpuscles gravitate and form a reddish brown flocculent precipitate at the bottom. When blood is present in urine, a slight amount of albumin, derived from the plasma of cells is present called Spurious Albuminuria.

Test. (i) First of all, test the reaction, if it is neutral or alkaline, acidulate it with acetic acid. Take some tincture guaiacum (and not tincture guaiacum ammoniata) in a test tube and add an equal quantity of fresh liquor hydrogen

peroxide or ozonic ether or old turpentine. Note whether there is any blue colour, if not, the reagents are all good and then the further step can be proceeded with, otherwise fresh solutions should be taken. If the reagents are all good, gently layer with a pipette some urine by the side of the test tube. If a blue colour due to oxidation of guaiacum appears at the junction it indicates blood. Iodides taken internally will also produce a blue colour, but it is slow to develop and is more diffuse in character and not limited at the junction of the fluids.

This test can also be done on a filter paper as in the case of bile pigments. Soak a filter paper in urine. Pour near each other one or two drops of tincture guaiacum and ozonic ether or old turpentine or fresh liqour hydrogen peroxide. When these drops run together, a blue colour will form at the junction if the urine contains blood.

Always prepare tincture guaiacum fresh with rectified spirit and keep it in a blue or brown phial as it easily deteriorates in the tropics.

(ii) *Heller's Test.* Take some urine in a test tube and add one third its volume of potassium hydroxide, which will destroy the red blood cells, but will liberate the hæmoglobin. Gently boil the test tube a little, when a reddish brown coagulum due to precipitated earthy phosphate and hæmatin is thrown down at the bottom, if blood is present in the urine.

(iii) Take some urine in a test tube and add a few drops of acetic acid. Gently heat it, when a brownish red albuminous precipitate forms if the urine contains blood.

Blood may come with urine from any part of the urinary tract, either from kidney, bladder or urethra. The colour of the urine is the index of the quantity of blood present in it. If very slight, it does not affect the colour of the urine; if it is cloudy, then it indicates a small amount; and from red to dark red it indicates admixture of blood from moderate to a large amount. When blood comes from kidney, it is smoky, or slightly reddish brown in colour as it is intimately mixed

with urine. When it comes from bladder it is clotted or dark red and especially comes most freely, at the beginning or at the end of micturition. When it comes from urethra, it is clear and bright red in colour and may generally flow at the commencement of micturition or voided between the acts of micturition. The source of blood can also be ascertained microscopically by the detection of particular epithelia, renal, vesical and urethral. Physiologically, during mens in females, red blood corpuscles may appear in urine.

When blood comes with urine, it is due to trauma or injury and in all acute inflammations and congestion of the urinary apparatus, such as pyelitis, nephritis, cystitis, prostatitis and urethritis. It is also found in filariasis, (Filaria Sanguinis Hominis) in acute specific and eruptive fevers, tuberculosis, in black water fever, after severe burns, in purpuria, scurvy, after large internal hæmorrhage and in hæmorrhages due to irritation of stones and tumours. It is sometimes found after poisoning by

hæmolytic substances such as turpentine, cantharidis and carbolic acid &c.

Pus. When pus cells or degenerated leucocytes are present in urine, they make it turbid and minute white specks are seen floating in it and on standing for sometime a white cloudy sediment collects at the bottom. When pus is present in urine, a slight amount of albumin, derived from the nucleo-albumin of the cells is present called 'spurious albuminuria.' When pus is present in moderate amount it is detected chemically but when in minute trace, a microscopical examination of the deposit for pus cells is necessary.

Test. (i) Take some urine in a test tube, preferably from the bottom where sediment has collected, either with the help of a pipette or decanting the upper supernatant urine. Add equal quantity of liquor potassæ and shake thoroughly well. In case of pus, the mixture becomes gelatinous and ropy. Now slowly throw the urine from the test tube, when it does not fall drop by drop but run in ropes and in long threads like a fluid jelly.

(ii) Take some urine from the bottom where sediment has collected and drop a small piece of caustic potash and stir well. The sediment will become slimy and tough if pus is present; while in case of mucus, it will pass more or less into solution.

(iii) Take some fresh urine in a test tube and add a few drops of caustic potash solution and gently heat it. A ropy gelatinous precipitate insoluble in acetic acid indicates pus.

(iv) Take some urine in a test tube and stratify a few drops of tincture guaiacum. A greenish blue colour appears at the point of contact after a few minutes, which disappears on heat, if pus is present in urine.

When pus comes from the kidney, it is intimately mixed with urine and the reaction is generally acid, but when it comes from the bladder, it is thready and collects as a floculent precipitate at the bottom and generally flows at the end of micturation. The reaction of urine may be alkaline or acid. But when the re-

action of urine is acid and pus generally comes at the beginning of micturation, it comes from the urethra.

Like blood the source of pus can also be ascertained by the detection of particular epithelium of the genito-urinary tract in the urine under the microscope.

When pus cells are present in urine it is called Pyuria. It indicates an inflammation or suppuration in the genito-urinary tract, hence it is present in cystitis, pyelitis, prostatitis, gonorrhœa, leucorrhœa etc. It is present also in B. Coli infection.

The sudden gush of pus during micturation denotes perinephritic, pelvic or any other abscess has found its way into the urinary canal.

Indican. About 4 to 16 milligrammes of indican are daily excreted in health; but when in excess it is detectable by ordinary chemical tests. It comes on from indol, within the intestine, during intestinal putrefaction and after bacterial infection, generally above the cæcum and is oxidised as indoxyl. Indoxyl combines with potas-

sium sulphate and forms potassium indoxyl sulphate or indican. Like potassium indoxyl sulphate or indican, small quantity of potassium skatoxyl sulphate or methyl indican, derived from skatol may also be present in urine.

Before testing for indican, albumin if present should be removed by acidifying the urine with acetic acid, boiling and filtering off the coagulated proteid.

Test. (i) Take a little nitric acid in a test tube and slowly stratify a little urine by the side of it with a pipette. An indigo ring at the junction of the two fluids denotes the presence of indican.

(ii) Take a little urine in a test tube, add a few dorps of acid hydrochloric dil and a few drops of lime water and then add a few drops of chloroform. If indican is present in excess, chloroform drops are tinged to indigo colour.

(iii) Take some urine in a test tube, add a little hydrochloric acid and then add a few drops of nitric acid. Add some crystals of potassium chlorate (or a freshly prepared solution of potassium chlorate).

Then add a few drops of chloroform and shake up the mixture. Chloroform drops dissolve the products of oxidation and will be tinged blue, due to formation of indigo blue or less frequently violet, due to indigo red, if excess of indican is present.

(iv) *Jaffe's Test.* Take one inch of urine in a test tube and add an equal quantity or a little more of strong hydrochloric acid and about half an inch of chloroform. Add a single drop of 5% potassium chlorate solution and mix. Allow the chloroform to settle and examine the colour. If it be blue, indican is present; if not add another drop of the chlorate solution and mix again. If still no blue colour be found in the chloroform, indican is absent.

Note. The extraction with chloroform is best done by repeatedly pouring the mixture from one tube to the other. It is essential to add at least an equal volume of strong hydrochloric acid to liberate free indoxyl. This is oxidised to indigo blue, which is soluble in chloroform.

(v) *Mac Munn's Test.* Boil equal volumes of urine and hydrochloric acid with a few drops of nitric acid, cool and

then shake with chloroform, the chloroform becomes more or less violet, according to the quantity of indican present.

It is affected by food, a meat diet increases, while vegetable diet diminishes it. The quantity of indican eliminated gives a rough idea of intestinal putrefaction and bacterial decomposition of body protein within the intestine. It is excessively produced in liver diseases, which interfere with bile formation and thus leads to intestinal putrefaction and other fermentative processes. It is found when there is a deficiency of hydrochloric acid in the stomach, or when there is an obstruction to the proper flow of the contents of the small intestine, as in intestinal obstruction, in various intestinal disorders, such as colitis, peritonitis, appendicitis, enteritis, cancer of the stomach and liver, in chronic constipation, dysentry and in intestinal tuberculosis. It sometime occurs in purulent diseases of albuminous putrefaction, such as in dyspepsia of meat eaters, in peurperal women with intestinal troubles, in

pulmonary gangrene, putrid bronchitis, empyema and in early stages of cholera.

Acetone or Dimethyl Ketone. ($C_3 H_6 O$).

Acetone and Diacetic acid are found in urine in ketosis also called acidosis, but may be present in very minute trace in normal urine. When acetone is present in minute quantity, it is not always detectable by ordinary chemical tests. Urine containing acetone bears a sweet fruity smell and reduces Fehling's solution.

(i) *Rothera's Test.* Take some urine in a test tube. Add enough crystalline ammonium chloride till some remains undissolved at the bottom. Add one or two crystals of sodium nitroprusside and wait for sometime to dissolve the crystals. Layer over it some liquor ammon fortis. An intense violet ring develops slowly at the junction, if acetone is present in urine.

(ii) *Lieben's Idoform Test.* Take some urine in a test tube and add a few drops of caustic soda or liquor potassæ. Warm gently to body heat and then

add one or two crystals of iodum or a few drops of iodine solution. Now shake up the liquid, which now becomes brownish yellow, and then add liquor potassæ drop by drop, till the colour vanishes; when iodoform is precipitated at the bottom as yellowish crystals, which is recognised by its peculiar smell, if acetone is present.

(iii) *Legal's Test.* Take some urine in a test tube, add equal quantity of 10% caustic soda solution or enough liquor sodæ or potassæ to make it perfectly alkaline. Then add one or two crystals of sodium nitroprusside, when a red colour forms from creatinin. Add a few drops of acetic acid, when it will turn into a deep red colour, if acetone is present; otherwise the red colour fades into pale yellow colour.

Diacetic acid is oxidised in the bladder into acetone and is present in moderate amount, when there is increased destructive proteid metabolism and fatty food and tissues disintegrate, as in high

fevers; such as typhoid and pneumonia. It is found in nephritis, delayed ether alcohol and chloroform poisoning, grave anæmia, cancer, hydrophobia, asthma, some brain diseases, eclampsia, prolonged starvation, some gastro-intestinal disorders and in defective protein and fat metabolism. It is of special importance, when it occurs in the last stage of diabetes, called diabetic acetonuria and generally precedes and foretells a grave disorder coma. In coma acetone diminishes but the amount of hydroxybutric acid increases. The more the amount of acetone, the severe is the case and the worse is the prognosis.

Diacetic Acid. $= C_3 H_6 O + CO_2$
($C_4 H_6 O_3$) (acetone) (carbon dioxide)

Diacetic acid or aceto-acetic acid or acetone and carbon dioxide.

It is found only in fresh unboiled urine. It decomposes within 6 to 12 hours into acetone and carbon dioxide hence it should be tested as soon as possible after voiding

Gerhardt's Ferric Chloride Test.

Take some fresh or recently voided urine in a test tube and add drop by drop a few drops of liquor ferri perchloride, when a yellowish white precipitate of ferric phosphate falls to the bottom. Filter the urine and thus remove all the ferric phosphates. Now to the filtrate add a few more drops of liquor ferri perchloride, when it turns into Bordeux-red (deep purple red) which disappears on boiling if diacetic acid is present. Salicylates, salol, phenacetin, aspirin, antipyrin, carbolic acid or any other coaltar derivatives also produce this red colour but does not disappear on boiling, while on the contrary intensifies on heating.

Gerhardt's test is not very sensitive, hence if positive, it indicates a moderate amount of diacetic acid and denotes a threatening coma, hence active measures should be taken to prevent the development of coma.

When diacetic acid is present in the urine, it is of graver significance than

acetone and threatens a diabetic coma. Diacetic acid in large quantity is present in the blood in diabetic coma or when symptoms of it will manifest. Coma sets in due to acidosis brought about by the defective elimination of carbon dioxide from the body and though the blood remains alkaline, the balance of acid and bases in the blood is disturbed. Diacetic acid has no direct relation with the quantity of sugar eliminated in the urine, since its appearance often precedes diminution of sugar.

It is mostly found in advanced cases of diabetes, sometimes in typhoid fever, nervous disorders with excitement, some gastro-intestinal disorders, cancer and inanition. It is found in most cases where acetone is present.

Fat or Chyle. When minute fat globules are in abundance and albumin is present in urine, it is called Chyluria, but when large fat globules predominate and albumin is absent, it is called Lipuria. When the lymph gain entrance to the urinary system, chyle appears in urine.

The urine is turbid and milky in colour and on settling three layers will be seen; the upper layer creamy or oily, the middle milky, as the fat cells are seen floating with thick oily consistency, while at the bottom, pinkish deposit forms, consisting of blood and epithelium. The specific gravity of this urine is generally lowered as the quantity of urea and chlorides are lessened but albumin and phosphates are generally present in it.

Test. Shake up a little urine with ether (and a few drops of potassium hydroxide, if need be) when fat will be dissolved and the urine will be clear.

It is generally found in the morning urine. It is present in persons with highly fatty diet and in pregnant women, in nephritis, cystitis and in fat embolism. Chyluria suddenly occurs in Filaria Sanguinis Hominis due to their migration from the lacteals to the urinary tract.

CHEMICAL EXAMINATION.

(QUANTITATIVE).

Urea Urea can be estimated, when there is no albumin or sugar present in the urine and chlorides are normal. If albumin is present in the urine, it should first be removed by addition of a little acetic acid, then boiling and filtering. If there is sugar in the urine, the amount of nitrogen is increased due to the chemical action of glucose and hypobromite solution. The principle of urea estimation is that alkaline hypobromite solution decomposes urea and gives out nitrogen gas, carbon dioxide and water. The carbon dioxide gas is absorbed by the excess of alkalies, while the nitrogen gas rises to the top of the closed tube and the amount of urea is calculated.

Urea is roughly estimated from the specific gravity; by dividing the last two digits of the specific gravity by 10 *e.g.* specific gravity of urine is 1020, then the

urine contains 2% of urea. This is true of a single voiding in normal urine, when chlorides are normal and there is no pathological ingredients such as sugar, albumin, etc.

It is accurately estimated by ureometers; (1) Doremus ureometer.

(2) Gerrard's ureometer.

Doremus Ureometer. It consists of a stout tube with graduations at its upper portion and closed at the top. It bends at the bottom to a thistle funnel shaped opening towards the left. Close to the bottom, along its side, another smaller graduated tube, with its upper end open is attached through a biway glass stopper. The whole instrument is fixed to a wooden stand.

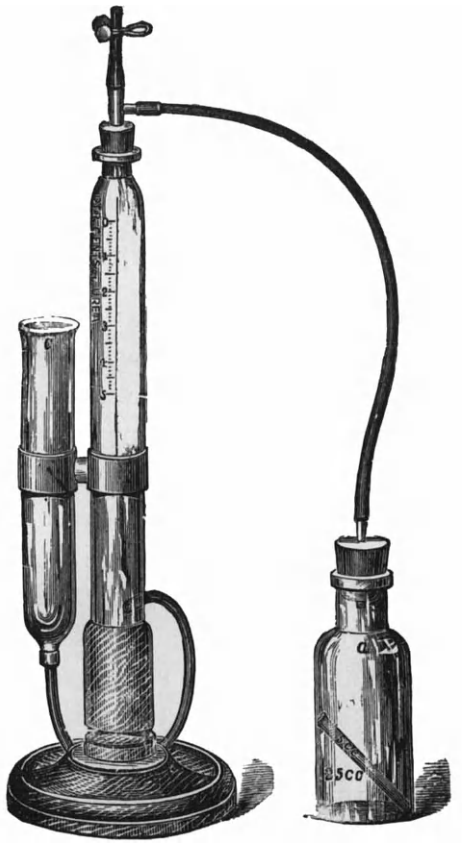
Ureometer (Doremus).

Fill in sodium hypobromite solution completely on the long left side tube, up

to the mark at the bend of the bulb, leaving the bulb almost empty; tilting the whole apparatus to the right. Then stand it erect and keeping the stopcork closed, fill in urine on the small tube up to the mark 0. Now very slowly turn the stopcork and run exactly 1 c.c. of urine. Wait about 20 minutes, when all bubbles have ceased to come off and nitrogen gas is liberated and collected at the top of the long tube, pushing the hypobromite solution downwards; then read off the gradations marked on the tube. Each larger division indicates ·01 gramme *i.e.* one centigramme; and the smaller divisions indicate milligrammes of urea. For example, if the solution stands at the level of ·02 and nitrogen gas is filled up above it and 1 c.c. of urine was run in, then it indicates ·02 gramme of urea in 1 c.c. of urine or 2 centigramme of urea in 1 c.c.; hence multiplying by 100; we get 2%.

Gerrard's Ureometer. It consists of a stout graduated glass cylinder, one end of which is placed on a wooden stand,

while the other end tapers, to which is



Ureometer (Gerrard's).

attached a rubber stopcork, through the slit of which passes a T-shaped glass

tubing; to the upper end of which is attached a small piece of rubber tubing closed with a metal clip. To the other end of the tube is attached a rubber tube, which ends in a glass tube and is inserted in the rubber stopper of a wide mouth glass phial of 6 ounce capacity, with a small glass test tube in it. From the lower part of the main glass cylinder, a rubber tube passes and is attached to the lower part of a short glass cylinder, which is open at its upper end and which acts as a water reservoir. It can be slipped up and down, along the measured cylinder with the help of the metal ring.

Put 25 c.c. of freshly prepared hypobromite solution, in the wide mouth six ounce phial and place 5 c.c. of albumin free urine in the miniature test tube (provided with the instrument) and lower it in the wide mouth phial, with the help of a forceps or with the tip of one of your finger. Care should be taken that the urine does not mix with the hypobromite solution and so the small tube should stand up against the side of the wide mouth

glass phial. Tightly close the stopper of the phial. Now put water on the outer cylinder and see that the water comes on the main cylinder at the level of zero-mark. Tilt the metal ring and keep water low down in the outer cylinder, for there may be an overflow, when water will be driven to it, from the main tube by pressure of nitrogen gas. When everything is complete and the stopcocks are tightened; mix the urine and the hypobromite solution by tilting and gentle movement of the phial; when nitrogen gas will be liberated with great effervescence. Wait for about a quarter of an hour, when all effervescence have ceased and nitrogen gas has collected on the upper end of the main tube, then make the water on the two cylinders on the same level, by lowering the outer cylinder, with the help of the metal ring. Now ascertain the gas collected on the main tube and read off the scale, which indicates the percentage of urea present. To get to grains per ounce, multiply the figure by 4·375 or roughly by 4½.

Albumin. The total quantity of

albumin excreted daily in Europeans is about 8 grammes or 120 grains i.e. about $\frac{1}{2}$% of albumin; but amongst the Bengalees it is slightly less.

Take some fresh urine, filter it and acidulate, if it is alkaline by acetic acid, and note the specific gravity. If it is high, dilute the urine with water, noting the dilution, till the specific gravity falls to 1008 or lower.

It can roughly be estimated by the following method. Fill two-third of a test tube with urine and boil it vigorously for a few minutes. Allow it to cool, when precipitate will slowly gravitate to the bottom of the test tube. When all the precipitate has collected say after an hour, calculate as follows :—

No precipitate (but the urine is turbid)—	
Less than	·01%
Slight precipitate at the bottom of the test tube	·05%
Precipitate $\frac{1}{10}$th of the volume of urine ...	·1%
Precipitate ¼th ,, ,, ,, ...	·25%
Precipitate ⅓rd ,, ,, ,, ...	·5%
Precipitate ½ of ,, ,, ,, ...	1%
Whole fluid a compact mass ...	2 to 3%

It is accurately done in two ways

(i) Esbach's Albuminometer.

(ii) Aufrecht's Albuminometer.

Esbach's Albuminometer. It consists of a stout graduated test tube, with several empirically marked graduations corresponding to parts per thousand in the bottom and also two marks U and R on the side of it.

Albuminometer (Esbach's)

Take urine up to the mark U and then add the reagent up to the mark R. Close the tube with the rubber stopper and mix the two liquids by repeated inversions about a dozen times, without shaking (shaking generates heat, which may dissolve albumin) and let it stand vertically in natural position for twenty-four hours in an even temperature. First of all a yellow precipitate appears, floating about, which gradually settles down. Then read off the precipitated proteid collected on the scale at the bottom. The graduations indicate

grammes of dried albumin in a litre of urine (1000 c.c.). Hence the precentage is got by dividing with 10, or only putting a decimal point before the figure. Now if the urine has been diluted, multiply the figure by the number of dilutions. Precipitate stands at 2, it means 2 grammes of albumin in 1000 c.c. of urine, i.e. ·2%. Now the urine is diluted 5 times; then $\cdot 2 \times 5 = 1 \cdot 0\%$ or $4\frac{1}{2}$ grains per ounce of urine.

If the amount of albumin be less than ·05% it cannot be accurately measured by this method. Further if the level of the coagulum be above the graduation mark 4, another sample of urine must be further diluted and the process of estimation repeated.

Aufrecht Albuminometer. It consists of a graduated conical tube, drawn out into a blunt cone at the closed end and two marks U and R marked on it. It is now generally used, as it is more advantageous than Esbach's, on account of its accuracy, no dilution of urine is necessary and the work is finished in

2 or 3 minutes instead of twenty-four hours.

Fill urine up to the mark U and add the reagent up to the letter R. Close the tube with the rubber cork and mix the two liquids by tilting it gently upside down several times without shaking. Now put the albuminometer in the centrifuge and use it for two or three minutes, when precipitate of albumin will fall to the bottom. The graduated scale indicates exactly the percentage of albumin present.

Albuminometer (Aufrecht's)

Sugar. It can roughly be ascertained by experienced observers from the qualitative test; first in determining the rapidity which took the Fehling's solution to be reduced and brick dust precipitate to be formed; and secondly the amount of urine added when boiled.

Another rough, though not accurate method to estimate the sugar is to take

4 c.c. of Fehling's solution and diluting with 20 c.c. of water, put it in a large stout test tube and boil. Add to it urine drop by drop. If 4 drops reduce the solution wholly, sugar is about 10%; 8 drops 5%; 16 drops 2·5% and so on.

It is accurately estimated by:—

(i) Fehling's solution. (This is the most simple, convenient and is in frequent use).

(ii) Benedict's solution.

(iii) Fermentation test with yeast cum Saccharometer (rarely used).

The urine should be fresh and if it contains albumin, it should be removed by adding acetic acid, then boiled and filtered.

Fehling's Method.—Take 5 c.c. of Fehling's solution-A and 5 c.c. of Fehling's solution-B in a c.c. measure glass. Mix the two solutions, by slightly shaking the two liquids and pour it in a clean porcelain evaporating dish, placed over an iron tripoid stand with a piece of wire gauze. Dilute the Fehling's solution with

water, (preferably recently boiled water) firstly to allow any change of colour to be more distinctly seen and secondly to guard against undue evaporation of the solution. Now dilute the urine in any proportion as you like. The higher the specific gravity, the greater the amount of sugar present in the urine and hence the greater should be the dilution for accuracy; but the author's usual practice is to take in a measure glass 5 c.c. of urine and then add 45 c.c. of water, making a total of 50 c.c., i.e. the dilution is 1 in 10. Close the upper end of the measure glass with the palm of your hand or with a cork and mix the water and the urine thoroughly. Pour the diluted urine in a burette, (preferably 50 c.c.) fitted with a ground glass stopper, fixed in a stand. Now boil the Fehling's solution in a spirit lamp, before adding any urine and see that it is all right (i.e. the blue colour of the Fehling's is not faded). Note the point in the burette, where the diluted urine stands. Cautiously unloosen the stopcock, so that the diluted urine falls

drop by drop on the Fehling's solution in the porcelain evaporating dish. Boil the Fehling's solution, while so adding the diluted urine and stir with a glass rod, throughout the entire tritation. A brick dust precipitate forms on the porcelain dish due to cuprous oxide. Gently heat till the blue colour of the Fehling's solution has entirely disappeared. Towards the end, remove the spirit lamp and stop the urine flowing for a few seconds, so that the brick dust precipitate may settle. When all trace of the blue colour is discharged, tighten the stopcock of the burette, so that not a single drop falls on the porcelain evaporating dish. Then read off the burette where the liquid stands and calculate as follows—10 c.c. of Fehling's (5 c.c. of A and 5 c.c. of B) is neutralised or completely reduced by ·05 gramme of sugar or ·77 grains of sugar. Now for example, the diluted urine stood in the burette, before letting out at 6; and after flowing the urine in the porcelain dish, when the Fehling solution was completely reduced, it stood at 26 in

the burette. Hence 20 c.c. of diluted urine was required to neutralise 10 c.c. of Fehling's. But the urine was diluted 1 in 10; therefore 2 c.c. of actual urine was required to neutralise 10 c.c. of Fehling's; i.e. 2 c.c. of actual urine contains ·05 grammes of sugar or ·77 grains of sugar. Now find out, the percentage of sugar; or how many grammes of sugar, in 100 c.c. of urine?

If 2 c.c. of actual urine contains ·05 gramme of sugar.
∴ 1 c.c. of actual urine contains ·025 gramme of sugar.
∴ 100 c.c. of actual urine contains (·025 × 100).
$= 2{\cdot}5$ grammes of sugar ; i.e. $2\frac{1}{2}\%$.

or

$$2 : 100 :: \tfrac{5}{100} : x \; ; \; \therefore x = \frac{100 \times 5}{100 \times 2} = \tfrac{5}{2} = 2\tfrac{1}{2}\%.$$
$= 2\frac{1}{2}$ grammes in 100 c.c.

Then put it in grains per ounce, multiply the figure by 4·375 or roughly by $4\frac{1}{2}$ i.e. $\frac{9}{2} \times \frac{5}{2} = \frac{45}{4} = 11\frac{1}{4}$ grains per ounce of urine.

End point. Now how to ascertain the end point or when the last trace of the blue colour of the Fehling's solution

has disappeared, which is somewhat difficult for a tyro, as the whole fluid assumes a brownish red colour. Remove the spirit lamp, underneath the porcelain cup for a few minutes and when the red brownish deposit has gravitated to the bottom, then judge by any of the following methods.

Physical:—

(i) By simply looking at the fluid on the surface, particularly on the edges, after the reddish brown precipitate has settled at the bottom.

(ii) Slowly tilt the porcelain cup to one side and then see the fluid on the surface and the edges, against the white surface of the porcelain cup, which will serve as a back ground, when the slightest blue tint can be detected.

(iii) Take a piece of clean cloth or a white filter paper and soak one drop of the fluid from the surface and not from the reddish brown sediment collected at the bottom and see whether there is any trace of blue colour still present.

Chemical:—

(iv) Soak a filter paper with potassium ferrocyanide solution and acetic acid. (Copper sulphate solution will be coloured reddish brown on it.) Drop on it the fluid from the surface and not from the bottom where sediment has collected, with a stirring rod and note whether there is any reddish brown colour. If so, add urine drop by drop, till no reddish brown colour is stamped on it; that is the end point.

Benedict's Method. Take 25 c.c. of Benedict's solution (quantitative) in a porcelain evaporating basin, instead of 10 c.c. as in Fehling's solution. Add a little powered pumice or talcum (to avoid bumping) and dilute it with water if need be. Run in the diluted urine, from the burette to the porcelain basin, while the solution is being heated, as in Fehling's method. When the last trace of blue colour of the reagent vanishes and a chalk white precipitate of copper sulphocyanate forms, then tighten the stopcock of the

burette and calculate as follows—25 c.c. of this solution is completely reduced by ·05 gramme of glucose (while in Fehling's 10 c.c. is required).

Benedict's method has got the following advantages over Fehling's.

(i) It is highly reliable, as it is very sensitive to sugar and is not reduced by creatinine, uric acid, xanthin and glycoronic acid.

(ii) The solution can be kept for a long time as there is little chance of its deteriorating on long standing.

(iii) Copper is reduced here as cuprous sulphocyanate, a white substance. In this method, when the solution becomes white and the last trace of blue colour disappears, can easily be noticed and thus facilitates the end point.

Table of Sugar calculation.

Dilution—1 c.c. of urine + 9 c.c. of water = 1 in 10. Ascertain the number of c.cs. from the burette reading, required to neutralise 10 c.c. of Fehling's solution,

or 25 c.c. of Benedict's solution. Then ascertain as follows :—

10 c.c. of Fehling's solution or 25 c.c. of Benedict's solution } = ·05 grammes of Glucose.

Diluted urine required to neutralise.	Actual urine required to neutralise.	% of sugar.	Grains per ounce.
1 c.c.	·1 c.c.	50%	218·5
2 c.c.	·2 c.c.	25%	109·2
3 c.c.	·3 c.c.	16·6%	72·8
4 c.c.	·4 c.c.	12·5%	54·6
5 c.c.	·5 c.c.	10%	43·7
6 c.c.	·6 c.c.	8·3%	36·4
7 c.c.	·7 c.c.	7·1%	31·2
8 c.c.	·8 c.c.	6·21%	27·3
9 c.c.	·9 c.c.	5·5%	24·3
10 c.c.	1 c.c.	5%	21·85
11 c.c.	1·1 c.c.	4·5%	19·88
12 c.c.	1·2 c.c.	4·1%	18·23
13 c.c.	1·3 c.c.	3·8%	16·83
14 c.c.	1·4 c.c.	3·5%	15·62
15 c.c.	1·5 c.c.	3·3%	14·58
16 c.c.	1·6 c.c.	3·1%	13·67
17 c.c.	1·7 c.c.	2·9%	12·86
18 c.c.	1·8 c.c.	2·7%	12·15
19 c.c.	1·9 c.c.	2·6%	11·51
20 c.c.	2 c.c.	2·5%	10·93
21 c.c.	2·1 c.c.	2·3%	10·41
22 c.c.	2·2 c.c.	2·2%	9·94
23 c.c.	2·3 c.c.	2·1%	9·51

Diluted urine required to neutralise.	Actual urine required to neutralise.	% of sugar.	Grains per ounce.
24 c.c.	2·4 c.c.	2·08%	9·11
25 c.c.	2·5 c.c.	2·0%	8·74
26 c.c.	2·6 c.c.	1·9%	8·41
27 c.c.	2·7 c.c.	1·8%	8·09
28 c.c.	2·8 c.c.	1·78%	7·81
29 c.c.	2·9 c.c.	1·72%	7·5
30 c.c.	3 c.c.	1·6%	7·29
32 c.c.	3·2 c.c.	1·5%	6·83
34 c.c.	3·4 c.c.	1·4%	6·43
36 c.c.	3·6 c.c.	1·38%	6·07
38 c.c.	3·8 c.c.	1·31%	5·75
40 c.c.	4 c.c.	1·25%	5·46
42 c.c.	4·2 c.c.	1·19%	5·20
44 c.c.	4·4 c.c.	1·13%	4·96
46 c.c.	4·6 c.c.	1·08%	4·75
48 c.c.	4·8 c.c.	1·04%	4·55
50 c.c.	5 c.c.	1%	4·37

If 50 c.c. of diluted urine or 5 c.c. of actual urine does not completely reduce either the Fehling's or the Benedict's solution, it can be expressed as "*Sugar-Less than 1%*."

Further in such cases, it may be necessary to find out the actual amount of sugar present, when the urine need not be

diluted, or if diluted, a burette and a measure glass of 500 c.c. capacity should be used; and then ascertain as follows :—

Diluted urine required to neutralise.	Actual urine required to neutralise.	% of sugar.	Grains per ounce.
55 c.c.	5·5 c.c.	·9%	3·93
60 c.c.	6 c.c.	·83%	3·64
70 c.c.	7 c.c.	·71%	3·12
80 c.c.	8 c.c.	·62%	2·73
90 c.c.	9 c.c.	·55%	2·43
100 c.c.	10 c.c.	·5%	2·18
110 c.c.	11 c.c.	·45%	1·98
120 c.c.	12 c.c.	·41%	1·82
130 c.c.	13 c.c.	·38%	1·68
140 c.c.	14 c.c.	·35%	1·56
150 c.c.	15 c.c.	·33%	1·46
160 c.c.	16 c.c.	·31%	1·36
170 c.c.	17 c.c.	·29%	1·28
180 c.c.	18 c.c.	·27%	1·21
190 c.c.	19 c.c.	·26%	1·15
200 c.c.	20 c.c.	·25%	1·09
210 c.c.	21 c.c.	·23%	1·04
220 c.c.	22 c.c.	·22%	·99
230 c.c.	23 c.c.	·21%	·95
240 c.c.	24 c.c.	·208%	·91
250 c.c.	25 c.c.	·2%	·87
260 c.c.	26 c.c.	·19%	·84
270 c.c.	27 c.c.	·18%	·81
280 c.c.	28 c.c.	·178%	·78

Diluted urine required to neutralise.	Actual urine required to neutralise.	% of sugar.	Grains per ounce.
290 c.c.	29 c.c.	·172%	·75
300 c.c.	30 c.c.	·16%	·73
320 c.c.	32 c.c.	·15%	·68
340 c.c.	34 c.c.	·14%	·64
360 c.c.	36 c.c.	·138%	·60
380 c.c.	38 c.c.	·131%	·57
400 c.c.	40 c.c.	·125%	·54
420 c.c.	42 c.c.	·119%	·52
440 c.c.	44 c.c.	·113%	·49
460 c.c.	46 c.c.	·108%	·47
480 c.c.	48 c.c.	·104%	·45
500 c.c.	50 c.c.	·100%	·43

Fermentation Method. Mix thoroughly 1 gramme of yeast, (preferably German) and 15 c.c. of urine in a mortar and completely fill in an Einhorn's saccharometer, noting that no air bubbles remain at the top of the graduated tube. Keep it erect for 12 hours, in a warm place to ferment, when carbon dioxide gas will rise to the top of the tube. Now read off the graduated scale, which means the percentage of sugar. The result is too inaccurate, hence it is not generally used.

Chloride Estimation. (Mohr's method).

The pinciple is, chloride combines with silver nitrate and forms silver chloride, a white insoluble compound, which precipitates at the bottom. When all the chlorides have combined with silver nitrate, any further addition of silver nitrate combines with chromate, forming silver chromate, which is pink in colour. Take in a porcelain cup 10 c.c. of urine (albumin should be free, if there be any) and dilute it with 50 c.c. of distilled water. Then add a few drops of 5% potassium chromate solution and a pinch of calcium carbonate, just to neutralise any free acid, which may be present. Fill in a burette, fixed in a stand, standard silver nitrate solution and note the point where it stands. Allow it to run on the porcelain cup, drop by drop, constantly stirring all the time with a glass rod. Each drop produces a red colour, which disappears on stirring, for chlorine combines with silver to form silver chloride, a white precipitate. When all the chlorides have been precipitated, silver

then combines with chromate, thus forming silver chromate, which is pink in colour and it remains so, inspite of vigorous stirring. When a permanent pink colour appears, on the porcelain cup, then tighten the stopcock and read off the burette and calculate as follows.

Now note down the number of c.c. of silver nitrate solution used. Deduct ½ c.c. from it, if the specific gravity of urine is low, say below 1015; and 1 c.c. if the specific gravity is high, say over 1015; for there are other substances besides chromate and chlorides, such as phosphates, with which silver may unite. Then calculate, every remaining c.c. as equal to ·01 gramme or 10 milligrammes of sodium chloride. Suppose 15 c.c. of silver nitrate solution was used, deduct 1 c.c. from it, then 14 c.c. is left; therefore 14 c.c. × ·01 gramme = ·14 gramme of chloride. Now 10 c.c. of urine was taken, so it contains ·14 gramme of chloride; therefore 100 c.c. contains 1·4 grammes or 1400 milligrammes of chloride or 1·4%. Now

calculate on the quantity of urine sent for examination, or on the total collection in twenty-four hours.

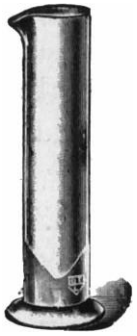
MICROSCOPICAL EXAMINATION.

Always test the reaction of urine, as soon as it is received. If it is alkaline, fermentation will set in, so centrifugalise and examine it as soon as possible; but if the reaction is acid, the urine may be examined at leisure, after 3 or 4 hours, by double sedimentation method. Sediments may be deposited by the three following methods :—

(i) **Centrifugal Method.** Take some urine, in a centrifugal or sedimentation tube and centrifugalise it, in a centrifuge for a few minutes and transfer a drop or two from the bottom, with a pipette on the centre of a clean glass slide and cover it with a cover glass. Soak and clear off the urine, surrounding the cover glass, with a filter paper and then examine it under a microscope.

(ii) **Gravitation Method.** Allow the urine to stand, for about 3 or 4 hours, preferably in a conical urine sediment

glass, when sediment will deposit by gravity. Carefully decant the supernatant fluid, or introduce a pipette through the clear supernatant fluid down into the sedimentary layer and draw up some sediment into the pipette by capillary action, and discarding the first drop, put the second drop, on the centre of a clear glass slide and cover it with a cover glass; (or the cover glass may be dispensed with in case of casts, as it crushes them) and examine it under a microscope.



Urine Sediment Glass.

(iii) **Double Sedimentation Method.** This is the best and the ideal method, as it combines gravity and the centrifugal machine for acid urine; (but in alkaline urine fermentation sets in soon, so the urine should be examined as soon as possible). Set aside the acid urine as it is, or put it in a conical urine sediment glass for a few hours, say 3 or 4 hours. It should be corked or covered with a piece

of paper to avoid contamination. A sediment forms by gravity. Draw some urine and the sediment from the bottom, with a pipette, a few times and put them in a centrifugal or sedimentation tube and to better advantage, centrifugalise it for a few minutes; when a slight thick sediment, collects at the bottom of the tube. Draw it with a pipette and put it on the centre of a clean glass slide and covering it with a cover glass, examine it under a microscope.

Usual system for general practitioners. Urine examination should be done at noon or in the evening, as they see their patients in the morning; hence a sample of urine should be covered and set aside till noon or evening, when they are free to do the examination and thus sufficient time has been allowed to form sediment by gravity. The supernatant fluid is carefully decanted and used for chemical tests, while the sediment is centrifugalised and a thick portion is drawn up from below with a pipette and put on the centre of a clean glass slide and then covered

with a cover glass and examined under a microscope.

How to use the microscope. In urine examination, always use the concave reflecting mirror and then by partly narrowing the diaphragm, examine the slide, first with a $\frac{2}{3}$rd and then by a $\frac{1}{6}$th inch objective.

Urinary Sediments.

(i) Unorganised or chemical.

(ii) Organised, anatomical or morphological. (These are of more serious import, than the unorganised or chemical sediments).

Sediments are present both in acid and alkaline urine and are :—

(i) Crystalline—Formed into a definite shape.

(ii) Amorphous—Shapeless.

Unorganised Sediments.

A. In Acid Urine.

Common

Crystalline—

1. Calcium Oxalate.
2. Uric acid.

Amorphous—

3. Urates.

Rare

Crystalline—

4. Cystin.
5. Leucin.
6. Tyrosin.

B. In Alkaline Urine.

Common

Crystalline—

1. Triple Phosphate or Ammonio-Magnesium Phosphate.

Amorphous—

2. Ammonium Urate.
3. Amorphous Phosphate.

Rare

Crystalline—

4. Simple Phosphate or Calcium Phosphate.
5. Calcium Carbonate.

ORGANISED SEDIMENTS.

A. Endogenous
1. Red Blood Cells.
2. Pus Cells.
3. Mucus.
4. Spermatozoa.
5. Epithelial Cells.
6. Tube casts.

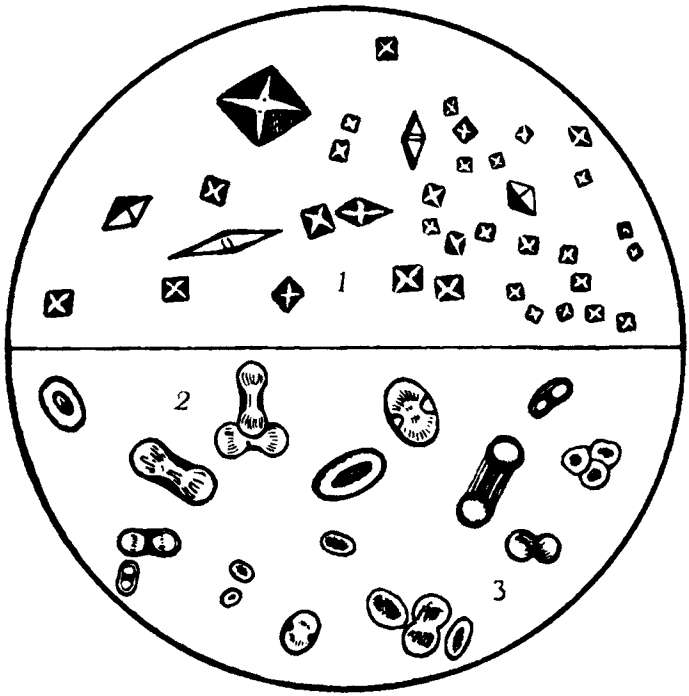
B. Exogenous
1. Parasites.
2. Micro-organisms
3. Foreign bodies.

UNORGANISED SEDIMENTS.

Calcium Oxalate. The sediment is mainly crystalline and occurs as colourless refractile octahedral or square envelope shaped crystals, the cross lines of them being of moderate refraction. Sometimes it forms like small squares; occasionally dumb-bells, or hour glass shape in the form of figure 8, rarely biscuit shaped discs.

It is found in sentimental people with sedentary habits, between the ages of sixteen to forty, owing to dietetic error, in sluggish and torpid liver, in people suffering from chronic dyspepsia, from

CALCIUM OXALATE.



1. Octahedral or Square envelope shaped crystals.

2. Dumb-bell shaped crystals.

3. Biscuit shaped discs.

URIC ACID

nervous overstrain, mental anxiety, in convulsive nervous diseases, such as hysteria, epilepsy, tetanus and apoplexy. If in excess, the crystals irritate the urinary passages; and when they are aggregated together, they foretell the formation of hard mulberry stone. (The passage of which gives much dragging pain to the patient). It is immaterial if little is voided, as it is derived from vegetable food, but if the specific gravity of urine is raised and it is present in moderate amount, it indicates diseases due to bacterial decomposition of food.

When calcium oxalate crystals are found in urine, moderately or in fair numbers, regular outdoor exercises should be advised, which has a beneficial effect on the system and thus lessens these crystals.

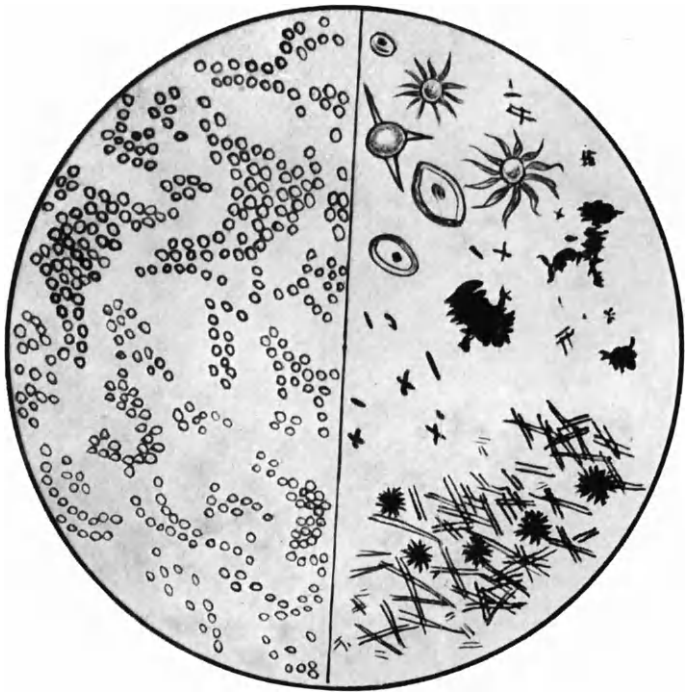
Uric Acid. It is always found in moderately acid urine. Uric acid crystals are mostly pigmented, slightly yellow or reddish brown from urochrome, or they may be coloured dark brown by bile, indican, various pigments and drugs.

They are the only coloured crystals found in urine. Uric acid crystals are of various shapes and owing to the presence of pigments, colloidal material etc. in the urine, these crystals are transformed from rhombic prisms to lozenges and lanceolate stones. Sometime some of these crystals are aggregated together and become spherical, forming rosettes. In rare cases, the final derivative form—dumb-bells, formed from the original rhombic prisms, may also be seen.

Urates. They may be amorphous or crystalline. Potassium, calcium and magnesium urates are amorphous, consist of minute granules, running together into moss like clumps and are pigmented pink or slightly reddish brown; but when they are colourless, they resemble a deposit of phosphates, but they are much smaller than the phosphates.

Sodium Urate. It may be amorphous or crystalline. When amorphous, it looks like moss with small granules, either isolated or in clusters. When crystalline it looks like fan shaped clusters or

URATES.



Left—Amorphous urate
Right—Crystalline urate. (Hedgehog and thorn apple crystals).

prismatic needles, generally known as hedgehog crystals.

Ammonium Urate. It is crystalline and slightly darker and more opaque than the sodium urate. It is seen as small concentric yellowish brown globules, either singly or in groups, sometimes plain or with numerous short spines, so called thorn apple crystals.

Cystin, Leucin and Tyrosin. *They are of rare occurrence.* They indicate perverted protein metabolism and diagnostic of hepatic disorders.

Cystin. It is found in acid urine, but disappears when the urine decomposes. It is a regular colourless, six sided crystalline plate, may be single or superimposed in layers. It is insoluble in acetic acid, but soluble in alkalies. It occurs in articular rheumatism, but mainly in stone formation. It is liable to occur in some members of the same family and indicates hereditary perverted protein metabolism, but is of no pathological importance.

Leucin. The crystals are flat, refractile, yellow or light brown spherical

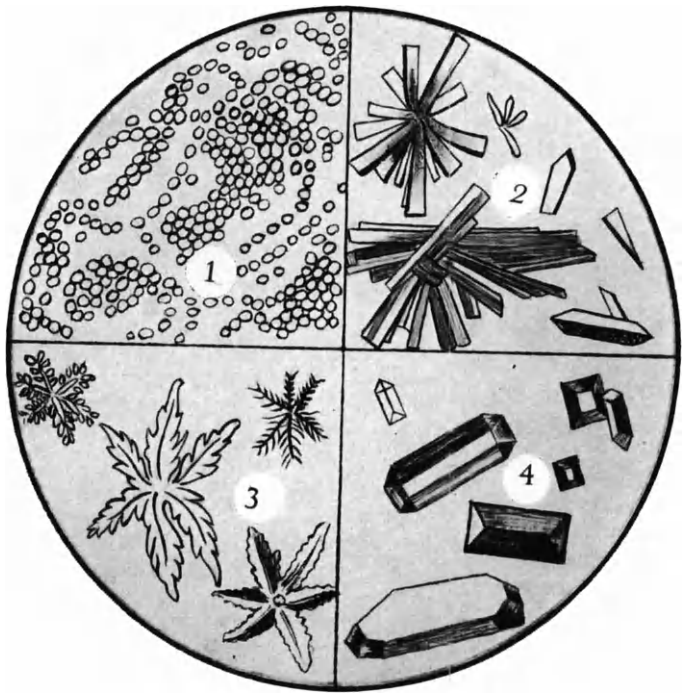
bodies, often concentrically striated, separate or joined together, looking like oily balls.

Tyrosin. The crystals look like tufts of fine needles, as if a fine broomstick with a constriction in the middle, or star crossing at different angles. It is coloured yellow or light brown or may appear as black.

Leucin and tyrosin are the terminal products of albuminous metabolism. They are found in diseases of liver degeneration, such as catarrhal jaundice, gall-stone, cancer, cirrhosis and acute yellow atrophy of the liver and phosphorus poisoning. They may be occasionally found in pernicious anæmia, bronchitis, cystitis, nephritis, tuberculosis and typhoid fever.

Phosphates. They are of various kinds, some of them are amorphous and some crystalline. All of them are colourless and soluble in acetic acid. Alkaline phosphates are readily soluble and are amorphous. Earthy or common phosphate is readily deposited and is both amorphous

PHOSPHATES.



1. Amorphous phosphate.

2. Stellar phosphate.

3 and 4. Triple phosphate.

3. Feathery form.

4. Wedge prism form.

and crystalline. Triple phosphate is crystalline.

Amorphous phosphate. It is shapeless and colourless; may be deposited singly or in clusters of fine particles or granules.

Crystalline phosphate. (i) *Triple phosphate or ammonio-magnesium phosphate.* It is colourless, shines with moderate refraction and looks like wedge prisms, commonly known as coffin lid crystals or knife rests, from their resemblance to the pest-board cover of office knives; or look like rectangular office envelopes. Rarely, in markedly ammoniacal urine, it looks like a feather or the cut end of a leaf of a tree, known as feathery phosphate.

(ii) *Common, earthy or calcium phosphate.* It is rosette or star shaped, occurring singly or in clusters, commonly known as stellar phosphate; the crystalline prisms arranged as stars, the slender ends of the spicules are in the centre.

Carbonate. Calcium carbonate is a rare deposit. The deposit is white in

colour and occurs when amorphous, as fine granules, sometime it is seen in the form of dumb-bells or crystalline radiating spheres.

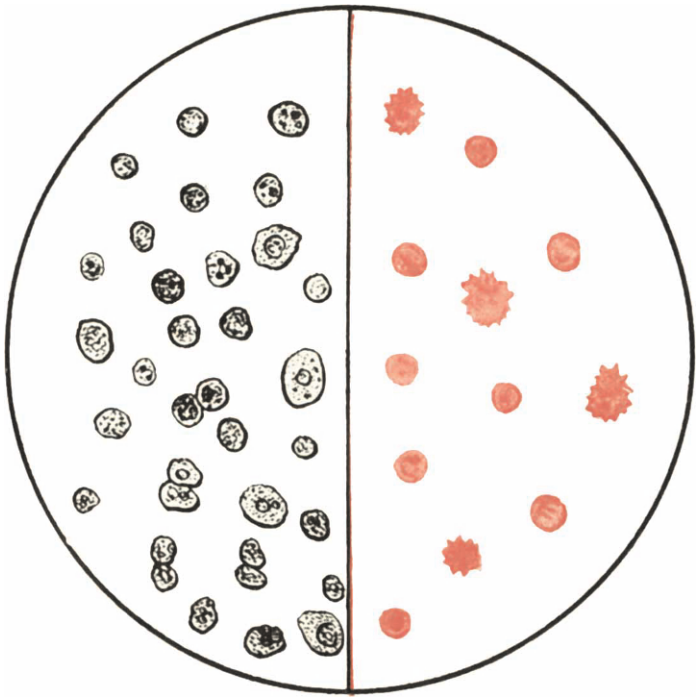
Organised Sediments.

Red Blood Cells. In fresh urine, red blood corpuscles are seen under the microscope, as red biconcave circular disc. Sometimes they are irregular in outline, may be contracted, swollen, crenate and deformed. They may be scattered or aggregated together. When the red blood corpuscles contain hæmoglobin, they are seen coloured, but sometime in alkaline urine when they lose their colouring matter, they are seen as colourless disc.

Blood Clots. They are sometimes found in urine and are composed of distorted aggregated red cells. They are generally of rusty brown colour.

Hæmin crystals. Put on a microscopic slide, a drop or two of the urine from the bottom (where sediment has collected or after being centrifugalised). Add one or two crystals of sodium chloride

PUS CELLS
and
RED BLOOD CELLS
with
FOUR CRENATED CORPUSCLES.



and two or three drops of glacial acetic acid. Heat the slide gently in a spirit lamp. When the slide is cool, examine it under the microscope, when reddish brown rhomboidal crystals of hæmin will be seen.

Pus cells. During inflammation leucocytes show degenerative changes and are converted into pus cells. When the reaction of urine is acid, the white blood cells are seen under the microscope, as small white circular granular bodies, with one or more nuclei and almost twice as large as red blood corpuscles. They may lie singly or in groups. But when the reaction of urine is alkaline, these pus cells are swollen and become transparent, and when degeneration has proceeded, the cells lose their outline and become a mass of debris. The strength or constitution of a patient, can be ascertained by the nature of the granulations in pus cells. The granulations indicate the amount of living matter in those cells. The more coarser the granulations, the better is the constitution; and inversely the finer the granu-

lations, the poorer the constitution. Pus cells when present in large numbers indicate gonorrhœa and leucorrhœa. In leucorrhœa besides pus cells, epithelial cells and sometime a number of thread filaments are present.

Mucus. Mucus generally appears in two forms.

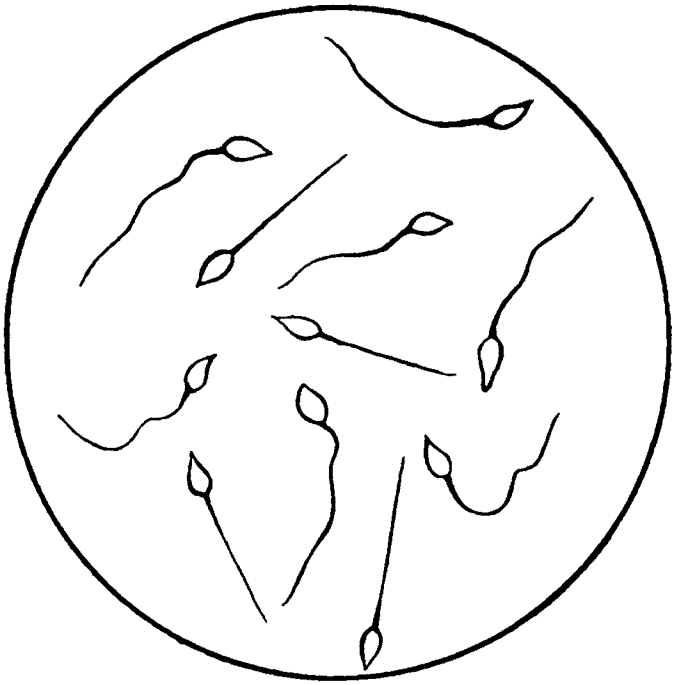
(i) Mucus corpuscles.

(ii) Mucus shreds.

(i) Mucus corpuscles are generally distorted mucus cells and are sometimes mistaken for pus cells; the differences are, while mucus corpuscles are pale, irregular in outline, but finely granular and non-nucleated; pus corpuscles are shining, regular and nucleated. When normal leucocytes and mucus corpuscles appear in urine, albumin is not detected, but when urine is mixed with pus cells, albumin is detected.

(ii) Mucus shreds are the mucus fibres aggregated together. They are irregular, of unequal thickness, often twisted or folded like a pale ribbon and consist of fine longitudinal striations, tapering at

SPERMATOZOA
OR
SEMINAL ANIMALCULES.



one or both ends. They are generally called cylindroids or mucus casts. When mucus is in abundance these mucus casts appear in urine.

Mucus appears more or less in every urine. It is fairly present in all inflammatory conditions of genito-urinary tract of both sexes, in fevers, and in some wasting diseases such as carbuncle etc.

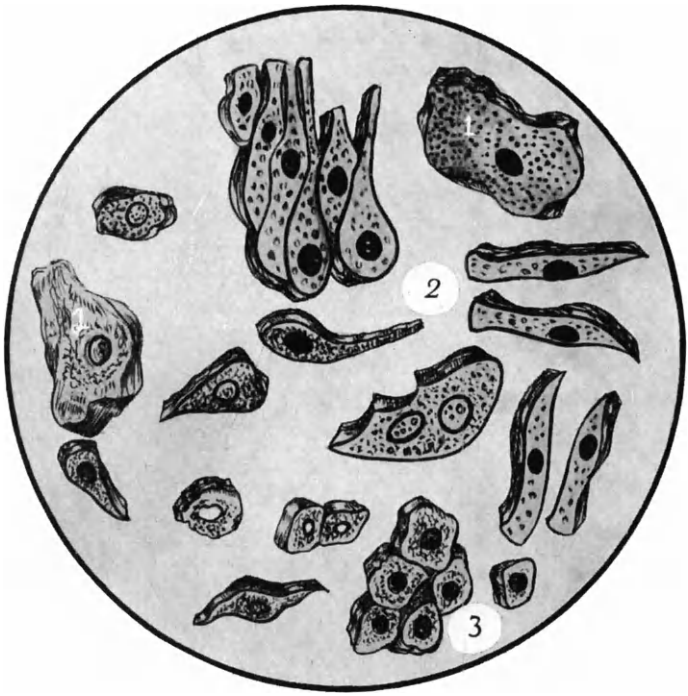
Spermatozoa (or Seminal Animalcules). They are seen under the microscope as small tadpoles. Each spermatozoon contains a small ovoid head, a short neck, a body and a long delicate wavy tapering tail with an end piece. Each is about $\frac{1}{600}$th inch in length.

Spermatozoa are found in spermatorrhœa, after emissions, in testicular inflammations and after sexual intercourse. They are sometimes found after convulsive attacks of hysteria and epilepsy and when there is any irritating constituent in the urine, such as calcium oxalate. Urine containing spermatozoa is slightly hazy and on standing forms a white flocculent precipitate.

Epithelial Cells. These cells may come from any part of the genito-urinary tract, from kidney to the urinary meatus; but a few epithelial cells from the urethra may be normally present in a specimen of urine. All epithelia are flat, granular and may be desquamated from the superficial layer or from the deeper layers of the same organ; so different kinds of epithelia are found in the urine at the same time. To diagnose the location from the morphological characters of the epithelia are next to impossible, as they are often distorted in shape and appearance. The following varieties of epithelium are chiefly found in urine.

I. Pavement or Squamous Epithelium. It is a large flat cell, irregular in outline, with one central nucleus. It may lie singly or appear in groups. The cells desquamate generally from the vagina and are present in the urine of females, more so, who have borne children. They are occasionally present though slightly in mens' urine, being voided from the urethral mucous membrane. If present

EPITHELIAL CELLS.



1. Pavement or Squamous Epithelium.
2. Columnar and Transitional Epithelium.
3. Spherical and Cubical Epithelium.

in moderate amount and mixed with pus corpuscles, they denote an inflammation or catarrh of the genito-urinary tract.

II. Columnar and Transitional Epithelium. They are generally known as 'Epithelia—spindle form.' They consist of oval cells with relatively large nuclei and tenuous extremities and are generally voided from the bladder, ureter and urethra. When voided in large numbers, they indicate cystitis, pyelitis etc.

III. Spherical and Cubical Epithelium They are generally known as 'Epithelia—small round.' They are small, circular and generally a trifle larger than ordinary leucocytes with large distinct nuclei. They come from the kidney and when present in large numbers, with or without casts, generally indicate grave disorders of the kidney.

IV. Smegma. The epidermal scales from the clitoris or vagina clustered together are occasionally found in the urine of females.

TUBE CASTS :—(i) True casts.
(ii) Pseudo or false casts.

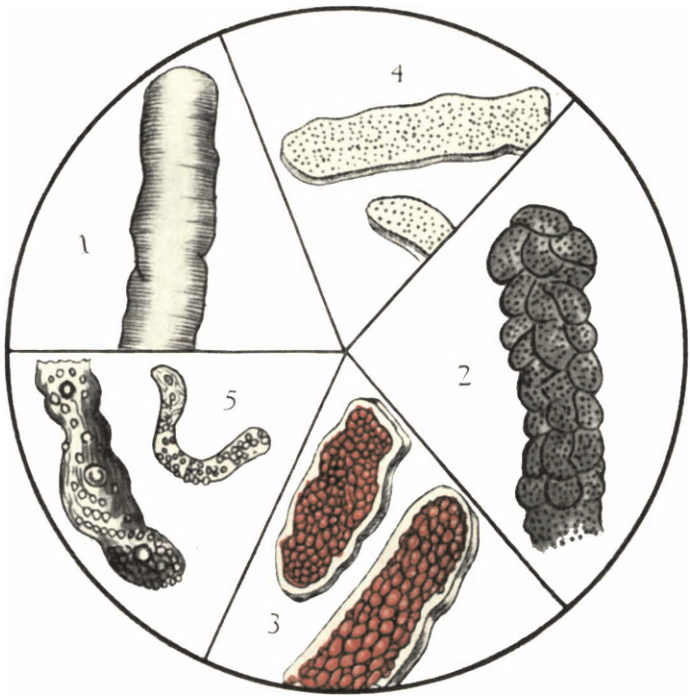
True Casts. These are casted or thrown off portions from the uriniferous tubules of the kidney, due to albuminous exudation from the blood vessels. They are short, cylindrical structures, with parallel margins, having distinct definite outline. They are of uniform breadth, with one end somewhat rounded, while the other end is sharply broken off. They are found in all forms of nephritis, brain diseases and in debility. Their presence indicates some kidney disorder and when albumin is present with them, the outlook is grave.

Varieties :—(i) Simple—One variety of cast only.

(ii) Mixed—Different varieties of cast intermingle together ; e.g., granular at one end, epithelial at the other.

(iii) Compound—True morphological elements are studded or incorporated over hyaline casts e.g. red blood cells, pus cells, epithelia &c.

TUBE CASTS.



1. Hyaline Cast.
2. Epithelial Cast.
3. Blood Cast.
4. Granular Cast.
5. Fatty Cast.

True casts may be :—

A. Primary.
B. Secondary.

A. Primary. (Appears in acute inflammation)	1. Hyaline Cast. (i) Narrow. (ii) Broad. 2. Epithelial Cast. 3. Blood Cast. (i) Erythrocytes. (ii) Leucocytes.
B. Secondary. (Appears in chronic inflammation)	4. Granular Cast. (i) Fine. (ii) Coarse. 5. Fatty Cast. 6. Waxy Cast.

1. Hyaline Cast. It is composed of a transparent homogeneous substance and are refractive to light. It is generally pale and almost invisible, for nothing is imbedded in it, but when compound, it contains fine granules or studded with epithelium or pus cells. It is found in the earliest and in the recovering stages of parenchymatous and interstitial nephritis. In rare cases one or two may be voided in apparently healthy urine.

2. Epithelial Cast. It is the agglutination of renal epithelial cells and looks like some scales of a fish in tube like form. Some time the basement membrane is made up of hyaline cast with epithelial cells embedded in it. The outline of the cells with the granules are sometimes distinctly visible. It is found in acute and chronic nephritis.

3. Blood Cast. Blood corpuscles (erythrocytes and leucocytes) are thickly studded together and borne upon a hyaline or fibrinous matrix. It indicates a hæmorrhagic process and is found in diffuse acute congestive nephritis. Moreover pus cells, sometime appear over any of the true cast, when it is called a mixed cast.

4. Granular Cast. Fine or coarse granules of light brown colour derived from the residual matter of degenerated renal epithelium may lie over a hyaline matrix; or the granules are packed together and shed in the form of a cast. It is found in various types of nephritis, but

commonly found in chronic parenchymatous nephritis.

5. Fatty Cast. It contains fat globules. It is the intermediary product of fatty degeneration of epithelial and granular casts. It is highly refractive. It indicates fatty degeneration of the kidney.

6. Waxy Cast It is plain, of moderate refraction, wavy, constricted and fluted in appearance at places and shows brittleness. It indicates waxy degeneration of the kidney. *It is of rare occurrence.*

The location of the cast can be roughly ascertained by the size of it. The narrowest originate from the narrow tubules, the intermediary ones from the convoluted tubules; while the largest are from the straight collecting tubules. The larger the size of the cast, the graver the prognosis.

Pseudo-casts or False Casts. These are not thrown off portions from the tubules of the kidney, but are formed or substances aggregated together accidentally and look like casts in the slide. In reality these are not true casts and

hence have no clinical significance. They can be easily differentiated from the true casts by their irregular or undefined outline. They are generally of greater length and have no definite cells. When in doubt, the easy method to differentiate is to warm the slide a little over a spirit lamp, when pseudo-casts will disappear while true casts will remain.

1. Cylindroids or Mucus Cast. It is the aggregation of mucus and not a true cast.

2. Urate Cast. Sodium and ammonium urates clustered together, look like a cast. Moreover when they are aggregated over a hyaline cast, the diagnosis lies between a granular cast or urates, but their yellowish brown colour will at once prove its accidental formation.

3. Bacterial Cast. After the urine has stood for sometime, micrococci and bacteria are massed together and look like a cast.

4. Pus Cast. Pus cells aggregated together look like a cast.

5. Gonorrhœal threads. When they collect into a mass, they look like a cast.

6. Prostatic threads. They are seen as thin curling cotton threads, floating or sinking in the urine. These are mucus threads, in which are embedded pus cells and epithelium and look like a cast. They are found in chronic gonorrhœa, gleet and prostatitis.

7. Connective tissue fibres. These are formed generally in bundles, which run in wavy direction and look like a cast. They are found in urine, when there is intense inflammation in the genito-urinary tract.

Parasites.

(i) *Trichomonas or Pentatrichomonas.* It is very motile. The body of the organism is oval or spindle shaped with the posterior pole tapering, while from its anterior end four or five small filaments or flagella are given off and one of them is turned towards the back. Two or three minute bodies resembling nucleus are seen in the cell body. It is commonly found in the urine of females in Bengal,

who are suffering from leucorrhœa or from any acid discharges. It is mostly non-pathogenic and inhabit the external genitals.

(ii) *Filaria Sanguinis Hominis* (*Filaria Bancrofti*). It is very motile. It is a thread like cylindrical body, a blunt round head with a pointed tail. It is mostly found in the early morning urine, as the parasite is active during night, i.e. the resting hours of the patient. It is found in hæmaturia, chyluria and in elephantisis. In all cases of chyluria, filaria embryos should be searched for.

Ecchinococcus, Bilharzia Hæmatobia, Ascaris Lumbricoides, Oxyuris Vermicularis are rarely found in urine.

Micro-organisms. In healthy urine when voided no micro-organisms can be detected, but when urine has stood and exposed to the air for sometime, bacteria and cocci develop. They decompose the urine and make it turbid and ammoniacal. Some of the micro-organisms present in urine are harmless but others are pathogenic, of which streptococci, staphy-

lococci, gonococci, tubercle bacillus and Bacillus coli communis are the most important.

To detect them fully, it is proper in the female to take a catheter specimen, while in the male only cleaning the glans penis with soap and water is sufficient. The first portion is generally rejected, while the latter portion is collected into a sterile bottle.

Strepto and staphylococci are examined with the help of methylene blue stain. They can be recognised by their peculiar form, the former is in chain, while the latter is grouped in clusters.

Gonococcus. To find it out, allow the urine 2 or 3 hours to stand, when sediment will gravitate and then pipette off some of it and centrifugalise and select a thick portion of the sediment from the bottom. Draw it up with a pipette and put a drop or two in the centre of a clean glass slide and spread it out evenly. Allow it to dry and fix it by passing it a few times over a spirit lamp, keeping the specimen

side upwards. Drop a few drops of methylene blue solution, (preferably Lœffler's methylene blue solution) on the slide and wait for a few minutes. Wash in water, dry it in air and examine it under an oil immersion lens. Gonococci are kidney shaped diplococci lying side by side, their flattened surfaces facing each other. They are mainly intracellular, i.e. lying within the pus cells, but not invading the nuclei. They are at times extracellular—outside the pus cells, hence free. Frequently several groups of two, three, four or more pairs may be seen. They are stained deeper blue than the cell nuclei.

The best plan to detect gonococcus is to take fresh pus by touching a slide directly from the orifice of the urethra by gently squeezing the penis in male and from the genitals of the females and spreading and staining it in the usual method above described, as the examination from the urinary sediment is not always satisfactory.

Tubercle Bacillus. To detect the

tubercle bacillus from urine is an uphill work and is not worth the trouble, but in suppuration of the genito-urinary tract, tubercle bacillus should be searched from urine. In those cases always insist or take a catheter specimen under strictly aseptic conditions to avoid contamination from external genitals and to be free from smegma bacillus. The urine should be examined fresh. Centrifugalise it and then take a drop from the bottom, spread it thinly on a slide. Now hold the slide with a slide forceps, fix it by passing over the flame of the spirit lamp about three or four times and flood it with carbol fuchsin and holding it on a convenient distance over the spirit lamp, steam it on the flame for about five minutes, taking care not to dry it up; or take a small quantity of carbol fuchsin in a test tube and boil it over a spirit lamp and flood it on the film while still boiling and keep it for about five minutes. Now wash it in a tap or in a bowl of water. Drop a few drops of about 25% dilute sulphuric acid, till decolourised; (i.e. the red colour

is removed, but a faint pink colour is seen). Wash again in water. Counter-stain by dropping on the slide Lœffler's methylene blue for a minute. Wash, dry and examine under a 1/12th inch oil immersion lens; when bright, slightly curved red rods, either straight or beaded with rounded ends will be seen, if tubercle bacilli are present.

Bacillus Coli Communis. A surmise can be had of coli infection, if the urine is acid in reaction and there is pus in it. The easiest method to detect B. Coli is by Mac Conky's neutral red bile salt lactose media. Take in a test tube, some of this media and also place an inverted Durham's fermentation tube in it. Now add a little of the urine to be examined and incubate for 18 to 24 hours in an incubator at 37°C. If the urine contains B. Coli, (i) gas will be collected inside Durham's tube, due to fermentation; (ii) the media will turn pink, due to the formation of acid.

Foreign Bodies. These are innumerable, but are generally common from the following sources :—

(i) *Pubic Hair.* It is a common accompaniment in urine and can easily be recognised by the thin cylindrical elongated shape, the epidermic cells, pigments and lastly by the medulla.

(ii) *Wearing Apparel.* Fibres of cotton, wool and silk from the wearing apparel may be found in urine and their different shapes and character will diagnose the element.

Cotton—Twisted and wavy appearance.
Wool—Plain and striated.
Silk—Homogeneous with high refraction.

(iii) *Dusting Powder.* Starch granules and other medicinal substances used as dusting powder in the private parts may come with urine.

(iv) *Receptacle.* Fat or oil globule and any other substance, which the unclean receptacle may contain. Fat cells are recognised by their yellow colour, generally circular in outline and of moderate refraction. Any other subs-

tance, which the receptacle contained can be ascertained with a little care and diligence.

(v) *Atmosphere.* Dust particles, vegetable debris, scales, feathers etc., may contaminate the urine from the atmosphere.

(vi) *Slides and cover glasses.* Flaws and artefacts are sometime seen in slides and cover glasses during microscopical examination of urine. They have no bearing whatsoever.

Ehrlich's Diazo Reaction. The diazo reaction never takes place in normal urine, but when the urine contains some aromatic substances which yield a red colour in the presence of sulpho-diazo-benzol, produced by the action of nitrous acid with sulphanilic acid, the reaction is said to be positive.

Test. First of all, the two reagents should always be freshly prepared.

A. A saturated solution of sulphanilic acid to which is added 5% of hydrochloric acid.

B. ½% of sodium nitrite.

Mix equal quantity of urine and sulphanilic acid solution and pour 3 or 4 drops of nitrite solution and shake it thoroughly, till frothy. Now add liquor ammonia to make it strongly alkaline. If the reaction is positive, the solution is turned into a bright red colour, which is apparent also in the foam, which is pink

or rose red. On allowing the fluid to stand for twenty-four hours a precipitate forms, the upper margin of which is green or greenish black.

It is present when there is increased destruction and excretion of tissues containing nuclein and purin; hence it is found in cases of advanced typhoid fever, from the second week onwards, but in suspected or mild cases of enteric, the reaction may be absent. It is also found in pneumonia, typhus, advanced cases of tuberculosis, meningitis and in acute rheumatic fever. It was a means of diagnosis to old practitioners in place of Widal's in typhoid and for tubercle bacillus in sputum of phthisis.

Russo's Test. Take 5 c.c. of urine in a test tube and add 4 drops of a 1 in 1000 watery solution of methylene blue. If positive, the fluid will assume an emerald green colour, without any trace of blue. For control, take a similar test tube with 5 c.c. of ordinary water and add 4 drops of ·1% aqueous solution of methylene blue. Compare the two and declare your result.

It is generally found positive in typhoid, but may be found also in phthisis, measles and small-pox.

Urea concentration test of Mac Lean. The theory of this test is to surcharge the blood with urea and then to note the eliminating power of the kidney. The bladder is emptied and the patient is given by the mouth 5 grammes of urea dissolved in 100 c.c. of water. Urea has an unpleasant taste, so it can be masked by flavouring with some sweet or pleasant draught along with it. After an hour, all the samples of urine passed by the patient is preserved for urea estimation. According to MacLean, the percentage of urea in a normal person in the first sample should be at least 1·5%, the second 2%. The usual concentration is 2%; hence anything below 2% denote renal inefficiency. In nephritis, the elimination of urea becomes markedly diminished. It does not always prove that the kidney is not functionating well as it has got a fallacy. Urea is absorbed by the intestine hence anything impairing its absorption

will naturally lead to a lesser output by the kidney.

Urobilinogen Test. Di-methyl-para-amino-benzaldehyde solution 3% in hydrochloric acid. One or two drops of this solution should be added to 1 to 2 c.c. of filtered urine when it will turn into a pink colour; or heat the lower part if necessary to produce the colour.

This indicates defective liver function. It is present in influenza and kala-azar. It is sometimes found in pernicious anæmia, malarial cachexia and in lead and phosphorus poisoning.

APPENDIX I.

Solutions and Reagents.

Benedict's solution (Qualitative).

Copper sulphate (pure)	... 17·3	grammes
Sodium citrate (pure)	... 173	,,
Sodium carbonate (crystalline)	200	,,
or		
Sodium carbonate (anhydrous)	200	,,
Distilled water ...	... 1000	c.c.

The citrate and carbonate are dissolved with the aid of heat, in 700 c.c. of distilled water. The copper sulphate is dissolved in 100 c.c. of distilled water, in another phial. Mix the two solutions with constant stirring, cool and make it up with distilled water to 1000 c.c. Filter and keep it in a stoppered phial.

Benedict's solution. (Quantitative).

Copper sulphate (pure)	... 18	grammes
Sodium citrate (pure)	... 200	,,
Sodium carbonate (crystalline)	... 200	,,
or		
Sodium carbonate (anhydrous)	... 100	,,
Potassium thiocyanate	... 120	,,
Potassium ferrocyanide 5%	... 5	c.c.
Distilled water ...	... 1000	,,

The citrate, carbonate and thiocyanate are dissolved with the aid of heat, in 800 c.c. of distilled water. The copper sulphate is dissolved in 100 c.c. of distilled water, in another phial. Mix the two solutions with constant stirring, when cool add the ferrocyanide solution and make it up with distilled water to 1000 c.c. Filter and keep it in a stoppered phial.

Hypobromite solution.

Dissolve 100 grammes of caustic soda in 250 c.c. of distilled water. Label it caustic soda solution (stock solution). Now take 25 c.c. of this caustic soda solution and mix with 2·5 c.c. of bromine just before use. (Bromine is generally kept in sealed glass capsule, as it is caustic and care should be taken in handling it).

Standard silver nitrate solution.

Dissolve 29·063 or 30 grammes of pure fused silver nitrate in 1000 c.c. of water. The solution should be freshly prepared or when prepared should be wrapped up in a blue paper.

Loeffler's methylene blue solution.

Dissolve one soloid (B. W & Co) of methylene blue—0·1 gramme in 7 c.c. of absolute alcohol and add 25 c.c. of distilled water to which one drop of liquor potassæ B. P. has been added.

Carbol fuchsin solution.

Fuchsin ...	... 1 part.
Absolute alcohol	... 10 parts.
Carbolic acid 5% (aqueous solution)	... 90 ,,

Barium chloride solution.

Barium chloride	... 4 parts.
Hydrochloric acid	... 1 part.
Distilled water	... 16 parts.

Caustic soda solution.

4 grammes in 100 c.c.

Caustic potash solution.

5·6 grammes in 100 c.c.

Esbach's reagent.

Picric acid	... 10 grammes.
Citric acid	... 20 ,,
Distilled water (boiling)	800 c.c.
Distilled water (cold)	... 200 ,,

Aufrecht reagent.

Picric acid	... 15 grammes.
Citric acid	... 30 ,,
Distilled water	... 1000 c.c.

In these two reagents, picric acid coagulates the albumin, while citric acid dissolves the phosphates.

Nylander's reagent.

Bismuth subnitras	...	2 grammes.
Soda tartarata	...	4 ,,
Sodium hydroxide	...	10 ,,
Distilled water	...	100 c.c.

Mac Conky's media.

Sodium taurocholate	...	0·5%
Peptone ...	...	2%
Neutral red solution (1%)	...	0·5%
Lactose ...	...	0·5%
Aqua	Q.S. ...	100 parts.

APPENDIX II.

Conversion Tables.

To convert grammes per 100 c.c., into grains per ounce—multiply by 4·375.

To convert grammes per 100 c.c., into grains per gallon—multiply by 700.

To convert grammes per 1000 c.c. (litre), into grains per gallon—multiply by 70.

To convert grains per gallon, into grammes per litre—divide by 70.

To convert grammes to ounces avoirdupois—multiply by 20 and divide by 567.

To convert litres to pints—multiply by 88 and divide by 50.

Nylander's reagent.

Bismuth subnitras	...	2 grammes.
Soda tartarata	...	4 ,,
Sodium hydroxide	...	10 ,,
Distilled water	...	100 c.c.

Mac Conky's media.

Sodium taurocholate	...	0·5%
Peptone ...	...	2%
Neutral red solution (1%)	...	0·5%
Lactose ...	...	0·5%
Aqua	Q.S. ...	100 parts.

APPENDIX II.

Conversion Tables.

To convert grammes per 100 c.c., into grains per ounce—multiply by 4·375.

To convert grammes per 100 c.c., into grains per gallon—multiply by 700.

To convert grammes per 1000 c.c. (litre), into grains per gallon—multiply by 70.

To convert grains per gallon, into grammes per litre—divide by 70.

To convert grammes to ounces avoirdupois—multiply by 20 and divide by 567.

To convert litres to pints—multiply by 88 and divide by 50.

APPENDIX III.

RELATION OF WEIGHTS AND MEASURES.

1 metre = 39·37 inches. It is the ten-millionth part of a line drawn from the Equator to the Pole.

1 d.m. = 3·93 inches—4 inches.

1 c.m. = ·393 inch = $\frac{2}{5}$th of an inch.

1 m.m. = ·039 inch = $\frac{1}{25}$th of an inch.

u = micron = $\frac{1}{1000}$ millimetre.

1 inch = 2·5 c.m. = 25·4 millimetre.

1 grain = ·064 gramme = 64·8 milligramme.

1 gramme = 15·432 grains. It is the weight of a cubic centimetre of water at 4°c in Paris.

1 decigramme = 1·543 grain = 1½ grain.

1 centigramme = ·154 grain = $\frac{3}{20}$th grain.

1 milligramme = ·0154 grain = $\frac{3}{200}$th grain.

1 c.c. = 16·9 minim—17 minims = one millilitre. The measure of 1 gramme of water at 4°c.

1 drachm = 3·89 grammes.

1 ounce = 28·35 grammes.

1 lb. = 7000 grains = 453·6 grammes.

1 minim = ·059 c.c. = ·91146 grain.

1 fl. drachm = 3·54 c.c. = 54·68 grains.

1 fl. ounce = 28·39 c.c. = 437·5 grains.

1 fl. pint = 567·92 c.c. = 8750 grains.

1 fl. gallon = 4545·96 c.c. = 70000 grains.

1 litre = 1000 c.c. = 35·21 fluid ounces.

APPENDIX IV.

FORM OF URINE ANALYSIS.

Report on the Examination of Urine.

Of .....

Sent on .....*and examined the same day.*

Physical Examination:—

Quantity (sent for examination) ...
Colour ...
Appearance ...
Odour ...
Sediment ...
Specific Gravity ...

Chemical Examination:—

Qualitative—

Reaction ...
Albumin ...
Mucin ...
Excess of Phosphate
Carbonates ...
Sugar ...
Acetone ...
Diacetic Acid ...
Blood ...
Pus ...
Bile ...
Chyle ...
Indican ...

Quantitative—

(Not done unless asked and paid for)

Sugar ...
Albumin ...
Urea ...
Chlorides ...
Other Constituent ...

Microscopical Examination (of Centrifugalised Deposit.)

Unorganised deposits

Crystalline—

Uric Acid ...
Urates ...
Calcium Oxalate ...
Triple Phosphate ...
Cystin, Leucin and Tyrosin ...

Amorphous—

Urates ...
Phosphates ...

Organised deposits

Red Blood Corpuscles
Pus Corpuscles ...
Mucus ...
Spermatozoa ...

Epithelia—

Small round ...
Spindle form ...
Pavement form ...

Tube Casts—

Hyaline ...
Epithelial ...
Bloody ...
Granular ...
Fatty ...
Falsecasts ...

Filaria Sanguinis Hominis ...
Micro-Organisms ...
Other Products ...

Remarks :—

Dated..... .....*19* .